EVALUATION OF APPROPRIATE STORAGE CONDITIONS OF LIQUID BREWER'S YEAST AS FEED SUPPLEMENT FOR LACTATING DAIRY COWS AND ITS EFFECT ON MILK QUALITY

PETER ALPHONCE OBUONG ALARU

A thesis submitted to the Graduate School in partial fulfilment for the requirements of the Master of Science degree in Food Science of Egerton University

EGERTON UNIVERSITY

MAY, 2019

DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and to the best of my knowledge has not been presented for the award of any another degree elsewhere.

Signature _____ Date _____

Peter Alphonce Obuong Alaru

Reg. No. KM16/3383/12

Recommendation

This research thesis has been submitted with our approval as University supervisors.

Signature _____ Date _____

Prof. Alfred A. Shitandi, PhD

Department of Dairy, Food Science and Technology

Egerton University, Njoro, Kenya

Signature _____ Date _____

Prof. Symon M. Mahungu, PhD

Department of Dairy, Food Science and Technology

Egerton University, Njoro, Kenya

DEDICATION

This work is dedicated to my beloved wife Mrs. Jane Cherono Obuong and the entire family for commitment and support throughout the program.

ACKNOWLEDGEMENT

This is to express sincere gratitude to the Almighty God for providing me with strength and knowledge to accomplish this work. Appreciation to Egerton University's Department of Dairy and Food Science and Technology for having accepted to offer me a chance to pursue postgraduate studies at the institution. I sincerely appreciate mentorship, valuable input and constructive comments by my supervisors Prof. Alfred A. Shitandi, PhD and Prof. Symon M. Mahungu, PhD through their rich expansive knowledge and experience that enabled the success of this study. I thank Dr. J.M Muia for mentorship on research activities and continued guidance throughout the study period. I wish to appreciate the financial support by Eastern Africa Agricultural Productivity Project (EAAPP) through their Master's degree scholarship program and Kenya Agricultural and Livestock Research Organization (KALRO) for granting me full time study leave. I am grateful to the department of Dairy and Food Science and Technology for provision of facilities, the chairman Prof. A. Faraj for granting permission to access laboratory facilities past working hours and weekends and the entire support of departmental staff.

I salute staff of Happy Feeds Limited led by Mr Elisha Ondieki and their distributors for provision of liquid brewer's yeast samples and all farmers who participated in the study for finding time to provide information on dairy production and accepting on-farm trials. It is my hope that the outcome of this study will add value to dairy farming in the area and improve their economic status. Thanks to Githunguri Dairy Farmers Cooperative Society staff led by Manager Mr. Albert Mwangi, Extension Officers Mr. Paul Njoroge, Mr. Johan Chege, Mr. Moses Muigai and Laboratory technician Madam Joyce Wangari. I acknowledge participation of the Ministry of Agriculture, Livestock and Fisheries state department of Livestock Githunguri Sub-County officials Madam Virginia W. Karanja, Kihanya J. Mwangi and Ann W. Muriithi.

ABSTRACT

Milk production in Kenya is dominated by smallholder dairy farmers who are faced with challenges on feeding dairy cows. This is generally due to inadequate and low quality feeds and high cost of inputs. Thus, utilization of inexpensive nutrient dense feed supplement such as liquid brewer's yeast (LBY) is inevitable. However, LBY is rarely used due to its short shelf life and lack of technical information on effect on milk quality. This study was performed to investigate the appropriate storage conditions of LBY and to ascertain its suitability for use as alternative feed source for dairy cows without compromising on milk quality in smallholder dairy farms in Githunguri Sub-County, Central region in Kenya. First phase of the study involved collection of samples from three source of LBY (supplier, distributors and farmers), stored under aerobic condition at 10, 20 and 30 °C, then tested at days 0, 7, 14 and 21 in a 3 x 3 x 4 factorial arrangement. The parameters tested were total viable counts (TVC), total coliform counts (TCC), lactic acid bacteria (LAB), yeast and mould. The second phase entailed evaluation of milk for physicochemical: butter fat, milk protein, solid not fat, lactose, ash, freezing point depression, electrical conductivity (EC) and microbiological: TVC and TCC parameters. Thirty farms were randomly selected from three milk delivery routes. A longitudinal survey was conducted where farms were nested within routes and equal number of farms selected per route based on supplementation of lactating cows with either LBY or commercial dairy meal (CDM). A repeated measure analysis was performed using linear mixed models methodology by PROC MIXED of SAS for milk quality and questionnaire data summarized using descriptive statistics. The results revealed statistically significant levels ($p<0.05$) on sources and storage time of LBY while the interventions were insignificant ($p>0.05$). The results on milk quality indicated significantly ($p<0.05$) higher milk protein levels and low freezing point depression for milk from LBY supplemented cows (3.07 ± 0.03% and -0.532 ± -0.005 °C) than those supplemented with CDM (2.99 ± 0.03% and -0.516 ± -0.005 °C) respectively. This was an indication of increase in total solids. The study concluded that appropriate storage time of LBY is seven days, thereafter, a significant increase in microbial load is observed. Conversely, the use of LBY as a protein feed supplement improved milk quality. The research recommends capacity building to enable stakeholders in the dairy industry appreciate the importance of hygienic storage and utilization of LBY as alternative protein source for dairy cows.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND ACRONYMS

ADF	Acid Detergent Fibre
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
BGA	Brilliant Green Agar
BPW	Buffered Peptone Water
CAC	Codex Alimentarius Commission
CDM	Commercial Dairy Meal
CLA	Conjugated Linoleic Acid
CP	Crude Protein
DBG	Dried Brewers' Grains
DLPO	District Livestock Production Officer
DM	Dry Matter
DMI	Dry Matter Intake
EABL	East Africa Breweries Limited
EC	Electrical Conductivity
EFSA	European Food Safety Authority
ELISA	Enzyme Linked Immunosorbent Assay
EMB	Eosin Methylene Red
EU MRL	European Union Maximum Residue Limit
FAMEs	Fatty Acid methyl Esters
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration of United States
IMViC	Indole Methyl Red Voges-Proskauer Citrate Test
KALRO	Kenya Agricultural and Livestock Research Organization
LAB	Lactic Acid Bacteria
LBY	Liquid Brewer's Yeast
LST	Lauryl Sulfate Tryptose Broth
MBA	Methyl Blue Agar
MPN	Most Probable Number
MRT	Milk Ring Test
NIRS	Near- Infra- Red Spectrotrophy
PCA	Plate Count Agar
SARA	Sub Acute Ruminal Acidosis

SAS	Statistical Analysis Systems Software
SNF	Solid Not Fat
TCC	Total Coliform Count
TVC	Total Viable Count
WBG	Wet Brewers' Grains
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

1.1. Background

Kenya has experienced spectacular growth in the dairy sub-sector both in terms of the number of dairy cattle and milk production since its liberalization in 1992 (Republic of Kenya, 2010). The dairy industry accounts for about 4% of Gross Domestic Product (GDP). The sub-sector has a herd of over 3.5 million heads of pure bred dairy cattle and cross breeds and 9.3 million indigenous cattle, with annual production estimated at 5 billion litres of milk (FAO, 2011; Muia *et al.,* 2011; Gichohi, 2014), making it the most developed in Sub-Saharan Africa. Milk production in Kenya is dominated by smallholder dairy farmers estimated at over 1.8 million distributed all over the country and contribute more than 80 percent of the total milk produced in the country (Republic of Kenya, 2010). The sub-sector provides employment opportunities both in the formal and informal sectors starting at the farm level to the processing and marketing sectors, thereby contributing directly to poverty reduction and improved household income (Muriuki, 2011). This notwithstanding, food insecurity, low income and poverty are still major challenges among smallholder dairy farmers mainly due to inadequate and low quality feeds, and high costs of inputs. The high cost of commercial dairy meal (CDM) has led to low levels of supplementation among smallholder farmers, leading to low milk production.

In order to improve productivity in smallholder farms, there is need to feed inexpensive and nutrient dense feeds to the dairy cattle. However, supplementation with the available conventional protein sources such as cotton seed cake, soya bean meal, fish meal and sunflower seed cake which can be used to formulate feed rations for dairy cattle is hampered by the high costs. Therefore, use of alternative high protein feed supplement such as liquid brewer's yeast (LBY) that is four times cheaper than the conventional protein sources is inevitable. If proven to be a viable option, then introduction of such in-expensive protein source can play a significant role in enhancing dairy development in the country as it can be used by resource poor smallholder dairy farmers.

By-products from the brewing process include wet and dry brewer's grain, brewer's condensed soluble, and liquid and dry brewer's yeast which pose serious disposal challenges to the industry (Kerby and Vriesekoop, 2017). The use of sewage lines and landfills as methods of brewers' by-products waste disposal are expensive and unsustainable options (Kerby and Vriesekoop, 2017). In order to reduce waste disposal costs, brewing industries sell these by-products as feedstuffs for both ruminants and non-ruminant nutrition (Westendorf *et al*., 2002).

Brewer's yeast is an excellent source of protein of high biological value and digestibility and water soluble vitamins (Kerby and Vriesekoop, 2017). However, Liquid brewers' yeast (LBY) is seldom used as it spoils quickly.

The production of LBY by East Africa Breweries Limited (EABL) is estimated at 20,000 litres per day but only 10% is dried because of the high costs involved and the rest is sold in liquid form. The LBY supply-chain originates from the EABL Ruaraka factory to Happy Feeds and Distributors then to farmers. It is estimated that over 15% of farmers in Githunguri supplement dairy cattle with LBY. However, technical information on appropriate storage methods of LBY under our local conditions and its effect on milk quality is non-existent. This study examined the effect of handling practices, temperature and time on microbial growth during storage of LBY through analysis of different samples obtained along the supply chain from supplier, distributors and farmers. Evaluation of milk quality from cows supplemented with LBY was carried out by investigation of both physicochemical and microbiological quality of milk.

1.2. Statement of the problem

Liquid brewer's yeast is a by-product from the brewing industry used as a supplement for dairy cows. However, there is concern on liquid brewer's yeast short shelf life that presents a problem on its storability, and past experience by local dairies showed that its use as feed supplement for lactating dairy cows led to production of milk with inferior quality that negatively impacted on processability of milk products such as yoghurt, cheese and butter. Information regarding storability and the effect on milk quality is lacking. This study sought to establish appropriate temperature/time combination for storage of LBY and its effect on milk quality.

1.3. Objectives

1.3.1. General objective

The study was to develop appropriate storage conditions of liquid brewer's yeast as feed supplement for lactating dairy cows and contribute to food security by improving on milk quality.

1.3.2. Specific objectives

The specific objectives of this study were:

i. To determine the effect of temperature and storage period on microbial growth on liquid brewer's yeast.
ii. To determine the effect of feeding liquid brewer's yeast on milk quality.

1.4. Hypotheses

The following null hypotheses were tested at 0.05 significant levels:

i. Temperature and storage period has no effect on microbial growth on liquid brewer's yeast.
ii. Feeding liquid brewer's yeast has no effect on milk quality.

1.5. Justification

Milk production in Kenya is dominated by smallholder dairy farmers who are faced with challenges on feeding dairy cows. This is generally due to inadequate and low quality feeds and high cost of inputs. Thus, utilization of inexpensive nutrient dense feed supplement such as liquid brewer's yeast (LBY) is inevitable. However, LBY is seldom used due to its short shelf life and lack of technical information on effect on milk quality. This study will contribute to enhancement of the improved storage condition in liquid form and minimize spoilage of liquid brewer's yeast. The findings are of great importance to food process industry, dairy sub-sector and have sustainable and environmentally sound management through appropriate technologies use. The technical information would contribute to an enhanced storage life which would reduce the cost of liquid brewer's yeast compared to conventional protein feeds and lead to cost effective production of milk with improved quality.

CHAPTER TWO
LITERATURE REVIEW

2.1. Brewing process

Brewing is a fermentation process which involves starch hydrolysis and the use of *Saccharomyces cerevisiae* resulting in ethanol and resulting by-products (Wunderlich and Back, 2009; Hemalatha *et al*., 2015; Walker and Stewart, 2016). The brewing process starts with malting, barley germination, stabilization and product flavour developed (Briggs *et al.,* 2004; Gupta *et al*., 2010; Milala and Addy, 2014; Zhang and Li, 2017). The malt is milled, mixed with warm or hot water and wort filtered (Szwajgier, 2011; Milala and Addy, 2014). The process enhance maximum extraction and fermentation in minimum time (Sakamoto and Konings, 2003; Szwajgier, 2011; Sammartino, 2015). The duration is 1-2h with a varied temperature range of 30 - 72°C to facilitate different enzyme reaction. Filtration enhances removal of coarse non soluble malt components and spent grains (Lewis and Young, 2001; Sammartino, 2015). The resulting wort is boiled with hops to inactivate enzymes and microorganisms from raw materials, modify flavour (Bokulich and Bamforth, 2013), colour and precipitate haze precursors within 0.5-1.5h at elevated temperatures of over 100 °C. The brewery adjuncts and process aids can be added at this point to supplement part of the malt starch (Sammartino, 2015). The boiling extracts hops and gives beer its typical bitterness which inhibits growth of many Gram-positive bacteria (Briggs *et al.*, 2004; Bokulich and Bamforth 2013; Walker and Stewart, 2016). Wort clarification may be performed by straining, settling or centrifugation to remove spent hops in < 1h at 80-100 °C. The wort is cooled and oxygenated by passing through a heat exchanger to 12-18 °C in < 1h and oxygen injected to create favourable condition for yeast growth during fermentation (Sakamoto and Konings, 2003; Sammartino, 2015).

During fermentation stage, the brewer's yeast strains which are traditionally divided into lager or bottom fermenting (*Saccharomyces pastorianus*) cultivated at 8-14°C and ale or top fermenting (*Saccharomyces cerevisiae*) cultivated at 15-26 °C (Wunderlich and Back, 2009; Stewart, 2016) are added to wort. The two strains convert fermentable sugars to ethanol, carbon dioxide, production of flavour compounds and purge unwanted volatiles such as hydrogen sulphide (Hornsey, 2013; Walker and Stewart, 2016) by evolution of carbon dioxide along with a drop in pH from 5.0-5.2 to 3.8-4.0 in 2-7 days (Sakamoto and Konings, 2003; Sammartino, 2015). Yeast for further fermentation is obtained, desired specific gravity controlled and green beer produced from wort (Sammartino, 2015) which requires 7-21 days at 1-14 °C to mature during aging and conditioning stage. The beer develops typical aroma,

flavour and carbon dioxide levels adjusted (Priest and Campbell, 2003; Walker and Stewart, 2016). Yeast and suspended solids are removed during clarification through filtration at -1-0 °C for 1-2h of mature beer to obtain bright beer which is subjected to biological stabilization through either sterilized filter at -1-0 °C or flash pasteurization of bulk product at 73-75 °C for 1-2min or bottle pasteurization at 70-72 °C for 20-25min to remove or destroy any microorganisms (Lewis and Young, 2001: Priest and Campbell, 2003). In bottle pasteurization is a more severe process and ensures microbial stability when properly performed as post-pasteurization contamination is contained (Lewis and Young, 2001).

The mature pasteurized beer has a combination of antimicrobial factors. It is acidic (pH 3.8–4.7) and contains ethanol 0.5–10%, w/w, hop bitter compounds *ca*. 17–55 ppm iso-α-acids and sulphur dioxide, has low levels of oxygen < 0.1 ppm and nutrients as well as a high content of carbon dioxide 0.5%, w/w (Sakamoto and Konings, 2003; Sammartino, 2015).

The three main by-products from brewing process of significant use as feedstuffs in the ruminant nutrition industry are brewer's condensed soluble, brewer's yeast and wet or dry brewer's grain (Westendorf *et al.,* 2002). The by-products are removed from the brewing process at different stages starting with brewer's condensed soluble, wet and dried brewer's grains are then removed from the brewing process before addition of yeast to start fermentation process; as a result, the first two by products contain no brewer's yeast. Fermentation is thus allowed to continue upon completion of which cooling of beer takes place and the yeast drops to the bottom of the fermentation vessel where it is drained from the beer (Rijnders *et al*., 2000).

2.2. Yeasts

Yeasts are unicellular fungi classified based on cell, ascospore, colony (Schneiter, 2004; Brandt and Warnock, 2015; Goddard and Greig, 2015) and physical characteristics that include ability to ferment sugars for production of ethanol and resulting by-products (Wunderlich and Back, 2009; Azhar, *et al*., 2017). Phylum *Ascomycetes*, class *Hemiascomycetes* are true fungi comprising of budding yeasts whereas true yeasts are in main order *Saccharomycetale* (Schneiter, 2004; Brandt and Warnock, 2015). They are found in natural habitats such as soil, plant leaves and flowers, salt water, skin surfaces or intestinal tracts of warm blooded animals in symbiotic if not parasitic forms (Azhar *et al.,* 2017). Multiplications are by single cell division (budding) as in *Saccharomyces cerevisiae* or direct division (fission) in *Schizosaccharomyces* or simple irregular filaments (mycelium) (Schneiter, 2004; Azhar *et al.,* 2017). Nutrient requirements include carbon, nitrogen, phosphate, sulfur and amino acids (Ljungdahl and Daignan-Fornier, 2012). The growth and behavior of yeast

cells can change in concurrence to available nutrients and are able to alter the length of cell cycle over at least 10 fold ranges (Petti *et al.,* 2011). They have several developmental programs engagements depending on the particular nutritional requirement ranging from rapid mitotic growth in rich media to filamentous growth under limited nutrient conditions (Boer *et al.,* 2010; Petti *et al.,* 2011; Broach, 2012).

The strain *Saccharomyces cerevisiae* is the most studied eukaryotic model organisms in molecular and cell biology (Briggs *et al.,* 2004; Azhar *et al.,* 2017). Their cellular mechanism of replication, recombination, cell division and metabolism are typically conserved between yeasts and larger eukaryotes (Jouhten *et al.*, 2016). Yeast has been used widely in baking, winemaking and brewing since ancient times (Hornsey, 2013; Jouhten *et al.,* 2016). Nonetheless the use of yeast of the genus *Saccharomyces* as part of nutritional supplement and health food realms in human (Llopis *et al.,* 2014) and animals is of interest due to their well-known probiotic effect (Jakobsen and Narvhus, 1996; Lourens and Viljoen, 2001; Buchl *et al.,* 2010; Moslehi-Jenabian *et al.,* 2010; Llopis *et al.,* 2014). Commercially used yeast strains for humans are *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae var boulardii* (Sargent and Wickens, 2004; Czerucka *et al.,* 2007; McFarland, 2010; Azhar *et al.,* 2017). Food and Drugs Administration U.S. FDA, (2018) has indicated that *Saccharomyces cerevisiae* possess the generally recognized as safe (GRAS) status with the same approval of its five probiotic strains (NCYC SC 47, NCYC 1026, CNCM I-1077, CNCM I-1079 and MUCL 39885) by the European Union for application in animal feeds (Buchl *et al*., 2010; European Food Safety Authority, 2014). Additional yeasts such as *Candida pintolopesii, Candida utilis* and *Candida saitoana* (Bovill *et al*., 2001; Leuschner *et al.,* 2004) and *Aspergillus* species are used in animal feeds (Lee *et al.,* 2006) with approval of GRAS status for *Aspergillus oryzae* by FDA and supported by the World Health Organization (FAO and WHO, 1987).

2.3. Food spoilage

Spoilage of food is a metabolic process which makes food unacceptable or undesirable for consumption due to changes in sensory characteristics (Rawat, 2015), but may not necessarily cause illness in absence of toxins or disease causing pathogens. Food spoilage may be caused by infestation of food by insects and rodents, visible parasites which render food undesirable, degradation of pigments, fats and proteins (off-flavors and odors) (Hammond *et al.,* 2015). Production of stimulating pigments (greening in potatoes) caused by light, microbial growth and metabolization of food, presence of air especially oxygen oxidizing lipids producing strong off-flavors and odors are known to have negative effect on food (Rawat, 2015). Less moisture

and physical effect of excessive heat or freezing temperature can affecting texture and breaking emulsions of food. Phenol compounds or pectin degradation from plants oxidized by endogenous enzymes are also known to cause food spoilage (Doyle, 2007). The factors are interrelated as increase in water activity, oxygen and temperature can speed up endogenous enzyme and microbial activities (Hamad, 2012).

Successful utilization of nutrients in a food are determined by several factors which include water activity and type of solute, pH, oxygen and carbon dioxide levels, temperature (storage and processing), available nutrients, solid or liquid state of food and preservatives (Hamad, 2012). Designing of prediction models normally target spoilage microbials on different foods and specific organisms examined singly or in combination (Hammond *et al*., 2015) to predict the commencement and course of spoilage process. The determinants for microbial growth in food are water activity, food matrices, temperature, pH, additives and food preservatives (Hamad, 2012).

2.3.1. Bacteriology of beer and brewing by-products spoilage

Beer is prone to microbial spoilage at every stage of production from various sources (Briggs *et al.,* 2004; Bokulich and Bamforth, 2013; Muller-Auffermann *et al.,* 2015). The microbial contaminations are divided into primary, originating from production area and secondary from filing area (Back, 2005; Vriesekoop *et al.,* 2012). Primary contamination may present a major challenge in the brewing industry and can lead to spoilage of the whole production batch (Bokulich and Bamforth, 2013) whereas secondary contamination mostly affect un-pasteurized beer that may only be some packages in the brewing process (Muller-Auffermann *et al.,* 2015). The possible spoilage microorganisms during beer production process vary in range and types depending on nutrients, oxygen and natural antimicrobials level (Wunderlich and Back, 2009; Vriesekoop *et al.,* 2012). When wort is converted to beer, anaerobiosis condition is created and microbial survival level reduced in finished beer. *Lactobacillus* and *Pediococcus* strains are hop-resistant and may account for most spoilage cases (Back, 2005; Suzuki *et al.,* 2008 Vriesekoop *et al.,* 2012; Muller-Auffermann *et al.,* 2015). The changes in beer may range from minor to gross off-flavours and aroma defects, turbidity, reduced yeast crops and abnormal attenuation rates (Bokulich and Bamforth, 2013).

Traditionally beer was considered to be a microbiologically safe beverage as standard beer does not support growth of food pathogens (Azhar *et al.,* 2017). However, Haakensen and Ziola, (2008) reported presence of *Bacillus cereus* and *Bacillus licheniformis* with strains able to cause food poisoning in home-brewed beers. The strains which were isolated grew in

commercial beer at pH of 4.8-5.2 and alcohol level of 4-5% v. Harmful metabolites like N-nitrosamine or biogenic amines may be produced by some spoilage bacteria during brewing process or in finished beer (Priest and Campbell, 2003; Van Vuuren and Priest, 2003).

2.4. Dairy cattle nutrition

2.4.1. Utilization of liquid brewer's yeast in Kenya

The production of Liquid brewer's yeast (LBY) by East Africa Breweries Limited (EABL) is about 20,000 litres per day in Kenya and 10,000 litres per day in Uganda thus a total of 30,000 litres per day. The by-product is subjected to autolysis at 80°C for 45 seconds-1 minute to destroy all yeast cells and cooled to a temperature of 50°C. Thereafter, viability test is carried out to ensure that all viable cells are destroyed. The autolysis process is repeated should the test turn positive. The aim is to safeguard use of the by-product by unscrupulous persons for production of other alcoholic beverages and protect animals against plasma ethanol toxicity that can occur when animals are fed more than 2.3 litres of live yeast cells. The by-product is then supplied to distributors and farmers under brand name of 'Chachu' (Muema, 2018).

Happy Feeds Limited has sole access to the by-product from EABL. The supply chain is from EABL to Happy Feeds Ltd, distributors and finally to farmers. However, large scale farmers collect the by-product directly from Happy Feeds Ltd depot at EABL in Ruiru. The price of Chachu at EABL is Kes 2.50 per litre. Happy Feeds Ltd sell it to distributors within 42 Kilometre radius from Ruiru depot at Kes 5.00 and Kes 6.00 for distance above 42 Km or places within the depot proximity but with heavy traffic that can lead to slow traffic movement like Ngong. The specific days of LBY supply to individual distributors vary depending on the sale which is affected by weather and season. Maximum utilization of LBY is during dry season when there is shortage of green fodder and farmers generally feed hay, silage and crop residues to animals while miminal use is during cold weather. Distributors mainly clear their stock in two to three days and refilling done thereafter. However, there are some middlemen who purchase the by-product in bulk from distributors and then sell to farmers. This may compromise the quality as adulteration of the by-product may take place before re-sell to farmers by the middlemen (Muema, 2018).

The by-product is used for ruminant and non-ruminant feeding in Kenya. However, the main use of the by-product in Githunguri is cattle feeding. Lactating dairy cows are fed 4 litres per day thus 2 litres per milking while other herds of cattle are fed 2 litres per day. It is a cheap protein source with crude protein level of between 32.8%-33% and this is the main reason for

its attractiveness in the region. Farmers in Githunguri acknowledged optimum milk production upon LBY supplementation (Muema, 2018).

2.4.2. Probiotics in animal nutrition

Degradation of dietary compounds in the rumen is by anaerobic microorganisms mainly bacteria and protozoa found in rumen fluid (Castillo-Gonzalez *et al.*, 2014). The ecosystem of rumen has a significant effect on ruminant response to diet (Desnoyers *et al*., 2009; De Nardi *et al.,* 2016). The current definition adopted by FAO and WHO defines Probiotics as foods containing live microorganisms which when consumed confer health benefits on the host (Vibhute *et al.,* 2011; Nagpal *et al.,* 2012; Fijan, 2014). The use of microorganisms as probiotics in animal nutrition was due to its verified effectiveness on the gut flora (Flint *et al.*, 2012; Yáñez-Ruiz *et al.,* 2015). The application of strains separately and in combination improved efficacy of feed intake, conversion rate, daily weight gain and total body weight in non-ruminants and ruminants (Musa *et al.,* 2009; Retta, 2016). It is important to understand the way probiotics exert their beneficial effects in target species. Their application in feeds for various animals is to substitute nutritive antibiotics or chemotherapeutics (Musa *et al.,* 2009; Retta, 2016; Khan *et al*., 2016). Probiotic bacteria are both host and strain specific and a combination of different strains can increase a range of protective actions (Lima-Filho *et al.,* 2000; Fijan, 2014; Uyeno *et al.,* 2015). Appropriate selection of probiotics for use in animal feeds is regarded as primary requirement and multiple strain use has shown active broad spectrum action against micro-organisms (Musa *et al.*, 2009). According to study by Uyeno *et al.* (2015), effective action of bacterial probiotics are towards non ruminants and pre-ruminants calves while yeasts and fungal such as *Saccharomyces cerevisiae* and *Aspergillus oryzae* have superior results in adult ruminants. Vibhute *et al.* (2011) indicated that animals fed with probiotic supplements have improved benefits on milk yields, milk protein and solid-non-fat components. *Aspergillus oryzae* and *Saccharomyces cerevisiae* indicated increase in milk production, milk solid-not-Fat (SNF) and tended to boost milk protein percentage in dairy cows (Yu *et al.,* 1997; Kalmus *et al.,* 2009; Shreedhar *et al.,* 2016).

2.4.3. Application of probiotics in lactating dairy cows ration

The use of yeast in both fundamental and biotechnology research is vast and *Saccharomyces cerevisiae* is the most used eukaryotic model (Briggs *et al.,* 2004: Dikicioglu *et al.,* 2013; Giannattasio *et al.,* 2013). The relevance of this microorganism is derived from its ability to trigger stress response to adapt to new adverse environmental conditions (Sousa *et al.,* 2012; Giannattasio *et al.,* 2013; Święciło, 2016). Subclinical ruminal acidosis has been

reported upon feeding high-concentrate diets and in rumen at reduced pH < 6.25 (Sauvant *et al.*, 2006: Krizova *et al.*, 2011). Longer periods of low rumen pH inhibit feed intake (Owens *et. al.*, 1998; Hernandez *et al.*, 2014) and low acetate-to-propionate ratios alter volatile fatty acids profile in the rumen (Owens *et al.*, 1998; Kleen *et al.*, 2003; Sauvant *et al.*, 2006; Hernandez *et al.*, 2014). According to Castillo-González *et al*. (2014), accumulation of lactic acid bacteria was observed at low pH. Improved nutritional value of poor quality forages and high grain diet, increase in numbers of rumen lactate-consuming bacteria, prevention of lactate accumulation and drop in rumen pH was reported on addition of *Saccharomyces cerevisiae* to feeds (Arambel *et al.*, 1990; Newbold *et al.*, 1996; Beauchemin *et al.*, 2003; Krizova *et al.*, 2011; Castillo-González *et al.*, 2014). The number of cellulolytic bacteria, fiber degradation and changes in Volatile fatty acid (VFA) in the rumen trigger benefits in milk production (Martin and Nisbet, 1990; Chaucheyras-Durand *et al.*, 2012; Poppy *et al.*, 2012). The ability of yeast to induce superior results had been previously reported by Auclair, (2001). Marsola *et al.* (2010) however, stated reduction on ruminal lactate concentration upon feeding yeast probiotic to Holstein dairy cows during summer. Effective action of yeast on animals is clearly seen when animals are under heat stress through improvement on feed intake and milk yield (Arambel *et al.*, 1990; Huber, 1990; Schingoethe *et al.*, 2004; Giannattasio *et al.*, 2013; Święciło, 2016). The same results were shown on a study by Bruno *et al.* (2009) in which effect of feeding a culture of *Saccharomyces cerevisiae* to lactating cows on performance during heat stress was evaluated and improved milk yield and composition of cows on late lactation was achieved.

In an elaborate meta-analysis study by Desnoyers *et al*. (2009) on 110 papers, 157 experiments and 379 treatments aimed at investigating quantitative effect of live yeast supplementation on intake, rumen fermentation and milk production and to identify major differences in experimental conditions between studies that can affect response to treatments, the result indicated an average increase on rumen pH of 0.03 and volatile fatty acid concentration of 2.17m*u.* A similar average increase was observed on dry matter intake (DMI) of 0.44g/kg of body weight (BW), total-tract organic matter digestibility of 0.8%, milk yield of 1.2g/kg BW and milk fat content at 0.05% while no influence was recorded on acetate-to-propionate ratio and milk protein content. A study by Vibhute *et al.* (2011) in which four multi-strain probiotics were used with two bacteria strains: *Lactobacillus acidophilus* and *Propionibacterium frendenreichii* and two fungi strains: *Saccharomyces cerevisiae* and *Saccharomyces boulardii,* the findings of improved milk production and composition were confirmed.

2.4.4. Probiotics mechanism of action

A summary of four mechanisms which enable probiotics to effect protective action were given by Musa *et al.* (2009) as antagonism through production of antimicrobial substances, competition with pathogens for adhesive sites or nutritional source, immunomodulation of host and inhibition of the production of bacterial toxins. The first three mechanisms explain the action of lactic acid bacteria whereas the last two are characteristics of yeast action (Musa *et al.,* 2009). Several activities take place in rumen by inhabitant micro-organisms that may lead to an increase in fermentation and acid production that increases ruminal pH. Unless buffering system counter the acids rise, it may lead to, reduced feed intake, microbial metabolism and negative nutrient degradation results like inflammation, acidosis, diarrhea, laminitis and low milk fat (Chaucheyras-Durand *et al.,* 2012). Fungal probiotics effect benefit by stimulation of inhabitant fungi (Huber, 1990; Matsubara *et al.,* 2016) and an increase in cellulolytic bacteria in the rumen (Dawson *et al.,* 1990; Retta, 2016). This mode of action was also supported by findings reported by Robinson and Erasmus, (2009) in a systemic review of the literature from peer review publication since 1990 on use of *Saccharomyces cerevisiae* (yeast products) on lactating Holstein cows. The findings indicated that it allows rumen microbes to increase fiber fermentation, and decreased lactic acid concentration which reduces rumen pH thereby improving rumen metabolism. Microbial activities are stimulated by probiotics which leads to increased nitrogen use by rumen (Newbold *et al.,* 1998; Goto *et al.,* 2016; Retta, 2016). The efficiency involves ammonia use to microbial protein, altered metabolism of endogenous nitrogen and improved flow and acid absorption (Erasmus *et al.,* 1992; Goto *et al.,* 2016).

Adequate balance between soluble nitrogen and carbohydrates supply enables live yeast to enhance microbial growth ensuring incorporation of digested carbohydrates into microbial mass and optimized fermentation achieved with no wastage in form of volatile fatty acids (Chaucheyras-Durand *et al.,* 2008; chaucheyras-Durand *et al.,* 2012). A study by De Ondarza *et al.* (2012), in which sodium bicarbonate was used as buffer to counter pH fall in dairy cows, higher pH retention was indicated on cows fed live yeast as compared to control at 6.22 vs. 6.03. The pH was stable and any drop below 5.6 was within a short time. However, Marden *et al.* (2008) in an elaborate study in which a comparison between live yeast (*Saccharomyces cerevisiae*) and Sodium bicarbonate effect on stabilization of ruminal pH, mode of action and total tract apparent digestibility in dairy cows was determined, mean pH was greater in sodium bicarbonate and live yeast compared to control, an indication of stabilizing effect in both additives. In the same trial a lower redox potential (Eh) and Clark's exponent (rH) was shown

on live yeast. The result indicated ability of *Saccharomyces cerevisiae* to prevent accumulation of lactate and facilitates better fiber digestion while only exogenous buffering effect was shown on sodium bicarbonate.

2.4.5. Safety aspects in eukaryotic probiotics use

Probiotics are generally safe but may sometimes cause complications and side effects on susceptible individuals. The European Food Safety Authority assigned most yeast probiotics Qualified Presumption of Safety Status (Huys *et al.,* 2013; Laulund *et al.,* 2017). They are considered safe products with unusual association to outbreaks or food borne illness (Huys *et al.,* 2013). Few cases however have been reported in immunosuppressed and debilitated humans using biotherapeutic products containing *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae var boulardii* (Niault *et al.,* 1999; Rijnders *et al.,* 2000; Cassone *et al.,* 2003; Huys *et al.,* 2013).

Low to moderate nature of virulence has been reported in animal model studies of *Saccharomyces cerevisiae* and *Saccharomyces cerevisiae var boulardii* (McCullough *et al.,* 1998; Perez-Torrado and Querol, 2016). The ability of *Saccharomyces cerevisiae* to penetrate intestinal mucosa of animals to reach other organs was evidenced in immunosuppressed mice trial (Llopis *et al.,* 2014). The immunosuppression and disruption of mucosal integrity by *Saccharomyces cerevisiae* was indicated in a study by Llopis *et al.* (2014) which are comparable to risk factors emerging in human infections. Nonetheless, findings by Pereyra *et al.* (2011) detected presence of fungal and mycotoxin contamination in malt and brewers' grains that can pose risk to animals and human health.

2.5. Influence of nutrition on milk production and its composition

2.5.1. Influence of brewing by product on milk production

Milk production is driven by several factors including dry matter intake and the quality of feedstuffs used (Alqaisi *et al.,* 2014). Earlier research had indicated no significant influence on milk production on supplementing or completely replacing the protein source in the diet with wet brewer's grain (Hoffman *et al.,* 1988). The findings were supported by West *et al.* (1994) upon replacement of a portion of the ground corn/soy bean meal concentrate mix and found similar milk production among all treatments with no differences in milk production on replacing forage with wet brewer's grain (West *et al.,* 1994). Brewing by-products inclusion level in the diet could have an effect on milk production due to possible reduction in dry matter intake (DMI) (Faccenda *et al.,* 2017) but inclusion of up to 30% of the diet DMI with wet brewer's grain did not indicate differences in milk production in studies conducted by West *et*

al. (1994). However, a significant (P <0.10) increase in milk production was recorded when liquid brewers' yeast was added along with 30% wet brewer's grain compared to 30% wet brewer's grain (West *et al.,* 1994). The increase was attributed to possible enhanced ruminal environment from the yeast and the numerical increase in DMI (West *et al.*, 1994). Despite the fact that there were no significant differences in milk production, there were variations in milk protein or fat produced from cows supplemented with wet brewer's grain in some research. A study by Faccenda *et al.* (2017) recorded increase in milk production on feeding dried brewers' grain (DBG) compared to soybean meal (SBM) which was due to reduction on DMI, crude protein and non-fiber carbohydrates with increase on levels of DBG in the diet. However, a decreased milk protein percentage was observed in heat stressed cows receiving WBG at either 15 or 30% with a dietary ether extract of 3.5% and 4.2% respectively, compared to 3.2% for the 0% WBG fed cows (West *et al.,* 1994). The increased dietary fat content of WBG diets could be the contributing factor to decreased milk protein percentage. Davis *et al.* (1983) found similar results when pressed brewers grains were fed at 40% and then compared to the control diet, 0% pressed brewers' grains. Conversely, a comparison on amount of fat, protein and total solids by Faccenda *et al.* (2017) revealed linear reduction with increase on DBG. Yet, earlier comparison of percent and amount of fat by Polan *et al.* (1985) indicated no significant differences when comparing milk fat percent, but yield of milk fat (kg) produced was significantly higher when WBG was compared to the basal diet with considerable overall interaction in milk fat percent and a trend in fat yield for the high protein level. The trend was different according to a study by Miyazawa *et al.* (2007) in which 9.3% of diet DM as WBG had a tendency to have higher milk fat percentages but not milk fat quantity. In two trials with pressed brewer's grains, higher milk fat percentage was reported in diets with up to 40% inclusion compared to the control diet (Davis *et al.,* 1983).

2.5.2. Fatty acids and influence on nutritional factor

Milk fat is the most variable component of milk both in concentration and composition with animal diet being a major determinant factor (Roca-Fernandez, 2014; Odle *et al*., 2017). Concentration is reduced by feeding readily fermentable carbohydrates (starch) and unsaturated fat diets while feeding rumen inert fats can increase milk fat percentage (Palmquist, 2006; Lock *et al.,* 2013). Nonetheless, greater variations are shown on *de novo* synthesized fatty acids especially C12 to C16 and oleic acid C18:1 when supplemental fats are fed to ruminants (Staples, 2006; Pérez, 2011; Howes *et al.,* 2014). The great diversity of component fatty acids in ruminant milk fat derived from ruminal biohydrogenation on dietary unsaturated

fatty acids and range of synthesized fatty acids *de novo* in the mammary glands makes it unique in composition with forty to sixty percent being long chain fatty acids (predominantly C18) (Song and Kennelly, 2003; Hoffmann, *et al*., 2013). However, the amount of fat in the diet is a major contributing factor (Palmquist, 2006; Hoffmann *et al.,* 2013). Several literature reviews (Dewhurst *et al.,* 2006; Elgersma *et al.,* 2006; Kalac and Samkova, 2010; Roca-Fernandez, 2014) have extensively documented the positive effects of forage with emphasis on fresh herbage in ruminants' diet on increase of proportion of unsaturated fatty acids of milk fats. The saturated fatty acids mainly C12:0, C14:0 and C16:0 have a risk factor for coronary heart disease due to their ability to elevate serum cholesterol levels in the body (Ohlsson, 2010; Briggs *et al.,* 2017). The unsaturated fatty acids especially polyunsaturated fatty acids have positive impact on human health which makes them favourable in the diet with special attention on conjugated linoleic acids due to its anticancer and other health benefits (McGuire and McGuire, 2000; Collomb *et al.,* 2006; Ohlsson, 2010; Briggs *et al.,* 2017) and linolenic acid (C18:3n-3) on beneficial properties against coronary heart disease (De Caterina and Zampolli, 2001; Kristensen *et al.,* 2001; Briggs *et al.,* 2017).

2.5.3. Influence of nutrition on long chain fatty acid profiles

People have become more concerned with health and implication of food eaten on health matters are intensely assessed by the population (Ohlsson, 2010; Briggs *et al.,* 2017). Milk is a nutritious, widely-consumed food that has potential to become more healthful if saturated fat can be decreased (Vannice and Rasmussen, 2014). Conjugated linoleic acid (CLA) occurs naturally in foods with principal dietary source being dairy products and other derived foods from ruminant animals (Pariza *et al.,* 1997; Lehnen *et al.,* 2015). CLA has cis-9, trans-11 octadecadienoic acid as primary isomer which accounts for more than 82% of the total CLA isomers in dairy products (Chin *et al.,* 1992; Lehnen *et al.,* 2015). It has been shown to have anticarcinogenic properties in humans (Vannice and Rasmussen, 2014; Kim *et al.,* 2016) and effective in reducing tumor in model animals. However, the typical amount consumed by humans is lower than the anti-tumor effective dose. The content in milk varies from 3.0 – 5.5 mg/g of fat. Increased consumption of foods of ruminant origin or increase in milk and meat can increase intake of CLA. Increase in ruminant products is a practical approach with potential of increasing the nutritive and therapeutic value of milk.

The anticancer and anti-obesity property that has been shown by conjugated linoleic acid warrants the pressure for increased research in the area (Kim *et al.,* 2016). According to Miyazawa *et al.* (2007) fatty acid profiles of milk can be altered by feeding by-products that

have highly digestible fiber which may modify the rumen through biohydrogenation (Nudda *et al*., 2014). The observation was supported by Hur *et al.* (2017) as increased CLA in milk fat was detected when diets high in linoleic acid were fed to dairy cows. However, inconsistency in CLA had been reported on feeding wet brewer's grain to lactating dairy cows. Miyazawa *et al.* (2007) reported an increase of CLA upon feeding 9.3% to dairy cows but a numerical decline was observed from cows fed either 15% wet brewer's grains or 15% dried brewer's grain (Dhiman *et al.,* 1999)

Alteration of LCFA, most notably C18:0, C18:1, C18:2 and C18:3, had been shown when wet brewer's grain was fed to dairy cows (Miyazawa *et al.,* 2007). However, the result differed with findings of Dhiman *et al.* (1999). In the study, C18:0 and C18:1 were the same with significantly low C18:2 and C18:3 on feeding wet brewer's grains compared to dried brewer's grains though no explanation was given by both researchers for the reductions as indicated in their results.

2.5.4. Metabolic problems associated with the by-products

There is limited cited information on negative metabolic and clinical effect on feeding LBY to lactating dairy cows. However, improper feeding regimes of most by products can lead to potential negative effects. Grierus *et al.* (2005) reported fast ruminal fermentation rates and low pH (3.8-4.8) on ruminants fed large amount of WBG due to particle size and high soluble carbohydrates. It supported the earlier finding by Owens, (1959) of susceptibility of animals fed large amount of WBG to ruminal acidosis. Moreover, the same condition was also reported by Kwatra *et al.* (1983) on buffaloes presented by clinical signs such as ataxia, dehydration, glazed eyes and diarrhea. In addition, latent ruminal acidosis was reported in feedlot steers fed WBG (Grierus *et al.,* 2005). Furthermore, increased incidences of lameness were reported on dairy cows fed WBG compared to non WBG diet of 47.8 Vs 24.0 % (Okwee-Acai and Acon, 2005). Cattle fed W BG at 57% of the diet and were not allowed to graze in Uganda also reported claw lessons and lameness (Okwee-Acai and Acon, 2005). In a study to determine toxicity of WBG, it was observed that feeding spoilt WBG predisposed animals to more incidences of lactic acid poisoning which decreased the rumen pH below acceptable levels (Owens, 1959). Contamination with toxic agents is possible under unsuitable storage conditions. Wadhwa *et al.* (1995) isolated *Aspergillus flavus* from diets containing WBG that was fed to hepatotoxicity diagnosed buffaloes. Nevertheless, aflatoxin of between 1-3 g/kg was found on one third of WBG samples collected at dairy farms during the study (Simas *et al.,* 2007). On the other hand, positive results of decreased rumen keratosis and liver abscesses

were observed by Preston *et al.* (1973) in growing and finish cattle fed dried BG at three levels, 0%, 25% or 50% of the ration compared to a high corn ration. In Kenya, there are no reported findings on metabolic problems associated with feeding the by-products.

2.5.5. Techniques in microbiological analysis

The traditional way of detecting and identifying bacteria from food, or other samples, is based on culturing, enumeration, and isolation of presumptive colonies for further identification analysis (Gracias and McKillip, 2004; Lopez-Campos *et al.*, 2012). This may require the food sample to be homogenized, concentrated, and/or pre-enriched prior to culturing (Zhao and Doyle, 2001; Wiedmann *et al.*, 2014). Bacterial cells can become injured or viable but nonculturable (VNC) due to the sub lethal stressors, such as heat, cold, acid, and osmotic shock, during the food processing steps (Hakovirta, 2008). However, such bacterial cells still pose a threat in the food industry that has led to development of improved methods to detection levels of the injured cells (Ray, 1986). Nonetheless, even the improved methods are not able to detect all bacterial cells, especially those that are viable but nonculturable. Bacteria pre-enrichment in a food sample can be performed by a selective or non-selective broth culture Zhao and Doyle, (2001), by the selective agar superimposed technique to resuscitate the injured cells Ray, (1986) or concentration of the food sample by filtration or centrifugation prior to plating. Immunomagnetic and metal hydroxide based separation are modern methods of concentration or even selecting specific bacteria from heterogeneous or polluted samples (Gracias and McKillip, 2004). Culture media of different forms such as non-selective, selective and differential media can then be used to plate the pre-treated food samples (Gracias and McKillip, 2004). The media are named based on nature of use and detection levels. Non-selective media or standard methods agar, such as the aerobic plate count, is used to detect and count the amount of bacteria in the sample while Selective medium has specific compound, such as bacteriocin, an antibiotic, or a growth nutrient, which selectively inhibits or promote the growth of specific microorganisms. Differential medium which contains an indicator, such a chromogenic or fluorogenic substrate is able to differentiate bacteria by a variety of chemical reactions during growth (Manafi, 2000). Incorporation of enzyme substrates such as fluorogenic or chromogenic into a selective media that are based on production of specific and exact enzymes for substrate by bacteria will aid in identification of microorganisms directly without further sub culturing or biochemical tests (Manafi, 2000). The action of enzyme on the substrate, fluorogenic or chromogenic, will enhance the bacterial growth to fluoresce or change color, respectively. A review on developments on chromogenic

and fluorogenic culture media for the enumeration and identification of *Escherichia coli*, *Salmonella*, *Clostridium perfringens*, *Bacillus spp.* and *S. aureus* has been completed as reported by Manafi (2000).

CHAPTER THREE
MATERIALS AND METHODS

3.1. Study area

The study was conducted at purposively selected farms in Ikinu, Thakwa and Kigumo locations in Githunguri sub-county within Kiambu County, Central Kenya. The area is located at about 1600 m above sea level and lies between latitude 1° 05´´ and 1° 06´´ South of the Equator and longitude 36° 53´´ and 36° 55´´. The soils are deep, well drained dark reddish to brown, friable clay, with a bimodal rainfall regime that starts in mid-March with a peak in April-May while the second starts in mid to end of October with annual average of about 1065mm. The mean maximum monthly temperature in the region vary from 22.4 °C to 27.6 °C while the mean minimum temperature ranging from 11.3 °C to 14.9°C. The farmers were systematically selected based on availability of lactating Holstein Friesian dairy cows, feeding trends, accessibility of the farm and willingness to participate. The feeding system practiced in the area was cut-and-carry stall feeding system where Napier grass and crop residues are cut and fed to cows in stalls, commonly known as zero grazing. The milking cows were supplemented with either CDM or LBY.

3.2. Evaluation of appropriate storage conditions of liquid brewer's yeast

3.2.1. Sampling and storage of liquid brewer's yeast

The samples of LBY were taken from three different sources (one Supplier, three distributors and fifteen farmers) in order to identify the possible contamination source along the supply chain and to develop the most appropriate storage conditions. The distributors and farmers were systematically selected thereby each distributor supplied five farmers with LBY that gave a total of fifteen farmers who took part in the study. Samples were obtained from supplier's storage tank immediately after receiving LBY from the breweries out let. Sampling at distributors' level was conducted immediately after delivery of the by-product by the supplier and at farmer's point on the day of purchase. Sampling was done in 250 ml containers then immediately cooled and transported to the laboratory for analysis. For each sampling time, microbiological evaluation was performed.

3.2.2. Microbiological analysis

Microbiological analysis methods were carried out as outlined in Mamo *et al.* (2016) protocol. The morphology of microorganisms, enzyme reaction (metabolism), staining and a range of selective and differential media was used. This involved in brief, serial dilution of the

sample, incubation on appropriate agar plate, enrichment step, solid media use, suspected colonies on the agar plate examined under microscope, staining and biochemical tests were performed.

3.2.3. Total viable counts (TVC)

Total viable counts was carried out by standard procedures as described by Carter and Cole Jr, (2012) using pour plate method which gave an indication of the initial microbial load in liquid brewer's yeast sample. Plate count agar (oxoid) was used. In brief one millilitre sample of liquid brewer's yeast was serial diluted to buffered peptone water (oxoid) up to 10^{-6} and 1ml of homogenate sample was transferred using each sterile pipette into sterile Petri dishes marked in duplicates as per the dilution index. Twenty ml of initially autoclaved Plate Count Agar at 121 °C for 15 minutes, cooled and tempered in water bath at 45 °C was then poured into the duplicate petri dishes. The media and sample was mixed gently by swerving in a figure eight manner. The petri dishes were left to cool at room temperature, followed by incubation at 37 °C for 48 hours in an inverted manner.

3.2.4. Total coliform counts (TCC)

Coliforms were enumerated by most probable number (MPN) and presumptive test used. The procedure described by Carter and Cole Jr, (2012) was used and selection of lactose fermenters was through the use of MacConkey agar (oxoid). In brief, one millilitre (1ml) of liquid brewer's yeast sample was diluted seven-fold using buffered peptone water (oxoid) followed by seven serial dilutions in which one millilitre of liquid brewer's yeast sample was diluted in 9 ml of peptone water. Twenty ml of initially autoclaved MacConkey agar (oxoid) at 121 °C for 15 minutes, cooled and tempered in water bath at 45 °C was poured into the duplicate petri dishes. The media and sample was mixed gently by swerving in a figure eight manner. The Petri dishes were left to cool at room temperature, followed by incubation at 37 °C for 48h in an inverted manner. A small portion of the culture from each positive presumptive tube of LST was re-cultured in Brilliant Green Lactose 2% Bile (BGLB) broth and incubated at 35 ± 0.5 °C for 48 ± 2 hours. Production of gas after the incubation time was taken as a positive indicator of coliform bacteria.

3.2.5. Gram staining and biochemical tests for lactic acid bacteria isolates

Gram Staining

The gram reaction of the isolates was determined by light microscopy after Gram staining. Lactic acid bacteria (LAB) are gram positive and gave blue-purple colour by gram

staining. Cultures were grown in MRS at 37 °C for 24 h under anaerobic conditions using anaerobic cylinder jars. Cells from fresh cultures were used for Gram staining. After incubation, cultures were transferred aseptically into 1.5 ml eppendorf tubes and centrifuged for 5 minutes at 6000 rpm. The supernatant removed and cells re-suspended in sterile water. Gram staining procedure was applied as per Carter and Cole Jr (2012). Bacterial suspension heat-fixed on a glass slide and the smear flooded with crystal violet for 1 minute before washing off the excess stain with distilled water followed by complete cover of the smear with Gram's iodine for about 60 seconds and then washed. Decolourization was carried out by adding five drops of 95% ethyl alcohol for a few seconds and the slide rinsed with water. Finally, the smear was counter-stained with safranin for 30 seconds, washed and air dried then observed under a light microscope (Carter and Cole Jr, 2012).

3.2.6. Yeast and mould counts

Enumeration by surface spread plate technique of diluted samples was used on potato dextrose agar and incubated at 25 °C for 5 days.

3.2.7. Temperature and pH Measurements

The pH was determine using a previously calibrated digital pH meter (Knick, Portamess, Germany) while temperature was measured using a thermometer.

3.2.8. Experimental design

The treatments had a factorial nature. It was a 3 x 3 x 4 factorial experiment with the following factors; three LBY Sources (Happy Feeds, Distributors, and Farmers), three interventions (10, 20 and 30 °C) and four Storage periods (0, 7, 14, and 21 days). In order to increase precision in the trial, LBY samples were obtained in two different periods from the sources. The two periods or runs independently formed blocks.

The microbial load was determined by results of total viable counts, total coliform counts, lactic acid bacteria, yeast and mould counts which were converted to base ¯ 10 logarithm of colony forming units per millilitre of LBY sample. The transformed values were fitted separately as dependent variable with independent variable being temperature and storage length of LBY. The effect of independent variables on the dependent variables was tested using the general linear model of SAS version 9.1.3 (2006).

Hence, the model was;

$$Y_{ijkl} = \mu + \beta_i + \gamma_j + \lambda_k + Tl + (\Upsilon\lambda)_{ij} + (\gamma T)jl + (\lambda T)_{kl} + (\Upsilon\lambda T)_{ijk} + \varepsilon_{ijkl}$$

Where,

μ = Overall mean

β_i = effect of i^{th} block, I = 1,2

Υ_j = effect of j^{th} level of LBY source, j = Happy Feeds, Distributor, Farmer

λ_k = effect of k^{th} level of intervention, k = 10, 20 and 30 °C

T_l = effect of l^{th} level of storage period (days), l = 0, 7, 14, and 21

$(\Upsilon\lambda)_{jk}$ = 2-factor interaction between LBY source and intervention

$(\Upsilon T)_{jl}$ = 2-factor interaction between LBY source and storage period

$(\lambda T)_{kl}$ = 2-factor interaction between intervention and storage period

$(\Upsilon\lambda T)_{jkl}$ = 3-factor interaction between LBY source, intervention and storage period

$\varepsilon_{ijkl} \sim N(0,\delta^2)$ is the error term

3.3. Evaluation of milk quality in lactating dairy cows using liquid brewer's yeast as on farm feed supplement

3.3.1. Survey of sampled farmers on the study routes

The researcher obtained a list of farmers in all the nine milk delivery routes from Githunguri Sub-County. For the purpose of this study, three representative milk delivery routes were randomly selected based on feeding regime. All the farmers were purposively selected in the three milk delivery routes who were supplementing cows with either CDM (control groups) or LBY (test group) which formed the sampling frames. In each route 10 farms were selected (CDM =5; LBY=5) using systematic random sampling technique. For each route and group within route, the first farm was randomly selected from the sampling frame and the others were selected after skipping a predetermined number of farms (determined based on number of farms in the sampling frame and sample size required). Therefore, a total of 30 farms (CDM=15 farms; LBY=15 farms) from three centres along three milk delivery routes were selected to take part in the study.

3.3.2. Questionnaire development

The questionnaire (Appendix 1) mainly focussed on a few sections that could enhance in-depth understanding of livestock nutrition, milk composition and hygienic quality such as; feeding systems of dairy cattle, livestock feed conservation methods, estimation of forage feeds fed to dairy cattle, strategy for supplementation of lactating cows, and sources of water.

3.3.3. Selection of farmers

The farmers were systematically selected based on availability of lactating Holstein Friesian dairy cows, feeding regime, accessibility of the farm and willingness to participate.

The feeding system practiced in the area was cut-and-carry stall feeding system where Napier grass and crop residues are cut and fed to cows in stalls, commonly known as zero grazing. The milking cows were supplemented with either commercial dairy meal or LBY.

Sample collection and preparation

The milk samples were collected from the 30 systematically identified farms once per week during morning milking for a period of 4 weeks with a total of 120 samples for analysis. Sample collection was conducted as per (AOAC 925.20) procedures. In brief samples size necessary for analysis was collected. Two hundred and fifty ml sample of milk was collected for components and 60 ml for microbiological analyses. The milk was first stirred for not less than 30 sec and sample placed in nonabsorbent air tight containers completely filled, stoppered tightly, identified and kept cold but above freezing temperature until examined. The samples were transported in the ice cooled boxes to the Guildford Institute Laboratories of Egerton University for analysis.

Physicochemical analysis

The samples were prepared as per (AOAC 925.21). In brief samples temperature were raised to 20 °C, mixed until homogeneous sample was achieved by pouring into clean receptacle and back repeatedly and promptly measured to test portions. In case any lumps of cream did not disperse, the sample was warmed in water bath at 38 °C and mixed until homogeneous to ensure no cream remained adhering to container or stopper and cooled to 20 °C before transferring to test portions.

Analysis of milk fat, protein, lactose, total solids, solid not fat, density, added water, conductivity and freezing point was performed as per mid-infrared spectroscopic method (AOAC 972.16) using Lactoscan MCC30 which uses infrared spectrophotometer measuring principle and provides milk components results through infra-red light measurement in about 30 sec. Comparisons for milk fat percentage was by Garber method, protein by Kjeldahl method, total solids by Standard method for examination of dairy products and lactose by difference and were nearly the same as lactoscan results.

3.3.4. Hygienic quality

Total viable counts

Total viable counts was carried out by standard procedures as described in section 3.2.3.

Total coliform counts

Coliforms were enumerated by most probable number (MPN) and presumptive test used as described in section 3.2.4.

3.3.5. Statistical analysis

Statistical package for social scientists (SPSS) version 20 data and descriptive statistics was used to analyse the Data obtained from the farmers who participated in the study survey.

The experiment was laid out as a completely randomized block design (CRD) and mean comparison conducted using LSD at $\alpha = 0.05$.

$$Y_{ijk}=\mu + W_i + S_j + R_k + SR_{jk} + e_{ijk}$$

Where:

Y= Represents observation $_{ijk}$

μ = Represents the overall mean

W_i= Represents effect of weeks, i=1, 2, 3 and 4

S_j= Represents effect of supplementation regime, j=CDM or LBY

R_k= Reprenents milk delivery route, k= 1, 2 or 3

SR_{jk}= Represents two factor interactions between supplementation regime and milk delivery route

Eijk= N~ $(0,\delta^2)$ is the error term

The statistical analysis was performed using SAS software generalized linear model procedure (SAS, 2006).

CHAPTER FOUR

RESULTS

4.1. Evaluation of appropriate storage conditions of liquid brewer's yeast

This study evaluated the effect of different temperatures and storage period on microbiological growth on liquid brewer's yeast that could affect the shelf life thereby reducing its suitability for use as a feed supplement for lactating cows under smallholder systems in Kenya. The samples were taken from three different sources (supplier, distributors and farmers) in order to identify the possible contamination source along the supply chain and to develop the most appropriate storage condition.

4.1.1. Mean square values and levels of significance of effects that influence microbial growth.

Mean square values and levels of significance of effects included in the analysis of variance for microorganisms investigated are presented in Table 1. Source of LBY significantly influenced ($p<0.05$) variation in all the parameters tested (LAB, yeast, mould, TVC and TCC), storage time significantly affected variation in all parameters except TCC whereas temperature intervention had no effect in all the parameters tested. Interaction between day and source had significant effect on LAB and yeast while the other factors did not affect the microbial growth parameters.

Table 1: Mean square values and levels of significance of effects that influence microbial growth.

Effects	Mean square values of microbial load (log_{10} cfu/ml)				
	LAB	Yeast	Mould	TVC	TCC
Temperature	0.0123 ^{ns}	0.5997 ^{ns}	11.8184^{ns}	0.8087^{ns}	0.3953^{ns}
Days	99.6348***	50.8916***	73.2520***	88.3863***	6.6507^{ns}
Source of LBY	8.2689***	30.2258***	69.0169***	16.1128***	45.0143***
Temperature*source	0.0658 ^{ns}	0.3737 ^{ns}	4.6212^{ns}	0.2624^{ns}	0.4336^{ns}
Days*source	4.2396***	7.1654***	1.7432^{ns}	0.7929^{ns}	5.4995^{ns}
Temperature*days	0.0392 ^{ns}	0.2980 ^{ns}	3.4298^{ns}	0.2688^{ns}	0.5603^{ns}
Temperature*days*source	0.0787 ^{ns}	0.2157 ^{ns}	3.4394^{ns}	0.1989^{ns}	1.1051^{ns}

LAB: lactic acid bacteria; TVC: total viable counts; TCC: total coliform counts; ns: not significant; ***$p<0.001$; **$p<0.01$ and *$p<0.05$

4.1.2. Effect of sampling source on microbial growth

The least square means (lsm) for the effect of different LBY sources on growth of microorganisms are presented in table 2. The analyses showed significant levels in Yeast, mould TVC as well as TCC across the supply chain whereas LAB counts for distributors and farmers were similar. Farmers recorded highest counts followed by distributors while supplier recorded lower values. Growth of LAB for supplier was significantly lower compared to distributor and farmers whereas distributor and farmers counts were statistically similar. The

trend was different for mould growth where supplier recorded least count followed by distributors while samples from farmers had the highest counts. Generally, a steady rise in microbial counts was reported between supplier and distributors than between distributors and farmers. There was no significant difference in pH across the three sources. Overall mean pH for the sources was 4.09. The values are for samples as taken from the LBY sources.

Table 2: Effect of liquid brewer's yeast from three sources on microbial growth (log_{10} cfu/ml).

Source	pH	LAB	Yeast	Mould	TVC	TCC
Supplier	4.13 ± 0.07^{a}	7.26 ± 0.23^{b}	6.75 ± 0.25^{c}	3.03 ± 0.46^{a}	7.11 ± 0.14^{a}	1.56 ± 0.33^{c}
Distributors	4.10 ± 0.07^{a}	8.02 ± 0.13^{a}	8.13 ± 0.14^{b}	4.12 ± 0.27^{b}	8.03 ± 0.08^{b}	2.66 ± 0.19^{b}
Farmers	4.06 ± 0.07^{a}	8.12 ± 0.07^{a}	8.39 ± 0.07^{a}	5.12 ± 0.13^{c}	8.28 ± 0.04^{c}	3.36 ± 0.10^{a}

Different superscript in the same column indicate statistical significant differences ($p<0.05$). Supplier 1, distributors 3, farmers 15. LAB: Lactic Acid Bacteria, TVC: Total Viable Counts, TCC: Total Coliform Counts.

4.1.3. Effect of storage temperature on growth of LAB, yeasts, Mould, TVC and TCC

The findings on the effect of temperature on growth of microorganisms are shown in table 3. Overall results indicated no significant differences in microbial counts for LAB, yeast, TVC and TCC across the three interventions except for samples of LBY from farmers source that recorded significant levels of mould counts between at10, 20 and 30 °C. Generally, the trend depicted microorganisms increase from supplier to farmers across all temperatures.

Table 3: Effect of temperature on microbial growth in liquid brewer's yeast (log_{10} cfu/ml)

Temp °C	**Source**	**LAB**	**YEAST**	**Mould**	**TVC**	**TCC**
10	Supplier	7.18 ± 0.39^{cb}	6.85 ± 0.42^{c}	2.72 ± 0.80^{d}	7.05 ± 0.25^{a}	1.78 ± 0.57^{ad}
10	Distributors	8.03 ± 0.23^{abc}	8.02 ± 0.25^{b}	3.62 ± 0.46^{cd}	7.82 ± 0.14^{c}	2.58 ± 0.33^{ae}
10	Farmers	8.08 ± 0.11^{ab}	8.48 ± 0.12^{a}	4.39 ± 0.23^{c}	8.14 ± 0.07^{c}	3.41 ± 0.16^{bg}
20	Supplier	7.28 ± 0.39^{c}	6.90 ± 0.43^{c}	3.22 ± 0.80^{cd}	7.21 ± 0.25^{a}	1.51 ± 0.57^{ac}
20	Distributors	7.98 ± 0.23^{a}	8.24 ± 0.25^{ab}	4.37 ± 0.46^{cd}	7.98 ± 0.14^{c}	2.70 ± 0.33^{bcde}
20	Farmers	8.15 ± 0.11^{abc}	8.41 ± 0.12^{a}	4.93 ± 0.23^{b}	8.34 ± 0.07^{b}	3.48 ± 0.16^{fg}
30	Supplier	7.32 ± 0.39^{c}	6.50 ± 0.43^{c}	3.17 ± 0.80^{cd}	7.08 ± 0.24^{a}	1.39 ± 0.57^{a}
30	Distributors	8.04 ± 0.23^{a}	8.14 ± 0.25^{ab}	4.37 ± 0.46^{cd}	8.28 ± 0.14^{bc}	2.70 ± 0.33^{bcde}
30	Farmers	8.13 ± 0.11^{a}	8.28 ± 0.12^{ab}	6.05 ± 0.23^{a}	8.38 ± 0.07^{b}	3.19 ± 0.16^{beg}

Different superscript in the same column indicate statistical significant differences ($p<0.05$). LAB: Lactic Acid Bacteria, TVC: Total Viable Counts, TCC: Total Coliform Counts, Temp: Temperature.

Least square means of the effects included in the analysis of variance for pH on temperature are presented on figure 1. Temperature significantly influenced ($p<0.0001$) variation in pH across the three levels of intervention tested. The highest pH was recorded at 10 °C followed by 30 °C while the lowest pH was reported at 20 °C (Figure 1).

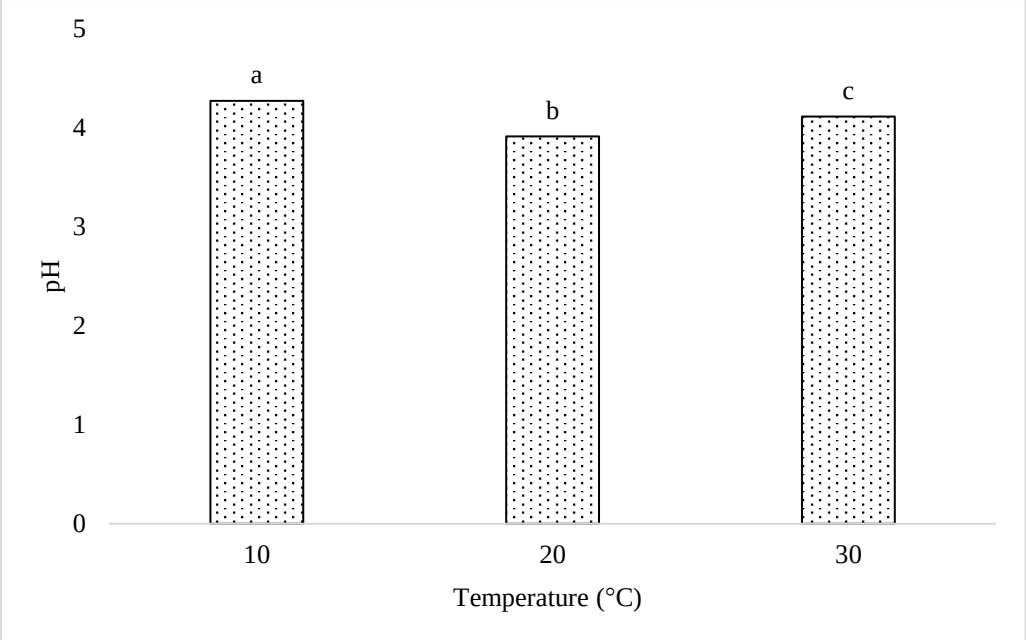

Figure 1: Effect of storage temperature on pH of liquid brewer's yeast across the three sources.

4.1.4. Effect of storage period on the growth of LAB, yeast, mould, TVC and TCC

The study on the effect of time on microbial growth on LBY from different sources are shown in table 4. The result revealed significant ($p<0.05$) differences in LAB, Yeast and TVC on day, 0, 7 and 14 while day 14 and 21 were statistically similar. A different growth trend was detected on growth of mould where a steady increase was observed from day 0 up to day 21 on samples across the three LBY sources tested. Total coliform counts increased significantly between days 0-7, whereas a decline trend was observed from day 7-21. Generally, maximum growth was detected on day 14 for LAB and TVC across the supply chain, Yeast had maximum counts at day 21 for samples from distributors and farmers while maximum counts for supplier was recorded at day 14. Samples from distributors and farmers had the highest TCC on day 7 while maximum count for supplier was at day 14.

Table 4: Effect of time (days) on microbial growth in liquid brewer's yeast (log_{10} cfu/ml)

Time	Source	LAB	YEAST	Mould	TVC	TCC
0	Supplier	3.89 ± 0.45^{g}	4.51 ± 0.50^{e}	1.44 ± 0.93^{i}	4.98 ± 0.29^{a}	0.85 ± 0.66^{a}
0	Distributors	6.09 ± 0.26^{f}	7.00 ± 0.29^{c}	2.50 ± 0.54^{dfi}	5.64 ± 0.16^{c}	1.87 ± 0.38^{ad}
0	Farmers	6.54 ± 0.13^{e}	7.82 ± 0.14^{b}	2.93 ± 0.27^{dfi}	6.23 ± 0.08^{d}	3.26 ± 0.19^{beg}
7	Supplier	7.79 ± 0.45^{cd}	5.56 ± 0.50^{d}	2.69 ± 0.93^{defi}	6.87 ± 0.29^{e}	1.13 ± 0.66^{a}
7	Distributors	7.87 ± 0.26^{d}	7.86 ± 0.29^{b}	3.83 ± 0.54^{defg}	8.38 ± 0.16^{b}	3.37 ± 0.38^{be}
7	Farmers	7.88 ± 0.13^{d}	8.02 ± 0.14^{b}	5.03 ± 0.27^{bc}	8.56 ± 0.08^{b}	4.24 ± 0.19^{f}
14	Supplier	8.68 ± 0.45^{abcd}	8.83 ± 0.50^{a}	3.67 ± 0.93^{bcgi}	8.49 ± 0.29^{bf}	2.44 ± 0.66^{ae}
14	Distributors	9.35 ± 0.26^{ab}	8.77 ± 0.29^{a}	4.43 ± 0.54^{be}	9.09 ± 0.16^{fg}	2.74 ± 0.38^{bcde}
14	Farmers	9.26 ± 0.13^{a}	8.77 ± 0.14^{a}	5.91 ± 0.27^{a}	9.21 ± 0.08^{f}	3.21 ± 0.19^{be}
21	Supplier	8.68 ± 0.45^{abcd}	8.09 ± 0.50^{b}	4.34 ± 0.93^{be}	8.11 ± 0.29^{b}	1.82 ± 0.65^{ac}
21	Distributors	8.76 ± 0.26^{abc}	8.93 ± 0.29^{a}	5.71 ± 0.54^{ac}	9.00 ± 0.16^{fg}	2.65 ± 0.38^{bcde}
21	Farmers	8.80 ± 0.13^{b}	8.95 ± 0.14^{a}	6.60 ± 0.27^{a}	9.14 ± 0.08^{gf}	2.73 ± 0.66^{bce}

Different superscript in the same column indicate statistical significant differences ($p<0.05$). LAB: Lactic Acid Bacteria, TVC: Total Viable Counts, TCC: Total Coliform Counts.

Least square means of the effects included in the analysis of variance for pH on storage time (days) are presented on Figure 2. Overall, storage time significantly influenced ($p<0.05$) variation in pH across the storage levels tested. The pH levels reported from days 0-14 was insignificant while a significant reduction was observed on comparison of day 21 to all other days.

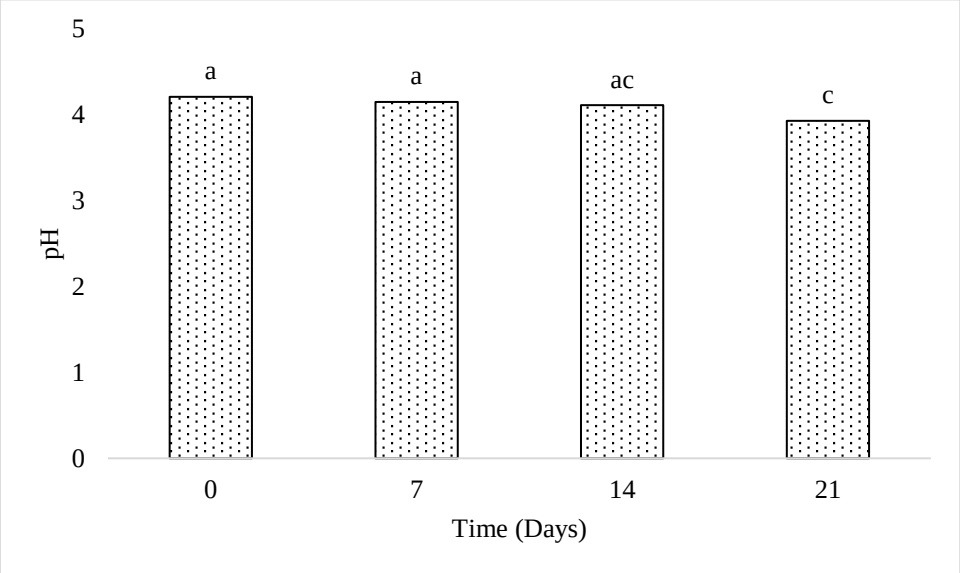

Figure 2: Effect of storage time on pH of liquid brewer's yeast across the three sources

4.2. Evaluation of milk quality in lactating dairy cows using liquid brewer's yeast as on farm feed supplement

4.2.1. Livestock management and feeding trend and physicochemical criteria of milk

The study findings indicated that most of the participating farmers (93%) practiced stall feeding while only 7% of the farmers combined stall feeding with grazing. Some of the most popular forage conservation methods among the farmers were silage (30%) and crop residue drying (30%). Only 13% of the farmers conserved feed as hay, 3% used both silage and hay as the preferred feed conservation methods, while the remaining 33% of the households did not conserve any feeds. The feed conservation trend was quite noticeable at farm level during the study period which coincided with dry season in the area.

About 37% of the farmers used feed troughs to estimate feeds offered to dairy cattle, 27% of them used gunny bags while 33% of the farmers did not estimated feeds at all. The findings indicate that most farmers (60%) supplement lactating cows at a uniform rate, 33% of them based level of supplementation on milk production but only 7% of the farmers supplemented cows using own assessment based on their levels of experience on dairy production. The levels of CDM supplementation was at 2 kg per cow per milking. However, LBY was supplemented by farmers at a uniform daily rate of 4 litres per cow per day (2 litres per milking).

Among the participating farmers, water used for domestic consumption and livestock was sourced based on priority from boreholes (70%), rivers (13%), rain water (10%) and dam (7%). Treated piped water was not mentioned by any farmer, an indication that water from the same sources as pointed out above is used in all operations like washing of equipment and cleaning of the cows' udder as indicated in table 5.

Table 5: Livestock management and feeding trends

Parameters	Categories	Number (n)	Percent (%)
Feeding systems	Stall feeding	28	93.0
	Stall feeding and grazing	2	7.0
Forage conservation methods	Hay	4	13.0
	Silage	9	30.0
	Hay and silage	1	3.0
	Crop residue drying	9	30.0
	No conservation	7	23.0
Forage feed estimation	Do not estimate	10	33.0
	Gunny bags	8	27.0
	Feed troughs	11	37.0
	Others	1	3.0
Strategy of supplementation	Uniform rate	18	60.0
	Based on milk production	10	33.0
	Others	2	7.0
Sources of water	Borehole	21	70.0
	Dam	2	7.0
	Rain	3	10.0
	River	4	13.0

4.2.2. Physicochemical criteria of milk

The determination of physicochemical components in foods and especially in dairy products is important for both regulatory and nutritional information purposes. Table 6 indicate results on physicochemical composition of milk samples obtained under different supplementation regimes.

The results indicate that feeding trends mainly influenced protein and freezing point of milk. Dairy cows supplemented with LBY produced milk of superior protein quality (3.07 ± 0.03) compared to (2.99 ± 0.03) for CDM fed cows. Conversely LBY fed cows produced milk of lower freezing point (-0.532 ± 0.005) than CDM supplemented (-0.516 ± -0.005) dairy cows. The remaining parameters tested in milk were not significantly influenced by the feeding regimes, although higher levels were observed on LBY supplemented diets than CDM diets.

Table 6: Effect of liquid brewer's yeast on milk composition (Mean±SD)

Parameter	Supplement		Overall	
	CDM	**LBY**	**Mean**	**RMSE**
BF (%)	3.69 ± 0.06[a]	3.75 ± 0.06[a]	3.72	0.5
Protein (%)	2.99 ± 0.03[a]	3.07 ± 0.03[b]	3.03	0.19
SNF (%)	8.25 ± 0.06[a]	8.38 ± 0.06[a]	8.32	0.46
Ash (%)	0.67 ± 0.004[a]	0.68 ± 0.004[a]	0.67	0.04
Lactose (%)	4.50 ± 0.03[a]	4.58 ± 0.03[a]	4.54	0.24
FP (°C)	-0.516 ± -0.005[a]	-0.532 ± 0.005[b]	-0.524	-0.041
Density (g/ml)	1.029 ± 0.000[a]	1.029 ± 0.000[a]	1.029	0.001
EC (mS/cm)	5.22±0.07[a]	5.28 ± 0.07[a]	5.25	0.56

Different superscript in the same row indicate statistically significant differences ($p<0.05$) Kenya Bureau of Standards (KeBS) recommended raw milk components. Added water: 0%; Fat content not less than 3.25%; Protein content: not less than 3.5%; Solids Not fat (SNF); not less than 8.5%; Density range between 1.028g/ml to1.036g/ml; Freezing point (FP): Between -0.525 °C to -0.550 °C. Electrical conductivity (EC) range between 4.0 mS/cm to 5.5 mS/cm at 25 °C.

.

In the study, milk delivery routes and the supplementation regimes did not affect milk quality in all the parameters tested. However, notable interaction between routes and supplementation trends was recorded in milk fat, protein, lactose and freezing point. Conversely no interaction was observed on milk density, ash, solid not fat and added water.

Table 7: Effect of LBY supplementation on milk quality from different delivery routes (Mean ± SD)

Parameters (%)	**Supplementation**					
	Route 1		**Route 2**		**Route 3**	
	CDM	**LBY**	**CDM**	**LBY**	**CDM**	**LBY**
Butter fat (%)	3.46 ± 0.34^{a}	3.92 ± 0.61^{d}	3.85 ± 0.50^{bd}	3.58 ± 0.30^{abce}	3.77 ± 0.50^{c}	3.76 ± 0.55^{a-e}
Protein (%)	2.95 ± 0.25 ^{a}	3.17 ± 0.28 ^{b}	3.03 ± 0.13 ^{ac}	2.97 ± 0.07 ^{acde}	2.99 ± 0.13 ^{acd}	3.06 ± 0.19 $^{a-e}$
Solid not fat (%)	8.24 ± 0.32 ^{a}	8.61 ± 0.75 ^{b}	8.30 ± 0.35^{ac}	8.15 ± 0.18^{ac}	8.20 ± 0.34^{ac}	8.39 ± 0.52^{abc}
Ash (%)	0.66 ± 0.03 ^{a}	0.70 ± 0.06 ^{b}	0.67 ± 0.03 ^{ac}	0.66 ± 0.01 ^{acd}	0.67 ± 0.02 $^{a-d}$	0.68 ± 0.04 ^{acd}
Lactose (%)	4.46 ± 0.21^{a}	4.71 ± 0.41^{b}	4.54 ± 0.19 ^{ac}	4.46 ± 0.10 ^{acd}	4.50 ± 0.14 ^{acd}	4.59 ± 0.28 $^{a-d}$
Freezing point (°C)	-0.50 ± 0.064 ^{a}	-0.549 ± 0.056 ^{c}	-0.526 ± 0.025 ^{bc}	-0.515 ± 0.013 ^{abde}	-0.520 ± 0.020 ^{abde}	-0.532 ± 0.038 $^{b-e}$
Density (g/ml)	1.029 ± 0.001 ^{a}	1.030 ± 0.003 ^{b}	1.029 ± 0.001^{ac}	1.029 ± 0.001 ^{acde}	1.029 ± 0.001^{ac}	1.029 ± 0.001^{acde}
EC (mS/cm)	5.25 ± 0.90 ^{a}	5.29 ± 0.59 ^{a}	5.14 ± 0.38^{a}	5.27 ± 0.34^{ab}	5.27 ± 0.37 ^{a}	5.27 ± 0.60 ^{b}
Added water (%)	0.93 ± 1.02 ^{a}	0.54 ± 0.84 ^{ab}	0.67 ± 0.96 ^{a}	1.24 ± 0.97 ^{ac}	0.84 ± 1.05 ^{a}	0.84 ± 1.11 ^{a}

Different superscript in the same row indicate statistically significant differences ($p<0.05$)

CDM: commercial dairy meal, LBY: liquid brewer's yeast, EC: electrical conductivity

SD: standard deviation

4.2.3. Hygienic criteria of milk

Total viable counts (TVC) and Total coliform counts (TCC)

Milk samples were collected during morning milking and immediately cooled. Enumeration of Total viable count was used to reflect the general aerobic microbiological quality of milk samples.

The results shown in table 8 indicate that TVC for LBY and CDM diets had a mean of 6.66 ± 0.59 versus 6.49 ± 0.7 $\log_{10}$ cfu/ml respectively. The analysis of variance results of the data indicated no statistical significant difference ($p>0.05$) between the mean TVC of milk samples from different trial diets. In this study, TVC levels did not differ along the study routes and in the interaction between routes and diets.

The total coliform counts of milk samples for the LBY showed a mean of 4.75 ± 0.62 as compared to control diet which was 4.68 ± 0.58 log10cfu/ml. The counts were essentially the same for diets ($p>0.05$) and interaction between diet and routes ($p>0.05$). The results suggest that there was no effect of diets on TCC on tested samples.

Table 8: Total viable counts ($\log_{10}$ cfu/ml) in trial diets and routes on raw milk samples

		n	TVC	TCC
Trial diets	LBY	60	6.66 ± 0.59	4.75 ± 0.62
	CDM	60	6.49 ± 0.7	4.68 ± 0.58
Routes	Githunguri I	40	6.63 ± 0.78	4.68 ± 0.66
	Githunguri II	40	6.45 ± 0.59	4.56 ± 0.56
	Githunguri III	40	6.64 ± 0.57	4.91 ± 0.49

The recommended total viable counts (TVC) and total coliform counts (TCC) for raw milk by Kenya Bureau of Standards (KeBS) are: TVC: 0-1,000,000 cfu/ml for very good quality milk; 1,000,000-2,000,000 cfu/ml for good quality milk and figures >2,000,000 cfu/ml denotes bad quality milk. TCC: 1-1,000 cfu/ml for very good quality milk; 1,000-50,000 cfu/ml for good quality milk whereas figures > 50,000 cfu/ml indicates bad quality milk (Kabui *et al.*, 2015). LBY: liquid brewer's yeast, CDM: commercial dairy meal, n: number of farms (sampling units per week for four weeks) and three milk delivery routes (sampling frame with ten samples per route weekly for four weeks).

CHAPTER FIVE

DISCUSSION

5.1. Evaluation of appropriate storage conditions of liquid brewer's yeast

5.1.1. Effect of sampling source on the growth of lactic acid bacteria, yeast, mould, total viable counts and total coliform counts

The results of this study on lactic acid bacteria (LAB) were below those reported for fermented liquid feed and close to the non-fermented liquid feed as reported by Canibe and Jensen (2003). In their research, they reported LAB to be 9.4 log cfu/g and 7.2 log cfu/g for fermented and non-fermented liquid feeds, respectively. The values of the present study are comparable to those reported by Wang and Nishino (2008) for wet brewers' grain exiting the factory. In the research, LAB population was reported to be log 7cfu/g. While studying total mixed ration containing wet brewers grains preserved as silage, Wang and Nishino (2009), found that the viable numbers of LAB were above 10^7cfu/g throughout the storage period and were not influenced by the method of ensilage. In the research, they confirmed that wet brewer's grain was a favorable substrate for lactic acid bacteria due to the high amount of lactic acid in the wet brewer's grains even before ensiling.

In the present study of LBY, a by-product from the brewing industry with low pH levels as shown in table 2, lactic acid bacteria growth could have been favoured over other microorganisms that explain the high level in the by-product. In another research by Filya *et al.* (2000), lactic acid bacteria proliferated to reach a level of 7.2 Log cfu/g when whole crop wheat silage was under storage. Furthermore, the findings of this research indicated that LAB ($\log_{10}$ cfu/ml) was lowest at supplier level but increased within the supply chain at distributors and farmers level. The observed increase were statistically significant between supplier and distributors and supplier and farmers with insignificant observation between distributors and farmers, an indication that quality of LBY from supplier was better than distributor and farmers. This demonstrates that possible deterioration of quality could possibly occur at distributors' level and is carried over by farmers during purchase of LBY or at farm level due to poor storage conditions.

On the other hand, yeast counts were 6.75±0.25, 8.13±0.13, and 8.39±0.07 log cfu/ml at supplier, distributor and farmer level, respectively. Counts of yeasts at the supplier level were comparable to 6.9 ± 0.69 log cfu/ml reported by Canibe and Jensen (2003). Similar to the growth of LAB, yeast cells increased gradually along the supply chain from supplier to farmer. Despite the fact that yeast cells were lower than LAB count at supplier level, a significantly higher level of yeast counts than LAB count was recorded at farmer level, a demonstration of

possible yeast development under aerobic condition as reported by De Souza *et al.* (2012). Increase in LAB and yeast corresponds to an increase in acidity and the corresponding decrease in pH. Such conditions have the potential to enhance the growth of yeast and hence the greater increase in yeast counts.

Aggelis *et al.* (1998), indicated that, the organic acids accumulated due to LAB biochemical metabolism may eventually stimulate growth of yeasts. This may result in deterioration of the feeds on exposure to air (Driehuis *et al*., 2001; Nishino and Touno, 2005; De Souza *et al.*, 2012). The LBY samples used on the trial were stored under aerobic condition during study period in order to reflect the ideal farm storage conditions; this could have precipitated the higher yeast cell counts.

The lactic acid bacteria counts was statistically similar between distributors and farmers. However, yeast counts were significantly different between the three sources, an indication that the level of yeast contamination increased along the supply chain. In a previous study in smallholder dairy farms in Kenya, on-farm production and handling of animal feeds in peri-urban dairy farms where intensive management predominate was reported to impair the quality of feeds (Mwende *et al*., 2016a). A study by Čabarkapa and Ivanov (2009) similarly reported that agricultural and storage practices determine the microbial feed safety hazards.

Mould count reported in this work for supplier as indicated on table 2, were lowest followed by distributors and finally farmers. The values from distributors are comparable to values reported by Mwende *et al*. (2016b), while higher values were observed on LBY obtained from farmers compared to their findings. They stated that commercial feeds had mould count of 4.2 log CFU/g while farm-sourced feeds had counts of 4.3 log CFU/g. In addition, they reported that feed concentrates had the highest fungal count of log 4.92 ±0.4 CFU/g compared to log 3.99±0.9 CFU/g for forages. These values are much higher compared to LBY from supplier source indicating that LBY may be safer compared to the concentrates and forages used by small holder dairy farmers. However, handling practices of the by-product along the supply chain could hasten shelf life thereby compromising its suitability as feed supplement as detected at distributors and farmers points. The counts obtained from suppliers can be classified as relatively safe, distributors fall under transition zone whereas the by-product from farmers is unsafe and cautionary measures are required (Cooporative Resource International, 2016). According to its classification, mould counts between 1.0 - 4.0 log CFU/g can be categorized as relatively safe, between 4.0-5.0 log CFU/g as being in the transition zone and counts between 5.0-7.0 log CFU/g as being unsafe and cautionary measures advised. The observed increase on mould load was statistically significant across the supply chain. The results indicated that the

quality of LBY from supplier was good. This demonstrates that deterioration of quality could possibly occur at distributors' level and is carried over by farmers during purchase of LBY or at farm level due to poor storage conditions. According to Čabarkapa *et al.* (2009), agricultural and storage practices determine the microbial feed safety hazards. Unhygienic handling containers used by distributors as well as farmers could have a direct impact on the microbiological quality of feeds. Due to its high moisture content, LBY stored under such handling conditions may result in contamination with mycotoxin producing fungi (Mussatto *et al.*, 2006). According to Makau *et al.* (2016b) there is a dire need to improve on storage and handling conditions of animal feed. The study concluded that animal feed from commercial sources as well as those formulated on the farm required attention in order to reduce exposure of dairy animals to aflatoxins which end up in the milk consumed by human beings. *Aspergillus* spp. have been known to produce aflatoxins in food and feed (Mangal *et al.*, 2016; Kocsubé *et al.*, 2013; Reddy *et al.*, 2010; Sweeney and Dobson, 1998). Earlier studies found the optimum pH for aflatoxin production by the *Aspergillus* spp. as 3.5 to 8.0 (Oviedo *et al.*, 2011). Sivakumar *et al.* (2014) observed the optimum pH range for growth of *Aspergillus flavus* and *Aspergillus fumigatus* at 4-4.5. The pH levels observed in this research fall in this range and thus the potential for aflatoxin production in the LBY is high if the feed were to be contaminated by fungi. However, the findings differ with other researchers who reported optimum growth at pH levels between 5.5 to 6.5 (Al-Gabr *et al.*, 2013). The presence of mycotoxicogenic fungi in animal feeds increases the risk of mycotoxin food poisoning in animals and in turn in human beings (Sivakumar *et al.*, 2014). The existing but grossly ignored challenge of mycotoxin contamination of dairy feeds can be addressed by utilization of fungal free raw materials for animal feeds processing or safer brewing by-products like LBY and ensuring good storage conditions of the agricultural produce along the supply chain up to final use.

The values of TVC in this work as indicated in table 2 were higher as compared to TVC reported for commercial concentrates and forages by Mwende *et al*. (2016a). In the study, TVC for commercial concentrates and forages reported was 5.99 and 5.01 ($\log_{10}$ cfu/Ml), respectively. Liquid brewer's yeast is a by-product of fermentation from the brewing industry which is an excellent source of protein, vitamins and minerals with high moisture content that makes it ideal for microbial growth (Canibe and Jensen, 2012). In the current research, the high TVC counts can be attributed to low pH and high dominance of the by-product by LAB as reported in this study. Canibe and Jensen (2012) indicated that proliferation of lactic acid bacteria leads to production of lactic acid which in turn reduces the pH of the resulting medium.

The low pH in turn impedes proliferation of coliforms. The pH <4.5 is required to inhibit growth of pathogenic microorganisms (Canibe and Jensen, 2012), the overall pH reported in this research was 4.09 and that could explain the low TCC in LBY.

Nevertheless, coliforms are indicator organisms and their presence may be indicative of contamination during handling of the feeds. According to Prasad *et al.* (2016), fecal contamination of feeds is widespread on farms; it is an important route for exposure of cattle to coliforms and other organisms. Handling of containers used along the supply chain is very important especially by distributors as well as farmers that could have a direct impact on the microbiological quality of the by-product. The containers as well as the handlers can act as the main sources of contamination of the LBY. Čabarkapa *et al.* (2009) indicated that agricultural practices determine the microbial content as well as safety of the feed. In the study area, storage methods for the LBY varied along supply chain. Happy Feeds Limited, the sole supplier of the by-product store 'Chachu' (brand name of LBY) in stainless steel tanks at their depot and transport it to distributors in stainless steel tankers. Distributors use 2,300 litres capacity white plastic tanks for storage of the LBY under a roof. However, at farm level, storage methods range from 20 litres capacity plastic containers (common jerry cans) to larger capacity containers that are either closed or open and put on floor, to concrete troughs at cow sheds under the same conditions at room temperature that makes 'Chachu' prone to microbial contamination. Wafula *et al.* (2016) indicated that plastics are difficult to clean, even after thorough cleaning of plastic jerry cans, mean microbial residual load for TVC and TCC levels were found to be 3.84 ± 0.92 and 3.64 ± 0.80 $\log_{10}$ cfu/cm^2 , respectively. In addition, the scratches on plastic containers harbor bacteria which would contaminate the fresh LBY delivered to distributors or farmers. The condition is worsened by the mode of cleaning practiced by distributors as well as farmers in the area. Distributors manually clean the 2,300 litre capacity plastic containers using hand brushes to scratch through the plastic container walls after which they rinse with water. Wafula *et al.* (2016) pointed out that the use of scourers on plastic containers is less effective in reducing the microbial load on the container surfaces. Thus, it appears that during the washing process, more scratches are made on the plastic surfaces that harbor more microorganisms leading to increased contamination of subsequent consignment. This may be elucidated by the high presence of microbial load along the supply chain. The condition was made worse at the farm level because LBY was viewed as animal feed that can be stored in any container and even washing of such containers before refilling was less important. This could explain the high levels of TVC and TCC in the by-product at the farmers' level as shown in table 2.

5.1.2. Effect of storage temperature on growth of lactic acid bacteria, yeast, mould, total viable counts and total coliform counts

In the present study, yeast growth increased insignificantly with increase in temperature. This could suggest contamination of LBY with other yeast strains some of which could be more psychrophilic along the supply chain. The yeast cells remained viable at lower storage temperatures. This explains the lack of influence of temperature on the growth of yeast in LBY stored at 10, 20 and 30 °C and the slight decrease in mean counts of yeast at 30 °C as shown in table 3. The findings have further demonstrated that dominance of LAB is unaltered by the lower trial temperatures used during the extended storage periods. The same trend was reported on LAB growth rate on meat under chill temperatures (Korkeala *et al.,* 1989). In a previous research, Canibe and Jensen (2003) reported similar results. In their study, fermented liquid feed stored at 20 °C had LAB and yeast count reaching 6.9 ± 0.32 and 9.4 ± 0.32, respectively. Lactic acid bacteria have been reported to grow at temperatures as low as 10° C reaching 10^8cfu/g within 7 to 12 days (Hamasaki *et al.,* 2003). This explains the spoilage witnessed in beer and cooked meat by Lactic acid bacteria under refrigeration irrespective of non-detectable levels of bacteria below 10 cfu/g (Hamasaki *et al.,* 2003). The bacteria can as well multiply under very high temperatures that can be above 40 °C (Adamberg *et al.,* 2003). In the present study, the counts of LAB increased gradually with increase in temperature from 10 °C to a maximum of 30 °C across the three LBY sources. The research findings may explain spoilage trends reported by farmers in the study area under different temperature regimes. The same trend of increase of LAB was reported with increase in temperatures between 25 - 38 °C (Adamberg *et al.,* 2003).

Liquid brewer's yeast is the last by-product removed during beer manufacture. The by-product is normally subjected to autolysis to destroy any viable yeast. However, yeast in the by-product reported in the study could be acquired along the supply chain from unhygienic storage containers that eventually multiply under favorable conditions during storage. This informed the choice of the temperature range tested in the study that was to determine the most possible appropriate intervention level to prevent deterioration of LBY under on-farm storage conditions.

Nonetheless, the three temperature intervention range tested in this work did not affect the multiplication of yeast. The findings demonstrated that *Saccharomyces* genus and non-*Saccharomyces* yeast that could originate from LBY storage containers or other feeds in the farm could grow under a wider temperature range. The minimum temperature to support growth of yeast in the *Saccharomyces* genus has been reported at between 1.3 °C for

Saccharomyces cariocanus and 4.3 °C for *Saccharomyces kudriavzevii*. *Saccharomyces cerevisiae* was adapted to grow at higher temperature optimum 32.3 °C and maximum 45.4 °C (Salvado *et al.,* 2011). Similarly, the growth rate of yeast was reported to increase with increase in temperature and the cell biomass increased within the optimal temperature range (Charoenchai *et al.,* 1998). The cellular processes such as protein synthesis and substrate transport to enzymes are also influenced by changes in temperature and at lower range; enzyme kinetics is greatly reduced (Tai *et al.,* 2007).

In this study, overall result indicated that the growth of mould was not significantly affected by temperature range tested. This may be an indication that LBY is an ideal product for fungal species that can grow under a wider temperature range. The occurrence and magnitude for growth of mould varies with geographical and seasonal factors as well as conditions under which a food or feed crop is grown, harvested and stored (Lanyasunya *et al.*, 2005). The set temperatures for the present study were 10 - 30 °C to practically cover temperature range for most dairy farming regions in Kenya. Various fungal species can grow at a wide range of temperatures. According to Sivakumar *et al.* (2014), higher temperatures, 28 - 30 °C is optimal for the growth of *Aspergillus* species of fungi while temperatures as low as -2 °C are reported to support growth of some *Fusarium* species such as *Fusarium sporotrichioides* (Sweeney and Dobson, 1998). Thus, the range of temperatures between 10 - 30 °C would favour growth of a wide range of fungal species that may possibly contribute to contamination of LBY in the event that appropriate storage conditions are not observed. Previous studies found out that the optimum growth temperatures for fungi could be higher than the temperatures tested in this research. *Aspergillus niger* one of the producers of mycotoxins in agricultural produce was found to grow well at temperatures between 7.0 - 45.7 °C with an optimum at 34.9 °C (Kocsubé *et al.,* 2013; Dagnas *et al.,* 2014), however, the optimum pH for *Aspergillus niger* growth is 7-7.5 °C (Sivakumar *et al.,* 2014). Liquid brewer's yeast is more acidic (overall mean temperatures pH reported in this work is 4.09) therefore; optimum growth can be inhibited by reduction in pH. Other researchers had previously reported temperature range of between 10 - 12 °C to 42 - 43 °C with an optimum growth at 32 - 33 °C for *Aspergillus flavus* and *Aspergillus parasiticus.* This may explain the insignificant but gradual increase in microbial counts at temperatures between 10 - 30 °C in this trial. Nevertheless, growth at these temperatures would still be noticeable as the fungi can grow at temperatures as low as 4 °C (Gougouli and Koutsoumanis, 2012) or 9 °C (Dagnas *et al.*, 2014). The overall mean pH reported in this study is 4.09. Previous work by Sivakumar *et al. (* 2014) indicated that the pH range of 4 - 4.5 is appropriate for *Aspergillus flavus* and *Aspergillus*

fumigatus that present a challenge in storage conditions for LBY due to high probability for the species growth. Furthermore, the temperature and pH reported in this research would still permit the production of aflatoxins as this mycotoxin can be produced at temperatures between 15 - 37 °C (ICMSF, 1996; Koehler *et al.*, 1985) and pH as low as 3.0 with an optimum at 6.0 (ICMSF, 1996). The optimum temperature for aflatoxin production as reported by Joffe and Lisker (1969) is 24 °C which is about the room temperature in the study area.

The outcome of the results indicated no significant differences in TVC and TCC across the three storage temperatures investigated at day 0, 7, 14 and 21 in the present study. That can be attributed to growth of various bacterial species at different optimum temperatures based on the composition of the medium (Adamberg *et al.,* 2003). Lack of significant difference in the growth levels at different temperatures tested may mean that different strains of bacteria were present in LBY, some of which would thrive at 10, 20 and 30 °C. The ability of bacteria to grow optimally at the three different storage temperatures in LBY point out at the possibility of huge diversity of bacteria that can thrive in LBY. This is in agreement with Maciorowski *et al.* (2007), that animal feeds may be carriers of a wide variety of microorganisms. Thus, irrespective of storage temperatures, bacteria would grow in LBY resulting in spoilage or contamination with toxins (Maciorowski *et al.,* 2007). Nevertheless, these values of TVC are higher than those reported by Mwende *et al.* (2016a) for other commercial feeds as well as farm sourced feeds indicating that higher moisture content of LBY and storage condition could hasten bacteria multiplication.

The rate of activity of lactic acid bacteria has also been reported to increase with increase in temperature. Since LBY has been found to allow proliferation of lactic acid bacteria, this may explain significant reduction in pH of the LBY with increase in temperature from 10 °C to 20 °C. On the other hand, aerobic fermentation in feed raises the pH of the medium (Prasad *et al.,* 2016). The high pH may in turn encourage growth of pathogenic bacteria like *Listeria monocytogenes* (McDonald *et al.,* 1991; Prasad *et al.,* 2016) when the pH rises above 5.5 (D'Mello, 2004). This may explain the significant increase in pH with increase in temperature from 20 °C to 30 °C despite the fact that, there were no significant differences in microbial counts.

5.1.3. Effect of storage period on the growth of lactic acid bacteria, yeast, mould, total viable counts and total coliform counts

There was a gradual increase in population of LAB from days 0 up to a maximum level at day 14 and then a decrease at day 21, an indication of possible depletion of nutrients due to

LAB multiplication during the storage period or yeast growth and possible competition with LAB for nutrients. Lactic acid bacteria have been reported to grow at temperatures as low as 10° C reaching 10^8 cfu/g within 7 to 12 days (Hamasaki *et al.* 2003). The trend of growth during the storage period on this work was observed in a previous research involving storage of feeds containing wet brewer's grains. In the research, Wang and Nishino (2009) found lactic acid to be 6.57 log cfu/g at the beginning of the research, increasing to 8.86 ± 0.033 log cfu/g at day 14.

Wang and Nishino (2008) reported a different trend in lactic acid growth when wet brewer's grains were stored alone. In the research, lactic acid bacteria were found to be 6.91 log cfu/g at the beginning of the study, increasing to 6.94 log cfu/g at day 14. The difference in the findings may be attributed to means of storage which were different in both studies. Whereas Wang and Nishino's, (2008) work involved vacuum packaging, the current research was conducted under aerobic storage conditions that could have influenced faster increase in LAB counts. Although, Wang and Nishino, (2008) reported aerobic deterioration occurrence within 2 days for wet brewers grains stored alone, other researchers found that homofermentative LAB impaired the aerobic stability of silages of mature cereal crops; wheat, sorghum, maize (Weinberg *et al.*, 1993).

A gradual increase from day 0 to day 14 followed by slight reduction at day 21 of yeast counts was reported in the current study as shown in table 4. The same progress on growth was reported by Wang and Nishino (2009), yeasts increased from 5.21 log cfu/g at the beginning of the experiment, to 5.86 ± 0.087 log cfu/g at day 14. Similar trend on gradual increase in yeast with storage period followed by a reduction with time was observed in this research. However, mean counts for yeast reported in this work are higher than those reported by Wang and Nishino (2009). This could be attributed to significant decline in pH with increase in storage days that could have favored yeast growth in LBY. Growth of LAB results in a decrease in pH as the acidity increases. In the present research, the mean pH of the LBY was 4.09 across the storage period tested. Previous research had reported growth of LAB at a pH of 4.2 (Ni *et al.*, 2015). The organic acids accumulated due to LAB biochemical metabolism may eventually stimulate growth of yeasts (Aggelis *et al.,* 1998).

According to a study by Wang and Nishino (2008), aerobic deterioration occurred within 2 days for wet brewers grains stored alone to a level of 7.08 and 5.2 log cfu/g, for LAB and yeasts, respectively. Thus, LBY would be prone to spoilage by the LAB as well as the yeasts. Weinberg *et al.* (1993) indicated that homofermentative LAB impaired the aerobic stability of silages of mature cereal crops (wheat, sorghum, maize), this was evident from

intensive CO_2 production and development of yeasts and molds. Yeast activity on feed during storage can eventually results in spoilage of the feeds (Weinberg *et al.*, 1993). It appears that during storage, fermentation occurs resulting in an increase in the levels of lactic acid bacteria, yeasts, and lactic acid and a corresponding decrease in pH. With time, nutrients are depleted while accumulation of metabolites (toxins) in the medium occurs. Thus, growth of the microorganisms stop and a decline trend starts (Juška, 2011).

Overall, there was statistically significant levels of interaction between days and source ($p<0.05$) as indicated in table 1. This suggests that the effect of day on LAB and yeast growth was dependent on the source of LBY. Clearly, the levels of LAB and yeast at day 0 from the three sources were significantly different, an indication of possible contamination of the LBY within the supply chain. However, no interactions were observed upon comparison of temperature and days and temperature and source ($p>0.05$). The findings suggest that the three temperature intervention levels have no significant effect on the growth of LAB and yeast on LBY irrespective of the source. Conversely, there was a significant increase in the levels of LAB and yeasts during storage and thus the possibility of deterioration with increase on storage duration (Weinberg *et al.*, 1993). This concurs with other research on the effect of temperature on LAB (Adamberg *et al.*, 2003) and yeast (Charoenchai, *et al.*, 1998) growth at different temperatures.

This study revealed a significant increase in growth of mould during storage from day 0 up to a maximum level at day 21 as shown in table 4. This continuous increase in mould counts can be attributed to storage conditions by actors especially the distributors and farmers at the supply chain and the fact that mould can tolerate harsh conditions than other microorganisms. Despite the fact that the supplier uses steel tanks to transport LBY to the distributors, distributors and farmers use plastic containers to store LBY. Plastic containers are difficult to clean, which may lead to accumulation of mould at every batch both at distributors and farmers levels. Moreover, there is no any appropriate quantifiable cleaning mode practiced by distributors and farmers. This may result in contamination of LBY by mycotoxigenic fungi leading to poisoning of livestock when they consume the contaminated feeds.

According to Makau *et al.* (2016b), high aflatoxin contamination in feeds in peri-urban dairy farms may be attributed to prolonged storage of animal feeds (hay, concentrates and silage) under precarious conditions in small stores. This is so because the peri-urban farmers practice stall feeding as opposed to grazing. Thus, the farmers have to buy feeds and any form of supplements such as concentrates or brewing by-products like LBY and store for daily use.

Inappropriate storage conditions as well as handling of the feeds may contribute to multiplication of mould and eventual production of mycotoxins in feeds.

The gradual reduction in pH with increase in storage period can be attributed to acid producing lactic acid bacteria which are common in LBY. Presence of lactic acid bacteria may also result in production of antifungal substances that may curb the growth of mould (Rouse *et al.,* 2007; Asurmendi *et al.,* 2016). The decrease in pH would result in a decrease in the rate of growth of mould. Again the optimum growth pH for mould is around 6.0 (ICMSF, 1996), that may explain the low mould counts in this study

The trend of TVC load in the results obtained in the present study are similar to those reported for wet brewer's grain by Wang *et al*. (2014). In the research, wet brewers grain stored for three days attained peak counts of between 7 - 8 log cfu/g for samples stored at 25 °C within 2 days and 6 - 7 cfu/g for samples stored at 15 °C in less than 2 days. It appears that the rate of deterioration was faster for these samples than what was found out in the present research where such levels of deterioration were attained between 7-14 days of storage.

On the other hand TCC increased significantly from day 0, until day 7 attaining a maximum count and a drop thereafter from days 7 to 21 as shown on table 4. This is possibly due to competition between the bacteria growing in LBY, the depletion of nutrients therein as well as the gradual reduction in pH of the medium (Canibe and Jensen, 2012). The significant decrease in pH is detrimental to coliforms since they cannot survive in environments with low pH levels (Canibe and Jensen, 2012). Moreover, as a result of the reduction in TCC at day 21, there was no significant difference between the counts of coliforms between days 0 and 21 indicating that LAB out competed coliforms at ambient temperature (Canibe and Jensen, 2012).

5.2. Livestock management and feeding trend

The feeding system practiced in the area was cut-and-carry stall feeding system where Napier grass (*Pennisetum purpureum*) and crop residues are cut and fed to cows in stalls, commonly known as zero grazing. The animals were fed with Napier grass and crop residues ad libitum to meet both maintenance and production requirements. Lactating cows were supplemented twice at the time of milking with either CDM (at the rate of 2 kg per milking) or LBY (at the rate of 2 L per milking). The cows were milked twice daily at 4.30 am and 2.30 pm during the experimental period.

5.2.1. Physicochemical criteria of milk

Milk protein percentage of Holstein cows fed LBY based diets was significantly ($p<0.05$) higher than cows supplemented with CDM but lower than Kenya Bureau of Standards (KeBS) of proteins not less than 3.5%. A study by Poppy *et al.* 2012 indicated higher protein levels on cows supplemented with yeast culture that is in concurrence with the present study. The trial was conducted at a time when farmers relied mainly on purchased animal feeds or conserved feedstuff, mostly dried crop residues and hay as shown in table 5. The high protein levels in milk from this study could be associated with improved nutritional value of poor quality forages, increase in numbers of rumen lactate-consuming bacteria, prevention of lactate accumulation and drop in rumen pH as reported by Beauchemin *et al.* (2003) in an experiment where lactating cows were fed on yeast based diets. There was no significant differences in protein levels within the 3 routes ($p>0.05$), which could be an indication of nearly the same management practices. Significant levels of differences ($p<0.05$) were recorded when supplementation regimes were compared and in the interaction between routes and supplementation regimes ($p<0.05$), which suggested that any significant changes observed in milk protein levels was nutritionally dependent.

There was a significant difference ($p<0.05$) in milk FP among supplementation regimes and their interactions with milk delivery routes. However, no changes ($p>0.05$) were recorded in milk FP among the milk delivery routes. The FP range was within KeBS recommendation of between (-0.525 °C to -0.550 °C). Liquid brewer's yeast supplemented cows' milk had a decrease in milk FP, an indication of an increase in total solids that positively translates to higher yield of dairy products such as cheese leading to increase in profit margin by the processors. The findings of this study is in concurrence with a study by Shreedhar *et al.* (2016) that reported a decrease in milk FP for cows supplemented with *Saccharomyces cerevisiae*. Addition of water to milk is one of the most likely factors that could affect milk freezing point as it influences the concentration of water soluble components in milk. Earlier studies by Harding (1999) indicated that adulterated milk will have a higher FP closer to zero degrees centigrade. In case no addition of water is detected, then the difference in FP could be due to different levels of concentration of milk components in the aqueous phase (Bjerg and Rasmussen, 2005). Concentration of milk components can be influenced by other factors such as nutrition, water intake and stage of lactation or subclinical mastitis (Bjerg and Rasmussen, 2005). It can also be influenced by concentration of carbon dioxide in milk, region and seasonal, climatic condition and the breed of dairy cow (Slaghuis, 2001). Lactose concentration and pH of milk have also been reported to have a significant influence in FP of milk (Brouwer,

1981). In this study, the FP of milk from cows supplemented with LBY was significantly ($p<0.05$) lower than those on control supplement. This is an indication of positive effect of trial diet on milk components. A study by Ayad *et al.* (2013) reported stability in blood glucose level in normal range on cows supplemented with yeast based diets but not in the control group. Steady supply of glucose is essential for production of lactose which is responsible for 53.8% of milk FP (Brouwer, 1981). Nutritionally related milk FP problem may only be possible in a situation where the cow is either starved or fed on very poor quality diets with little or no grains (Bowman, 2005). However, such feeding condition will automatically lead to extremely low milk production and deterioration of animal body condition which might raise animal welfare concern. Farmers in the study area practiced stall feeding with proper guidelines on balanced nutrition under constant supervision from agricultural extension staff thereby ruling out the possibility of malnutrition. The significant differences in MFP discovered in the interaction between supplementation regimes and milk delivery routes could be due to different management practices and slight variations of climatic conditions in different routes that may not be necessarily associated with supplementation regimes.

The results indicated overall mean 3.72% BF that is higher than KeBS recommendation of not less than 3.25% BF. Although there was a higher milk BF% on cows supplemented with LBY as compared to cows supplemented with CDM, the difference was not significant ($p>0.05$). The effects of LBY supplementation on milk BF content concurs with the findings of Harris and Webb (1990) who reported higher milk BF% on lactating dairy cows fed LBY based diet. Studies by Putnam *et al.* (1997) also reported high BF content in milk of cows fed yeast based diets. Martin and Nisbet (1990) associated the positive effect on milk BF% with increase in number of cellulolytic bacteria which enhances fiber degradation, thereby improving the digestibility of the diet and increase in proportion of acetic acids among the fermented Volatile fatty acids in the rumen.

The level of SNF on supplementation regimes and milk delivery routes was the same ($p>0.05$). This can be explained by the fact that milk tested in the study was from cows of the same breed that would generally be uniform in genetic composition. A relatively uniform level of SNF can be achieved as long as diets are balanced in nutrients with adequate roughages. However, feeding of high fiber and low energy rations can depress SNF content (Harris and Bachman, 2003). Majority (93%) of the participating farmers practiced stall feeding as shown in Table 4. Under this feeding system, all cows are fed as a single group that may result in either underfeeding the high producing cows or over-feeding the low producing cows. This notwithstanding, there was a significant difference ($p<0.05$) in the interaction between the LBY

supplementation and the milk delivery routes, which could be an indication of differences in LBY supplementation trends between the milk delivery routes. However, the SNF (8.32) percentage recorded in this study is slightly lower than KeBS of not less than (8.5%).

The result shows that cows supplemented with LBY produced milk with slightly higher ash as compared to cows supplemented with CDM. Interaction between the diets and routes was statistically significant ($p<0.05$) but no significant difference was reported among supplementation regimes and milk delivery route ($p>0.05$).

There was no significant difference ($p>0.05$) on lactose when the two supplementation regimes were compared. However, a slightly higher lactose level for cows supplemented with LBY was observed. One possible explanation regarding the increase in lactose could be because the study was conducted at a time of limited forage in the area and farmers had to purchase varied hay types from different sources with diverse quality to feed cows. The LBY supplementation could have contributed to stimulation of cellulolytic bacteria in the rumen, increase in fiber digestion and flow of microbial protein from the rumen as reported in a study by Jouany and Morgavi (2007). This further confirms the suggestion by Bruno *et al.* (2009) that feeding of yeast based diets improved milk lactose as compared to cows on control diets. In this study, there was no significant difference ($p>0.05$) in milk Lactose percentage among the two supplementation regimes and along the different milk delivery routes. The results may suggest that apart from the difference in the supplementation regimes, forage quality and quantity fed within the sample routes were essentially the same and could not generally affect lactose percentage. However, significance differences ($p<0.05$) in the levels of lactose was observed in the interaction between supplementation regimes and milk delivery routes. This shows the complexity in identification of response possibilities of dairy cows to the LBY due to differences in dietary composition and yeast source as reported in a study by Alshaikh *et al.* (2002) and management practices in different milk delivery routes within the study area. The discrepancies in response to LBY in different studies could be associated with breed differences, type of forage, stage of lactation, the source of LBY and feeding strategies.

Milk density was similar among supplementation regimes, milk delivery routes and their interactions. This is one of the parameters used to assess milk quality since milk density increases when milk has lower water content, which can be an indication of higher presence of milk solids. Measurement of this parameter is important in the study because farmers delivered milk to collection points or processing plants. Any adulteration of milk by farmers would adversely affect quality of dairy products processed from the supplied milk. There was a strong

indication from this study that feeding trends were generally similar in the three milk delivery routes. The average milk density levels were within the standard limits recommended by KeBS.

Electrical conductivity (EC) of milk was determined at 25 °C in milliSiemens (mS/cm) and was essentially the same for trial diets ($p>0.05$), routes ($p>0.05$) and interaction between diets and route ($p>0.05$). The results indicated that milk EC was not affected at all by the cows' diet and study routes. The determination of milk EC is by the anions and cations concentration (Kitchen, 1981). Mastitis condition causes variation of ionic concentration in the infected dairy cows' udder quarter which increases EC in milk thereby making it a main diagnostic indicator in detection of subclinical mastitis (Hamann and Zecconi, 1998; Goodling *et al.,* 2001; Sloth *et al.,* 2003). During udder infection with mastitis, the concentration of lactose and potassium decrease and citrates, bicarbonates and sodium and chloride concentrations increase in milk due to increased permeability of the mammary epithelium (Ogola *et al.,* 2007). Electrical conductivity has been expressed in several studies as a maximum value for each quarter or each milking (Goodling *et al.,* 2001; Norberg *et al.,* 2004; Janzekovic *et al.,* 2009). The average EC (5.25 mS/cm) of study samples were within the acceptable levels of between 4.0 mS/cm and 5.5 mS/cm at 25 °C. According to Gargouri *et al.* (2013), the test is rapid, easy and inexpensive that can indirectly be used to determine somatic cell count (SCC) and essentially diagnose subclinical mastitis in milk. This system is rarely used in Kenya; however, most dairy cooperative societies have invested in purchase of lactoscans that can rapidly be used to measure EC. Nevertheless, mastitis is not the only condition that alters the ionic concentration of milk. Non mastitis correlated variations in EC like temperature, fat percentage, milking interval, stage of lactation and breed are major drawback to diagnostic value of EC (Janzekovic *et al.,* 2009). Holstein Friesians were used during the work to manage breed variation effect. Studies have shown that heritability for susceptibility of cows to mastitis is low at 1 to 3% while for the somatic cells is noticeably higher at 10 to 15% (Janzekovic *et al.,* 2009). However, other factors like stage of lactation, milking intervals and disease conditions of each individual cows were not assessed. Bulk milk from each individual farms were used that could had variations. Moreover, milk samples were obtained from morning milking time. Previous works by Norberg *et al.* (2004) had shown that within-milk variation in EC of milk from an infected quarter may be larger than variations in EC of milk from healthy cows possibly due to physical changes in mastitic milk that may affect milk flow; therefore, a cow suffering from mastitis may not necessarily show increased EC. The findings of this work could not then authoritatively conclude that increases in EC could have been attributed to cows suffering from mastitis conditions. The study considered the aforementioned drawbacks and variability of

milk as a product and decided to utilize other screening tests like individual bulk milk cultures (TVC and TCC) to determine the milk quality alongside EC in order to adopt a multiple subjective interpretation of the results.

According to KeBS, all milk in the dairy industry, whether raw or processed is supposed to contain no amount of added water. In the present study, incidences of added water in raw milk samples were low, with an average of 0.81±1.0 and 0.87±0.99 for milk produced from LBY and CDM supplemented cows respectively. There was no significant difference among supplements, routes and their interactions in the level of milk adulteration. This study demonstrates that adulteration was not commonly practiced in the study area.

5.2.2. Hygienic criteria of milk

Total viable counts (TVC)

The results shown on table 8 indicate that the TVC for milk from LBY and CDM fed animals were not significantly different ($p>0.05$). In this study, TVC levels did not differ along the study routes and in the interaction between routes and diets. The findings suggest that farmers across tested routes applied ideally the same management skills. Recorded overall mean for TVC in this study was slightly higher than the recommended (>$\log_{10}$ 6.3 cfu/ml) for raw milk set by KeBS. The results were in concurrence with the findings of earlier studies carried out in Kiambu by Mwangi *et al.,* (2000) but inconsistent with findings of Kabui *et al.* (2015) that reported levels within recommended standards by KeBS. Several studies such as that by Grimaud *et al.* (2007) in Uganda, Shitandi and Kihumbu (2004) in Malawi, Kivaria *et al.* (2006) and Karimuribo *et al.* (2005) in Tanzania and Mamo *et al.* 2016 in Ethiopia had indicated such high levels of TVC. The higher TVC values could be attributed to inadequate procedures during equipment cleaning and milking (Ksontini *et al.*, 2011). Gargouri *et al.* (2014) indicated that the origin of such contamination could either be from milking utensils with milk residues on surfaces which can act as nutrient source for growth and multiplication of bacteria on subsequent milking, poor quality cleaning water, udder surface or milk filtering materials.

Total coliform counts (TCC)

The total coliform count of milk samples for the LBY fed cows showed a mean of 4.75 ± 0.62 $\log_{10}$cfu/ml as compared to control diet fed cows which was 4.68 ± 0.58 $\log_{10}$cfu/ml. The counts were essentially the same for diets ($p>0.05$) and interaction between diet and routes ($p>0.05$). The results suggest that there was no effect of diets on TCC on tested samples. Studies by Janzekovic *et al.* (2009) suggested that fast changing of feed rations, feeding either

muddy, moldy, rotten, frozen feed or lush pastures could cause diarrhea, thereby increasing micro-organisms count in milk. Coliform count was higher than recommended by KeBS (log_{10} 4.7 cfu/ml), an indication of either non-sanitary production conditions or poor handling practices during production and storage of raw milk. This further corroborates the findings of some earlier studies in Kiambu that reported relatively higher coliform counts compared with the threshold set by the national and international standards for raw milk (Mwangi *et al.*, 2000; Fakudze *et al.*, 2001). However, Kabui *et al.* (2015) reported coliform count levels within the recommended limits by KeBS. The presence of coliforms is more prevalent in the environment. According to Mwangi *et al.* (2013), coliforms can be present in plant materials and soil, get dispersed into atmosphere by dust and ends up into raw milk or fecal contamination during production and storage. Such contamination may possibly occur either due to poor hygienic conditions of the zero grazing units, unhygienic milk handling, or different sources of water used by farmers in the area as shown on table 10. Subclinical mastitis and inadequate cooling of milk might as well be responsible for such higher reported counts.

CHAPTER SIX
CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

i. The study identified major sources of contamination of LBY with spoilage microorganisms as unhygienic handling by distributors and farmers. Appropriate supplementation period of LBY to dairy cows is seven days.

ii. The two supplementation regimes CDM and LBY affected physicochemical quality of milk in the study area where higher protein levels and low freezing point was recorded in milk from cows supplemented with LBY compared to cows on CDM based diet. Moreover, the hypothesized theory that the use of LBY would lead to production of low grade milk for processing into different dairy products was nullified by the findings. The study indicated that LBY can be used successfully as a protein feed supplement in the dairy industry.

6.2. Recommendations

i. The research recommends storage of the by-product in hygienic containers and to avoid pooling of fresh LBY with previously supplied product. Storage in a cool and less humid environment, regular cleaning of feed troughs to prevent contamination of fresh feeds, are some of the good handling practices that need to be observed along the supply chain. This will enable prevention of major risk factors that can contribute to microbial contamination of LBY.

ii. There is need for capacity building to enable stakeholders in the dairy industry appreciate the importance of utilization of LBY as a cheaper protein source for dairy cows.

6.3. Areas for further research

i. A detailed study on bio-deterioration phenomena of the yeast strains at molecular level that would support a more insight knowledge on the degradation pathways and molecular adaption strategies to changing environmental conditions linked to temperature fluctuations which could enable more effective control avenues.

REFERENCES

Adamberg, K., Kask, S., Laht, T. M., & Paalme, T. (2003). The effect of temperature and pH on the growth of lactic acid bacteria: a pH-auxostat study. *International Journal of Food Microbiology, 85*(1), 171-183.

Aggelis, G., Athanassopoulos, N., Paliogianni, A., & Komaitis, M. (1998). Effect of a Teucrium polium L. extract on the growth and fatty acid composition of Saccharomyces cerevisiae and Yarrowia lipolytica. *Antonie Van Leeuwenhoek, 73*(2), 195-198.

Al-Gabr, H. M., Ye, C., Zhang, Y., Khan, S., Lin, H., & Zheng, T. (2013). Effects of carbon, nitrogen and pH on the growth of Aspergillus parasiticus and aflatoxins production in water. *Journal of Environmental Biology, 34*(2), 353.

Alqaisi, O., Torsten, H., Martin, H., & Andreas, S. (2014). Nutritional and ecological evaluation of dairy farming systems based on concentrate feeding regimes in semi-arid environments of Jordan. *Saudi Journal of Biological Sciences, 21*(5), 41-55.

Alshaikh, M. A., Alsiadi, M. Y., Zahran, S. M., Mogawer, H., & Aalshowime, T. A. (2002). Effect of feeding yeast culture from different sources on the performance of lactating holstein cows in Saudi Arabia. *Asian-Australasian Journal of Animal Science, 15*(3), 352-356.

AOAC. (1990). *Official Methods of Analysis, 15th Edition Volume II.* Association of Analytical Chemists: Washington DC.

Arambel, M. J., & Kent, B. A. (1990). Effect of yeast culture on nutrient digestability and milk yield response in early to mid lactation dairy cows. *Journal of Dairy Science, 73*(6), 1560-1563.

Asurmendi, P., García, M. J., Ruíz, F., Dalcero, A., Pascual, L., & Barberis, L. (2016). Biological control of AFB1-producing Aspergillus section Flavi strains isolated from brewer's grains, alternative feed intended for swine production in Argentina. *Journal of Environmental Science and Health, Part B, 51*(7), 477-481.

Auclair, E. (2001). Yeast as an example of the mode of action of probiotics in monogastric and ruminant species. In J. Brufau, *Feed manufacturing in Mediterranean region. Improving safety: From feed to food* (pp. 45-53). Zaragoza: CIHEAM.

Ayad, M. A., Benallou, B., Saim, M. S., Smadi, M. A., & Mezaine, T. (2013). Impact of feeding yeast culture on milk yield, milk components, and blood components in Algerian dairy herds. *Journal of Veterinary Science and Technology, 4*(2), 1-5.

Azhar, S. H., Rahmath, A., Siti, A. J., Hartinie, M., Jualang, A. G., Ainol, A. M., & Kenneth, F. R. (2017). Yeasts in sustainable bioethanol production: A review. *Biochemistry and Biophysics Reports, 10*(7), 52-61.

Back, W. (2005). Colour Atlas and Handbook of Beverage Biology. In W. Back, *Brewery* (pp. 10-112). Numbergy, Germany: Fachverlag Hans Carl, Germany.

Beauchemin, K. A., Yang, W. Z., Morgavi, D. P., Ghorbani, G. R., & Kautz, W. (2003). Effect of bacterial direct-fed microbial and yeast on site and extent of digestion, blood chemistry and subclinical acidosis in feedlot cattle. *Journal of Animal Science, 81*(6), 1628-1640.

Bjerg, M., & Rasmussen, M. D. (2005). Freezing point of bulk tank milk in Denmark. *Proceedings NMC 44th Annual meeting, Orlando, FL.* Verona: National Mastitis Council.

Boer, V. M., Christopher, A. C., Patrick, H. B., David, B., & Joshua, D. R. (2010). Growth-limiting Intracellular Metabolites in Yeast Growing under Diverse Nutrient Limitations. *Molecular Biology of the Cell, 21*(1), 198-211.

Bokulich, N. A., & Bamfortha, C. W. (2013). The Microbiology of Malting and Brewing. *Microbiology and Molecular Biology Reviews, 77*(2), 157-172.

Bovill, R., Bew, J., & Robinson, S. (2001). Comparison of selective media for the recovery and enumeration of probiotic yeasts from animal feeds. *International Journal of Food Microbiology, 67*(1), 55-61.

Bowman, M. (2005). *Significant of nutritional Effect on the freezing point of Milk.* Ontario Agri-business Association Nutrition Committee.

Brandt, M., & Warnock, D. (2015). Taxonomy and Classification of Fungi. In J. Jorgensen, M. Pfaller, K. Carroll, G. Funke, M. Landry, S. Richter, & D. Warnock, *Manual of Clinical Microbiology, Eleventh Edition* (pp. 1935-1943). Washington, DC: ASM Press.

Briggs, D. E., Boulton, P. A., & Stevens, B. (2004). *Brewing: Science and Practice.* Abington Hall: Wood head Publishing Limited Abington Cambridge CBI 6AH, England.

Briggs, M. A., Kristina, S. P., & Penny, M. K.-E. (2017). Saturated Fatty Acids and Cardiovascular Disease: Replacements for Saturated Fat to Reduce Cardiovascular Risk. *Healthcare, 5*(2), 1-29.

Broach, J. R. (2012). Nutritional control of growth and development in yeast. *Journal of Genetics, 192*(1), 73-105.

Brouwer, T. (1981). Calculations concerning the determination of freezing depression of milk. *Netherlands Milk and Dairy Journal*, 35, 159-175.

Bruno, R. G., Rutigliano, H. M., Cerri, R. L., Robinson, P. H., & Santos, J. E. (2009). Effect of feeding Saccharomyces Cerevisiae on performance of dairy cows during summer heat stress. *Animal Feed Science and Technology, 150*(3), 175-185.

Buchl, N. R., M, H., Mietke-Hofmann, H., Wenning, M., & Scherer, S. (2010). Differentiation of probiotic and environmental Saccharomyces cerevisiae strains in animal feed. *Journal of Applied Microbiology, 109*(3), 783-791.

Čabarkapa, I. S., & Ivanov, D. S. (2009). Screening of mycotoxins in animal feed from the region of Vojvodina. *Matica Srpska Proceedings for Natural Sciences, 117*(1), 87-96.

Čabarkapa, I., Kokić, B., Plavšić, D., Ivanov, D., & Lević, J. (2009). Microbiological safety of animal feeds. *Biotechnology in Animal Husbandry, 25*(5), 1155-1162.

Canibe, N., & Jensen, B. B. (2003). Fermented and nonfermented liquid feed to growing pigs: effect on aspects of gastrointestinal ecology and growth performance. *Journal of Animal Science, 81*(8), 2019-2031.

Canibe, N., & Jensen, B. B. (2012). Fermented liquid feed-Microbial and nutritional aspects and impact on enteric diseases in pigs. *Animal Feed Science and Technology, 173*(1), 17-40.

Carter, G. R., & Cole Jr, J. R. (2012). *Diagnostic procedure in veterinary bacteriology and mycology* (5th Edition ed.). New York: Academic Press.

Cassone, M., Scaletti, S., Pastella, E., & Venditti, M. (2003). Outbreak of Saccharomyces cerervisiae subtype boulardii fungemia in patients neighbouring those treated with a probiotic preparation of the organism. *Journal of Clinical Microbiology, 41*(11), 5340-5343.

Castillo-González, A. R., Burrola-Barraza, M. E., & Domínguez-Viveros, J. C.-M. (2014). Rumen microorganisms and fermentation. *Archivos de Medicina Veterinaria, 46*(3), 341-361.

Charoenchai, C., Fleet, G. H., & Henschke, P. A. (1998). Effects of temperature, pH, and sugar concentration on the growth rates and cell biomass of wine yeasts. *American Journal of Enology and Viticulture, 49*(3), 283-288.

Chaucheyras-Durand, F., Eric, C., Cécile, M., & Evelyne, F. (2012). Use of Yeast Probiotics in Ruminants: Effects and Mechanisms of Action on Rumen pH, Fibre Degradation,and Microbiota According to the Diet. In *Probiotic in Animals* (pp. 119-152). licensee InTech.

Chaucheyras-Durand, F., Walker, N. D., & Bach, A. (2008). Effects of active dry yeasts on the rumen microbial ecosystem: past, present and future. *Animal Feed Science and Technology, 145*(1), 5-26.

Chin, S. F., Liu, W., Storkson, J. M., & Ha, Y. L. (1992). Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognised class of anticarcinogens. *Journal of Food Composition and Analysis, 5*(3), 185-197.

Collomb, M., Schmid, A., Sieber, R., Wechsler, D., & Ryhanen, E. L. (2006). Conjugated linoleic acids in milk fat: Variations and physiological effect. *International Dairy Journal, 16*(11), 1347-1361.

Cooporative Resource International. (2016, December 29). *Mold/ Yeast/ Mycotoxin Testing.* Retrieved December 3, 2018, from Cooporative Resource International: http://agsource.crinet.com/page3189/MoldYeastMycotoxinTesting

Czerucka, D., Piche, T., & Rampal, P. (2007). Review article: yeast as probiotics-Saccharomyces boulardii. *Alimentary Pharmacology & Therapeutics, 26*(6), 767-778.

D'Mello, J. P. (2004). *Microbiology of animal feeds. In Assessing Quality and Safety of Animal Feeds, Issue 160.* Geneva: Food and Agriculture Organization of the United Nations, (FAO).

Dagnas, S., Onno, B., & Membré, J. M. (2014). Modeling growth of three bakery product spoilage molds as a function of water activity, temperature and pH. *International Journal of Food Microbiology, 186*(9), 95-104.

Davis, C. L., Greenwalt, D. A., & McCoy, G. C. (1983). Feeding value of pressed brewers grains for lactating dairy cows. *Journal of Dairy Science, 66*(1), 73-79.

Dawson, K. A., Newman, K. E., & Boling, J. A. (1990). Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. *Journal of Animal Science, 68*(10), 3392-3398.

De Caterina, R., & Zampolli, A. (2001). -n-3 fatty acids and inflammatory response- Biological background. *Journal of Lipids, 3*(6), 69-78.

De Nardi, R., Giorgio, M., Shucong, L., Ehsan, K., Kees, J. C., Plaizier, M. G., . . . Severino, S. (2016). Metagenomic analysis of rumen microbial population in dairy heifers fed a high grain diet supplemented with dicarboxylic acids or polyphenols. *BMC Veterinary Research, 12*(29), 1-9.

De Ondarza, M., Sniffen, C., Dussert, L., Chevaux, E., Sullivan, J., & Walker, N. D. (2010). Case study:Multiple-Study analysis of the effect of live yeast on milk yield, milk

component content and yield, and feed efficiency. *The Professional Animal Scientist, 26*(6), 661–666.

De Souza, L. C., Zambom, M. A., Magali, P. S., Marcela, A. N., Ana, C. R., Liliane, B., . . . Simoni, G. (2012). Development of microorganisms during storage of wet brewery waste under aerobic and anaerobic conditions. *Revista Brasileira de Zootecnia, 41*(1), 1-9.

Desnoyers, M., Giger-Reverdin, G., Bertin, G., Duvaux-Ponter, C., & Sauvant, D. (2009). Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. *Journal of Dairy Science, 92*(4), 1620-1632.

Dewhurst, R. J., Shingfield, K. J., Lee, M. R., & Scollan, N. D. (2006). Increasing the concentration of the beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. *Animal Feed Science and Technology, 131*(3), 168-206.

Dhiman, T. M., Anand, G. R., Satter, L. D., & Pariza, M. W. (1999). Conjugated linoleic content of milk from cows fed different diets. *Journal of Dairy science, 82*(10), 2146-2156.

Dikicioglu, D., Pir, P., & Oliver, S. G. (2013). Predicting complex phenotype-genotype interactions to enable yeast engineering: Saccharomyces cerevisiae as a model organism and a cell factory. *Biotechnology Journal, 8*(9), 1017-1034.

Doyle, M. E. (2007). *Microbial Food Spoilage — Losses and Control Strategies. A brief review of literature.* Madison WI 53706: Food Research Institute, University of Wisconsin–Madison.

Driehuis, F., Oude, E. S., & Van Wikselaar, P. G. (2001). Fermentation characteristics and aerobic stability of grass silage inoculated with Lactobacillus buchneri, with or without homofermentative lactic acid bacteria. *Grass and Forage Science, 56*(4), 330-343.

Elgersma, A., Tamminga, S., & Ellen, G. (2006). Modifying milk composition through forage. *Animal Feed Science and Technology, 131*(3), 207-225.

Ellin, D. M. (2007). *Microbial Food Spoilage — Losses and Control Strategies. A brief review of literature.* Madison: Madison WI 53706: Food Research Institute, University of Wisconsin.

Erasmus, L. J., Botha, P. M., & Kistner, A. (1992). Effect of yeast culture supplement on production, rumen fermentation and duodenum nitrogen flow in dairy cows. *Journal of Dairy Science, 75*(11), 3056-3065.

European Food Safety Authority. (2014). Scientific Opinion on the safety and efficacy of MycoCell (Saccharomyces cerevisiae) as a feed additive for dairy cows. *European Food Safety Authority Journal, 12*(9), 1-12.

Faccenda, A., Maximiliane, A. Z., Deise, D. C., & André. (2017). Use of dried brewers' grains instead of soybean meal to feed lactating cows. *Brazilian Journal of Animal Science, 46*(1), 39-46.

Fakudze, F. M., & Dlamini, A. M. (2001). Compositional and hygienic quality of consumer milk from shops in Swaziland. *UNISWA Research Journal af Agriculture, Science and Technology, 5*(2), 169-176.

FAO. (2011). *Global food losses and waste. Extent, causes and prevention.* Rome: FAO.

FAO, World Health Organization, & WHO Expert Committee on Food Additives. (1987). *Principles for the safety assessment of food additives and contaminants in food.* Geneva: FAO.

Fijan, S. (2014). Microorganisms with Claimed Probiotic Properties: An overview of Research Literature. *International Journal of Environmental Research and Public Health, 11*(5), 4745-4746.

Filya, I., Ashbell, G., Hen, Y., & Weinberg, Z. G. (2000). The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. *Animal Feed Science and Technology, 88*(1), 39-46.

Flint, H. J., Karen, P. S., Sylvia, H. D., Petra, L., & Evelyne, F. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut Microbes, 3*(4), 89-306.

Gargouri, A., Hamed, H., & Elfeki, A. (2013). Analysis of raw milk quality at reception and during cold storage: combined effects of somatic cell counts and psychrotrophic bacteria on lipolysis. *Journal of Food Science, 78*(9), 1405-1411.

Gargouri, A., Hamed, H., Ben, A. B., Elfeki, A., & Gdoura, R. (2014). Evaluation of Tunisian milk quality in dairy herds: Inter-relationship between chemical, physical and hygienic criteria. *Animal Science Journal, 85*(6), 714-721.

Giannattasio, S., Guaragnella, N., Maša, Ž., & Ersilia, M. (2013). Molecular mechanisms of Saccharomyces cerevisiae stress adaptation and programmed cell death in response to acetic acid. *Frontiers in Microbiology, 4*(33), 1-7.

Gichohi, M. (2014). *Status of the Kenya Dairy industry.* MD, Kenya Dairy Board.

Goddard, M. R., & Greig, D. (2015). Saccharomyces cerevisiae: a nomadic yeast with no niche? *FEMS Yeast Research, 15*(2), 1-6.

Goodling, R. C., Rogers, G. W., Cooper, J. B., & Rune, B. (2001). Genetic relationships among electrical conductivity of milk, somatic cell scores and mastitis. *Journal of Dairy Science, 84*(Suppl 1), 484.

Goto, H., Abdul, Q. Q., Yo-Han, K., Kentaro, I., Toshihiro, I., & Shigeru, S. (2016). Effects of a bacterial probiotic on ruminal pH and volatile fatty acids during subacute ruminal acidosis (SARA) in cattle. *Internal Medicine, 78*(10), 1595-1600.

Gougouli, M., & Koutsoumanis, K. P. (2012). Modeling germination of fungal spores at constant and fluctuating temperature conditions. *International Journal of Food Microbiology, 152*(3), 153-161.

Gracias, K., & McKillip, J. (2004). A review of conventional detection and enumeration methods for pathogenic bacteria in food. *Canadian Journal of Microbiology, 50*(11), 883-890.

Grierus, M., de Jonge, L., & Meijer, G. A. (2005). Physico-chemical characteristics and degr. *Livestock Production Science, 97*(2), 219-225.

Grimaud, P. S., & Grillet, N. (2007). An evaluation of milk quality in Uganda: Value chain assessment and evaluation. *African Journal of Food, Agriculture, Nutrition and Development, 7*(5), 16-24.

Gupta, M., Abu-Ghannam, N., & Gallaghar, E. (2010). Barley for Brewing: Characteristic Changes during Malting, Brewing and Applications of its By-Products. *Comprehensive Reviews in Food Science and Food Safety, 9*(1), 318-328.

Haakensen, M., & Ziola, B. (2008). Identification of novel horA- harbouring bacteria capable of spoiling beer. *Canadian Journal of Microbiology, 54*(4), 321-325.

Hakovirta, J. (2008). *Modern Techniques in Detection, Identification and Quantification of Bacteria and Peptides from Food.* Dissertation, University of Helsinki.

Hamad, S. H. (2012). Factors Affecting the Growth of Microorganisms in Food. In S. H. Hamad, B. Rajeev, K. A. Abd, & P. Gopinadhan (Eds.), *Progress in Food Preservation 1* (pp. 405-427). Wiley Online Publisher.

Hamann, J., & Zecconi, A. (1998). Evaluation of the electrical conductivity of milk as a mastitis indicator. *International Dairy Federation, Bullet 334.* Brussels, Belgium.

Hamasaki, M., Noda, T., & Ohsumi, Y. (2003). The early secretory pathway contributes to autophagy in yeast. *Cell Structure and Function, 28*(1), 49-54.

Hammond, S., James, H. B., Joseph, R. B., Tatiana, P. F., Trevor, S. F., Norman, M.-S., . . . Jordan, G. O. (2015). Food Spoilage, Storage, and Transport: Implications for Sustainable Future. *BioScience, 65*(8), 758-768.

Harding, F. (1999). Milk quality. *Aspen Publication*, (pp. 62-67). Gaithersburg.

Harris, B. J., & Webb, D. (1990). The effect of feeding a concentrated yeast culture product to lactating dairy cows. *Journal of Dairy Science, 73*(1), 266.

Harris, B., & Bachman, K. C. (2003, June 1). Nutritional and management factors affecting solids-non fat, acidity and freezing point on milk. *IFAS Extension*, pp. 1-5.

Hemalatha, R., Saravanamurugan, C., Meenatchisundaram, S., & Rajendran, S. (2015). Comparative Study of Bioethanol Production from Agricultural Waste Materials Using Saccharomyces cerevisiae (MTCC 173) and Zymomonas mobilis (MTCC 2427) by Enzymatic Hydrolysis Process. *International Journal of Microbiological Research, 6*(2), 74-78.

Hernandez, J., José, L. B., Angel, A., & Cristina, C. (2014). Ruminal Acidosis in Feedlot: From Aetiology to Prevention. *The Scientific World Journal, 2014*(1), 1-9.

Hoffman, P. C., & Armentano, L. E. (1988). Comparison of brewers wet and dried grains and soybean meal as supplements for dairy cattle. *Nutritional Reports International, 38*(1), 655-663.

Hoffmann, A., Herbert, S., Margit, S., Helga, T., Jara, K. H., Eva, W., & Rainer, M. (2013). Changes in fatty acid composition of various full fat crushed oilseeds and their free oils when incubated with rumen liquor in vitro. *Archives of Animal Nutrition, 67*(1), 77-92.

Hornsey, I. (2013). *Brewing 2nd Edition.* Thomas Graham House, Science Park, Milton Road, Cambridge CB4 OWF, UK: Royal Society of Chemistry.

Howes, N. L., Bekhit, A. E.-D., David, J. B., & Anna, W. C. (2014). Opportunities and Implications of Pasture-Based Lamb Fattening to Enhance the Long-Chain Fatty Acid Composition in Meat. *Comprehensive Reviews in Food Science and Food Safety, 14*(12), 22-36.

Huber, J. T. (1990). The fungal and yeast culture story in lactating dairy cows. *International proceedings South West Nutrition manage* (pp. 87-94). Tempe AZ.

Hur, S. J., Kim, H. S., Bahk, Y. Y., & Park, Y. (2017). Overview of conjugated linoleic acid formation and accumulation in animal products. *Livestock Science, 195*(1), 105-111.

Huys, G., Nadine, B., Frank, D., Luc, D. V., Marc, H., Bruno, P., . . . Georges, D. (2013). Microbial characterization of probiotics–Advisory report of the Working Group "8651 Probiotics" of the Belgian Superior Health Council (SHC). *Molecular Nutrition and Food Resources, 57*(1), 1479-1504.

ICMSF. (1996, January 1). *Microorganisms in Foods 5: Characteristics of Microbial Pathogens.* Retrieved from International Commission on Microbiological Specifications for Food: https://books.google.co.ke/books?id=lxycHnaPfCYC

Jakobsen, M., & Narvhus, J. (1996). Yeast and their possible beneficial and negative effects on the quality of dairy products. *International Dairy Journal, 6*(8), 755-768.

Janzekovic, M., Brus, M., Mursec, B., Vinis, P., Stajnko, D., & Cus, F. (2009). Mastitis detection based on electric conductivity of milk. *Journal of Achievements in Materials and Manufacturing Engineering, 34*(1), 39-46.

Joffe, A. Z., & Lisker, N. (1969). Effects of light, temperature, and pH value on aflatoxin production in vitro. *Applied Microbiology, 18*(3), 517-518.

Jouany, J., & Morgavi, D. (2007). Use of 'natural' products as alternative to antibiotic feed additives in ruminant production. *Animal, 1*(10), 1443-1466.

Jouhten, P., Ponomarova, O., Gonzalez, R., & Patil, K. (2016). Saccharomyces cerevisiae metabolism in ecological context. *FEMS Yeast Research, 16*(7), 1-8.

Juška, A. (2011). Minimal Models of Growth and Decline of Microbial Populations. *Journal of Theoretical Biology, 269*(1), 195–200.

Kabui, K., Arimi, S., Kang'ethe, E., Omore, A., Makokha, S., Nduhiu, G., . . . Macharia, J. (2015). A determination of raw milk quality and the most suitable microbiological test at the milk collection level in two regions of Kenya. *International Journal of Veterinary science, 4*(2), 55-59.

Kalac, P., & Samkova, E. (2010). The effect of feeding various forages on fatty acid composition of bovine milk fat: A review. *Czech Journal of Animal Science, 55*(12), 521-537.

Kalmus, P., Toomas, O., Andres, W., Raivo, L., & Kalle, K. (2009). Effect of yeast culture on milk production and metabolic and reproductive performance of early lactation dairy cows. *Acta Veterinaria Scandinavica, 51*(32), 1-7.

Karimuribo, E. D., Mdegela, R. H., Kusiluka, L. J., & Kambarage, D. M. (2005). Assessment of drug usage and antimicrobial residues in milk on smallholder farms in Morogoro, Tanzania. *Bulletin of Animal Health and Production in Africa, 53*(4), 234-241.

Kerby, C., & Vriesekoop, F. (2017). An Overview of the Utilisation of Brewery By-Products as Generated by British Craft Breweries. *Beverages, 3*(24), 1-12.

Khan, R. U., Shabana, N., Kuldeep, D., Karthik, K., Ruchi, T., Mutassim, M. A., . . . Arshad, Z. (2016). Direct-Fed Microbial: Beneficial Applications, Modes of Action and

Prospects as a Safe Tool for Enhancing Ruminant Production and Safeguarding Health. *International Journal of Pharmacology, 12*(3), 220-231.

Kim, J. H., Kim, Y., Kim, Y. J., & Park, Y. (2016). Conjugated Linoleic Acid: Potential Health Benefits as a Functional Food Ingredient. *Annual Review of Food Science and Technology, 7*(1), 221-244.

Kitchen, B. J. (1981). Review of the progress of dairy science: bovine mastitis, milk compositional changes and related diagnostic tests. *Journal of Dairy Research, 48*, 167-188.

Kivaria, F. M., Noordhuizen J, P., & Kapaga, A. M. (2006). Evaluation of the hygienic quality and associated public health hazards of raw milk marketed by smallholder dairy producers in the Dar es salaam region of Tanzania. *Tropical Animal Health and Production, 38*(3), 185-195.

Kleen, J. L., Hooijer, G. A., Rehage, J., & Noordhuizen, J. P. (2003). Subacute ruminal acidisis (SARA): A review. *Journal of Veterinary Medicine Series A, 50*(8), 406-414.

Kocsubé, S., Perrone, G., Magistà, D., Houbraken, J., Varga, J., Szigeti, G., . . . Samson, R. A. (2016). Aspergillus is monophyletic: Evidence from multiple gene phylogenies and extrolites profiles. *Studies in Mycology, 85*(9), 199-213.

Koehler, P. E., Beuchat, L. R., & Chhinnan, M. S. (1985). Influence of temperature and water activity on aflatoxin production by Aspergillus flavus in cowpea (Vigna unguiculata) seeds and meal. *Journal of Food Protection, 48*(12), 1040-1043.

Korkeala, H., Alanko, T., Mäkelä, P., & Lindroth, S. (1989). Shelf-life of vacuum-packed cooked ring sausages at different chill temperatures. *International Journal of Food Microbiology, 9*(3), 237-247.

Kristensen, S. D., Bach, I. A., & Schmidt, E. B. (2001). -n-3 polyunsaturated fatty acids and coronary thrombosis. *Journal of Lipids, 36*(1), 79-82.

Krizova, L., Richter, M., Trinacty, J., Riha, J., & Kumprechtova, D. (2011). The effect of feeding live yeast cultures on ruminal pH and redox potential in dry cows as continuously measured by a new wireless device. *Czech Journal of Animal Science, 56*(1), 37-45.

Ksontini, H., Kachouri, H., & Hamdi, M. (2011). Microflora distribution and assessment of microbiological quality milk from Tunisia collection centres. *African Journal of Microbiology Research, 5*(12), 1484-1491.

Kwatra, M. S., Nem, S. S., & Oberoi, M. S. (1983). Spontaneous brewers' grains toxicity in buffaloes. *Indian Journal of Animal Science, 53*(1), 330-331.

Lanyasunya, T. P., Wamae, L. W., Musa, H. H., Olowofeso, O., & Lokwaleput, I. K. (2005). The risk of mycotoxins contamination of dairy feed and milk on smallholder dairy farms in Kenya. *Pakistan Journal of Nutrition, 4*(3), 162-169.

Laulund, S., Anette, W., Patrick, M. F., & Véronique, Z. (2017). Regulatory and Safety Requirements for Food Cultures. *Microorganisms, 5*(28), 1-14.

Lee, K., Lee, S., & Lee, B. (2006). Aspergillus oryzae as probiotic in poultry: a review. *International Journal of Poultry Science, 5*(1), 1-3.

Lehnen, T. E., Marcondes, R. d., Augusto, C., Aline, M., & Alexandre, M. L. (2015). A review on effects of conjugated linoleic fatty acid (CLA) upon body composition and energetic metabolism. *Journal of the International Society of Sports Nutrition, 12*(36), 1-12.

Leuschner, R. G., Bew, J., Fourcassier, P., & Bertin, G. (2004). Validation of the official control method based on polymerase chain reaction (PCR) for identification of authorised probiotic yeast in animal feed. *Journal of Systematic and Applied Microbiology, 27*(4), 492-500.

Lewis, M. J., & Young, T. (2001). *Brewing.* New York: Kluwer Academic Plenum Publishers.

Lima-Filho, J. V., Vieira, E. C., & Nicoli, J. R. (2000). Saccharomyces boulardii and Escherichia coli combinations against experimental infections with Shigella flexneri and Salmonella enteritidis subsp.Typhimurium. *Journal of Applied Microbiology, 88*, 365-370.

Ljungdahl, P. O., & Daignan-Fornier, B. (2012). Regulation of amino acid, nucleotide, and phosphate metabolism in Saccharomyces cerevisiae. *Genetics, 190*(3), 885-929.

Llopis, S., Carolina, H.-H., Lucı´a, M., Amparo, Q., Marı´a, M., & Marı´a, F.-E. (2014). Pathogenic Potential of Saccharomyces Strains Isolated from Dietary Supplements. *PLoS One, 9*(5), 1-21.

Lock, A. L., Preseault, C. L., Rico, J. E., DeLand, K. E., & Allen, M. S. (2013). Feeding a C16:0-enriched fat supplement increased the yield of milk fat and improved conversion of feed to milk. *Journal of Dairy Science, 96*(10), 6650–6659.

Lopez-Campos, G., Mónica, A.-U., Victoria, L. A., & Joaquín, V. M.-S. (2012). *Microarray Detection and Characterization of Bacterial Foodborne Pathogens.* Berlin: Springer Science & Business Media.

Lourens, A., & Viljoen, B. C. (2001). Growth and survival of a probiotic yeast in dairy products. *Journal of Food Research International, 34*(9), 791-796.

Maciorowski, K. G., Herrera, P., Jones, F. T., Pillai, S. D., & Ricke, S. C. (2007). Effects on poultry and livestock of feed contamination with bacteria and fungi. *Animal Feed Science and Technology, 133*(1), 109-136.

Makau, J. M., Irungu, P., Nyikal, R. A., & Kirimi, L. W. (2016). An assessment of the effect of a national fertilizer subsidy programme on farmer participation in private fertilizer markets in the North Rift Region of Kenya. *African Journal of Agricultural and Resource Economics, 11*(4), 292.

Mamo, J., Kumera, B., & Asmamaw, M. (2016). Evaluation of microbiological quality of raw milk, homemade Ergo and homemade Ayib in North Shoa District, Amhara, Ethiopia. *Pakistan Journal of Food Science, 26*(2), 83-91.

Manafi, M. (2000). New developments in chromagenic and fluorogenic culture media. *International Journal of Food Microbiology, 60*(2), 205-218.

Mangal, M., Khan, F., Bansal, S., & Oberoi, H. S. (2016). Validation of PCR based detection system for aflatoxin producing molds. *Indian Journal of Experimental Biology, 54*(1), 472-476.

Marden, J. P., Julien, C., Monteils, V., Auclair, E., Moncoulon, R., & Bayourthe, C. (2008). How does live yeast differ from sodium bicarbonate to stabilize ruminal pH in high-yielding dairy cows? *Journal of Dairy Science, 91*(9), 3528-3535.

Marsola, R. S., Favoreto, M. G., Silvestre, F. T., Shin, J. C., Walker, N., Adesogan, A., . . . Santos, J. E. (2010). Effect of feeding live yeast on performance of holstein dairy cows during summer. *Journal of Dairy Science, 93*, 432-432.

Martin, S. A., & Nisbet, D. J. (1990). Effects of Aspergillus oryzae fermentation extract on fermentation of amino acids and starch by mixed ruminal microorganisms invitro. *Journal of Animal Science, 68*(7), 2142-2149.

Matsubara, V. H., Bandara, H. M., Marcia, P. A., & Lakshman, P. S. (2016). Probiotics as Antifungals in Mucosal Candidiasis. *Clinical Infectious Diseases, 62*(9), 1143-1153.

McCullough, M. J., Clemons, K. V., McCusker J, H., & Stevens, D. A. (1998). Species identification and Virulence attributes of Saccharomyces boulardii. *Journal of Clinical Microbiology, 36*(9), 2613-2617.

McDonald, P., Henderson, A. R., & Heron, S. J. (1991). *The Biochemistry of Silage.* Marlow: GEN, Chalcombe Publications.

McFarland, L. V. (2010). Systemic review and meta-analysis of Saccharomyces boulardii in adult patients. *World Journal of Gastroenterology, 16*(18), 2202-2222.

McGuire, M. A., & McGuire, M. K. (2000). Conjugated linoleic acids (CLA): A ruminant fatty acid with beneficial effects on human health. *Journal of Animal Science, 77*(E-Supl), 1-8.

Milala, M. A., & Addy, E. O. (2014). Hydrolytic enzyme levels in malted cereals. *Advances in Biochemistry, 2*(5), 76-79.

Miyazawa, K., Sultana, H., Mirata, T., Kanda, S., & Itabashi, H. (2007). Effect of brewers grain in rumen fermentation, milk production and milk composition in lactating dairycows. *Journal of Animal Science, 78*(5), 519-526.

Moslehi-Jenabian, s., Pedersen., L., & Jespersen, L. (2010). Beneficial effects of probiotic and food borne yeasts on human health. *Nutrients, 2*(4), 449-473.

Muema, V. (2018, April 27). *Importance of chachu (Brewer's Yeast)*. Retrieved from Happy Feeds Limited: www.happyfeeds.co.ke

Muia, J. M., Kariuki, J. N., Mbugua, P. N., Gachuiri, C. K., Lukibisi, L. B., Ayako, W. O., & Ngunjiri, W. V. (2011). Smallholder dairy production in high altitude Nyandarua milk-shed in Kenya: Status, challenges and opportunities. *Livestock Research for Rural Development, 23*(108), 1-12.

Muller-Auffermann, K., Grijalva, F., Jacob, F., & Hutzler, M. (2015). Nisin and its usage in breweries: a review and discussion. *Journal of the Institute of Brewing, 121*(5), 309-319.

Muriuki, H. (2011). *Dairy development in Kenya.* Food and Agricultural Organization of the United Nations, Rome.

Musa, H. H., Wu, S. L., Zhu, C. H., Seri, H. I., & Zhu, G. Q. (2009). The Potential Benefits of Probiotics in Animal Production and Health. *Journal of Animal and Veterinary Advances, 8*(2), 313-321.

Mussatto, S. I., Dragone, G., & Roberto, I. C. (2006). Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science, 43*(1), 1-14.

Mwangi, A., Arimi, S. M., Mbugua, S., Kangethe, E. K., & Omore, A. O. (2000). Assurance of marketed milk quality in Kenya. *Faculty of Veterinary Medicine Biennial Scientific Conference.* Nairobi: University of Nairobi, Kenya.

Mwangi, L., Matofari, J., Muliro, P., & Bebe, B. (2013). Handling practices and microbiological quality characteristics of traditional pastoral fermented milk (SUUSA). *11th African Crop Science Proceedings, Sowing innovations for sustainable food and nutrition security in Africa. II*, pp. 427-430. Entebbe: African crop science society.

Mwende, M. C., Wafula, M. J., Simiyu, P., & Omedo, B. B. (2016a). Association of on-farm feeds handling practices with fungal growth and mycotoxin production on feeds in smallholder dairy farms, Nakuru, Kenya. *African Journal of Agricultural Research, 11*(39), 3741-3750.

Mwende, M. K., Matofari, J. W., Muliro, P. S., & Bebe, B. O. (2016b). Aflatoxin B1 and deoxynivalenol contamination of dairy feeds and presence of aflatoxin M1 contamination in milk from smallholder dairy systems in Nakuru, Kenya. *International Journal of Food Contamination, 11*(39), 3741-3750.

Nagpal, R., Kumar, A., Kumar, M., Behare, P. V., Jain, S., & Yadav, H. (2012). Probiotics, their health benefits and applications for developing healthier foods: a review. *FEMS Microbiology Letters, 334*(1), 1-15.

Newbold, C. J., Mclntosh, F. M., & Wallace, R. (1998). Changes in the microbial population of a rumen stimulating fermenter in response to yeast culture. *Canadian Journal of Animal Science, 78*(2), 241-244.

Newbold, C. W. (1996). Mode of action of yeast Saccharomyces cerevisae as a feed additive for ruminants. *British Journal of Nutrition, 76*(2), 249-261.

Ni, K., Wang, Y., Cai, Y., & Pang, H. (2015). Natural lactic acid bateria population and silage fermentation of whole-crop wheat. *Asian-Australasian Journal of Animal Sciences, 28*(8), 1123-1132.

Niault, M., Thomas, F., Prost, J., Ansari, F. H., & Kalfon, P. (1999). Fungamia due to Saccharomyces species in a patient treated with enteral Sacharomyces boulardii. *Clinical Infectious Diseases, 28*(4), 930-930.

Nishino, N., & Touno, E. (2005). Ensiling characteristics and aerobic stability of direct-cut and wilted grass silages inoculated with Lactobacillus casei or Lactobacillus buchneri. *Journal of the Science of Food and Agriculture, 85*(11), 1882-1888.

Norberg, E., Hogeveen, H., Korsgaard, I. R., Friggens, N. C., Sloth, K. H., & Lovendahl, P. (2004). Electrical conductivity of milk: ability to predict mastitis status. *Journal of Dairy Science, 87*(4), 1099-1107.

Nudda, A., Gianni, B., Oscar, B. N., Antonello, C., Ana, H. D., Alberto, S. A., & Giuseppe, P. (2014). Feeding strategies to design the fatty acid profile of sheep milk and cheese. *Revista Brasileira de Zootecnia, 43*(8), 445-456.

Odle, J., Sheila, K. J., Dean, B. R., Dale, E. B., Russell, V. A., Fuller, W. B., . . . Andrew, C. S. (2017). The Potential Impact of Animal Science Research on Global Maternal and

Child Nutrition and Health: A Landscape Review. *Advances in Nutrition, 8*(2), 362-381.

Ogola, H., Shitandi, A., & Nanua, J. (2007). Effect of mastitis on raw milk compositional quality. *Journal of Veterinary Science, 8*(3), 237-242.

Ohlsson, L. (2010). Dairy products and plasma cholesterol levels. *Food and Nutrition Research, 54*(8), 1-9.

Okwee-Acai, J., & Acon, J. (2005). Claw lessons and lameness in Zerograzed cattle fed brewers' grains in Uganda. *Bulletin of Animal Health and Production Africa, 53*(2), 107-112.

Oviedo, M. S., Ramirez, M. L., Barros, G. G., & Chulze, S. N. (2011). Influence of water activity and temperature on growth and mycotoxin production by Alternaria alternata on irradiated soya beans. *International Journal of Food Microbiology, 149*(2), 127-132.

Owens, E. L. (1959). Observations of the toxicity of brewers' grains for dairy cows. *New Zealand Veterinary Journal, 7*(2), 43-46.

Owens, F., Secrist, D., Hill, W., & Gill, D. (1998). Acidosis in Cattle: A review. *Journal of Animal Science, 76*(1), 275-286.

Palmquist, D. L. (2006). *Milk Fat: Origin of fatty acids and influence of nutritional factors thereon* (3rd ed., Vol. 2). (P. F. Fox, & P. L. McSweeney, Eds.) New York, The Ohio State University, Wooster, OH 44691, USA: Springer.

Pariza, M. W., Yeonhwa, P., & Cook, M. E. (1997). Conjugated linoleic acid and the control of cancer and obesity. *Journal of Toxicological Science, 52*(12), 107-110.

Pereyra, M. L., Rosa, C. A., Dalcero, A. M., & Cavaglieri, L. R. (2011). Mycobiota and mycotoxins in malted barley and brewer’s spent grain from Argentinean breweries. *Letters in Applied Microbiology, 53*(4), 649-655.

Pérez, E. V. (2011). *Plasma Transport and Mammary Uptake of Trans Fatty Acids in Dairy Cows. PhD Thesis.* Leicestershire: University of Nottingham.

Perez-Torrado, R., & Querol, A. (2016). Opportunistic Strainsof Saccharomyces cerevisiae:A Potential Risk Sold in Food Products. *Frontiers in Microbiology, 6*, 1522.

Petti, A. A., Christopher, A. C., Joshua, D. R., & David, B. (2011). Survival of starving yeast is correlated with oxidative stress response and nonrespiratory mitochondrial function. *Proceedings of the National Academy of Sciences of the United States of America, 108*(11), E1089-E1098.

Polan, C. E., Herrington, T. A., Wark, W. A., & Armentano, L. E. (1985). Milk production responses to diets supplemented with dried brewers grains, wet brewers grains, or soybean meal. *Journal of Dairy Science, 68*(8), 2016-2026.

Poppy, G. D., Rabiee, A. R., Lean, I. J., Sanchez, W. K., Dorton, K. L., & Morley, P. S. (2012). A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of Saccharomyces cerevisiae on milk production of lactating dairy cows. *Journal of Dairy Science, 95*(10), 6027-6041.

Prasad, R. J., Souri, S., Sharma, A. K., Sharma, G., & Egrae, C. M. (2016). Contaminants and toxins in foods and feeds. *International Journal of Environmental Science and Technology, 1*(1), 82-88.

Preston, R. L., Vance, R. D., & Cahill, V. R. (1973). Energy evaluation of brewers' grains for growing and finishing cattle. *Journal of Animal Science, 37*(1), 174-178.

Priest, G. F., & Campbell, I. (2003). *Brewing Microbiology.* 233 Spring Street, New York, N.Y. 10013: Kluwer Academy/Plenum Publishers, Newyork.

Putnam, D. E., Schwab, C. G., Socha., M. T., Whitehouse, N. L., Kierstead, N. A., & Garthwaite, B. D. (1997). Effect of yeast culture in the diets of lactation dairy cows on ruminal fermentation and passage of nitrogen fractions and amino acids to the small intestine. *Journal of Dairy Science, 80*(2), 374-384.

Rawat, S. (2015). Food Spoilage: Microorganisms and their prevention. *Asian Journal of Plant Science and Research, 5*(4), 47-56.

Ray, B. (1986). Impact of bacteria injury and repair in food microbiology: its past, present and future. *Journal of Food Protection, 49*(8), 651-655.

Reddy, K. R., Raghavender, C. R., Reddy, B. N., & Salleh, B. (2010). Biological control of Aspergillus flavus growth and subsequent aflatoxin B 1 production in sorghum grains. *African Journal of Biotechnology, 9*(27), 4247-4250.

Republic of Kenya. (2010). *Kenya National Dairy Master Plan Volume 1: Situational analysis of the Dairy sub-sector.* Nairobi: Government printer.

Retta, K. S. (2016). Role of probiotics in rumen fermentation and animal performance: A review. *International Journal of Livestock Production, 7*(5), 24-32.

Rijnders, B. J., Van Wijngaerden, E., Verwaest, C., & Peetermans, W. E. (2000). Saccharomyces fungemia complicating Saccharomyces boulardii treatment in a non-immunocompromised host. *Journal of Intensive Care Medicine, 26*(6), 825-825.

Robinson, P. N., & Erasmus, L. J. (2009). Effect of analyzable diet components on response of lactating dairy cows to Saccharomyces serevisiae based yeast products: A systematic review of the literature. *Animal Feed Science and Technology, 149*(3), 185-198.

Roca-Fernandez, A. I. (2014). Animal factors condition milk performance and quality of grazing dairy cows. *Iranian Journal of Applied Animal Science, 4*(1), 1-20.

Rouse, S., Harnett, D., Vaughan, A., & Sinderen, D. V. (2007). Lactic acid bacteria with potential to eliminate fungal spoilage in foods. *Journal of Applied Microbiology, 104*(3), 915-923.

Sakamoto, K., & Konings, W. N. (2003). Beer spoilage bacteria and hop resistance. *International Journal of Food Microbiology, 89*(3), 105-124.

Salvado, Z., Arroyo-Lopez, F. N., Guillamon, J. M., Salazar, G., Querol, A., & Barrio, E. (2011). Temperature Adaptation Markedly Determines Evolution within the genus Saccharomyces. *Applied and Environmental Microbiology, 77*(7), 2292-2302.

Sammartino, M. (2015). Enzymes in Brewing. *Master Brewers Association of the Americas, 52*(3), 156-164.

Sargent, G., & Wickens, H. (2004). Brewers' yeast in C. difficile infection: probiotic or B-group vitamins? *Pharmaceutical Journal, 273*(7312), 230-231.

SAS Institute Inc. (2006). *Base SAS 9.1.3 Procedures Guide (2nd Edition, Volume 1,2,3 & 4).* Cary, North Carolina: SAS Institute Inc.

Sauvant, D., Giger-Reverdin, S., & Meschy, F. (2006). Control of latent ruminal acidosis. *Productions Animales-Paris-Institut National De La Recherche Agronomique, 19*(2), 69.

Schingoethe, D. J., Linke, K. N., Kalscheur, K. F., Hippen, A. R., Rennich, D. R., & Yoon, I. (2004). Feed efficiency of mid lactation dairy cows feed yeast culture during summer. *Journal of Dairy Science, 87*(12), 4178-4181.

Schneiter, R. (2004, January). Genetics, molecular and Cell Biology of Yeast. *Yeast Genetics*, pp. 2-86.

Shitandi, A., & Kihumbu, G. (2004). Assessment of the Califonia mastitis test usage in small holder dairy herds anbd risks of volatile antimicrobial residues. *Journal of Veterinary Science, 5*(1), 5-9.

Shreedhar, J. N., Manjunath, P., & Pradeep, K. (2016). Effect of Probiotics Supplementation on Milk Yield and Its Composition in Lactating Holstein Fresien and Deoni Cross Bred Cows. *Journal of Medical and Bioengineering, 5*(1), 19-23.

Simas, M. S., Botura, M. B., Correa, B., Sabino, M., Mallmann, C. A., Bitencourt, T. C., & Batatinha, M. J. (2007). Determination of fungal microbiota and mycotoxins in brewers' grains used in dairy cattle feeding in State of Bahia, Brazil. *Journal of Food Control, 18*(5), 404-408.

Sivakumar, V. K., Singaravelu, G., & Sivamani, P. (2014). Isolation, Characterization and Growth Optimization of Toxicogenic Molds from Different Animal Feeds in Tamilnadu. *International Journal of Current Microbiology and Applied Sciences, 3*(9), 430-445.

Slaghuis, B. A. (2001). The freezing point of authentic and original farm bulk tank milk in the Netherlands. *International Dairy Journal, 11*(3), 121-126.

Sloth, K. H., Friggens, N. C., Lovendahl, P., Andersen, P. H., Jensen, J., & Ingvartsen, K. L. (2003). Potential for improving description of bovine udder health status by combined analysis of milk measures. *Journal of Dairy Science, 86*(4), 1221-1232.

Song, M. K., & Kennelly, J. J. (2003). Biosynthesis of conjugated linoleic acid and its incorporation into ruminant's products. *Asian-Australian Journal of Animal Science, 16*(2), 306-314.

Sousa, M. L.-R. (2012). Stress and cell death in yeast induced by acetic acid. *In Cell Metabolism-Cell Homeostasis and Stress Response*, IntechOpen.

Staples, C. R. (2006). Milk Fat Depression in Dairy Cows . *Florida Ruminant Nutrition Symposiu* (pp. 1-17). Gainesville FL : Florida Ruminant Nutrition Symposiu.

Stewart, G. G. (2016). Saccharomyces species in the Production of Beer. *Beverages, 2*(34), 1-18.

Suzuki, K. A. (2008). Sake and beer spoilage lactic acid bacteria-A review. *Journal of the Institute of Brewing, 1144*(3), 209-223.

Sweeney, M. J., & Dobson, A. D. (1998). Mycotoxin production by Aspergillus, Fusarium and Penicillium species. *International Journal of Food microbiology, 43*(3), 141-158.

Święciło, A. (2016). Cross-stress resistance in Saccharomyces cerevisiae yeast--new insight into an old phenomenon. *Cell Stress Chaperones, 21*(2), 187-200.

Szwajgier, D. (2011). Dry and Wet Milling of Malt. A Preliminary Study Comparing Fermentable Sugar, Total Protein, Total Phenolics and the Ferulic Acid Content in Non-Hopped Worts. *Journal of the Institute of Brewing, 117*(4), 569-577.

Tai, S. L., Daran-Lapujade, P., Michael, C. W., Jack, T. P., & Jean-Marc, D. (2007). Acclimation of Saccharomyces cerevisiae to low temperature: A Chemostat-based Transcriptome Analysis. *Molecular Biology of the Cell, 18*(12), 5100-5112.

U.S. Food and Drug Administration. (2018, December 12). *Guidance Compliance Regulatory Information*. Retrieved January 2, 2018, from US Food and Drug Administration: www.fda.gov

Uyeno, Y., Suguru, S., & Takeshi, S. (2015). Effect of Probiotics/Prebiotics on Cattle Health and Productivity. *Microbes and Environments, 30*(2), 126-132.

Van Vuuren, H. J., & Priest, F. G. (2003). Gram- negative brewery bacteria. In G. C. Priest, *Brewing Microbiology, 3rd Edition* (pp. 119-245). New York, USA: Kluwer Academy/Plenum Publishers, New York.

Vannice, G., & Rasmussen, H. (2014). Position of the Academy of Nutrition and Dietetics: Dietary Fatty Acids for Healthy Adults. *Journal of the Academy of Nutrition and Dietetics, 114*(1), 136-153.

Vibhute, V. M., Shelke, R. R., Chavan, S. D., & Nage, S. P. (2011). Effect of probiotic supplementation on the performance of lactating crossbred cows. *Journal of Veterinary World, 4*(12), 557-561.

Vriesekoop, F., Krahl, M., Hucker, B., & Menz, G. (2012). 125th Anniversary Review: Bacteria in brewing: The good, the bad and the ugly. *Journal of the Institute of Brewing, 118*(1), 335-345.

Wadhwa, D., Randhawa, S., Nauriyal, D., & Singh, K. (1995). Clinico-biochemical and therapeutic studies on brewers' grains in buffaloes. *Indian Journal of Veterinary Medicine, 15*, 87-89.

Wafula, W. N., Matofari, W. J., Nduko, J. M., & Lamuka, P. (2016). Effectiveness of the sanitaion regimes used by dairy actors to control microbial contamination of plastic jerry cans' surfaces. *International Journal of Food Contamination, 3*(9), 1-8.

Walker, G. M., & Stewart, G. G. (2016). Saccharomyces cerevisiae in the Production of Fermented Beverages. *Beverages, 2*(30), 1-12.

Wang, B., Luo, Y., Myung, K. H., & Liu, J. X. (2014). Effects of storage duration and temperature on the chemical composition, microorganism density, and in vitro rumen fermentation of wet brewers grain. *Asian-Australas Journal of Animal Science, 27*(6), 832-840.

Wang, F., & Nishino, N. (2008). Ensiling of soybean curd residue and wet brewers grains with or without other feeds as a total mixed ration. *Journal of Dairy Science, 91*(6), 2380-2387.

Wang, F., & Nishino, N. (2009). Association of Lactobacillus buchneri with aerobic stability of total mixed ration containing wet brewers grains preserved as a silage. *Animal feed science and technology, 149*(3), 265-274.

Weinberg, Z. G., Ashbell, G., Hen, Y., & Azrieli, A. (1993). The effect of applying lactic acid bacteria at ensiling on the aerobic stability of silages. *Journal of Applied Bacteriology, 75*(6), 512-518.

West, J. E. (1994). Wet brewers' grain for lactating dairy cows during hot, humid weather. *Journal of Dairy Science, 77*(1), 196-204.

Westendorf, M. L., & Wohlt, J. E. (2002). Brewing by-products: their use as animal feeds. *Veterinary Clinics of North America: Food Animal Practice, 18*(2), 233-252.

Wiedmann, M., Siyun, W., Laurie, P., & Kendra, N. (2014). Assessment Criteria and Approaches for Rapid Detection Methods to Be Used in the Food Industry. *Journal of Food Protection, 77*(4), 670-690.

Wunderlich, S., & Back, W. (2009). Overview of Manufacturing Beer: Ingredient, Processes and Quality criteria. In V. R. Preedy, *Beer in health and disease prevention* (pp. 3-16). 35 Jamestown Road, London NW1 7BY, UK: Elsevier.

Yáñez-Ruiz, D. R., Leticia, A., & Charles, J. N. (2015). Manipulating rumen microbiome and fermentation through interventions during earlylife:areview. *Frontiers in Microbiology, 6*(10), 1-12.

Yu, P., Huber, J. T., Theurer, C. B., Chen, K. H., Nussio, L. G., & Wu, Z. (1997). Effect of steam-flaked or steam-rolled corn with or without Aspergillus oryzae in the diet on performance of dairy cows fed during hot weather. *Journal of Dairy Science, 80*(12), 3293-3297.

Zhang, Q., & Li, C. (2017). Comparisons of Copy Number, Genomic Structure, and Conserved Motifs for a-Amylase Genes from Barley, Rice, and Wheat. *Frontiers in Plant Science, 8*(10), 1-9.

Zhao, T., & Doyle, M. P. (2001). Evaluation of universal pre-enrichment broth for growth of heat-injured pathogens. *Journal of Food Protection, 64*(11), 1751-1755.

APPENDICES

Appendix 1: Questionnaire on dairy farming in Githunguri Sub-County, Kiambu County

PART A: TO BE FILLED BY THE ENUMERATOR

1.0 ENUMERATOR INFORMATION (please fill in all spaces provided)

1.1 Name of Enumerator……

1.2 P/No or ID No……..

1.3 Mobile No.....

1.4 Signature.....

1.5 Date of Interview.....

2.0 SITE INFORMATION (please fill in all spaces provided)

2.1 Division.....….....

2.2 Location.....….....

2.3 Sub-location……

2.4 Village……

PART B: RESPONDENT TO BE INTERVIEWED AND THE ENUMERATOR TO FILL THE QUESTIONNAIRE

1.0 HOUSEHOLD (Please fill or tick where appropriate)

1.1 Who is the respondent? (i) Husband [ ] (ii) Wife [ ] (iii) Farm manager [ ] (iv) Son [ ] (v) Daughter [ ] (vi) Other (Specify)……

1.2 What is the name of the farm……..

1.3 Name of respondent…… mobile no…….

1.4 Name of household head……...mobile no…….

1.5 Age of household head (i) ≤36 years [ ] (ii) 36-50 years [ ] (iii) >50 years [ ]

1.6 Formal education of household head (i) None [ ] (ii) Primary [ ] (iii) Secondary [ ] (iv) Post-secondary [ ] (v) Other [ ] (Specify)……..…

1.7 Are you a member of any farmer organization? (i) Yes [ ] (ii) No [ ]

1.8 If Yes, what is the name of the farmer organization……

2.0 DAIRY HERD AND BREEDS (Please fill or tick where appropriate)

Herd structure	Dairy breed							Total
	Friesian	Ayrshire	Guernsey	Jersey	Crosses	Zebu	Other (Specify)	
Milking cows								
Dry cows								
Bulls								
Heifers								

2.1 Dairy herd size, structure and breeds (Nos)

2.2 Which among the breed in 2.1 do you prefer?.....

2.3 Rank (1,2,3 etc) major reasons why you prefer the breed? (i) High milk yield [ ] (ii) Does not require a lot of feed [ ] (iii) Withstands disease challenges [ ] (iv) High milk butter fat [ ] (v) Other [ ] (Specify)……

3.0 PASTURE AND FODDER PRODUCTION (Please fill or tick where appropriate)

3.1 Do you have planted forages/fodder on your farm currently? (i) Yes [ ] (ii) No. [ ]

3.2 Farm utilization

Farm utilization	**Owned (Acres**	**Hired (Acres)**
Size of the farm		
Area of homestead		
Area under crop production		
Area under natural pastures/bushes		
Area under cultivated fodders and pastures		

3.3 Common pastures and fodders grown in the farm

Forages/fodder types	**Area (Acres) or No. cultivated**	**Production levels: (1.Poor; 2.Fair; 3.Moderate; 4. High; 5. Very high)**
Pasture grasses		
1.		
2.		
3.		
Herbaceous legumes		
1.		
2.		
3.		
Fodder grasses		
1.		
2.		
3.		
Fodder trees (Nos)		
1.		
2.		

3.4 How do you conserve feed for your livestock? (i) Hay [ ] (ii) Silage [ ] (iii) Hay and silage [ ] (iv) None [ ] (V) Crop-residues drying (e.g. Maize stovers) [ ] Others [ ] (Specify)……

4.0 FEEDING OF DAIRY CATTLE (Please fill or tick where appropriate)

4.1 Which is the feeding system of dairy cattle in your farm? (i) Stall feeding [ ] (ii) Stall feeding and grazing [ ] (iii) 3.Grazing [ ] (iv) Other [ ] (Specify)……

4.2 How do you estimate amount of forage to feed to dairy cattle? (i) Do not estimate [ ] (ii) Gunny bag [ ] (iii) Feed trough [ ] (iv) Weighing scale [ ] (v) Other [ ] (Specify)……...

4.3 Do you supplement dairy cattle? (i) Yes [ ] (ii) No [ ]

4.4 If No, rank (1, 2,3 etc) the major reasons why you do not supplementing dairy cattle. (i) High cost of supplement [ ] (ii) Low production of animals [ ] (iii) Unavailability of supplement [ ] (iv) Poor breed (v) Other [ ] (specify)……..…

4.5 If Yes, with which supplement? (i) Concentrate (e.g Dairy meal, Maize germ etc) [ ] (ii) Forages (e.g. sweet potato vines, Lucerne etc) [ ] (iii) Other [ ] (specify)……

4.6 How frequent is the supplementation? (i) 100% (All days) [ ] (ii) 75% of the days [ ] (iii) 50% of the days (iv) 25% of the days

4.7 Which major factor determines the amount of supplement offered to dairy cattle? (i) Milk production [ ] (ii) Availability of supplement [ ] (iii) Season [ ] (iv) Affordability of supplement [ ] (v) Other [ ] (Specify)……

4.8 What is your strategy for supplementation of lactating cows? (i) Uniform rate [ ] (ii) Based on milk production [ ] (iii) Other (specify) […..]

4.9 Do you steam up your cows prior to calving? (i) Yes [ ] (ii) No [ ]

4.10 Feeding of dairy cows (Major feeds only)

Class of feeds	**Specific feeds fed**	**Source of feed: 1.Own production 2.Purchased 3. Both**	**Season fed: 1. Wet 2.Dry 3.Both**
Total Mixed Ration (TMR) (Indicate the feed mixtures and their proportions)	1.		
	2.		
	3.		
Basal forages (e.g. Napier grass, Maize stover, Natural pastures etc)	1.		
	2.		
	3.		
Supplementary forages (e.g. Sweet potato vines, Lucerne, Calliandra etc)	1.		
	2.		
	3.		
Commercial concentrates (e.g Dairy meal, Maize germ, cotton seed cake etc)	1.		
	2.		
	3.		
Home-made concentrates (Indicate concentrate mixtures and their proportions)	1.		
	2.		
	3.		
Minerals (e.g. Unga high phosphorus, Baymix Maziwa etc)	1.		
	2.		
	3.		
Water	1 2		

(Indicate source of water e.g River, Dam, borehole, rain, vendors etc)r	3		
Others	1. 2.		

5.0 KNOWLEDGE ON RECORD KEEPING (Please fill or tick where appropriate)

5.1 Are you trained on record keeping? (i) Yes [ ] (ii) No [ ]

5.2 If yes, who trained you?.....

5.3 Do you keep dairy records? (i) Yes [ ] (ii) No [ ]

5.4 If yes which major records do you keep? (i) Breeding [ ] (ii) Milk production [ ] (iii) Live-weight [ ] (iv) Expenditure/ Revenue [ ] (v) All [ ] (vi) others [ ] (specify) ……

5.5 List (1,2,3 etc) in order of importance the major reasons why you keep record?

1……..……

2…….

3…….

5.6 If No why don't you keep records? (i) Time consuming [ ] (ii) Don't know how to do it [ ] (iii) Cumbersome [ ] (iv) Other [ ] (Specify)……

6.0 FARMERS EXPERIENCES WITH LIQUID BREWERS' YEAST (Please fill or tick where appropriate)

6.1 Are you aware of liquid brewers' yeast? (i) Yes [ ] (ii) No. [ ]

6.2 If yes who created the awareness on liquid brewers' yeast? (i) Extension workers [ ] (ii) Research institutions [ ] (iii) Other farmers [ ] (iv) Agro-vets [ ] (v) Media [ ] (vi) Agricultural shows [ ] (vii) Dairy cooperative [ ] (viii) Other [ ] (Specify)……..

6.3 Do you feed liquid brewers' yeast to your dairy cattle? (i) Yes [ ] (ii) No. [ ]

6.4 If Yes, when did you start feeding liquid brewers' yeast?.....

6.5 How is liquid brewers' yeast fed to dairy cattle? (i) Fresh [ ] (ii) After preservation [ ] (iii) Both [ ] (iv) Other [ ] (Specify)……

6.6 Where do you obtain liquid brewers' yeast from? (i) Happy feeds [ ] (ii) Dairy corporative [ ] (iv) Agro-vets [ ] (v) Distributors [ ] Other [ ] (specify……

6.7 Do you buy [ ] or is liquid brewers' yeast provided free of charge [ ]?

6.8 What is the cost in Ksh/ Kg of liquid brewers' yeast?.....

6.9 What quantity do you buy at a time (specify units)……..

6.10 How long (days) do you feed the quantity in 6.7 to your dairy herd?.....

6.11 Rank (1,2,3,…) in order of importance the major benefits of feeding liquid brewers' yeast to your dairy herd compared to other protein sources? (i) Low cost [ ] (ii) Readily available [ ] (iii) Increased animal production [ ] (iv) Good quality [ ] (v) Good taste [ ] (vi) Other [ ] (Specify)……

6.12 Class of cattle fed liquid brewers' yeast and daily quantities

Class of cattle	**Daily quantities fed (Specify)**
Milking cows	
Heifers	
Weaned calves	
Others (Specify)	

6.13 What information do you require on liquid brewers' yeast? (i) Quality [ ] (ii) Amount to feed [ ] (iii) Where to buy [ ] (iv) Preservation [ ] Others (Specify)……..

6.14 Have you been trained on how to feed liquid brewers' yeast? (i) Yes [ ] (ii) No [ ]

6.15 If yes who trained you? (i) MoALF Extension workers [ ] (ii) Corporative Extension workers [ ] (iii) Other farmers [ ] (iv) Other [ ] (Specify)……

6.16 Rank (1,2,3 etc) in order of importance the major challenges of feeding dairy cattle with liquid brewers' yeast and list the coping strategies for each challenge

Major challenges	**Rank**	**Coping strategies**
Short shelf live as compared to other protein sources		1.
		2.
Bulky and hence cumbersome to transport as compared to other protein sources		1.
		2.
Poor quality as compared to other protein sources		1.
		2.
Not readily available as compared to other protein sources		1.
		2.
Do not know appropriate quantities to supplement dairy cattle		1.
		2.
Dairy cattle do not like its taste as compared to other protein sources		1.
		2.
Others (Specify):		1.
		2.

The enumerator to thank respondent for time taken and patience during the interview

Appendix 2: Result output

The GLM Procedure

Dependent Variable: LAB

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Temp	2	0.0246377	0.0123188	0.01	0.9904
Days	3	298.9043657	99.6347886	77.77	<.0001
Source	2	16.5378263	8.2689131	6.45	0.0018
Temp*source	4	0.2631024	0.0657756	0.05	0.9951
Days*source	6	25.4373444	4.2395574	3.31	0.0035
Temp*days	6	0.2354050	0.0392342	0.03	0.9999
Temp*days*source	12	0.9439868	0.0786656	0.06	1.0000

Source	LAB LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
D	8.01894444	0.13339502	<.0001	1
F	8.12089236	0.06669751	<.0001	2
HF	7.25883333	0.23104695	<.0001	3

i/j	1	2	3
1		-0.68357 0.4947	2.849099 0.0046
2	0.683571 0.4947		3.584724 0.0004
3	-2.8491 0.0046	-3.58472 0.0004	

Temp	source	LAB LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
10	D	8.03191667	0.23104695	<.0001	1
10	F	8.08215625	0.11552347	<.0001	2
10	HF	7.32162500	0.40018505	<.0001	3
20	D	7.98462500	0.23104695	<.0001	4
20	F	8.15261458	0.11552347	<.0001	5
20	HF	7.27687500	0.40018505	<.0001	6
30	D	8.04029167	0.23104695	<.0001	7
30	F	8.12790625	0.11552347	<.0001	8
30	HF	7.17800000	0.40018505	<.0001	9

Days	source	LAB LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
0	D	6.09233333	0.26679004	<.0001	1
0	F	6.53812500	0.13339502	<.0001	2
0	HF	3.88900000	0.46209390	<.0001	3
7	D	7.87316667	0.26679004	<.0001	4
7	F	7.88258333	0.13339502	<.0001	5
7	HF	7.78700000	0.46209390	<.0001	6
14	D	9.34805556	0.26679004	<.0001	7
14	F	9.25876389	0.13339502	<.0001	8
14	HF	8.68350000	0.46209390	<.0001	9
21	D	8.76222222	0.26679004	<.0001	10
21	F	8.80409722	0.13339502	<.0001	11
21	HF	8.67583333	0.46209390	<.0001	

The GLM Procedure
Number of observations 384

Dependent Variable: Yeast

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	35	256.4417678	7.3269077	4.97	<.0001
Error	348	513.0425634	1.4742602		
Corrected Total	383	769.4843312			

R-Square	Coeff Var	Root MSE	Yeast Mean
0.333264	14.73841	1.214191	8.238279

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Temp	2	1.1994464	0.5997232	0.41	0.6661
Days	3	152.6747246	50.8915749	34.52	<.0001
Source	2	60.4515650	30.2257825	20.50	<.0001
Temp*source	4	1.4949854	0.3737464	0.25	0.9074
Days*source	6	42.9925669	7.1654278	4.86	<.0001
Temp*days	6	1.7881904	0.2980317	0.20	0.9760
Temp*days*source	12	2.5880046	0.2156670	0.15	0.9997

Source	Yeast LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
D	8.13270833	0.14309380	<.0001	1
F	8.38866667	0.07154690	<.0001	2
HF	6.75033333	0.24784574	<.0001	3

i/j	1	2	3
1		-1.5999 0.1105	4.83031 <.0001
2	1.599902 0.1105		6.350966 <.0001
3	-4.83031 <.0001	-6.35097 <.0001	

Temp	source	Yeast LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
10	D	8.02058333	0.24784574	<.0001	1
10	F	8.47848958	0.12392287	<.0001	2
10	HF	6.84937500	0.42928141	<.0001	3
20	D	8.23845833	0.24784574	<.0001	4
20	F	8.41204167	0.12392287	<.0001	5
20	HF	6.90187500	0.42928141	<.0001	6
30	D	8.13908333	0.24784574	<.0001	7
30	F	8.27546875	0.12392287	<.0001	8
30	HF	6.49975000	0.42928141	<.0001	9

Days	source	Yeast LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
0	D	6.97383333	0.28618761	<.0001	1
0	F	7.82279167	0.14309380	<.0001	2
0	HF	4.50750000	0.49569148	<.0001	3
7	D	7.85594444	0.28618761	<.0001	4
7	F	8.01609722	0.14309380	<.0001	5
7	HF	5.56233333	0.49569148	<.0001	6
14	D	8.77344444	0.28618761	<.0001	7
14	F	8.76906944	0.14309380	<.0001	8
14	HF	8.83433333	0.49569148	<.0001	9
21	D	8.92761111	0.28618761	<.0001	10
21	F	8.94670833	0.14309380	<.0001	11
21	HF	8.09716667	0.49569148	<.0001	12

The GLM Procedure

Number of observations 384

Dependent Variable: Mould

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	35	1156.725177	33.049291	6.40	<.0001
Error	348	1795.867627	5.160539		
Corrected Total	383	2952.592804			

R-Square	Coeff Var	Root MSE	Mould Mean
0.391766	47.29617	2.271682	4.803099

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Temp	2	23.6367335	11.8183667	2.29	0.1028
Days	3	219.7559904	73.2519968	14.19	<.0001
Source	2	138.0338745	69.0169373	13.37	<.0001
Temp*source	4	18.4849470	4.6212368	0.90	0.4667
Days*source	6	10.4595608	1.7432601	0.34	0.9167
Temp*days	6	20.5786953	3.4297825	0.66	0.6783
Temp*days*source	12	41.2726580	3.4393882	0.67	0.7835

Least Squares Means

Source	Mould LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
D	4.11837500	0.26772029	<.0001	1
F	5.12164583	0.13386015	<.0001	2
HF	3.03470833	0.46370515	<.0001	3

i/j	1	2	3
1		-3.35183	2.023878
		0.0009	0.0437
2	3.351829		4.324008
	0.0009		<.0001
3	-2.02388	-4.32401	
	0.0437	<.0001	

Temp	source	Mould LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
10	D	3.61529167	0.46370515	<.0001	1
10	F	4.39044792	0.23185257	<.0001	2
10	HF	2.71612500	0.80316088	0.0008	3
20	D	4.37416667	0.46370515	<.0001	4
20	F	4.92817708	0.23185257	<.0001	5
20	HF	3.21562500	0.80316088	<.0001	6
30	D	4.36566667	0.46370515	<.0001	7
30	F	6.04631250	0.23185257	<.0001	8
30	HF	3.17237500	0.80316088	<.0001	9

Days	source	Mould LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
0	D	2.50044444	0.53544058	<.0001	1
0	F	2.93369444	0.26772029	<.0001	2
0	HF	1.43800000	0.92741030	0.1219	3
7	D	3.83466667	0.53544058	<.0001	4
7	F	5.03394444	0.26772029	<.0001	5
7	HF	2.69266667	0.92741030	0.0039	6
14	D	4.43288889	0.53544058	<.0001	7
14	F	5.91413889	0.26772029	<.0001	8
14	HF	3.66533333	0.92741030	<.0001	9
21	D	5.70550000	0.53544058	<.0001	10
21	F	6.60480556	0.26772029	<.0001	11
21	HF	4.34283333	0.92741030	<.0001	12

The GLM Procedure
Number of observations 384

Dependent Variable: TVC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	35	653.8337029	18.6809629	38.18	<.0001
Error	348	170.2664271	0.4892713		
Corrected Total	383	824.1001300			

R-Square	Coeff Var	Root MSE	TVC Mean
0.793391	8.568367	0.699479	8.163508

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Temp	2	1.6174075	0.8087037	1.65	0.1930
Days	3	265.1589548	88.3863183	180.65	<.0001
Source	2	32.2256147	16.1128073	32.93	<.0001
Temp*source	4	1.0495708	0.2623927	0.54	0.7092
Days*source	6	4.7578020	0.7929670	1.62	0.1404
Temp*days	6	1.6130586	0.2688431	0.55	0.7703
Temp*days*source	12	2.3879210	0.1989934	0.41	0.9607

Source	TVC LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
D	8.02533333	0.08243443	<.0001	1
F	8.28570139	0.04121722	<.0001	2
HF	7.11170833	0.14278062	<.0001	3

t for H0: LSMean(i)=LSMean(j) / Pr > |t|

i/j	1	2	3
1		-2.82504 0.0050	5.541526 <.0001
2	2.825036 0.0050		7.899784 <.0001
3	-5.54153 <.0001	-7.89978 <.0001	

Temp	source	TVC LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
10	D	7.81520833	0.14278062	<.0001	1
10	F	8.13972917	0.07139031	<.0001	2
10	HF	7.04662500	0.24730329	<.0001	3
20	D	7.98158333	0.14278062	<.0001	4
20	F	8.33893750	0.07139031	<.0001	5
20	HF	7.21200000	0.24730329	<.0001	6
30	D	8.27920833	0.14278062	<.0001	7
30	F	8.37843750	0.07139031	<.0001	8
30	HF	7.07650000	0.24730329	<.0001	9

Days	source	TVC LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
0	D	5.64172222	0.16486886	<.0001	1
0	F	6.22918056	0.08243443	<.0001	2
0	HF	4.98266667	0.28556124	<.0001	3
7	D	8.37505556	0.16486886	<.0001	4
7	F	8.55933333	0.08243443	<.0001	5
7	HF	6.86616667	0.28556124	<.0001	6
14	D	9.08638889	0.16486886	<.0001	7
14	F	9.21426389	0.08243443	<.0001	8
14	HF	8.48583333	0.28556124	<.0001	9
21	D	8.99816667	0.16486886	<.0001	10
21	F	9.14002778	0.08243443	<.0001	11
21	HF	8.11216667	0.28556124	<.0001	12

The GLM Procedure
Number of observations 384

Dependent Variable: TCC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	35	229.300645	6.551447	2.52	<.0001
Error	348	905.420877	2.601784		
Corrected Total	383	1134.721522			

R-Square	Coeff Var	Root MSE	CC Mean
0.202077	51.77532	1.613005	3.115393

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Temp	2	0.79063359	0.39531679	0.15	0.8591
Days	3	19.95216075	6.65072025	2.56	0.0551
Source	2	90.02872154	45.01436077	17.30	<.0001
Temp*source	4	1.73466146	0.43366537	0.17	0.9552
Days*source	6	32.99739236	5.49956539	2.11	0.0512
Temp*days	6	3.36232596	0.56038766	0.22	0.9718
Temp*days*source	12	13.26090348	1.10507529	0.42	0.9534

		Standard		LSMEAN
D	2.65950000	0.19009443	<.0001	1
F	3.35889931	0.09504721	<.0001	2
HF	1.56100000	0.32925320	<.0001	3

i/j	1	2	3
1		-3.29079	2.889354
		0.0011	0.0041
2	3.290795		5.246314
	0.0011		<.0001
3	-2.88935	-5.24631	
	0.0041	<.0001	

Temp	source	CC LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
10	D	2.58425000	0.32925320	<.0001	1
10	F	3.40687500	0.16462660	<.0001	2
10	HF	1.78312500	0.57028328	0.0019	3
20	D	2.69658333	0.32925320	<.0001	4
20	F	3.47930208	0.16462660	<.0001	5
20	HF	1.50987500	0.57028328	0.0085	6
30	D	2.69766667	0.32925320	<.0001	7
30	F	3.19052083	0.16462660	<.0001	8
30	HF	1.39000000	0.57028328	0.0153	9

Days	source	CC LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
0	D	1.87366667	0.38018885	<.0001	1
0	F	3.25779167	0.19009443	<.0001	2
0	HF	0.84950000	0.65850641	0.1979	3
7	D	3.36672222	0.38018885	<.0001	4
7	F	4.24483333	0.19009443	<.0001	5
7	HF	1.12966667	0.65850641	0.0871	6
14	D	2.74272222	0.38018885	<.0001	7
14	F	3.20795833	0.19009443	<.0001	8
14	HF	2.44316667	0.65850641	0.0002	9
21	D	2.65488889	0.38018885	<.0001	10
21	F	2.72501389	0.19009443	<.0001	11
21	HF	1.82166667	0.65850641	0.0060	12

Appendix 3: Publications

Journal of Animal and Veterinary Sciences
2019; 6(2): 18-23
http://www.openscienceonline.com/journal/javs

Open Science

Physicochemical Quality of Milk from Dairy Cows Supplemented with Liquid Brewer's Yeast in Smallholder Dairy Farms

Peter Alphonce Obuong Alaru[1, 2, *], Alfred Anakalo Shitandi[1], Symon Maina Mahungu[1], John Muasya Kilumba Muia[2]

[1]Department of Dairy and Food Science and Technology, Egerton University, Egerton, Kenya

[2]Dairy Research Institute, Kenya Agricultural and Livestock Research Organization (KALRO), Naivasha, Kenya

Email address

peter.alaru@yahoo.com (P. A. O. Alaru)

[*]Corresponding author

To cite this article

Peter Alphonce Obuong Alaru, Alfred Anakalo Shitandi, Symon Maina Mahungu, John Muasya Kilumba Muia. Physicochemical Quality of Milk from Dairy Cows Supplemented with Liquid Brewer's Yeast in Smallholder Dairy Farms. *Journal of Animal and Veterinary Sciences*. Vol. 6, No. 2, 2019, pp. 18-23.

Received: January 9, 2019; **Accepted**: March 7, 2019; **Published**: April 16, 2019

Abstract

A study was conducted to evaluate physicochemical quality of raw milk from dairy cows supplemented with liquid brewer's yeast (LBY) in smallholder dairy farms. The milk was delivered from different routes to Githunguri Dairy Farmers' Cooperative Society in Kiambu County, Githunguri Sub-county, Kenya. The main objective was to ascertain suitability for use of LBY as alternative feed source for dairy cows without compromising on milk quality. Thirty farms (sampling units) were randomly selected from three milk delivery routes (sampling frame). A longitudinal survey was conducted where farms were nested within routes and equal number of farms selected per route based on supplementation of lactating cows with either LBY or commercial dairy meal (CDM). A repeated measure analysis was performed using the Linear Mixed Models methodology by PROC MIXED of SAS for milk quality and questionnaire data was summarized using descriptive statistics. Milk samples were analysed for physicochemical parameters such as butter fat (BF), protein, lactose, total ash, solid not fat (SNF), density and milk freezing point (MFP). The results indicated significantly ($p<0.05$) higher milk protein levels and lower freezing point for milk from LBY supplemented cows (3.07±0.03% and -0.532±0.005°C) compared to those supplemented with CDM (2.99±0.03% and -0.516±0.005°C). This was an indication of positive effect of LBY supplementation on the two parameters. The other physicochemical parameters were not significantly affected ($p>0.05$) by the type of supplementation regime, although higher levels were observed on LBY supplemented diets than CDM diets. The study indicates that LBY can be used as feed supplement for dairy cows without compromising on physicochemical quality of milk. In view of this, the research recommends use of LBY as a cost effective alternative protein source for dairy cows.

Keywords

Feed Supplement, Liquid Brewer's Yeast, Physicochemical Milk Quality, Smallholder Dairy Farms

Journal of Animal and Veterinary Sciences
2018; 5(4): 26-30
http://www.openscienceonline.com/journal/javs

Open Science

Effect of Handling Practices of Liquid Brewer's Yeast on Microbial Growth During Storage and Risk Unit Suitability as Feed Supplement in Smallholder Dairy Farms

Peter Alphonce Obuong Alaru1, [2, *], Alfred Anakalo Shitandi[1], Symon Maina Mahungu[1]

[1]Department of Dairy and Food Science and Technology, Egerton University, Egerton, Kenya

[2]Dairy Research Institute, Kenya Agricultural and Livestock Research Organization (KALRO), Naivasha, Kenya

Email address

peter.alaru@yahoo.com (P. A. O. Alaru
*Corresponding author

To cite this article

Peter Alphonce Obuong Alaru, Alfred Anakalo Shitandi, Symon Maina Mahungu. Effect of Handling Practices of Liquid Brewer's Yeast on Microbial Growth During Storage and Risk Unit Suitability as Feed Supplement in Smallholder Dairy Farms. *Journal of Animal and Veterinary Sciences*. Vol. 5, No. 4, 2018, pp. 26-30.

Received: October 17, 2018; **Accepted**: October 30, 2018; **Published**: December 21, 2018

Abstract

The study was conducted to determine by means of microbiological analyses, handling practices along the supply chain that could hasten deterioration of Liquid brewer's yeast (LBY); thereby compromising its suitability as feed supplement for lactating dairy cows under smallholder dairy farms. The trial evaluated effect of source of LBY, pH and temperature range on development of microorganisms during storage. Samples were collected from three sources (supplier, distributors and farmers), stored under aerobic condition at 10°C, 20°C and 30°C, then tested at day 0, 7, 14 and 21. Mean square values and levels of significance effect showed that storage time (days) and source of LBY significantly influenced ($p<0.05$) variation in total viable counts (TVC) whereas, only the source of LBY statistically affected variation in total coliform counts (TCC). Total viable counts was reported as 7.11±0.14, 8.23±0.08 and 8.28±0.04 (log10 CFU/ml) for supplier, distributors and farmers respectively. Total coliform count was highest at the farmers level with a mean of 3.36±0.10 (log10 CFU/ml), distributors was 2.66±0.19 (log10 CFU/ml) and lowest at supplier level with a mean of 1.56±0.33 (log10 CFU/ml). The levels of TVC and TCC were significant ($p<0.05$) during storage time. Major changes in pH were reported as 4.27±0.06, 4.11±0.06 and 3.91±0.06 at temperatures 10°C, 30°C and 20°C respectively. Findings of this study demonstrate that LBY can be successfully used to supplement dairy cows for a period of one week. Thereafter, a significant drop in pH and steady multiplication of microorganisms is possible. The study recommends need for hygienic handling of LBY by distributors and farmers to reduce risk of feed contamination in smallholder dairy farms.

Keywords

Liquid Brewer's Yeast, Feed Supplement, Handling Practices, Microbial Growth, Smallholder Dairy