Chemical composition and *in vitro* evaluation of the feeding value of brewery by-products for ruminants

An Ethiopian perspective

Dissertation

zur

Erlangung des Grades

Doktor der Agrarwissenschaften (Dr. agr.)

der Agrar-, Ernährungs- und Ingenieurwissenschaftlichen Fakultät

der Rheinischen Friedrich-Wilhelms-Universität Bonn

von

Ziade Abraha Kahsay, MSc

aus

Irob-Tigray, Äthiopien

Bonn

2025

Referent:	Prof. Dr. Karl-Heinz Südekum
Korreferentin:	Prof. Dr. Uta Dickhöfer
Tag der mündlichen Prüfung:	18.12.2024

Angefertigt mit Genehmigung der Agrar-, Ernährungs- und Ingenieurwissenschaftlichen Fakultät der Universität Bonn.

Dedicated to

My parents, my spouse, and my two kids

SUMMARY

Chemical composition and *in vitro* evaluation of the feeding value of brewery by-products for ruminants – an Ethiopian perspective

Livestock production plays a crucial role in food security and nutrition, especially in developing countries. In Ethiopia with its huge livestock population – more than two-thirds of population own livestock – lack of feed is a critical challenge, and the productivity of livestock remains very low. Feed scarcity in terms of quantity and quality is one of the key constraints for livestock to reach the potential productivity level. In this regard, utilisation of non-conventional feed resources efficiently becomes a considerable alternative to tackle feed scarcity and improve productivity. Use of cereals as ruminant feed in Ethiopia is unlikely considering its shortage and the direct competition with humans. Brewery by-products cost less than concentrate feeds and, as a supplement, could enhance protein and energy values of low-quality cereal crop residues and herbage from pastures. However, brewery by-products are highly variable feeds in terms of chemical composition and the nutrient value related to the brewing processes, and type of cereal grains used. These variables in turn may vary based on breweries and localities. Hence, the main goal of this thesis was to evaluate chemical composition and *in vitro* feeding value of brewery by-products – brewers grains (BG), and tella-atella (TA): a by-product of locally produced tella drink – obtained from Ethiopia; and investigate the potential variability within and between brewery by-products. The first part of the thesis comprises a review focusing on the chemical composition, feeding value, and storage and preservation conditions of BG as a ruminant feed in general and without restriction to Ethiopia. In addition, the review analyzes factors contributing to the variation of chemical composition of BG. Based on the findings of the review, the variables of the brewing processes, malt barley varieties used, and cereal grains added are main factors that contribute to the variation in chemical composition and nutrient value of BG. Particularly, several enzymes produced during malting and mashing processes, and use of several heat treatments (temperature regimes) are key factors of brewing processes influencing the components that hydrolyzed in to wort, and the components that remain in the BG. Both variables are interrelated, and the production and activity of the enzymes again depend on the specific optimal temperature used. In the second part, *in vitro* methods were applied to examine nutritive value of BG and TA samples with particular focus on crude protein (CP); and investigate the potential variability. A total of 17 samples of BG obtained from nine breweries, representing about 70% of the total available breweries in Ethiopia, and 3 TA samples were collected, and subjected to analysis of chemical composition and CP fractionation. Moreover, an *in vitro* rumen gas production technique was applied to estimate energy values, ruminally undegradable feed CP (RUP) and utilisable CP at the duodenum (uCP). The overall results show that BG and TA are relatively high in most nutrients, mainly containing high CP and neutral detergent fibre concentrations. Both by-products contain CP with low rumen degradability indicating great potential for milk production of dairy animals provided that the RUP is accessible in the small intestine. Hence, digestibility of RUP and its amino acid patterns in the small intestine of the animal require further investigation. However, substantial variations within BG, and compared with TA were observed in terms of chemical composition and nutritive value. The attempt to relate BG to the origin of cereal grains showed the tangible influence of the original grains on the nutrient values of BG. Particularly high starch concentrations in non-pure barley malt BG, and TA samples were most likely related to the brewing process and type of grains used. Considerable energy contents of these by-products make them also valuable energy supplements especially in regions like Ethiopia where the largest portion of the available feed is roughage. Overall, findings of the thesis underline that BG and TA, both as potential protein and energy supplements, could contribute to improved livestock productivity. Thus, considering the potential to the expansion of breweries in Ethiopia, both by-products could contribute to tackle the feed scarcity for ruminants. Due to the variability in terms of chemical composition and nutritive value, a periodic evaluation of feeding value is vital for efficient utilisation of the by-products. Further studies on the digestibility of RUP and composition of amino acids in the small intestine would also be essential to utilise the by-products efficiently in feed formulation. Moreover, utilisation of an additional agro-industrial by-product as a feed supplements should be considered to meet the existing huge feed demand in Ethiopia.

ZUSAMMENFASSUNG

Chemische Zusammensetzung und In-vitro-Bewertung des Futterwertes von Brauerei-Koprodukten für Wiederkäuer - eine äthiopische Perspektive

Die Viehzucht und Nutztierhaltung spielen eine entscheidende Rolle für die Lebensmittelsicherheit und die Ernährung, insbesondere in Entwicklungsländern. In Äthiopien, wo es einen riesigen Viehbestand gibt - mehr als zwei Drittel der Bevölkerung besitzen Vieh - ist der Mangel an Futtermitteln eine kritische Herausforderung, und die Produktivität des Viehbestands bleibt sehr niedrig. Futtermittelknappheit in Bezug auf Quantität und Qualität ist eines der Haupthindernisse für die Erreichung des potenziellen Produktivitätsniveaus in der Tierhaltung. In dieser Hinsicht ist die effiziente Nutzung nicht-konventioneller Futtermittel eine wichtige Alternative, um der Futtermittelknappheit entgegenzuwirken und die Produktivität zu steigern. Die Verwendung von Getreide als Wiederkäuerfutter in Äthiopien ist unwahrscheinlich, da es knapp ist und in direkter Konkurrenz zum Menschen steht. Brauerei-Nebenprodukte sind kostengünstiger als Konzentratfutter und könnten als Ergänzung den Protein- und Energiewert von energie- und nährstoffarmen Getreiderückständen (Stroh) und Weidegras verbessern. Brauerei-Nebenprodukte sind jedoch äußerst variable Futtermittel, was die chemische Zusammensetzung und den Nährstoffwert in Bezug auf die Brauverfahren und die verwendeten Getreidekörner betrifft. Diese Variablen können wiederum von Brauerei zu Brauerei und von Ort zu Ort variieren. Daher bestand das Hauptziel dieser Arbeit darin, die chemische Zusammensetzung und den In-vitro-Futterwert von Brauerei-Nebenprodukten - Biertreber (BG) und Tella-Atella (TA), einem Nebenprodukt des lokal hergestellten Tella-Getränks - aus Äthiopien zu bewerten und die potenzielle Variabilität innerhalb und zwischen Brauerei-Nebenprodukten zu untersuchen. Der erste Artikel der Arbeit ist ein Überblick über die chemische Zusammensetzung, den Futterwert sowie die Lagerungs- und Konservierungsbedingungen von BG als Wiederkäuerfutter im Allgemeinen und ohne Einschränkung auf Äthiopien. Darüber hinaus werden die Faktoren analysiert, die zur Variation der chemischen Zusammensetzung von BG beitragen. Ausgehend von den Ergebnissen der Untersuchung sind die Variablen der Brauverfahren, die verwendeten Gerstensorten und die zugesetzten Getreidekörner die Hauptfaktoren, die zu den Schwankungen der chemischen Zusammensetzung und des Nährwerts von BG führen. Insbesondere verschiedene Enzyme, die während des Mälzens und Maischens produziert werden, und die Anwendung verschiedener Wärmebehandlungen (Temperaturregime) sind Schlüsselfaktoren des Brauprozesses, die die Bestandteile, die in die Würze hydrolysiert werden, und die Bestandteile, die im BG verbleiben, beeinflussen. Beide Variablen sind miteinander verknüpft, und die Aktivität und Produktion der Enzyme hängt wiederum von der Verwendung der optimalen Temperatur für ein bestimmtes Enzym ab. Im zweiten Artikel wurden In-vitro-Methoden angewandt, um den Nährwert von BG- und TA-Proben mit besonderem Augenmerk auf den Proteinwert zu untersuchen und die mögliche Variabilität zu ermitteln. Insgesamt wurden 17 BG-Proben aus neun Brauereien, die etwa 70% aller in Äthiopien verfügbaren Brauereien ausmachen, und 3 TA-Proben entnommen und einer Analyse der chemischen Zusammensetzung und der Rohproteinfraktionierung (CP) unterzogen. Darüber hinaus wurde eine In-vitro-Pansengaserzeugungstechnik angewandt, um die Energiewerte, das im Pansen nicht abbaubare Rohprotein (RUP) und das verwertbare CP im Zwölffingerdarm (uCP) zu schätzen. Die Gesamtergebnisse zeigen, dass BG und TA reich an den meisten Nährstoffen sind und hauptsächlich hohe CP- und NDF-Konzentrationen aufweisen. Beide Nebenprodukte enthalten ein Protein mit geringer Pansenabbaubarkeit, was auf eine große Bedeutung für die Milchproduktion von Milchkühen hindeutet, vorausgesetzt, der RNP ist im Dünndarm zugänglich. Daher müssen die Verdaulichkeit von RNP und seine Aminosäuremuster im Dünndarm von Milchkühen weiter untersucht werden. Es wurden jedoch erhebliche Unterschiede innerhalb des BG und im Vergleich zum TA in Bezug auf die chemische Zusammensetzung und den Nährwert festgestellt. Der Versuch, BG mit den Ursprungsgetreidearten in Verbindung zu bringen, zeigte den spürbaren Einfluss der Ursprungsgetreidearten auf die Nährstoffwerte von BG. Die besonders hohen Stärkekonzentrationen in den Proben von nicht reinem Gerstenmalz BG und TA hängen höchstwahrscheinlich mit dem Brauprozess und der Art des verwendeten Getreides zusammen. Der beträchtliche Energiegehalt dieser Nebenprodukte macht sie auch zu einer wertvollen Energieergänzung, insbesondere in Regionen wie Äthiopien, wo ein großer Teil (mehr als 50 %) des verfügbaren Futters hauptsächlich aus Raufutter besteht.

Insgesamt unterstreichen die Ergebnisse dieser Arbeit, dass BG und TA als potenzielle Protein- und Energiezusätze zur Verbesserung der Produktivität der Viehbestände beitragen könnten. In Anbetracht des Potenzials für den Ausbau von Brauereien in Äthiopien tragen beide Nebenprodukte dazu bei, den Futtermittelmangel für Wiederkäuer zu beheben. Aufgrund der potenziellen Variabilität in Bezug auf die chemische Zusammensetzung und den Nährwert ist eine regelmäßige Bewertung des Futterwertes für eine effiziente Nutzung der Nebenprodukte unerlässlich. Weitere Studien über die Verdaulichkeit von RNP und die Zusammensetzung der Aminosäuren im Dünndarm wären für eine effiziente Verwertung der Nebenprodukte in der Futtermittelformulierung unerlässlich. Darüber hinaus sollte die Verwendung zusätzlicher agroindustrieller Nebenprodukte als Futtermittelergänzung in Betracht gezogen werden, um den bestehenden großen Futtermittelbedarf in Äthiopien zu decken.

TABLE OF CONTENTS

Summary I

Zusammenfassung II

Table of contents III

Figures IV

Tables V

Abbreviations VI

Chapter 1 1

General introduction 1

Chapter 2 11

Scope of the thesis

Chapter 3 14

A Review: Feeding value and affecting factors of brewers grains as a ruminant feed – Brewing processes, preservation, and utilization

CHAPTER 4 43

Chemical composition and *in vitro* characterization of energy and protein value of brewery by-products for ruminants - Ethiopian breweries

Chapter 5 81

General discussion, conclusions, and outlook

Acknowledgement 92

FIGURES

Chapter 3

Figure 1.1 Scheme of malting and brewing processes 27

Chapter 4

Figure 4.1. Relationship between ruminally undegraded crude protein at passage rate 0.03/h (RUP3; r=0.86; *left*), at 0.05/h (RUP5; r=0.80; *right*), and fraction C of brewers grains (0) + tella-atella (□) 56

Figure 4.2. Ruminally undegraded crude protein (RUP) and microbial crude protein (MCP) of brewers grains (BG) and tella-atella (TA)..... **Error! Bookmark not defined.**

Figure 4.3. Relationship between starch content and gas production after 2h (GP2; *left*), and after 24h (GP24; *right*) *in vitro* incubation of brewers grains (0) + Tella-atella (□) 61

Figure 4.4. Fermentation pattern of BG and TA samples over 96 h of incubation; the curves are plotted from calculated values based on the fermentation parameters estimated from Ørskov and McDonald (1979) equation $y = a + b(1 - e - ct)$); *A sample obtained from one Germen brewery as a reference prepared in the same manner. 61

TABLES

Chapter 3

Table 3.1. Chemical composition of brewers grains (g kg^{-1}, DM basis) from a number of relevant publications 19

Table 3.2. Mineral element composition of brewers grains 21

Chapter 4

Table 4.1. List of raw ingredients used and malt source of brewers grains (n=17) and tella-atella (n=3) samples 47

Table 4.2. Chemical composition of brewers grains and of traditional brewing by-products (tella-atella) 52

Table 4.3. Mineral concentrations of brewers grains and traditional brewing by-products (tella-atella) 54

Table 4.4. Concentration of neutral detergent insoluble CP (NDICP), acid detergent insoluble CP (ADICP) and crude protein (CP) fractions of brewers grains and traditional brewing by-products (tella-atella) 55

Table 4.5. *In vitro* estimations of effective ruminally undegraded crude protein (RUP) and effective utilizable crude protein at the duodenum (uCP) of brewers grains and traditional brewing by-products (tella-atella). 58

Table 4.6. *In vitro* gas production after 2h (GP2) and 24h incubation (GP24), maximum GP (a + b), gas production rate (C), digestibility of organic matter (OMD); metabolizable energy (ME) and net energy for lactation (NEL) of brewers grains and traditional brewing by-products (tella-atella) 60

ABBREVIATIONS

A	CP fraction, Non-protein Nitrogen multiplied by 6.25, soluble in Tungstic acid
AA	Amino acid
AAS	Atomic absorption spectroscopy
ADF	Acid detergent fibre
ADFom	ADF after incineration
ADICP	Acid detergent insoluble CP
ADIN	Acid detergent insoluble N
ADL	Acid detergent lignin
aNDFom	NDF after incineration and amylase treatment
B1	CP fraction, TP soluble in borate-phosphate buffer
B2	CP fraction, CP insoluble in borate-phosphate buffer minus NDICP
B3	CP fraction, NDICP minus ADICP
BG	Brewers grains
C	CP fraction, corresponds to ADICP
CL	Crude lipids
CP	Crude protein
DBG	Dried brewers grains
DM	Dry matter
DMI	DM intake
e.g.	Exempli gratia
FAO	Food and Agriculture Organization of the United Nations
FCM	Fat-corrected Milk
g	gram

GP	Gas production
GP2	GP after 2 hours incubation
GP24	GP after 24 hours incubation
h	Hour
kg	Kilogram
K_p	Ruminal passage rate
LTB	Local tella brewers, mostly women who brew tella drinks
MCLP	Mixed crop livestock production system in Ethiopia
MCP	Microbial crude protein
ME	Metabolisable energy
mg	milligram
MJ	Mega joule
mm	millimeter
modHGT	Modified Hohenheim gas test
n	Number
N	Nitrogen
NDF	Neutral detergent fibre
NDICP	Neutral detergent insoluble CP
NDIN	Neutral detergent insoluble N
NEL	Net energy for lactation
NFC	Non-fibre carbohydrates
NH_3-N	Ammonia N
NH_3-N_{blank}	NH_3-N from incubated blank sample
NH_3-N_{sample}	NH_3-N from incubated feed sample
n.i.	Not indicated

NRC	National research council
N_{sample}	Total N added to the syringe through the sample substrate
OM	Organic matter
OMD	Organic matter digestibility
pH	Potential of hydrogen (or power of hydrogen)
r	Simple linear correlation coefficient
RDP	Ruminally degradable feed CP
RUP	Rumen-undegraded feed CP
SAS	Statistical analysis system
SBM	Soybean meal
SD	Standard deviation of the mean
TA	Tella-atella
TP	True protein
uCP	Utilisable CP at the duodenum
uCP2	Effective utilisable CP at the duodenum at a passage rate of 0.02 hr^{-1}
uCP5	Effective utilisable CP at the duodenum at a passage rate of 0.05 hr^{-1}
VDLUFA	Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten (Association of German Agricultural Analytic and Research Institutes)
VFA	Volatile fatty acid
WBG	Wet brewers grains

CHAPTER 1

General introduction

Livestock production plays a critical role in food security and nutrition for humans, especially in developing countries. In Ethiopia, livestock constitutes an integral share of agriculture accounting for 45% of agricultural gross domestic products, and contributes to the livelihood of 14 million households or 70% of the population (FAO, 2019*a*). Ethiopia owns a large livestock population comprised of 70 million cattle, 52 million goats, 43 million sheep, 8 million camels, 57 million chicken, as well as equines in 2020 (CSA, 2021), mostly reared at smallholder farming, and subsistence oriented (Assefa *et al.*, 2016). Livestock is a major source of human food protein through the provision of meat and milk, draft power (for traction, transport and haulage), export commodities in form of leather and live animal, manure for farmland and household energy, security in times of crop failure, and means of social capital (FAO, 2019*a;* Management Entity, 2021). However, like in other Sub-Saharan African countries, the productivity is generally very low. This significantly hinders Ethiopia's self-sufficiency in production of milk and other dairy products despite its large livestock herd size.

In addition to low genetic performance of animals and prevalence of animal diseases, scarcity and low quality of available ruminant feed in Ethiopia is a primary factor that contribute to the very low productivity of livestock (Tolera *et al.*, 2012; FAO, 2018). About 80–90% of livestock feed in arid and semi-arid areas comes from natural pastures, and 40–50% of livestock feed in mixed farming system relies on crop residues (Negesse *et al.*, 2009). At the meantime, feeds produced during wet season are not enough to sustain for the long dry season considering the huge demand for feed. Limitations in feed conservation techniques is another prevalent problem (Tesfay *et al.*, 2016) which will not be addressed in this thesis. During the long dry season from October to May, both quality and quantity of the green forages are highly depleted (Gizachew and Smit, 2005; Negesse *et al.*, 2009). Hence, despite some variations across the regions, livestock feeding depends on low quality dry fodders, mainly crop residues such as straws, stovers and stubbles (Negesse *et al.*, 2009; Duncan *et al.*, 2016; Gizaw *et al.*, 2017). The shortage of feed is more escalating in the highlands of the country where more than 75% of both human and livestock populations are concentrated (Tolera *et al.*, 2012). For instance, in some zones in Tigray region, crop residues are

the main feed for livestock for 7–12 months, covering about 50–75% of the livestock feed (Tesfay *et al.*, 2016).

Cultivated forages and pastures are potential sources in Ethiopia to tackle the feed scarcity, and provide animals with essential nutrients. In the mid-1960s, thousands of tropical and temperate forage species were introduced from different parts of the world including South America, North America, Australia and some parts of Africa (Tolera *et al.*, 1999; Assefa *et al.*, 2016). For the last six decades, research on their agronomic practice, adaptability, herbage productivity, and feeding value in the several agro-ecological and production systems of Ethiopia has been conducted (Assefa *et al.*, 2016). Based on the suitability to the diverse agro-ecological condition of the country, the International Livestock Research Institute (ILRI) has recommended about 50 well-adapted forage crops for different agro-ecological zones (Turner *et al.*, 2019). However, despite the appreciable research efforts towards development of improved forages and pastures, and various forage development strategies, the adoption of improved forages by smallholder farmers and livestock producers has not been achieved to the desirable level (Tolera *et al.*, 2012). Lack of forage seed, quality, availability and associated institutional incapacities are key constraints in forage production among others suggesting further research directions (Turner *et al.*, 2019).

The feed processing industry in Ethiopia that has begun in 1960s is still at the inception stage in terms of its growth (Bediye *et al.*, 2018). The escalating prices of feed ingredients, limited financial capacities and awareness towards the feed have constrained the growth of the feed industry (Tolera *et al.*, 2012). As a result, the available feed processing plants are operating far below their annual capacities and caused to remain at the subsistence level. Though the production of compound feeds has doubled in the last decade, the quantity produced is still very low compared to the country's livestock size (Bediye *et al.*, 2018).

In general, the available feedstuffs lack to attain nutrient requirement of animals for maintenance and production purposes (Gizachew and Smit, 2005). For instance, an average quality wheat straw provides only 90% and 45% of the protein and energy maintenance requirement of low-producing ruminants, respectively (Assefa *et al.*, 2016). The organic matter (OM) digestibility of cereal crop residues is very low, usually less than 550 g/kg dry matter (DM; Gizachew and Smit, 2005).

Cereal grains that make a principal component of concentrate feeds for livestock in developed countries are of short supply and expensive in Ethiopia due to direct competition with human food uses (Negesse *et al.,* 2009). Utilisation of non-conventional animal feed sources and agro-industrial by-products for ruminant animals have been considered due to its comparative advantages over cereal grains (Negesse *et al.*, 2009). The term non-conventional feed resources may refer to all those feeds, mostly agro-industrial by-products, which have not been traditionally used for feeding livestock and or are not normally used in commercially produced rations for livestock (Devendra, 1993). In this regard, ruminants have an advantage over other animals in utilising resources that might not be used, otherwise, for human food consumption.

The major agro-industrial by-products in Ethiopia, with varying availability across the regions and proximity to the factories, are obtained from flour mills, modern and traditional oilseed processing units, breweries, and sugar refineries (FAO, 2019*b*). Due to their high price, and low availability, and lack of awareness by farmers, utilisation of agro-industrial by-products is mostly limited to commercial livestock farms and smallholder livestock producers in urban and peri-urban areas (Tolera *et al.*, 2012; Gizaw *et al.*, 2017). Agro-industrial by-products in Ethiopia mainly include wheat bran, wheat shorts/middlings, oilseed cakes, e.g., sesame cake, noug cake, groundnut cake and cotton seed cake, moreover molasses, brewery by-products, bagasse and sugarcane tops (FAO, 2019*b*). Supplementing locally available low-quality feeds with agro-industrial by-products, for instance urea and molasses, enhance dry matter intake, rumen fermentation, and nutrient digestibility in ruminants (Mekasha *et al.*, 2002; Negesse *et al.*, 2009).

Brewery by-products are a good example of non-conventional feedstuffs that have been used worldwide. Brewers grains (BG) – also termed brewers spent grains –, brewers hops, and brewers yeasts are the main by-products generated from the industrial beer brewing processes, BG being the major one. Once starch in the malted grains is converted into simple sugar during the mashing process of brewing, BG are separated from wort at the end of lautering process as by-product (Mussatto *et al.*, 2006). The global beer production in year 2018 was estimated to be 191.1 million hectolitre (Kirin Holdings Company, 2019), suggesting about 38.2 million tonnes of BG.

Due to its high concentration of crude protein (CP; up to 330 g/kg DM) and digestible neutral detergent fibre (Firkins *et al.*, 2002; Westendorf *et al.,* 2014), BG is suitable for ruminants and particularly for dairy cows (Dhiman *et al.,* 2003). Indeed, use of BG as livestock feed is a well-

established practice in many areas of the world, and several investigations have been undertaken to evaluate chemical composition and feeding value for dairy cows, beef cattle, horse, poultry and fish (Ademosun, 1973; Ngodigha *et al.*, 1994; Swain *et al.*, 2012). In addition, use of BG in human food, in energy production as a biofuel energy, and their chemical and biotechnological application have been reported earlier as reviewed by Mussatto (2014). Variability in chemical composition and feeding value, and difficulty in preserving for longer time due its high moisture content are often taken as a drawback of utilising brewing by-products consistently as animal feed (Dhiman *et al.*, 2003; Mussatto *et al.*, 2006; Aranguiz *et al.*, 2019).

The variability in BG in terms of feeding value is mainly attributed to the variability of grains used, and the industrial processes (Santos *et al.*, 2003). The use of different grains again might vary among countries, regions and companies depending on the intended beer type, availability and economic reasons (Meussdoerffer and Zarnkow, 2009). Nevertheless, barley malt is the main raw material and main starch source to be converted to sugars for fermentation in the brewing process (Kreisz, 2009). The German Purity Law still regulates that, for bottom-fermented beers, barley malt is the sole grain source for beer production (Meussdoerffer and Zarnkow, 2009). However, in many other countries nowadays this rule has been partly modified and malt from other grain sources are also used as carbohydrate sources in brewing depending on the type of beer. Moreover, considering the advancement in technologies and development in brewing (Iserentant, 2003), there could be possible variations from what we already know up to date in terms of chemical composition and feeding value of BG.

Brewery by-products in Ethiopia are obtained from both industrial beer production and traditional alcoholic drinks, namely, ‘tella’ and ‘areki’. Though consisitent and reliable research data on the amount of BG produced is lacking, the total annual production potential of BG from all 12 breweries found in Ethiopia in year 2016/2017 has been estimated to be more than 240,000 tons (Kitaw *et al.*, 2018; Mohammed *et al.*, 2019). The estimation of BG tonnage assumes that for every 100 liters of beer production up to 20 kg of BG (as is basis) is generated (Mussatto *et al.*, 2006).

Tella is a traditional and domestic alcoholic-beverage based on substrates such as barley, wheat, maize, millet, sorghum or teff (Tadesse and Yayneshet, 2011; Berhanu, 2014; Lee *et al.*, 2015); Areki is a colorless, clear, traditional alcoholic beverage which is distilled from fermentation products prepared in almost the same way as tella (Lee *et al.*, 2015). Traditional ‘tella’ brewing

and 'areki' distilling by-products – named as tella-atella (TA) and areki-atella, respectively – are very abundant in amount and diverse in different societies in Ethiopia (Tadesse and Yayneshet, 2011; Lee *et al.*, 2015). Most households, especially in Christian communities, brew tella. However, the compiled data on production at national level is limited. The raw grains and brewing procedure vary in different societies as well as slightly from brewer to brewer (Lee *et al.*, 2015). Nevertheless, the basic brewing steps remain similar. In order to get clear understanding of TA, it might be vital for the readers to understand the details of tella brewing steps at outset. Hence, the tella brewing steps are precisely described in Appendix 1 and added as additional information to this thesis.

In Ethiopia, TA is used traditionally for a long time to feed ruminants and poultry (Tesfay *et al.*, 2016; Aranguiz *et al.*, 2019), regardless of limited knowledge on its chemical composition and nutritional value. On the other hand, industrial brewery by-products (BG and brewers yeasts included) have been introduced in ruminant and poultry diets during the last two decades when brewing factories have expanded in the county (Tesfay *et al.*, 2016). Different cereal grains as carbohydrate substrates are included particularly in Ethiopian breweries in addition to barley malt due to economic reasons, and the intended beer type; thus, little is known about the extent of variability of the BG as by-product.

Most of the studies conducted on Ethiopian brewery by-products so far focussed on only the concentrations of crude nutrients of TA and/or considered mostly a BG sample from a single brewery and, therefore, provide limited information regarding the nutrient variability in BG (Aranguiz *et al.*, 2019). In Ethiopia and elsewhere, most of the previous publications generally lack information on the raw materials from which BG were derived, and the information on processing and standardization of the samples. In some cases, it was even not stated how and where the samples were taken. It is often not evident if the data represent analysis of a single sample, or several samples, or composites. Studies on the expected variation in the composition and nutritive value of BG obtained from different breweries attributed to various factors is lacking in Ethiopia (Aranguiz *et al.*, 2019). Moreover, detailed studies on the rumen degradation potential and intestinal digestibility of the traditional brewing by-products through digestive tract of the ruminants is vital.

In the light of the very recent growth of the Ethiopian brewing industry (Kitaw *et al.*, 2018), and limited research and knowledge of farmers on feeding value of its by-products (Gizaw *et al.*, 2017), brewing by-products in Ethiopia are underutilised or at least inefficiently used. Studies show that a significant quantity of these valuable resources is currently being wasted (FAO, 2018). Thus, full and efficient utilisation of brewing by-products in ruminant nutrition in Ethiopia requires knowledge on their nutritive value and the possible variations within these feed resources.

References

Ademosun AA (1973) Evaluation of brewers' dried grains in the diets of growing chickens. *British Poultry Science* **14**, 463–468.

Aranguiz AA, Berhanu T and Vernooij A (2019) Utilization and management of spent brewers grain in Ethiopian dairy farms: Report 1207. Wageningen, Netherlands: Wageningen Livestock Research. https://doi.org/10.18174/504103.

Assefa G, Mengistu S, Feyissa F and Bediye S (2016) Animal feed resources research in Ethiopia: Achievements, challenges and future directions. *Ethiopian Journal of Agricultural Sciences,* **EIAR 50th Year Jubilee Anniversary Special Issue**, 144–155.

Bediye S, Nemi G and Makkar HPS (2018) Ethiopian feed industry: current status, challenges and opportunities. https://www.feedipedia.org/content/ethiopian-feed-industry-current-status-challenges-and-opportunities.

Berhanu A (2014) Microbial profile of Tella and the role of gesho (Rhamnus prinoides) as bittering and antimicrobial agent in traditional Tella (Beer) production. *International Food Research Journal* **21**, 357–365.

CSA (2021) Agricultural Sample Survey 2020/21 [2013 E.C.]: Report on Livestock and Livestock Characteristics (Private Peasant Holdings): Statistical Bulletin 589. Addis Ababa: Central Statistical Agency (CSA), Federal Democratic Republic of Ethiopia. https://www.statsethiopia.gov.et/our-survey-reports/.

Devendra C (1993) *Sustainable animal production from small farm systems in South-East Asia*. Rome, Italy: Food and Agriculture Organization of the United Nations.

Dhiman TR, Bingham HR and Radloff HD (2003) Production response of lactating cows fed dried versus wet brewers' grain in diets with similar dry matter content. *Journal of Dairy Science* **86**, 2914–2921.

Duncan AJ, Bachewe F, Mekonnen K, Valbuena D, Rachier G, Lule D, Bahta M and Erenstein O (2016) Crop residue allocation to livestock feed, soil improvement and other uses along a productivity gradient in Eastern Africa. *Agriculture, Ecosystems & Environment* **228**, 101–110.

FAO (2018) *Ethiopia: Report on feed inventory and feed balance 2018*. Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO (2019*a*) *The future of livestock in Ethiopia: Opportunities and challenges in the face of uncertainty*. Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO (2019*b*) *Ethiopia: Availability and utilization of agroindustrial by-products as animal feed 2018*. Rome, Italy: Food and Agriculture Organization of the United Nations.

Firkins JL, Di Harvatine, Sylvester JT and Eastridge ML (2002) Lactation performance by dairy cows fed wet brewers grains or whole cottonseed to replace forage. *Journal of Dairy Science* **85**, 2662–2668.

Gizachew L and Smit GN (2005) Crude protein and mineral composition of major crop residues and supplemental feeds produced on Vertisols of the Ethiopian highland. *Animal Feed Science and Technology* **119**, 143–153.

Gizaw S, Ebro A, Tesfaye Y, Mekuriaw Z, Mekasha Y, Hoekstra D, Gebremedhin B and Tegegne A (2017) Feed resources in the highlands of Ethiopia: A value chain assessment and intervention options: LIVES Working Paper 27. Nairobi, Kenya: International Livestock Research Institute (ILRI). https://cgspace.cgiar.org/handle/10568/82636.

Iserentant D (2003) Beers: Recent technological innovations in brewing. In AGH Lea and JR Piggott (eds.). *Fermented Beverage Production*, pp. 41–58, Boston, USA: Springer.

Kirin Holdings Company (2019) Kirin Beer University Report Global Beer Production by Country in 2018. https://www.kirinholdings.co.jp/english/news/2019/1003_01.html.

Kitaw G, Tamir B, Assefa G and Animut G (2018) Production, preservation, and utilization patterns of brewery spent grain in Ethiopia. *Ethiopian Journal of Agricultural Sciences* **28**, 1–17.

Kreisz S (2009) Malting. In HM Esslinger (ed.). *Handbook of brewing: Processes, technology, markets*, pp. 147–164, Weinheim, Germany: Wiley-VCH.

Lee M, Regu M and Seleshe S (2015) Uniqueness of Ethiopian traditional alcoholic beverage of plant origin, tella. *Journal of Ethnic Foods* **2**, 110–114.

Management Entity (2021) Ethiopia's Livestock Systems. Gainesville, FL, USA: Feed the Future Innovation Lab for Livestock Systems. https://livestocklab.ifas.ufl.edu/media/livestocklabifasufledu/pdf-/LSIL_Livestock_Systems_Overview_Ethiopia_2021_08.pdf.

Mekasha Y, Tegegne A, Yami A and Umunna NN (2002) Evaluation of non-conventional agro-industrial by-products as supplementary feeds for ruminants. *Small Ruminant Research* **44**, 25–35.

Meussdoerffer F and Zarnkow M (2009) Starchy raw materials. In HM Esslinger (ed.). *Handbook of brewing: Processes, technology, markets*, pp. 43–83, Weinheim, Germany: Wiley-VCH.

Mohammed D, Addis D, Berhanu T and Vernooij A (2019) Mapping of spent brewers' grain supply chain in Ethiopia: Report 1210. Wageningen, Netherlands: Wageningen Livestock Research. https://library.wur.nl/WebQuery/wurpubs/fulltext/506451.

Mussatto SI (2014) Brewer's spent grain: a valuable feedstock for industrial application. *Journal of the Science of Food and Agriculture* **94**, 1264–1275.

Mussatto SI, Dragone G and Roberto IC (2006) Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science* **43**, 1–14.

Negesse T, Makkar HPS and Becker K (2009) Nutritive value of some non-conventional feed resources of Ethiopia determined by chemical analyses and an *in vitro* gas method. *Animal Feed Science and Technology* **154**, 204–217.

Ngodigha EM, Sese BT, Olaka OS and Iyayi EA (1994) Effect of brewers dried grain on growth performance and plasma amino acids of young pigs. *Journal of Applied Animal Research* **6**, 97–104.

Santos M, Jiménez JJ, Bartolomé B, Gómez-Cordovés C and Nozal MJd (2003) Variability of brewer's spent grain within a brewery. *Food Chemistry* **80**, 17–21.

Swain BK, Naik PK, Chakurkar EB and Singh NP (2012) Effect of feeding brewers' dried grain on the performance and carcass characteristics of Vanaraja chicks. *Journal of Applied Animal Research* **40**, 163–166.

Tadesse A and Yayneshet T (2011) Comparative chemical composition evaluation of local brewery and liquor by-products made from different ingredients. *Journal of Drylands* **4**, 307–309.

Tesfay Y, Gebrelibanos A, Woldemariam D and Tilahun H (2016) Feed resources availability, utilization and marketing in central and eastern Tigray, northern Ethiopia: LIVES Working Paper 11. Nairobi, Kenya: International Livestock Research Institute (ILRI). https://cgspace.cgiar.org/handle/10568/71089.

Tolera A, Berg T and Sundstøl F (1999) The effect of variety on maize grain and crop residue yield and nutritive value of the stover. *Animal Feed Science and Technology* **79**, 165–177.

Tolera A, Yami A, Mengistu A, Alemu D, Geleti D, Assefa G, Gizachew L, Bediye S and Woldesemayat Y (2012) *Livestock feed resources in Ethiopia: Challenges, opportunities and the need for transformation*: Ethiopian Animal Feeds Industry Association.

Turner M, Assefa G and Duncan AJ (2019) Forage seed quality in Ethiopia: Issues and opportunities: ILRI Project Report. Nairobi, Kenya: International Livestock Research Institute (ILRI). https://www.ilri.org/publications/forage-seed-quality-ethiopia-issues-and-opportunities.

Westendorf ML, Wohlt JE, Sniffen CJ and Ward RT (2014) Nutrient content of brewers grains produced at a commercial brewery. *Professional Animal Scientist* **30**, 400–406.

CHAPTER 2

Scope of the thesis

This thesis aimed to evaluate the chemical composition and feeding value of brewery by-products for ruminants in Ethiopia, as well as to assess the factors contributing to variations in the nutrient concentration of brewers' grains (BG). Accordingly, the research presented in this thesis includes a general introduction on livestock production, feed resources, their availability, and the significance of brewery by-products, highlighting the identified research gaps (Chapter 1). The main body consists of two manuscripts (Chapters 3 and 4) that focus on the feeding value and utilization of BG, with particular emphasis on its protein and energy content for ruminants. These manuscripts have been submitted to or prepared for publication in scientific journals and are presented in their original format, with only font adjustments made to ensure consistency throughout the thesis.

The first main part (Chapter 3) is a review focusing on the chemical composition and feeding value of BG for ruminants. First, relevant studies on chemical composition and feeding value across all regions of the world have been summarized to assess the feeding value and variability of BG for ruminants. Second, factors contributing to the variation in chemical composition and feeding value of BG with special focus on brewing processes and associated factors were addressed. In addition, utilisation and the possible preservation methods of BG were discussed.

The second main part (Chapter 4) dealt with evaluation of BG samples obtained from Ethiopian breweries. The majority, two-thirds, of the breweries found in Ethiopia are covered. Clear, standardized processing and drying procedures were followed to prepare the samples for the analysis. Accordingly, all the samples were analysed for chemical composition including proximate constituents, fibre fractions, and macro- and micro-minerals. The focus was given to protein value of BG. Crude protein fractions (A, B1, B2, B3, and C) based on Cornell Net Carbohydrate and Protein System were estimated. Utilisable crude protein at the duodenum (uCP) i.e. the sum of ruminally undegraded CP and microbial CP, and energy value (metabolisable energy, ME; net energy for lactation, NEL) using *in vitro* gas production methods were evaluated. Information on the characteristics of the raw ingredients of BG and TA samples was mostly pointed out; attempt was done to relate it to the feeding value of the respective by-product. Thus, the chemical

composition variability of BG and TA in general and in relation to the raw ingredients which BG and TA are derived from were addressed in this chapter as well as in the general discussion (Chapter 5).

The potential CP degradability, energy value in relation to starch content, organic matter digestibility (OMD), and gas production kinetics have been discussed in the last chapter (Chapter 5). Most interestingly, the relevance and possible efficient utilisation of BG from Ethiopian livestock production and feeding perspective have been discussed in this Chapter.

CHAPTER 3

A Review: Feeding value and affecting factors of brewers grains as a ruminant feed – brewing processes, preservation, and utilization

Ziade Abraha Kahsay[1], Christian Böttger[1,*], Karl-Heinz Südekum[1]

[1]University of Bonn, Institute of Animal Science, Endenicher Allee 15, 53115 Bonn, Germany; zkah@itw.uni-bonn.de (Z.A.K), Christian.Boettger@lwk.nrw.de (C.B.), ksue@itw.uni-bonn.de (K.-H.S.)

[*] Present address: Chamber of Agriculture of North Rhine-Westphalia, Ostinghausen – Haus Düsse, 59505 Bad Sassendorf, Germany

To be submitted

ABSTRACT

Brewing processes, malting barley varieties used and added cereal carbohydrate sources are the main factors that contribute to the variation in chemical composition and the nutritive value of brewers grains (BG) for ruminants. Chemical components that are not extracted into the wort during lautering are concentrated in BG. This review highlights how these components affect the nutritive value of BG for farm animals, partly based on studies reporting how the brewing processes directly affect wort extractions, and indirectly the chemical composition and nutritive value of the remaining BG. In addition, storage and preservation conditions, chemical composition, and feeding value of BG as a ruminant feed have been summarized in this review. Enzymes produced during the brewing processes and variation of temperature regimes mainly determine the components which are hydrolyzed into wort and the components that remain as BG. The activity of the enzymes varies and requires the optimum gelatinization temperature in the mashing process for starch extraction and protein solubility. In principle, approximately 65% of the malt proteins are retained in the BG whereas the rest is extracted into the wort. Brewers grains, mainly as a crude protein (CP ~19–34% of dry matter [DM]) and fibre source (neutral detergent fibre, NDF ~37.3–65.3% of DM), have been effectively utilized to replace concentrate mixtures and expensive protein sources like soybean meal in dairy rations. Ruminants might consume more DM with dried BG than wet BG. However, lactation response to both forms of BG as a supplemental feedstuff has been consistently similar. Apart from the use of additives and preservatives, recent research has focused on incorporating ensiled BG into a total mixed ration to extend its storage duration. Therefore, utilizing BG in animal nutrition is environmentally friendly and contributes to sustainable resource use.

Key words: brewers grains; brewing processes; chemical composition; feeding value

1 INTRODUCTION

Brewers grains (BG), also called brewers spent grains, are the main by-product of beer brewing. Brewers (spent) hops (Hot trub) and Brewers (spent) yeast are also by-products of brewing, which are not addressed in this review. Brewers grains comprise as much as 85% of total brewery by-products; and for every 100 litres of beer production, about 20 kg of BG is generated.[1] Approximately one ton of barley yields 800 kg malt[2]; and 100 kg of malt yields about 100-130 kg of fresh BG containing 70 to 80% water, equivalent to 21–22 kg BG per hectolitre of beer.[3] The global BG production was estimated to be 40.1 million tonnes in 2018, estimated from 1911 million hectoliters of beer produced in the same year with an increase of 0.6% from the previous year.[4] The first and second largest beer producing countries, China and USA, respectively, have decreased beer production by 2.2 and 1.7%, whereas Brazil (third largest beer producer) has increased beer production by 2.7% when compared to the year 2017. The annual beer production data from several countries are well documented by Kirin Beer University.[4]

Utilization of BG in ruminant feed is a viable option because of not only the high nutritive value but also because of the availability in a significant amount and fair price worldwide, as well as environmental safety.[5] Particularly in countries where cereal grains are more demanded for human food, BG might substitute other, grain-based concentrate feed sources.[6,7] However, the chemical composition and feeding value of BG for ruminants could vary owing to a number of factors associated with the diverse brewing experience and preferences of companies and societies all over the world. Chemical composition and feeding value of BG and the possible variation of the nutritive value always remain as a particular concern for animal nutrition researchers, traders in the feed industry and farmers. To our knowledge, only few comprehensive reviews on the chemical compositions and nutritive value of BG as ruminant feedstuff exist and have been mostly published before the 1990s.[8] Hence, an extensive literature search was performed for this review based mainly on databases of Google Scholar, Web of Science and Science Direct.

Brewing and malting processes, malt barley varieties used and added cereal grains mainly determine the nutritive value of BG.[9] While numerous scientific publications explore the effects of these factors on wort production and beer quality from a brewing process perspective, their impact on the nutritive value of BG for farm animals remains largely underreported. In fact, barley varieties are selected primarily in consideration of the wort quality[10], and not the

nutritional value of BG. Hence, very few studies have investigated the effect of barley cultivars on the nutritional composition of BG. However, chemical components that are not extracted into the wort during lautering obviously remain as a component of BG.[11] The variable components of the brewing processes, and limited information on the direct relationship between the brewing processes and malt characteristics and the chemical composition of BG precluded to perform a quantitative meta-analysis. Instead of that, we tried to figure out how brewing processes and associated factors affect chemical composition of BG partly based on the studies with a focus on how the brewing processes affect wort extractions.

Therefore, the present review aims to: (1) summarize the chemical composition, and feeding value of BG; (2) highlight how brewing processes and other associated factors relate to protein and energy value of BG; and (3) discuss appropriate preservation and storage conditions of BG as a ruminant feed.

2 CHEMICAL COMPOSITION OF BREWERS GRAINS

Brewers grains are primarily utilized as crude protein (CP) and fibre sources in ruminant nutrition.[6,7] The CP content of BG is higher than that of cereal grains such as maize, oats, rice, and barley – its foundation grain.[12] During the malting and mashing steps of the brewing process, starch in the barley is hydrolysed into fermentable sugars. As a result, the relative initial proportion of carbohydrates diminishes while the CP content increases in BG [13]. Hence, the CP content in BG doubles the concentration in the barley grain.[12] Table 1 shows a largely variable CP content of BG ranging on average from 186 to 339 g kg^{-1} dry matter (DM) reported by several studies. Robertson et al.[14] reported 10.3–24.0% CP of DM for BG samples collected from 10 breweries throughout the EU and South Africa; whereas Westendorf et al.[15] reported 29.6–37.4% CP of DM from 48 BG samples collected from the same brewery over a one-year period. The variabilities are more pronounced between than within breweries [9,14]. Recently, Naibaho and Korzeniowska [16] reported that all proximate analysis of moisture, ash, fat, protein, and carbohydrate contents varied among BG obtained from eight breweries. The differences in CP content of BG over years and between breweries that have been reported could be due to the barley malt varieties, several cereal grains included and steps in the brewing processes of individual breweries. Lynn et al.[17] reported that the CP concentration of BG obtained from microbreweries was 8 percentage units less than that of BG obtained from breweries (20.9 vs. 28.8% CP/DM) and, hence, this should be considered during diet formulation.

Essential amino acids (AA) in BG represent approximately 30% of the protein content with lysine (14.3% of protein) being the most abundant.[18] Other essential AA found in BG include leucine, phenylalanine, isoleucine, threonine and tryptophan. Non-essential AA comprise mainly histidine (26% of protein) and glutamine (17% of protein); the rest include aspartic acid, valine, arginine, alanine, serine, tyrosine, glycine, asparagine, glutamine and the non-proteinaceous AA, c-aminobutyric acid.[18]

Brewers grains are inherently a fibrous material with high content of neutral detergent fibre (NDF) and acid detergent fibre (ADF) ranging from 43.5–60.5% NDF of DM and from 20.9 to 31.8% ADF of DM (Table 1). Acid detergent lignin (ADL) in BG was estimated at 4.2– 4.5% of DM.[19,20] It is worthy to note that all fibre components, and particularly ADL, increase with the increase of drying temperature of the BG[19], and is associated with the Maillard reaction. Similarly, AD insoluble nitrogen (ADIN) in BG increases when a temperature greater than 100 °C is used for drying BG samples.[19]

In addition to fibre, a considerable content of crude fat (7–10% DM) and a variable and often low starch content (usually 1–13%) of BG, [5,14,15,21] contribute to total energy value for ruminants. The variation of starch content in BG is depending mainly on the type of cereal grains added and extraction efficiency of the respective brewery. Samples of BG with added cereal grains obtained from Ethiopian breweries, exhibited a starch concentration as high as 25% on DM basis (Chapter4, this thesis). From *in vitro* gas production studies, a metabolisable energy concentration of 10.3–11.3 MJ kg^{-1} DM was reported for BG tested in seven laboratories.[22] Expressed as total digestible nutrients, a measure still widely used in many countries, brewers' grains (BG) have values ranging from 71% to 80%, primarily due to their high digestible fiber content. [20,21,23,24]

Inorganic components of BG comprise varieties of macro and micro minerals [25] Phosphorus (~6 g kg^{-1}) and Ca (~3 g kg^{-1}) are the two most abundant mineral elements in BG.[25] Concentration of P lies far below 10 g kg^{-1} of the diet DM, the maximum tolerable concentration stated by NRC. [26] On the other hand, Ca and K levels in BG are lower than recommended to cover requirements of cattle.[24] Considerable quantities of vitamins including folic acid, niacin, biotin, thiamine, choline, pantothenic acid, riboflavin, and pyridoxine are also present in BG.[27]

Table 3.1. Chemical composition of brewers grains (g kg^{-1}, DM basis) from a number of relevant publications

Country of study/ Sample origin	n	Ash	CP	EE	NDF	ADF	ADL	Starch	Reference studies
Iran	n.i.		198	80	551	252			Aghajanzadeh-Golshani et al. [28]
USA	4	36 (29–42)	259 (238–269)	61 (50–75)	508 (387–582)	190 (155–204)	49 (40–62)		Arosemena et al.[29]
Belgium	n.i.	42	285	91	543	276	77	15	Brabander et al.[30]
Ireland	n.i., (from one batch ≥2)	32	231	135				14	Connolly et al.[31]
USA	1	42	269	75	549	201	47		DePeters et al.[32]
	1	29	238	50	387	155	40		
	1	38	262	55	514	201	62		
USA	10	43 (37–49)	270 (242–306)	63 (57–69)	373 (330–436)	180 (158–205)	58 (35–69)		DePeters et al.[33†]
USA	11	45 (39–50)	236 (225–246)	96 (91–101)	513 (491–527)	257 (230–314)	87 (63–122)		DePeters et al.[33‡]
Nigeria	n.i. (≥2)	110	244	35	535	471	134		Etela et al.[34]
Brazil	n.i. (≥2)	49	187	49	675				Faccenda et al.[35‡]
USA	n.i.	33	331	72[§]	526		42		Firkins et al.[36]
USA	n.i.	48	315			291	43		Johnson et al.[37]
USA	n.i.	44	188	35					Loosli and Warner [38]
USA	3		298	88	435	227			Moriel et al. a [21]
USA	n.i.		290	113	515	259			Moriel et al. b [39]
USA	6		234		566	215	45		Murdock et al.[20]
Brazil	1	46	153				48		Mussatto and Roberto[12]
Spain	3	39	257		605	215	42		Pereira et al.[19]
		48	290		585	180	100		
		47	272		593	184	55		

UK	n.i.		256		602	281	12	Phipps et al.[40]
USA	4		280			318		Polan et al.[41]
UK/EU and South Africa	10		186 (103–240)				67 (16–130)	Robertson et al.b[14]
Spain	8	34	242	39				Santos et al.[9]
Germany	10¶	41 (31–47)	273 (236–331)	107 (91–120)				Seifried et al.[42]
USA	163–1038	45	290 (248–336)	86 (75–101)	479 (414–552)	233 (207–288)		Westendorf and Wohlt[13††]
USA	48		336 (296–374)	89 (79–98)	485 (443–521)	209 181– 255	55 49–62	43 (21–75)	Westendorf et al.[15‡‡]
Japan	3	41	280	89	583	224			Xu et al.[43]

n= number of samples included in the study; n.i. = not indicated, CP = crude protein; CF = crude fat; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; † wet brewers grains; ‡ dried brewers grains; §expressed as fatty acids; ¶nine ensiled and one WBG samples; ††given by DairyOne Forage Laboratory in Ithaca, NY, between 1998 and 2001; ‡‡brewers grains are sampled once weekly for over 12 months.

The indicated mean values were according to the original source publication, otherwise values for each BG samples are listed where mean value was not calculated in the original source publication. Range (minimum–maximum values), if information is given in the original publication, is indicated in the parenthesis for all mean values of BG samples of n ≥3.

Table 3.2. Mineral element composition of brewers grains

Country of the study /origin of the sample	n	Macro-minerals (g kg^{-1} DM)							Micro-minerals (mg kg^{-1} DM)				Reference studies
		Ca	P	Mg	K	Na	Cl	S	Fe	Mn	Zn	Cu	
USA	4	2.7 (1.8–3.9)	4.4 (1.9–5.5)	2.8 (1.8–4.3)	1.1 (0.2–3.4)	0.3 (0.2–0.7)			148 (105–174)	50 (39–60)	97 (75–113)	24 (18-35)	Arosemena et al.[29]
USA	10	2.4 (1.9–2.8)	6.5 (5.9–7.6)	2.7 (2.5–3.2)	2.6 (1.9–3.4)	0.5 (0.2–0.9	0.6 (0.3–1.2)	3.1 (2.7–3.7)	138 (108–163)	49 (46–56)	88 (75–105	11 (7–18)	DePeters et al.[33†]
USA	11	2.3 (2.0–2.8)	6.3 (5.9–6.7)	2.5 (2.4–2.6)	3.6 (2.5–3.9)	0.2 (0.2–0.3)	1.1 (0.2–1.4)	2.4 (2.2–2.5)	123 (103–154)	49 (43–71)	94 (78–161)	17 (15–21)	DePeters et al.[33‡]
Ethiopia	17	2.5	5.3	2.1	0.8	0.3			185	60		8	Kahsay et al. (unpublished)
USA	2	3.2	6.3	1.9	0.7	<0.1		3.6	292	48	84	33	Moriel et al.a[21]
Brazil	1	3.5	5.2	2.0	0.3	0.3		2.0	193	51	178	18	Mussatto and Roberto[12]
n.i	n.i	3.5	5.9	2.1	4.7	0.1	1.2	3.3	247	49	91	9	NRC[26†]
n.i	n.i	3.0	6.7	2.6	5.0	0.4	0.7	3.8	224	45	85	11	NRC[26‡]
USA	250–595	3.5 (1.8–5.3)	6.8 (5.7–7.8)	2.3 (1.7–2.9)	1.6 (up to 3.8)	0.4 (up to 1.4)	0.8 (0.1–1.7)	3.3 (2.7–3.9)	207 (27–361)	50 (37–63)	97 (76–118)	8.3 (1.0–17)	Westendorf and Wohlt[13§]
USA	48	2.1 (1.7–2.6)	5.5 (5.0–6.2)	2.0 (1.6–2.4)	0.9 (0.7–1.3)	0.1 (0.1–0.1)	0.4 (0.1–1.6)	4.1 (3.7–4.4)	136 (111–163)	57 (46–72)	105 (82–139	13 (6–31)	Westendorf et al.[15¶]

n = number of samples included in the study; n.i. = not indicated; result for the wet brewers grains; ‡result for the dried brewers grains; §given by DairyOne Forage Laboratory in Ithaca, NY, between 1998 and 2001; ¶brewers grains are sampled once weekly for over 12 months.

The indicated mean values were according to the original source publication, otherwise values for each BG samples are listed where mean value was not calculated in the original source publication. Range (minimum–maximum values), if information is given in the original publication, is indicated in the parenthesis for all mean values of BG samples of n ≥3.

3 DRY MATTER INTAKE AND FEEDING VALUE OF BREWERS GRAINS

Brewers grains have been primarily utilized as a feedstuff for dairy cattle[13], and included in to the diets of beef and dairy cattle, sheep, swine, and poultry.[44–47] Over the past decades, a number of feeding trials have been undertaken on both wet and dried BG for dairy cows, beef cattle, and lambs; in this section, we review dry matter intake (DMI) and the performance of the ruminant animals fed on BG.

The DMI of the ruminants in general declines as moisture in the diets of the dairy cow increases.[48] This decline has been partly associated with increased bulkiness of feedstuffs caused by intracellular water and increased water intake.[48] An extremely high moisture content and the bulky nature of wet brewers grains (WBG) could limit the DMI of the animals when included at the level higher than 30% of diet DM. Both DMI and milk yield of the cows fed corn silage-based diets were considerably suppressed when WBG replaced soybean meal (SBM) at 30% or 40% of the diet.[23] Inclusion of BG in dairy diets up to 20 to 30% of diet DM, however, did not retard DMI and milk yield of the cow.[15,23,49,50] Particularly in hot humid conditions, inclusion of WBG up to 30% was also not detrimental to DMI of the cows[50], and was associated with the high environmental temperature and low moisture level of total mixed ration (TMR). It has been suggested that a TMR with moisture higher than 55% tended to decrease DMI in dairy cow depending on the environmental temperature.[48]

Animals are likely to consume more DM of dried brewers grains (DBG) than WBG due to less moisture content on the former one. However, as a supplemental feed, the difference in DM content of WBG and DBG does not affect DMI at ordinary inclusion levels (< 30%) in the diet. Both Armentano et al.[51] and Rogers et al.[52] reported similar DMI for diets including WBG and DBG: 2.8 vs 3 kg 100^{-1} kg body weight at inclusion level 20% of the diet DM; 1.97 vs 2.06% of body weight for steers at inclusion levels of 17% to 20% of the diet DM, respectively.

Brewers grains have been effectively utilized in dairy rations, and several studies indicate that BG have a potential to partially replace a concentrate mixture and expensive protein sources such as SBM.[20,37,41] Murdock et al. [20] suggested that BG could effectively substitute concentrate up to 30% DM in the diet of lactating dairy cows at least without any adverse effect on the milk production performance. No differences have been observed in milk yield, milk composition, rumen volatile fatty acids, or rumen pH of lactating cows when WBG replaced a concentrate

mixture at 15% and 30% of diet DM.[20] Others have observed that substituting the concentrate mixture with WBG at 10% of diet DM in multiparous dairy cattle led increased the molar percentage of acetic acid in the rumen.[53]

Diets supplemented with WBG exhibited similar or higher milk yields and similar milk quality characteristics compared with diets supplemented with SBM. Polan et al.[41] found higher milk yield from the cows fed on diets of BG and WBG up to 29% or DBG up to 24% of diet DM, when compared to SBM. Similarly, several lactation parameters such as milk yield, milk fat content, milk protein yield and milk lactose yield were increased with addition of WBG up to 20% of the diet partially replacing SBM.[49] Conversely, in hot humid weather no changes have been seen in DMI, milk yield and 4% fat-corrected milk (FCM) yield when heat-stressed Jersey cow received control versus WBG diets as well as for those receiving 15% versus 30% WBG of dietary DM.[50]

The positive effect of WBG on milk production is probably due to their high ruminally undegraded feed crude protein (RUP) content and intestinal availability of essential AA such as methionine and lysine. Belibasakis and Tsirgogianni[54] included WBG at 16% DM of the total diet by partially substituting WBG for maize silage, SBM, and wheat bran to change the RUP from 35% to 39% of CP for lactating cows during hot weather. The authors found a significant increase in actual milk yield, FCM yield, milk fat content and yield, and milk total solid concentration. Cozzi and Polan[55] evaluated lactation response of Holstein cows partially replacing SBM and corn by corn gluten meal and DBG as protein supplement in the diet to change the RUP from 34 to 42% of CP in the diet and, as a result, enhanced DMI and milk yield.

Characteristics of ruminal degradability of particular feed ingredients and subsequent flow of non-ammonia N, mainly in the form of AA bound in proteins, to the duodenum are of great importance for dairy animals to cover requiements for maintenance and milk production. Feeding a dairy cow with diets containing proteins resistant to rumen degradability increases the outflow of AA to the duodenum and, provided that the protein is digested in the small intestine, increase milk production.[51] In this regard, BG contain not only high CP, but also deliver more protein and DM to the duodenum.[51] The RUP that passes to intestinal tract should provide as much essential AA as possible and improve the quality of protein in the intestinal tract.[52] Only few studies investigated the digestibility of RUP of BG in the small intestine of ruminants.[19,56] Pereira et al.[19] found that BG had low rumen CP degradability and higher intestinal CP digestibility compared to

barley rootlets (mean value of digestible by-pass CP of 30% of total CP for BG vs 13% for barley rootlets).

Substitution of forage sources by BG has been questioned in the view of digestibility and ruminal fermentation (pH) characteristics when BG are utilized as energy source and replace forage NDF, or both forage NDF and non-fibre carbohydrate (NFC).[57] The authors noted that when DBG replace forage, DMI slightly increased but when DBG replaced concentrate, DMI declined significantly due to a combined filling effect of both fibrous feed sources (forage and DBG). Acetate to propionate ratio also decreases when cows feed on BG replacing forage in the diet.[57] Similarly, inclusion of WBG at the level up to 25% DM of diet by decreasing forage NDF and total NFC did not affect DMI, milk yield, body condition score, or body weight.[36]

Rooke et al.[58] included BG in a beef cattle diet to replace grass silage and have improved the performance of beef cows due to high protein concentration. Generally, due to high digestible fibre (cellulose and hemicellulose), CP, and RUP contents, BG are more suitable for ruminants, and particularly for dairy cows, than for non-ruminants.[59] A feeding rate of 13.6 to 22.6 kg of WBG per cow per day corresponding to 3.5 to 5.9 kg of DMI and 4 to 9 kg per calf per day corresponding to 1 to 2.4 kg of DMI was recommended.[24] Nevertheless, the inclusion level partly depends on the nutritive value of that particular feedstuff and complementing feed ingredients in the diet.

4 EFFECT OF BREWING PROCESSES AND ASSOCIATED FACTORS

The first process step that potentially affects the composition of BG is malting. Malting undergoes three phases: steeping (water absorption), germination (enzymatic modification of barley endosperm) and kilning (drying of malt through heating).[60,61] During the germination phase of barley or other cereal grains, enzymes such as amylases, proteases, ß-glucanases are activated and synthesized in the aleurone and starchy endosperm.[1] Physiological and biochemical changes occur to modify the structure of endosperm of the grains. Consequently, enzymes start to break down the complex starch into simple sugars and the CP into AA that are used partly for the embryo growth of the barley endosperm otherwise required in the following steps of brewing. The degradation of starch at the malting step is slow and only 5–10% of the starch of the grain is degraded during malting.[62] Nevertheless, most of the protein breakdown occurs during malting rather than mashing.[62] Hence, these changes occurring in the malting step affect the efficiency of extract during mashing and the nutrient composition of the remaining BG.

Moreover, the final phase of malting (kilning) involves variable heat treatment depending on the type of malt and the beer type intended. Most breweries use mainly a pale malt that is kilned under relatively low temperature of 50–85°C to achieve 4–5% moisture content in the final malt.[1,2] However, when specialty malts are produced, for instances dark malt, caramel malt, roasted malt, a malt can be even heated and roasted at 107–230°C.[2,60,63] Special malts are used in small quantity in different beer types to add to an individual beer aroma, color and flavor.[2] Hence, to decipher the entire effects of kilning on composition and feeding value of BG might require further research. Roasting or intensive heat treatment of malts reduce the activity of enzymes in the subsequent steps and, thereby, availability of the sugar and AA in the wort.[64] The AA level in the wort obtained from dark malt (roasted) was about half of the level in the wort obtained from normal (Pilsner) malt.[64] This can be associated to Maillard reactions at high temperature during kilning which may produce melanoidins.[64] The BG obtained from dark colored malt practically exhibited high ADL and CP fraction C in the recent work (Chapter4, this thesis), and this supports the argumentation. Moreover, treatment of the malts with varied temperatures will necessarily affect wort nutrient contents and subsequently, BG quality.

Mashing is the subsequent step where enzymatic degradation that has started during malting is completed.[65] During this step, malt starch is extensively degraded by different amylases into simple sugars that eventually are fermented to alcohols by yeast.[66] The non-starch polysaccharides of the cell wall of the malt are only partly solubilized and degraded and constitute later one of the main components of the BG. During both malting and mashing processes, about 35–40% of the CP content of malt is degraded, into AA and peptides by proteases in the endosperm of grains.[67] However, the activity of the enzymes highly depends on the temperature regimes used for mashing. Different breweries may follow different standard protocols of mashing depending on how the temperature is raised during mashing.[68] Heat treatment at varied intensity depending on the desired beer type, will likely affect the content and quality of protein in BG as it does in wort. In this regard, the existence of high RUP in BG might be related to temperature treatments occurring throughout the entire processes of malting, and mashing of malt.

Lautering – separation of mash into wort and BG by filtration – occurs after mashing. During lautering, a high protein concentration in the mash can affect filtration negatively. Particularly the aggregation of sulphur-amino acids in gel proteins occurring during mashing reduces filtration efficiency through forming cross-linked materials that precipitate and accumulate in BG.[69,70] This

aggregation obviously contributes positively to the protein value of the BG as a feedstuff. It is thus why brewers, for a greater wort extract yield, prefer a barley malt of optimum protein content rather than a high protein content. Therefore, the effect of malt type and processing on BG composition are interrelated to a certain extent. Lautering, in turn, is affected by many factors such as raw material quality, particle size, distribution of mash particles, the presence of barley-associated bacteria[71], or content of fibre and their degradation during mashing. These make the association of brewing processes and chemical composition of BG complex. In general speaking, the extent of protein hydrolysis into peptides and AA, the extent of entire conversion of starch (malt endosperm) into fermentable carbohydrates, as well as non-starch polysaccharide solubility obviously vary depending on the conditions of brewing processes, malt types, brewing technology and efficiency of the respective brewery. In principle, approximately 65% of the CP content of the malt retains in the BG while the remainder is extracted into the wort.[11,67] According to Langenaeken et al. [72], from the total starch of malt, about 75.3% is converted to fermentable sugar, 22.8% to dextrin and 1.8% remain in BG. A starch content of up to 25% DM has been reported for BG, however, when other than barley malt (unmalted cereals) grains were included during mashing (Chapter4, this thesis).

Barley malt variety and cereal grains used for brewing are other main factors affecting the chemical composition of BG. For at least about 70% of beers barley malt serves as starch source.[2] Two- and six-row barley provide two malt types commonly used for brewing, with two-rowed barley more common in Europe and six-row barley in USA and Canada.[2] Barley varieties are selected primarily in consideration of the wort quality, and not the nutritional value of BG. To our knowledge, no studies exist that relate barley types or cultivars directly to the chemical composition of BG as animal feed. However, few studies tried to relate barley cultivars to ß-glucan content of BG and its degradation.[73,74] Varieties with high ß-glucan and arabinoxylan contents are known to increase wort viscosity and negatively affect extract yield.[74] For instance, variable starch content and some CP concentration differences of BG have been reported for BG from two malt varieties.[73,74]

Depending on the intended beer type and availability, other grains such as sorghum, corn, rice, wheat, barley, oats, rye, millets, buckwheat, fonio, quinoa, and triticale are also added during mashing as starch sources for degradation and glucose fermentation.[13,75–77] For instance, during a recent study (Chapter4, this thesis), about 50% of Ethiopian breweries were using other than

barley malt cereal grain, i.e., raw barley, sorghum, or rice for beer production subject to local availability, season and economic benefits of the respective brewery. In this regard, different appropriate enzymes and hence optimum gelatinization temperatures are required for starch extraction and protein solubility from different cereals.[77] However, the extent of solubility and extraction of nutrients into wort obviously varies and contribute indirectly to the variation of chemical composition of BG. Therefore, the type and quality of grains added into brewing as well as the differences in barley varieties cause variation in nutritional value of BG.[1,9,75]

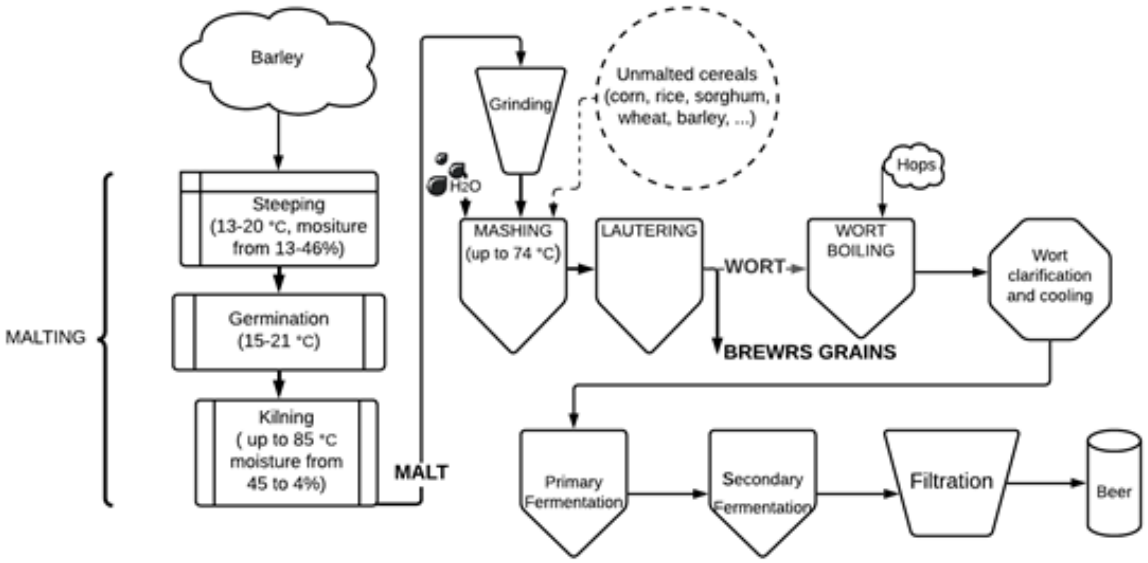

Figure 3.1. Scheme of malting and brewing processes

5 UTILIZATION AND PRESERVATION OF BREWERS GRAINS

Brewers grains are marketed mostly as wet (low DM concentration at about 20% of fresh weight) and sometimes dried (up to 90% DM). Most breweries sell BG fresh and wet to avoid the cost of energy required for drying.[37] The moisture content of WBG varies depending on the applied brewing technologes and ways of collection of all co-products generated during the processes.[13] It is common that breweries combine brewers yeasts and/or brewers hops with BG into a co-product that is also referred to as BG.[78–80] Subject to brewery, spent hops up to a rate of 5% may be added to BG mainly for disposal.[79,80] However, inclusion of hops is considered to affect the palatability and thus, overall sensory characteistics of of feed.[79] On the other hand, brewers yeast due to its

high CP content might enhance the protein value when included in BG. Typically BG from lauter-tuns, where other brewery by-products are excluded, contain higher moisture (≥ 78%) than BG obtained from mash filter (≤ 65%).[81]

Storage of WBG may be of great importance to provide a year-round nutritionally balanced feed, when there is excess supply.[49] It could be especially vital in temperate regions to preserve and store excess BG for winter feeding when fresh feed for animals is not available or only in very limted quantities.[82] However, to adequately utilise BG, preservation for longer storage periods is often a challenge for livestock farmers. The presence of high moisture content and low concentrations of fermentable sugars make WBG unsuitable for prolonged storage.[83] It can easily create molding, leading to offensive odor and excessive DM loss.[66] The possible duration of safe storage highly depends on the storage temperature. Mold growth was detected as early as after 36 hours storage at 25 °C and 12 hours storage at 35 °C, respectively.[84] Therefore, with aerobic storage, the shelf-life of WBG (80% moisture) or pressed WBG (70 - 75% moisture) does not exceed 7 to 10 days without additives.[12,23,37] Particularly during warm or hot weather or in tropical climate, the aerobic stability of WBG is only 2 or 3 days. A rapid protein and amino acid degradation occurs that is partly apparent by an intensive ammonia or fish smell.[85] The nutritional value (e.g., CP, NDF and water-soluble carbohydrate concentrations) decreased in response to increasing temperature up to 35 °C during 3 days of aerobic storage of WBG.[84] Hence, WBG is usually more preferred when utilized near breweries, immediately as fresh, and within no longer than a week depending on the environmental conditions. [24]

5.1 Preservation methods of brewers grains

Some possible preservation methods that have been proposed to maintain the BG for prolonged storage are discussed below:

Drying: Drying BG to a moisture level of below 10–12% is important to preserve and store for longer periods.[9,82] Drying minimizes the volume of the material, and hence reduces transportation costs and storage loses.[9,82] Oven drying, freeze-drying and freezing have been considered as being inappropriate and uneconomical to store large volumes.[83] Drying with direct rotary drum dryers traditionally is also very energy-intensive and not economically manageable.[86,87] Robertson et al.[83] have compared the effects of storage at ambient temperature against refrigeration for short-term storage and with autoclaving and freezing for long-term

storage. They concluded that refrigeration may provide short-term stabilization, while autoclaving could achieve longer-term stability. However, these methods are impractical for large-scale application due to high costs and challenges involved in preserving large volumes. Drying using superheated steam might be a better alternative for drying BG from an energy efficiency perspective.[86] The BG could successfully be dried without sticking using a superheated-steam rotary dryer at pilot-scale.[88] Compared to traditional drying operations (e.g., heated-air in rotary-drum dryers)[87], the drying with superheated steam has been suggested due to several advantages – increased efficiency, improved safety due to reduced risk for fire and explosions, free of odorous or particulate emissions, a possibility of combination of drying with material sterilization and pasteurization, and faster drying rates.[88,89] Nevertheless, superheated-steam drying could be energy-intensive next to oven drying, and requiring more complex drying equipment.[87] Hence, methods that are more convenient need to be developed to stabilize and optimize BG by developing a drying method that is economically profitable, allowing also processing of large quantities. Moreover, the methods should produce a biologically stable product to preserve the nutritional qualities of BG.

Ensiling with additives: Ensiling of BG is becoming nowadays a common practice of preserving BG at large scale in practical animal farming particularly in Western countries. However, the practice may not always result in quality silages due to several reasons.[66] The removal of the majority of fermentable carbohydrates (sugar and starch) during the brewing process negatively affects quality of BG silages. In addition, during lautering process, the sparging of mash – rinsing of mash with hot water prior to removal of BG – raises the mash temperature to 70–75°C. This high temperature causes to eliminate *Lactobacillus* species and maintain the spore forming *Clostridia* genera that usually are associated with poor ensiling quality of BG.[66] Nevertheless, several attempts have been made using several treatments and additives to safely ensile BG. Wet brewers grains could be preserved as high quality feed for 4 weeks when ensiled by treating with ammonia at 4% of DM.[37] An addition of 0.50% formic acid or 0.75% formic-propionic acid mixture maintained silage quality for 90 days.[66] Similarly, WBG silage prepared by addition of propionic acid at 0.40% of WBG has reduced total DM loss by approximately 5.8 percentage units compared with pure WBG.[21] Lilly et al. [82] conclude that WBG stabilized with molasses as well as potassium sorbate is more useful, and economical compared to DBG.

Ensiling as a total mixed ration: Recently, researchers were interested in ensiling BG as a TMR together with dry feeds to increase DM and fermentable carbohydrate level to improve storage of WBG.[90,91] However, controversial results have been seen on the effect of mixing the WBG with dry feedstuffs. Moriel et al [21] found that ensiling WBG with soybean hulls at 15 and 30% of TMR DM impaired the ensiling process, decreased CP and EE concentrations and digestibility (expressed as total digestible nutrients) of a mixture of WBG and soybean hulls; at the same time, DM loss, and NDF and ADF concentrations were increased. Ferraretto et al.[92] assumed that ensiling WBG with dry ground corn rehydrates corn for ensiling and as a result, starch digestibility improves and corn starch would enhance the level of fermentable carbohydrates for improved silage fermentation. However, improved WBG storage has been not achieved during their experiment. On the other hand, Wang et al.[91] recently reported that replacing common vetch, in ensiled TMR, with 20% WBG had no negative effect on fermentation quality, nutritive value, aerobic stability, *in vitro* gas production kinetics and digestibility. Further investigation might require understanding how BG ensiled with different locally available feeds and diet types affect the storage condition of the feed and performance of farm animals.

5. 2 Comparison of feeding value between wet, dried and ensiled brewers grains

Both WBG and DBG appear similar in terms of chemical composition. However, some variation has been shown particularly or rumnal CP and DM degradability.[51,52,23] Rogers et al.[52] compared WBG and DBG obtained from the same feedstock of a single brewery to determine the differences arising from heating during the drying process and found that CP in WBG is more ruminally degradable than that of DBG. Particularly, ADIN, an indicator of heat-damaged protein fractions, was 9% higher in DBG, and digestibility was higher in steers fed on WBG than DBG diets at an inclusion level of 22% of diet DM.[52] This suggests probably less availability of DBG protein post ruminally compared to WBG. Acid detergent insoluble nitrogen is described as N bound to artifact lignin (Maillard products)[93,94] which may occir as a result of overheating during brewing and drying processes. DePeters et al.[33] reported the unavailable CP (expressed as ADIN · 6.25) to be 22.5% of the total CP for DBG, and only 10% for WBG. Moreover, N intake, production of ruminal ammonia, apparent digestible nitrogen and nitrogen retained were higher for steers fed on diets containing WBG than diets containing DBG both included at 22% of DM of the diet.[52] Similarly, the ruminally undegraded DM portion (61% versus 51%) and undegraded nitrogen (58% versus 27%) has been estimated slightly higher for DBG versus WBG from in situ study at 0.05/h

rumen turnover rate.[51] It is questionable if the variations in ruminal fermentation result in digestibility variation of WBG and DBG in the small intestine of the animal. Further investigations are therefore recommended to understand the extent of digestion and availability of the protein from DBG in the small intestine of the animal.

However, the lactation response from both forms of BG has been consistently similar from most of the studies.[41,59] At an inclusion level of 15% DM of diet DM, cows fed diets containing DBG or WBG had similar DMI, energy intake, milk yield, energy output in milk, feed efficiency (FCM/DMI) and milk composition.[59] Similarly, feeding cows with diets containing WBG or DBG did not cause differences in ruminal pH, total volatile fatty acids (VFA) and molar ratios of VFA.[59]

A couple of studies revealed that the feeding value of ensiled WBG is more or less similar with WBG and DBG.[37,49] Johnson et al.[37] compared the feeding value of fresh WBG, WBG plus urea and ensiled WBG with SBM, and found that the different forms of BG could replace SBM similarly except that DM intake and milk yield were slightly lower for ensiled WBG. Imaizumi et al.[49] evaluated the performance of lactating cows fed on diets containing ensiled WBG with corn or WBG partially replacing SBM, and found that ensiled WBG did not affect milk yield and milk composition. However, the ensiling in this specific study lasted only for 14 days, and this might not be long enough to evaluate its long-term stability and hence, its long-term effects on milk production of dairy cattle. Recently, ensiled WBG with molasses as a replacement for cotton seed cake in the diet of lactating dairy cows has improved digestibility of the diet for DM, CP, NDF, and ADF and showed a positive response in milk yield.[95] Still, the studies on the feeding value of ensiled WBG appear linited both in number and validity.

Generally, a more pronounced interest lies on WBG than DBG and ensiled WBG, as it is more economically attractive because it reduces the cost incurred for drying. Moreover, the efficiency of protein utilization in the rumen as well as likely in the small intestine favors the use of WBG over DBG.[52] Otherwise, the difference between WBG and DBG in terms of chemical composition might be insignificant.

6 CONCLUSIONS

Brewers grains in different forms are accepted by animals and have shown favorable performance for all ruminant farm animals. It is a valuable and versatile feedstuff for all ruminants, and particularly for dairy animals due to its high CP and RUP content. Hence, BG can potentially

substitute expensive concentrate feedstuffs like SBM in the diet of dairy animals. However, due to its highly variable nutritional value—partially influenced by factors such as brewing processes, malt types and varieties, and the cereal grains used—it is essential to have a thorough understanding of its chemical composition and nutrient digestibility. Because of the fact that BG are a by-product, which is not produced considering the nutritional value for animals, brewing factors that affect the composition of BG make it complex for animal nutritionists to evaluate the overall quality of BG. Depending on the feed availability and animal farm situation, BG are utilized as fresh, dried and ensiled forms. Hence, consideration of additional energy cost for drying and appropriate economic decision should be applied for specific farm situation in practical feeding.

Acknowledgements: The first author would like to acknowledge KAAD (Katholischer Akademischer Ausländer-Dienst, Bonn, Germany) for a scholarship during the whole course of work.

References

1. Mussatto SI, Dragone G and Roberto IC. Brewers' spent grain: generation, characteristics and potential applications. *J Cereal Sci* **43**:1–14 (2006).
2. Kreisz S. Malting, in *Handbook of brewing: Processes, technology, markets*, ed. by Esslinger HM. Wiley-VCH, Weinheim, pp. 147–164 (2009).
3. Kunze W. *Technology Brewing & Malting*. VLB, Berlin (2004).
4. Kirin Holdings Company. Kirin Beer University Report Global Beer Production by Country in 2018. Available: https://www.kirinholdings.co.jp/english/news/2019/1003_01.html (2019 [4 June 2021].
5. Lynch KM, Steffen EJ and Arendt EK. Brewers' spent grain: a review with an emphasis on food and health. *J Inst Brew* **122**:553–568 (2016).
6. Mekasha Y, Tegegne A, Yami A and Umunna NN. Evaluation of non-conventional agro-industrial by-products as supplementary feeds for ruminants: *In vitro* and metabolism study with sheep. *Small Rumin Res* **44**:25–35 (2002).

7. Negesse T, Makkar HPS and Becker K. Nutritive value of some non-conventional feed resources of Ethiopia determined by chemical analyses and an *in vitro* gas method. *Anim Feed Sci Technol* **154**:204–217 (2009).
8. Udedibie ABI. Brewers' grains as feedstuff for milk production-A review. *J Anim Prod Res* **4**:43–55 (1984).
9. Santos M, Jiménez JJ, Bartolomé B, Gómez-Cordovés C and Nozal MJd. Variability of brewer's spent grain within a brewery. *Food Chem* **80**:17–21 (2003).
10. Evans DE, Redd K, Haraysmow SE, Elvig N, Metz N and Koutoulis A. The influence of malt quality on malt brewing and barley quality on barley brewing with Ondea Pro, compared by small-scale analysis. *J Am Soc Brew Chem* **72**:192–207 (2014).
11. Hennemann M, Gastl M and Becker T. Inhomogeneity in the lauter tun: A chromatographic view. *Eur Food Res Technol* **245**:521–533 (2019).
12. Mussatto SI and Roberto IC. Chemical characterization and liberation of pentose sugars from brewer's spent grain. *J Chem Technol Biotechnol* **81**:268–274 (2006).
13. Westendorf ML and Wohlt JE. Brewing by-products: their use as animal feeds: Their use as animal feeds. *Vet Clin Food Anim* **18**:233–252 (2002).
14. Robertson JA, I'Anson KJA, Treimo J, et al. Profiling brewers' spent grain for composition and microbial ecology at the site of production. *LWT-Food Sci Technol* **43**:890–896 (2010).
15. Westendorf ML, Wohlt JE, Sniffen CJ and Ward RT. Nutrient content of brewers grains produced at a commercial brewery: Variation in protein/nitrogen, fiber, carbohydrate, fat, and minerals. *Prof Anim Sci* **30**:400–406 (2014).
16. Naibaho J and Korzeniowska M. The variability of physico-chemical properties of brewery spent grain from 8 different breweries. *Heliyon* **7** (2021).
17. Lynn BA, Wohlt JE, Schoknecht PA and Westendorf L. Variation in nutrient content of microbrewers grains and effect on performance and carcass composition when fed wet to growing-finishing swine. *Prof Anim Sci* **25**:426–433 (2009).
18. Waters DM, Jacob F, Titze J, Arendt EK and Zannini E. Fibre, protein and mineral fortification of wheat bread through milled and fermented brewer's spent grain enrichment. *Eur Food Res Technol* **235**:767–778 (2012).
19. Pereira JC, Carro MD, González J, Alvir MR and Rodríguez CA. Rumen degradability and intestinal digestibility of brewers' grains as affected by origin and heat treatment and of barley rootlets. *Anim Feed Sci Technol* **74**:107–121 (1998).

20. Murdock FR, Hodgson AS and Riley Jr RE. Nutritive value of wet brewers grains for lactating dairy cows. *J Dairy Sci* **64**:1826–1832 (1981).
21. Moriel P, Artioli LFA, Poore MH and Ferraretto LF. Dry matter loss and nutritional composition of wet brewers grains ensiled with or without covering and with or without soybean hulls and propionic acid. *Prof Anim Sci* **31**:559–567 (2015).
22. Getachew G, Crovetto GM, Fondevila M, et al. Laboratory variation of 24 h *in vitro* gas production and estimated metabolizable energy values of ruminant feeds. *Anim Feed Sci Technol* **102**:169–180 (2002).
23. Davis CL, Grenawalt DA and McCoy GC. Feeding value of pressed brewers' grains for lactating dairy cows. *J Dairy Sci* **66**:73–79 (1983).
24. Thomas M, Hersom M, Thrift T and Yelich J. *Wet Brewers' Grains for Beef Cattle*. University of Florida, Institute of Food and Agricultural Sciences, Gainesville, FL, USA (2010). Available: https://journals.flvc.org/edis/article/view/118590 [5 February 2022].
25. Meneses NGT, Martins S, Teixeira JA and Mussatto SI. Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains. *Sep Purif Technol* **108**:152–158 (2013).
26. NRC. *Nutrient requirements of dairy cattle*, 7th edn. National Academies Press, Washington, D.C. (2001).
27. Ikram S, Huang L, Zhang H, Wang J and Yin M. Composition and Nutrient Value Proposition of Brewers Spent Grain. *J Food Sci* **82**:2232–2242 (2017).
28. Aghajanzadeh-Golshani A, Maheri-Sis N, Mirzaei-Aghsaghali A and Baradaran-Hasanzadeh A. Comparison of nutritional value of tomato pomace and brewer's grain for ruminants using *in vitro* gas production technique. *Asian J Anim Vet Adv* **5**:43–51 (2010).
29. Arosemena A, DePeters EJ and Fadel JG. Extent of variability in nutrient composition within selected by-product feedstuffs. *Anim Feed Sci Technol* **54**:103–120 (1995).
30. Brabander DL de, Boever JL de, de Am Smet, Vanacker JM and Boucqué CV. Evaluation of the physical structure of fodder beets, potatoes, pressed beet pulp, brewers grains, and corn cob silage. *J Dairy Sci* **82**:110–121 (1999).
31. Connolly A, Piggott CO and FitzGerald RJ. Characterisation of protein-rich isolates and antioxidative phenolic extracts from pale and black brewers' spent grain. *Int J Food Sci Technol* **48**:1670–1681 (2013).

32. DePeters EJ, Fadel JG and Arosemena A. Digestion kinetics of neutral detergent fiber and chemical composition within some selected by-product feedstuffs. *Anim Feed Sci Technol* **67**:127–140 (1997).
33. DePeters EJ, Fadel JG, Arana MJ, et al. Variability in the chemical composition of seventeen selected by-product feedstuffs used by the California dairy industry. *Prof Anim Sci* **16**:69–99 (2000).
34. Etela I, Oji UI, Kalio GA and Tona GO. Studies on sweet potato forage and dried brewers' grains as supplements to green panic for Bunaji cows. *Trop Grassl* **42**:245 (2008).
35. Faccenda A, Zambom MA, Avila ASd, et al. Nutrient digestibility and ruminal parameters of cattle fed dried brewers grains and Saccharomyces cerevisiae. *Livest Sci* **225**:109–115 (2019).
36. Firkins JL, Di Harvatine, Sylvester JT and Eastridge ML. Lactation performance by dairy cows fed wet brewers grains or whole cottonseed to replace forage. *J Dairy Sci* **85**:2662–2668 (2002).
37. Johnson C, Huber JT and King KJ. Storage and utilization of brewers wet grains in diets for lactating dairy cows. *J Dairy Sci* **70**:98–107 (1987).
38. Loosli JK and Warner RG. Distillers grains, brewers grains, and urea as protein supplements for dairy rations. *J Dairy Sci* **41**:1446–1450 (1958).
39. Moriel P, Artioli LFA, Poore MH and Fellner V. Effects of replacing ground corn with wet brewers grains on growth performance and concentrations of liver trace minerals and plasma fatty acids of preconditioning beef heifers fed medium-quality fescue hay. *Prof Anim Sci* **31**:425–433 (2015).
40. Phipps RH, Sutton JD and Jones BA. Forage mixtures for dairy cows: The effect on dry-matter intake and milk production of incorporating either fermented or urea-treated whole-crop wheat, brewers' grains, fodder beet or maize silage into diets based on grass silage. *Anim Sci* **61**:491–496 (1995).
41. Polan CE, Herrington TA, Wark WA and Le Armentano. Milk production response to diets supplemented with dried brewers grains, wet brewers grains, or soybean meal. *J Dairy Sci* **68**:2016–2026 (1985).
42. Seifried N, Steingass H, Leberl P and Rodehutscord M. *Feeding value of brewers´ grains for ruminants*. 21th International Scientific Symposium on Nutrition of Farm Animals, Radenci, Slowenien (2012). Available: https://www.uni-hohenheim.de/en/organization/publication/feeding-value-of-brewers-grains-for-ruminants.

43. Xu C, Cai Y, Moriya N and Ogawa M. Nutritive value for ruminants of green tea grounds as a replacement of brewers' grains in totally mixed ration silage. *Anim Feed Sci Technol* **138**:228–238 (2007).
44. Radzik-Rant A, Rant W, Niżnikowski R, et al. The effect of the addition of wet brewers grain to the diet of lambs on body weight gain, slaughter value and meat quality. *Arch Anim Breed* **61**:245 (2018).
45. Swain BK, Naik PK, Chakurkar EB and Singh NP. Effect of feeding brewers' dried grain on the performance and carcass characteristics of Vanaraja chicks. *J Appl Anim Res* **40**:163–166 (2012).
46. Ademosun AA. Evaluation of brewers' dried grains in the diets of growing chickens. *Br Poult Sci* **14**:463–468 (1973).
47. Ngodigha EM, Sese BT, Olaka OS and Iyayi EA. Effect of brewers dried grain on growth performance and plasma amino acids of young pigs. *J Appl Anim Res* **6**:97–104 (1994).
48. Robinson PH, Burgess PL and McQueen RE. Influence of moisture content of mixed rations on feed intake and milk production of dairy cows. *J Dairy Sci* **73**:2916–2921 (1990).
49. Imaizumi H, Batistel F, Souza J de and Santos FAP. Replacing soybean meal for wet brewer's grains or urea on the performance of lactating dairy cows. *Trop Anim Health Prod* **47**:877–882 (2015).
50. West JW, Lo Ely and Martin SA. Wet brewers grains for lactating dairy cows during hot, humid weather. *J Dairy Sci* **77**:196–204 (1994).
51. Armentano LE, Herrington TA, Polan CE, Moe AJ, Herbein JH and Umstadt P. Ruminal degradation of dried brewers grains, wet brewers grains, and soybean meal. *J Dairy Sci* **69**:2124–2133 (1986).
52. Rogers JA, Conrad HR, Dehority BA and Grubb JA. Microbial numbers, rumen fermentation, and nitrogen utilization of steers fed wet or dried brewers' grains. *J Dairy Sci* **69**:745–753 (1986).
53. Miyazawa K, Sultana H, Hirata T, Kanda S and Itabashi H. Effect of brewer's grain on rumen fermentation, milk production and milk composition in lactating dairy cows. *Anim Sci J* **78**:519–526 (2007).
54. Belibasakis NG and Tsirgogianni D. Effects of wet brewers grains on milk yield, milk composition and blood components of dairy cows in hot weather. *Anim Feed Sci Technol* **57**:175–181 (1996).

55. Cozzi G and Polan CE. Corn gluten meal or dried brewers grains as partial replacement for soybean meal in the diet of Holstein cows. *J Dairy Sci* **77**:825–834 (1994).
56. Merchen N, Hanson T and Klopfenstein T. Ruminal bypass of brewers dried grains protein. *J Anim Sci* **49**:192–198 (1979).
57. Younker RS, Winland SD, Firkins JL and Hull BL. Effects of replacing forage fiber or nonfiber carbohydrates with dried brewers grains. *J Dairy Sci* **81**:2645–2656 (1998).
58. Rooke JA, Duthie C-A, Hyslop JJ, Morgan CA and Waterhouse T. The effects on cow performance and calf birth and weaning weight of replacing grass silage with brewers grains in a barley straw diet offered to pregnant beef cows of two different breeds. *J Anim Physiol Anim Nutr* **100**:629–636 (2016).
59. Dhiman TR, Bingham HR and Radloff HD. Production response of lactating cows fed dried versus wet brewers' grain in diets with similar dry matter content. *J Dairy Sci* **86**:2914–2921 (2003).
60. Samaras TS, Camburn PA, Chandra SX, Gordon MH and Ames JM. Antioxidant properties of kilned and roasted malts. *J Agric Food Chem* **53**:8068–8074 (2005).
61. Holopainen-Mantila U. *Composition and structure of barley (Hordeum vulgare L.) grain in relation to end uses*. A Doctoral Thesis, Faculty of Biological and Environmental Sciences, University of Helsinki. Helsinki, Finland (2015).
62. Palmer GH (ed). *An Introduction to Brewing Science and Technology: Malting, Wort production and Fermentation*. The Institute of Brewing (1990).
63. Moreira MM, Morais S, Carvalho DO, Barros AA, Delerue-Matos C and Guido LF. Brewer's spent grain from different types of malt: Evaluation of the antioxidant activity and identification of the major phenolic compounds. *Food Res Int* **54**:382–388 (2013).
64. Coghe S, D'Hollander H, Verachtert H and Delvaux FR. Impact of dark specialty malts on extract composition and wort fermentation. *J Inst Brew* **111**:51–60 (2005).
65. Kühbeck F, Dickel T, Krottenthaler M, et al. Effects of mashing parameters on mash β-glucan, FAN and soluble extract levels. *J Inst Brew* **111**:316–327 (2005).
66. Allen WR and Stevenson KR. Influence of additives on the ensiling process of wet brewers' grains. *Can J Anim Sci* **55**:391–402 (1975).
67. Celus I, Brijs K and Delcour JA. The effects of malting and mashing on barley protein extractability. *J Cereal Sci* **44**:203–211 (2006).

68. Ogbonna AC. *Brewing science and technology: A comprehensive approach*, International Edition, Uyo-Nigeria (2011).
69. Buhler TM. *Effects of physical parameters in mashing on lautering performance*. A Doctoral Thesis, Loughborough University. Loughborough, UK (1996).
70. Moonen JHE, Graveland A and Muts GCJ. The molecular structure of gelprotein from barley, its behaviour in wort-filtration and analysis. *J Inst Brew* **93**:125–130 (1987).
71. Laitila A, Manninen J, Priha O, Smart K, Tsitko I and James S. Characterisation of barley-associated bacteria and their impact on wort separation performance. *J Inst Brew* **124**:314–324 (2018).
72. Langenaeken NA, Schepper CF de, Schutter DP de and Courtin CM. Carbohydrate content and structure during malting and brewing: A mass balance study. *J Inst Brew* **126**:253–262 (2020).
73. Viëtor RJ, Voragen AGJ and Angelino S. Composition of non-starch polysaccharides in wort and spent grain from brewing trials with malt from a good malting quality barley and a feed barley. *J Inst Brew* **99**:243–248 (1993).
74. Habschied K, Lalić A, Horvat D, et al. β-Glucan Degradation During Malting of Different Purpose Barley Varieties. *Fermentation* **6**:21 (2020).
75. Mussatto SI. Brewer's spent grain: a valuable feedstock for industrial application. *J Sci Food Agric* **94**:1264–1275 (2014).
76. Taylor JRN, Dlamini BC and Kruger J. 125th Anniversary Review: The science of the tropical cereals sorghum, maize and rice in relation to lager beer brewing. *J Inst Brew* **119**:1–14 (2013).
77. Meussdoerffer F and Zarnkow M. Starchy raw materials, in *Handbook of brewing: Processes, technology, markets*, ed. by Esslinger HM. Wiley-VCH, Weinheim, pp. 43–83 (2009).
78. Mohammed D, Addis D, Berhanu T and Vernooij A. *Mapping of spent brewers' grain supply chain in Ethiopia*. Wageningen Livestock Research, Wageningen (2019). Available: https://library.wur.nl/WebQuery/wurpubs/fulltext/506451 [27 April 2021].
79. Kerby C and Vriesekoop F. An overview of the utilisation of brewery by-products as generated by british craft breweries. *Beverages* **3**:24 (2017).
80. Karlović A, Jurić A, Ćorić N, Habschied K, Krstanović V and Mastanjević K. By-Products in the malting and brewing industries—Re-usage possibilities. *Fermentation* **6**:82 (2020).
81. O'Rourke T. Mash separation systems. *Brewer International* **3**:57–59 (2003).
82. Lilly V, Birch M and Garscadden B. The preservation of spent brewers' grains by the application of intermediate moisture food technology. *J Sci Food Agric* **31**:1059–1065 (1980).

83. Robertson JA, I'Anson KJA, Brocklehurst TF, Faulds CB and Waldron KW. Effect of storage conditions on the microbial ecology and biochemical stability of cell wall components in brewers' spent grain. *J Agric Food Chem* **58**:7266–7272 (2010).
84. Wang B, Luo Y, Myung KH and Liu JX. Effects of storage duration and temperature on the chemical composition, microorganism density, and *in vitro* rumen fermentation of wet brewers grains. *Asian Australas J Anim Sci* **27**:832 (2014).
85. LfL. *Biertreber Futterwert, Konservierung und erfolgreicher Einsatz beim Wiederkäuer*. Bayerische Landesanstalt für Landwirtschaft (LfL) (2008). Available: https://www.lfl.bayern.de/mam/cms07/publikationen/daten/informationen/p_29845.pdf [20 May 2021].
86. Tang Z, Cenkowski S and Muir WE. Modelling the superheated-steam drying of a fixed bed of brewers' spent grain. *Biosyst Eng* **87**:67–77 (2004).
87. Tang Z, Cenkowski S and Izydorczyk M. Thin-layer drying of spent grains in superheated steam. *J Food Eng* **67**:457–465 (2005).
88. Stroem LK, Desai DK and Hoadley AFA. Superheated steam drying of Brewer's spent grain in a rotary drum. *Adv Powder Technol* **20**:240–244 (2009).
89. van Deventer HC and Heijmans RMH. Drying with superheated steam. *Dry Technol* **19**:2033–2045 (2001).
90. Ferraretto LF, Fernandes T, Silva Filho WI, Sultana H and Moriel P. Dry matter loss, fermentation profile, and aerobic stability of wet brewers grains ensiled with various amounts of dry ground corn. *Prof Anim Sci* **34**:642–648 (2018).
91. Wang S, Zhao J, Yu C, et al. Nutritional evaluation of wet brewers' grains as substitute for common vetch in ensiled total mixed ration. *Ital J Anim Sci* **19**:1015–1025 (2020).
92. Ferraretto LF, Silva Filho WI, Fernandes T, Kim DH and Sultana H. Effect of ensiling time on fermentation profile and ruminal *in vitro* starch digestibility in rehydrated corn with or without varied concentrations of wet brewers grains. *J Dairy Sci* **101**:4643–4649 (2018).
93. Sanderson MA and Wedin WF. Nitrogen in the detergent fibre fractions of temperate legumes and grasses. *Grass Forage Sci* **44**:159–168 (1989).
94. Goering HK and van Soest PJ. *Forage fiber analyses (apparatus, reagents, procedures, and some applications)*. Agriculture Handbook No. 379. Agricultural Research Service, United States Department of Agriculture, Washington, D.C. (1970).

95. Getu K, Getachew A, Berhan T and Getnet A. Supplementary value of ensiled brewers spent grain used as replacement to cotton seed cake in the concentrate diet of lactating crossbred dairy cows. *Trop Anim Health Prod* **52**:3675–3683 (2020).

CHAPTER 4

Chemical composition and *in vitro* characterization of energy and protein value of brewery by-products for ruminants - Ethiopian breweries

Z.A. Kahsay[1], C. Böttger[1], K.-H. Südekum[1]*

[1]University of Bonn, Institute of Animal Science, Endenicher Allee 15, 53115 Bonn, Germany

* **Author for correspondence**:

Acknowledgements

The authors thank for all the breweries for the kind provision of BG samples.

Financial Support

The first author gratefully acknowledges Katholischer Akademischer Ausländer-Dienst (KAAD) for the scholarship grant during the course of work, and Institute of Animal Science (ITW), University of Bonn for the financial support.

Under review

Abstract

The current study examined a nutritive value of twenty samples of brewers grains (BG) and tella-atella (TA) – a by-product of locally produced tella drink – obtained from Ethiopia with primary focus on protein values; and investigated the potential variability. Samples were subjected to analysis of chemical, mineral, and crude protein (CP) compositions. *In vitro* gas test was used to determine energy value, ruminally undegraded feed crude protein (RUP) and utilisable CP at the duodenum (uCP). In BG, substantial contents of neutral detergent fiber (443–738 g/kg dry matter, DM), starch (43–255 g/kg DM), and ether extract (70–120 g/kg DM) contributed to mean values of 8.4 and 6.1 MJ/kg DM for metabolisable energy and net energy for lactation, respectively, with a considerable variability between breweries/samples. Particularly high starch concentrations in non-pure barley malt BG (43–255 g/kg DM), and TA (220–280 g/kg DM) samples were most likely related to the brewing process and type of grains used. BG exhibited variable but high content of CP (187–299 g/kg DM), RUP (569–772 g/kg CP), and uCP (111–198 g/kg DM). The relative higher portion of insoluble CP fraction (246 g/kg CP) in TA leads in to a question on the extent of availability in small intestine of the animals. The findings underline that both BG and TA can be utilised as supplements in ruminant diets and thus contribute to sustainable resource use. However, based on the variability observed in the current study, periodic evaluation of feeding value is vital for efficient utilisation of brewery by-products from Ethiopian breweries due to inconsistent use of raw ingredients and unmalted cereal grains. Digestibility of uCP and the amino acid patterns in the small intestine of the dairy animal require further investigation.

Key words: Brewers grains, rumen degradation, tella-atella, utilisable crude protein at the duodenum, Ethiopia

Introduction

Feed scarcity is a prominent issue in Ethiopia where more than two-third of the population own livestock making a country with one of the highest livestock population in Africa (FAO, 2019*b*). Identifying alternative feed sources and supplementing the available feeds with nutrient rich feed ingredients is important to improve livestock productivity. In this regard, utilizing non-conventional feed sources for ruminant animals can be considered due to its comparative advantages over concentrate feeds that have a direct competition with human food uses particularly in countries like Ethiopia (Negesse *et al.*, 2009). Protein, in particular, is of main importance in Ethiopian context where feeding of ruminants depends on low protein feedstuffs, such as cereal crop residues and natural pastures. Available cereal crop residues, for instance tef (*Eragrostis tef*) straw, grass pea hulls and maize stover are of poor nutritive quality – usually with low intake (50 g/ unit of metabolic body size ($kg^{0.75}$) or less), low organic matter digestibility (OMD, often less than 550 g/kg dry matter, DM), low crude protein (CP, 30–80 g/kg DM), high fiber (neutral detergent fiber, NDF >700 g/kg DM) and low mineral concentrations (Tolera *et al.*, 1999; Gizachew and Smit, 2005; Feyissa *et al.*, 2015). The *in vitro* dry matter digestibility of barley straw and stubbles is very low (440 and 320 g/kg DM, respectively; Bogale *et al.*, 2008). Brewery by-products cost less than concentrate feed in Ethiopia (Aranguiz *et al.*, 2019) and, as a supplement, could enhance protein and energy values of low quality forages and pastures.

Brewers grains (BG, also referred to as brewers spent grains), brewers hops and brewers yeasts are the main by-products generated from the beer brewing process and are used as a feed supplement for ruminants, horses, pigs and poultry (Westendorf and Wohlt, 2002). The total production potential of BG from all 12 breweries found in Ethiopia in 2018 was estimated to be around 240,000 tons on fresh basis (Mohammed *et al.*, 2019). The estimation was based on the assumption of a beer to BG ratio of 5:1 (Mussatto *et al.*, 2006). In addition, a brewing by-product known as tella-atella (TA), which is obtained from traditional 'tella' brewing is widely used as a livestock feed by smallholder livestock farmers in Ethiopia (Nurfeta and Abdu, 2014). Tella is a homemade alcoholic drink based on substrates such as barley, wheat, maize, millet, sorghum, teff or other cereals (Tadesse and Yayneshet, 2011; Lee *et al.*, 2015). Though there is no compiled data at national level, TA is produced in a significant amount as tella brewing is practiced by most households (Lee *et al.*, 2015). According to FAO (2019*a*), the amount of TA from local tella brewing and traditional distilling of liquor ("areki") obtained from only two regions is fivefold

higher than the BG production in the whole country. However, TA is diverse due to variation in used raw materials and production procedures in different societies (Lee *et al.*, 2015).

Both brewery by-products (BG and TA) have traditionally been used to feed ruminants and poultry in Ethiopia (Kitaw *et al.*, 2018; Mohammed *et al.*, 2019). Several studies evaluated chemical composition and DM or organic matter (OM) digestibility of TA (Mekasha *et al.*, 2002; Demeke, 2007; Negesse *et al.*, 2009; Nurfeta and Abdu, 2014), while other studies evaluated only chemical composition (Nurfeta, 2010; Tadesse and Yayneshet, 2011). However, there is no information on more specific characteristics of feed protein value, such as ruminal CP degradability. Demeke (2007) has reported chemical composition, energy value, and digestible DM contents of a BG sample obtained from a single brewery. Similarly, Feyissa *et al.* (2015) have determined the mineral profile of a BG sample obtained from a single brewery. Beyond this, there is no further data on the chemical composition and nutritional value of BG in Ethiopia as a ruminant feed.

The current study was conducted to systematically collect samples of BG and TA from Ethiopian breweries and traditional tella brewing, respectively, and determine chemical composition. Further, *in vitro* gas production was conducted to estimate ruminal CP degradability and utilisable CP at the duodenum (uCP) – a precursor of metabolisable protein. A second *in vitro* experiment used to estimate organic matter digestibility (OMD) and energy value. Finally, the potential variability attributed by differences in brewing processes and raw materials used was investigated.

Materials and Methods

Sampling and sample description

Samples of BG were obtained from single batch brewing of nine breweries. About two-third of all the breweries found in Ethiopia in year 2019 were included in the current study. A short interview with production managers of each company was conducted before sample collection to identify malt grains and any other cereal grains used in addition to barley malt in each beer type. Differences in beer type, malt grains and addition of other cereal grains were reasons to collect separate BG samples within one brewery. Three to four kg of each BG sample (n=16) from breweries were collected fresh directly after separation of wort and BG in the lauter tun or mash filter. Samples (n=3) of TA from traditional tella brewing; and a sample (n=1) from a commercial

German brewery, as a reference, were also included in the study. All samples from Ethiopian breweries were collected from November 2018 to March 2019, and German one in July 2019.

Based on the information obtained from the breweries, 9 BG samples were based on pure barley malt whereas 7 BG samples were based on barley malt plus barley grains or other cereal grains (denoted as non-pure barley malt BG for the purpose of the current study; Table 1). Added cereal grains include sorghum, rice and raw barley in different proportions. Tella-atella samples were mainly derived from maize or sorghum grains or a combination of both and a small amount of barley malt (usually about ratio of 0.03). Lee *et al.* (2015) describe the raw ingredients and steps of traditional tella brewing; however, some modifications depending on the cultural differences and regional experiences are to be expected. It was assumed that all BG samples have been produced through standard commercial brewing processes, except one sample from a micro-brewery was included. According to information obtained from companies, more than half of the barley malt used by all breweries was imported from European countries including Belgium, England, France, Germany, the Netherlands, and Poland.

Drying and milling

Immediately after sampling, uniformly mixed samples of BG or TA were weighed and distributed on aluminum plates in a thin layer (50 grams per 240 cm^2 plate area) and dried in a drying oven at 55 °C for 24-36 h. After assuring uniform oven drying of the samples, plates with samples were left at room temperature for 1 h and then weighed. Residual moisture was determined by drying subsamples at 103 °C for 12 h. Dried (55 °C) samples were packed and stored in airtight plastic bags at room temperature until further processing. Samples were ground in two steps using a cutting mill (5-mm screen; SM1, Retsch, Haan, Germany) and a centrifugal mill (1-mm screen; ZM 200, Retsch, Haan, Germany). To analyse starch concentration, material was additionally ground through a 0.2-mm screen (ZM 200, Retsch, Haan, Germany).

Table 4.1. List of raw ingredients used and malt source of brewers grains (n=17) and tella-atella (n=3) samples

Breweries	Brewery by-products code[a]	Raw ingredients[b]	Barley malt source[c]
		Brewers grains	
B1	BG1	Barley malt, barley grains (90,10),	imported (50,50)
B2	BG2[d]	Barley malt (100)	imported (50,50)
B3	BG3[d]	Barley malt (100)	imported (50,50)
B4	BG4	Barley malt (100)	imported (100)
B3	BG5	Barley malt, rice (80, 20)	imported (50,50)
B5	BG6	Barley malt, barley grains (90,10)	imported (70,30)
	BG8	Barley malt (100)	imported (100)
	BG9	Barley malt (dark colour), barley grains (90, 10)	imported (70,30)
B6	BG10	Barley malt , barley grains (68, 32),	100% imported
	BG11	Barley malt, sorghum (unknown)	100% imported
	BG12	Barley malt + sorghum + barley grains (unknown)	100% imported
B7	BG13[e]	Barley malt (100)	NA
	BG14[e]	Barley malt (100)	NA
B8	BG15[f]	Barley malt (100)	NA
	BG16[f]	Barley malt (100)	NA
B9	BG17[g]	Barley malt (100)	imported (100)
Tella-atella			
LTB1	TA1	Sorghum, maize, barley malt (48.5, 48.5, 3)	
LTB2	TA2	Sorghum, barley malt (97, 3)	
LTB3	TA3	Sorghum, barley malt (97, 3)	
German brewery sample			
B10	BG18[h]	Barley malt (100)	German brewery

NA, not applicable (lacks information); LTB, Local tella brewing; B, refer to breweries; TA, tella-atella.

[a]Anonymous code given to the samples.

[b]Information obtained through discussion with brewery production managers. Proportion of pure-barley malt and other grains are indicated in the parentheses in respective order.

[c] Proportion of imported barley malt and locally produced malt indicated in parentheses in respective order.

[d]Samples obtained from the same beer type in different location (different brewery).

[e]Samples obtained from the same brewery but at different time point.

[f]Samples obtained from the same brewery but at different time point.

[g]Sample obtained from micro-brewery.

[h]Sample obtained from German brewery.

General chemical analysis

Proximate analyses were carried out according to the Association of German Agricultural Analytic and Research Institutes (VDLUFA, 2012) as indicated by method numbers. Ash and crude lipids (CL) were analysed using methods 8.1 and 5.1.1, respectively. Crude protein was determined by Kjeldahl method (4.1.1) using a Vapodest 50 s carousel (Gerhardt, Königswinter, Germany) for automated distillation and titration. Neutral detergent fibre (aNDFom; 6.5.1; assayed with heat stable amylase and without sodium sulfite), acid detergent fibre (ADFom; 6.5.2), and acid detergent lignin (ADL; 6.5.3) were analysed using an Ankom^{2000} Fiber analyser (ANKOM Technology, Macedon, NY, USA). Analysis of aNDFom and ADFom was done separately and values were expressed exclusive of residual ash. Starch was quantified after enzymatic hydrolysis of starch to glucose as described by Brandt *et al*. (1987) using material ground through a 0.2 mm screen in a centrifugal mill (ZM 200, Retsch, Haan, Germany). Contents of K, Na, Mg, Fe, Mn, Zn, and Cu were determined using atomic absorption spectroscopy (AAS; AAnalyst 200, PerkinElmer, Waltham, MA, USA) according to VDLUFA (2012), applying methods 10.2.1, 10.1.1, 10.4.1, 11.1.2, 11.4.2, 11.5.2, and 11.3.2, respectively. Concentration of P was analysed using a photometric method (method 10.6.1; VDLUFA, 2012); Ca was determined using AAS following ISO 6869 standards.

Crude protein fractionation

Crude protein was partitioned into five fractions (A, B1,B2, B3, and C) based on the Cornell Net Carbohydrate and Protein System (Sniffen *et al.*, 1992), following a standardised procedure (Licitra *et al.*, 1996). Accordingly, True protein (TP), buffer insoluble CP, neutral detergent insoluble N (NDIN), and acid detergent insoluble N (ADIN) were specified using Kjeldahl digestion to determine N (method 4.1.1; VDLUFA, 2012). Neutral detergent insoluble CP (NDICP) and acid detergent insoluble CP (ADICP) were calculated by multiplying NDIN and ADIN, respectively, by 6.25. All analyses were carried out in triplicate. Samples containing more than 100 g/kg DM of CL were defatted in acetone before NDIN and ADIN analysis. Fraction A corresponds to the difference between CP and TP, and represents non-protein N multiplied by 6.25. Fraction B1 corresponds to TP soluble in borate-phosphate buffer. Fraction B2 corresponds to buffer insoluble CP minus NDICP. Fraction B3 corresponds to NDICP minus ADICP. Fraction C corresponds to ADICP.

Estimation of utilisable crude protein at the duodenum and ruminally undegraded feed crude protein (Experiment 1)

A modified Hohenheim gas test was applied to estimate uCP from ammonia nitrogen release upon *in vitro* incubation in rumen fluid-buffer solution based on the instructions of Menke and Steingass (1988). The method was extended to estimate feed CP degradability (Raab *et al.*, 1983). The principle of the method was described in detail by Südekum and Böttger (2019) and applied, for example, by Wild et al. (2019). Briefly, 130 mg of each BG sample were weighed into 100-ml glass syringes with and without addition of a carbohydrate mixture (130 mg) consisting of cellulose, maize starch, wheat starch and sucrose (40:20:20:20). Rumen fluid was obtained from at least two ruminally-cannulated sheep prior to morning feeding. The sheep received a diet of grass hay and compound feed, corresponding to their maintenance energy requirements. Samples were incubated in a rumen fluid-buffer solution for 8 and 48 h and gas production (GP) was recorded. Each sample was incubated once for each time point within one run, replicated each in four runs, using different batches of rumen fluid. Additionally, two blanks containing only rumen fluid-buffer solution were incubated for each time point within each run. The syringes were placed in a rotor inside the incubator (39 °C) randomly. Gas production was also recorded at 24 h for use in calculation of metabolisable energy (ME) concentration, and at both the 8 h and 24 h readings, the plunger was set back to 30 ml (not for the blank). After incubation, syringes were put on ice in order to stop fermentation and remained in the ice slurry for a minimum of 2 h until required for ammonia analysis. Amount of NH_3-N was measured in syringe contents for samples (NH_3-N_{sample}) and blanks (NH_3-N_{blank}) using automated steam distillation (Vapodest 50 s carousel, Gerhardt, Königswinter, Germany).

In vitro gas production kinetics and digestibility of organic matter (Experiment 2)

In vitro gas production was determined according to Menke and Steingass (1988). In short, samples (200-230 mg) of the oven-dry BG and TA were weighed into 100-mL glass syringes fitted with plungers. Rumen fluid was obtained and used in similar manner, and from the same sheep used in experiment 1. Syringes were filled with 30 mL of medium as described by Menke and Steingass (1988), except that the concentration of $NaHCO_3$ was reduced to 33 g/L and that of $(NH_4)HCO_3$ increased to 6 g/L to prevent a shortage in N during prolonged incubation times. Samples were

incubated in two consecutive runs using different batches of rumen fluid, each run involving duplicates of samples. Additionally, three blanks containing only rumen fluid-buffer solution were included in each run. Triplicates of a standard hay and a standard concentrate samples were included. The syringes were placed in a rotor inside the incubator (39 °C) randomly. The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72 and 96 h of incubation.

Calculations and statistical analysis

All data were analysed using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Contents of NH_3-N in syringes containing samples without added carbohydrates and blanks were used to calculate uCP (g/kg DM) at 8 and 48 h as follows:

$$uCP = ((N_{sample} - (NH_3\text{-}N_{sample} - NH_3\text{-}N_{blank}))/weight_{sample}) \times 6.25 \times 1000 \quad [1]$$

where N_{sample} = total N from the sample (mg), $weight_{sample}$ = weight of the sample incubated (mg DM) and other variables are as described above.

To estimate ruminally undegraded feed CP (RUP), a linear regression between NH_3-N and GP was calculated from the respective values of the samples with and without added carbohydrates:

$$NH_3\text{-}N = a + b \times GP \quad [2]$$

In this regression equation, the theoretical point of zero GP implies that no energy would be available to microbes and thus, only feed CP degradation but no microbial protein synthesis would occur. Subtracting $NH_3\text{-}N_{blank}$ from the intercept a yields N solely originating from the feed (ruminally degraded N, RDN, in mg). Ruminally undegraded feed CP was calculated at both 8 and 48 h as follows:

$$RUP\ (g/kg\ CP) = (N_{sample} - RDN) / N_{sample} \times 1000 \quad [3]$$

Then, microbial crude protein (MCP) was estimated as a component of uCP:

$$MCP\ (g/kg\ DM) = uCP - (RUP \times CP / 1000) \quad [4]$$

Linear regression of uCP and RUP values at 8 and 48 h to ln of time allowed for the calculation of effective uCP and RUP for assumed passage rate (Kp) of 0.03/h, and 0.05/h through calculating the function value of ln (33.3), and ln (20), respectively using the formula:

$$Effective\ uCP = y + a \times \ln (1 / kp) \quad [5]$$

Where *y* is the intercept and *a* is the slope.

Data on gas production kinetics were fitted to the nonlinear equation of (Ørskov and McDonald, 1979):

$y = a + b (1 – e^{-c \times t})$ [6];

where y is cumulative GP at time t, a is initial GP of the rapidly degradable fraction, b is potential GP of the slowly degradable fraction, c is the rate of GP, and (a + b) is potential GP.

The gas production parameters a, b and c were estimated using PROC NLIN in SAS 9.4. Short time GP was specified as cumulative GP at 2 h of incubation (GP2).

Organic matter digestibility, ME and net energy for lactation (NEL) were estimated from GP after 24 h *in vitro* incubation (GP24) using equations of (Menke and Steingass, 1988) as follows:

OMD = 9.00 + 09991 GP24 + 0.0595 CP + 0.0181 ash [7]

ME = 1.06 + 0.157 GP24 + 0.0084 CP + 0.022 CL − 0.0081 ash [8]

NEL = −0.36 + 0.1149 GP24 + 0.0054 CP + 0.0139 CL − 0.0054 ash [9], where ME and NEL are given in MJ/kg DM; CP, CL, and ash are given in g/kg DM, and GP24 is GP after 24 h given in ml/200 mg DM.

Simple linear correlation coefficient (*r*) was computed in excel to determine the relationship between RUP and fraction C at both passage rates, and between starch and GP (GP2 and GP24).

Results

Table 2 presents the chemical composition of BG and TA obtained from Ethiopian breweries and traditional tella brewing, respectively. The DM content of fresh BG varied with the range of 114 g/kg DM between the samples. The chemical composition analysis showed that aNDFom (443–738 g/kg DM), ADFom (195–299 g/kg DM) and CP (187–299 g/kg DM) constitute the major components of BG with substantial variation among the samples. All BG samples, except two, exhibited a CP concentration of ≥ 200 g/kg DM. The fiber fractions varied between the samples with more pronounced variation in ADL concentration (39–115 g/kg DM). Starch also showed notable variation both among the samples as well as between the two BG categories as the mean concentration of non-pure barley malt BG surpassed pure barley malt BG by 60% (43 g/kg DM). Three non-pure barley malt BG samples with sorghum or rice added to the malts, and a sample obtained from micro-brewery particularly exhibited the highest starch concentrations, ranging from 92 to 255 g/kg DM, and 136 g/kg DM, respectively, as well as the highest sugar concentrations. All TA samples consisted of starch concentrations higher than or equal to 220 g/kg DM whereas all BG, apart from two samples, consisted of less than 100 g/kg DM. Sugar content in general showed inconsistency in BG where three samples exhibited comparatively higher (48,

55.9 and 84.4 g/kg DM), five samples exhibited medium (16.3–26.6 g/kg DM), and eight samples exhibited very low or even below the detection level (≤1.7 g/kg DM). Comparatively TA showed slightly higher ADL content, ranged from 55.3 to 98.3 g/kg DM; and notably lower mean DM, CP and aNDFom concentrations than that of BG.

Table 4.2. Chemical composition of brewers grains and of traditional brewing by-products (tella-atella)

Brewery by-products	(g/kg) DM	g/kg DM							
		Ash	CP	EE	aNDFom	ADFom	ADL	Starch	Sugar
Brewers grains									
BG1	287	42	233	113	678	295	81.9	48.0	nd
BG2[a]	267	47	241	101	643	232	66.1	70.3	1.7
BG3[a]	282	41	256	120	690	263	73.6	44.9	0.6
BG4	310	42	249	107	676	270	78.1	79.7	16.3
BG5	279	42	299	105	633	235	46.5	92.1	22.4
BG6	246	42	251	115	693	265	72.7	46.8	nd
BG8	296	39	260	107	692	262	56.6	43.1	nd
BG9	276	44	274	95.4	697	265	115	43.2	0.5
BG10	266	45	187	91.9	707	280	65.2	85.4	48
BG11	272	31	206	81.3	514	195	38.5	255	55.9
BG12	273	32	254	117	443	208	40.9	232	84.4
BG13[b]	278	41	187	70.5	625	239	50.9	84.3	17.2
BG14[b]	313	38	200	101	671	244	64.2	88.5	26.1
BG15[c]	199	40	239	117	738	271	50.9	46.8	0.6
BG16[c]	201	39	238	112	725	299	97.6	48.3	0.8
BG17[d]	203	36	258	86.1	576	238	48.4	136	16.7
Mean	266	40	240	102	650	254	65.0	90.0	18.2
SD	35.9	4.3	31.0	14.2	79.0	28.7	20.9	65.2	24.9
Min	199	31	187	70.5	443	195	38.5	43.1	0.0
Max	313	47	299	120	738	299	115	255	84.4
BG18[e]	220	40	264	103	718	285	61.7	60.5	2.4
Traditional brewing by-products (tella-atella)									
TA1	139	42	219	102	422	226	98.3	220	6.7
TA2	160	35	197	95.6	444	231	86.3	241	3.6
TA3	144	27	160	85.2	400	202	55.3	289	2.2
Mean	148	35	192	94.4	422	220	80.0	250	4.2

DM, dry matter; CP, crude protein; EE, ether extract; aNDFom, neutral detergent fiber analysed with heat stable amylase and expressed exclusive residual ash; ADFom, acid detergent fiber expressed exclusive residual ash; ADL, acid detergent lignin; nd, not detected.

[a]Samples obtained from the same beer type in different locations (different brewery).

[b]Samples obtained from the same brewery but at different time point.

[c]Samples obtained from the same brewery but at different time point.

[d]Sample obtained from micro-brewery.

[e]Sample obtained from German brewery.

Table 3 provides mineral concentrations of both BG and TA. Phosphorus, Ca, and Mg, in decreasing order, were the predominant minerals in all BG samples. Most BG samples exhibited very low concentrations of Cu and Mn while in two samples Cu was not detected. All TA samples exhibited Ca concentration higher than 4.51 g/kg DM, whereas in none of the BG samples it exceeded 3.54 g/kg DM. Similarly, concentrations of K and Fe were notably higher (1.81–3.94 g/kg DM and 433–902 mg/g DM, respectively) in TA than in BG. Unlike in BG, Ca exceeded P in TA.

Table 4.3. Mineral concentrations of brewers grains and traditional brewing by-products (tella-atella)

Brewery by-products	(g/kg DM)			(mg/kg DM)				
	P	Ca	Mg	Na	K	Fe	Mn	Cu
Brewers grains								
BG1	5.14	2.50	1.99	0.63	0.38	284	71.0	4.0
BG2[a]	6.02	3.54	2.33	0.16	1.84	174	69.0	0.0
BG3[a]	5.33	2.45	1.95	0.54	0.61	155	59.0	3.0
BG4	6.14	2.32	2.45	0.11	0.83	126	67.0	0.0
BG5	6.24	3.02	1.98	0.66	0.85	118	73.0	4.0
BG6	5.89	2.80	1.98	0.45	0.57	167	64.0	13.0
BG8	5.72	2.83	2.02	0.41	0.40	116	55.0	11.0
BG9	6.60	2.93	2.56	0.39	0.82	117	61.0	12.0
BG10	4.65	2.94	1.99	0.17	0.72	266	71.0	14.0
BG11	4.33	1.97	2.37	0.15	1.16	238	36.0	9.0
BG12	4.78	2.16	1.99	0.16	0.86	318	55.0	10.0
BG13[b]	4.63	1.99	1.79	0.18	0.80	180	51.0	9.0
BG14[b]	3.47	1.19	1.76	0.23	0.92	248	54.0	10.0
BG15[c]	5.37	2.33	2.18	0.28	0.52	192	48.0	10.0
BG16[c]	4.38	2.24	2.00	0.17	0.58	204	60.0	8.0
BG17[d]	4.44	2.62	2.13	0.15	0.88	121	44.0	11.0
Mean	5.20	2.49	2.09	0.30	0.80	189	58.6	8.0
SD	0.86	0.54	0.23	0.18	0.35	65.0	10.6	4.4
Min	3.47	1.19	1.76	0.11	0.38	116	36.0	0.0
Max	6.60	3.54	2.56	0.66	1.84	318	73.0	14.0
BG18[e]	5.88	3.01	2.13	0.11	0.53	118	45.0	5.0
Traditional brewing by-products (tella-atella)								
TA1	3.38	4.61	1.77	0.31	3.94	818	31.0	8.0
TA2	2.96	4.51	1.49	0.48	2.86	902	33.0	8.0
TA3	2.33	4.88	1.09	0.54	1.81	433	24.0	5.0
Mean	2.89	4.67	1.45	0.44	2.87	718	29.0	7.0

[a]Samples obtained from the same beer type in different location (different brewery).

[b]Samples obtained from the same brewery but at different time point.

[c]Samples obtained from the same brewery but at different time point.

[d]Sample obtained from micro-brewery.

[e]Sample obtained from German brewery.

Data on CP fractions of BG and TA are given in Table 4. Fraction B2 and fraction B3 represented the first and second largest CP fractions of most BG samples, respectively, and constituted together a mean proportion of more than three-fourth of CP. Two non-pure barley malt BG samples particularly with sorghum grains added to the malts notably exceeded the other BG

samples in terms of fraction C. Fraction A varied among samples ranging from 8 to 141 g/kg CP. Fraction B1 was consistently low in all samples and averaged only 18 and 15 g/kg CP for BG and TA respectively. Both BG and TA showed generally similar CP fraction patterns except that in TA fraction C on average exceeded fraction B3 and was the second largest fraction. Fraction C was considerably higher for TA than BG.

Table 4.4. Concentration of neutral detergent insoluble CP (NDICP), acid detergent insoluble CP (ADICP) and crude protein (CP) fractions of brewers grains and traditional brewing by-products (tella-atella)

Brewery by-products	(g/kg DM)		(g/kg CP)				
	NDICP	ADICP	A	B1	B2	B3	C
Brewers grains							
BG1	92	31	8	24	572	264	132
BG2[a]	85	25	98	27	523	250	102
BG3[a]	118	29	69	36	436	345	114
BG4	102	30	37	11	542	291	119
BG5	164	32	23	2	427	441	107
BG6	106	33	26	33	519	294	129
BG8	118	30	32	25	491	335	117
BG9	137	44	100	2	396	341	161
BG10	91	22	47	41	424	367	120
BG11	107	49	23	3	458	280	237
BG12	88	59	68	0	589	112	233
BG13[b]	95	23	16	14	463	386	121
BG14[b]	94	24	57	34	439	352	118
BG15[c]	132	34	14	0	446	410	141
BG16[c]	120	31	56	34	406	373	131
BG17[d]	111	33	141	20	410	303	127
Mean	110	33	51	18	471	321	138
SD	21	10	37	16	61	77	40
Min	85	22	8	0	396	112	102
Max	164	59	141	41	589	441	237
BG18[e]	143	31	64	92	301	425	118
Traditional brewing by-products (tella-atella)							
TA1	90	63	7	16	566	124	286
TA2	85	53	50	0	517	162	270
TA3	61	29	29	28	565	197	182
Mean	79	48	29	15	549	161	246

DM, dry matter; CP, crude protein; A, B1, B2, B3 and C, crude protein fractions according to Licitra et al. (1996).

[a]Samples obtained from the same beer type in different location (different brewery).

[b]Samples obtained from the same brewery but at different time point.

[c]Samples obtained from the same brewery but at different time point.

[d]Sample obtained from micro-brewery.

[e]Sample obtained from German brewery.

Table 5 reports results on estimated RUP and uCP values of BG and TA at assumed passage rates of 0.03/h and 0.05/h. Effective RUP tended to show variation between BG samples at the range of around 200 g/kg CP at both assumed passage rates, and increased with the increase of passage rate. Effective RUP at both respective passage rates showed positive correlation (r = 0.86, 0.80) with fraction C (Fig. 1). Samples of non-pure barley malt BG showed an increment of 65% in terms of MCP at assumed passage rate of 0.05/h when compared to samples of pure barley malt BG. Whereas, MCP at assumed passage rate of 0.03/h was calculated as insignificant and even negative in most cases in both categories of samples. Two BG samples with sorghum grain added to the malts particularly exceeded the rest BG samples in terms of MCP at both respective assumed passage rates, but MCP was comparable to that of TA (Fig. 2).

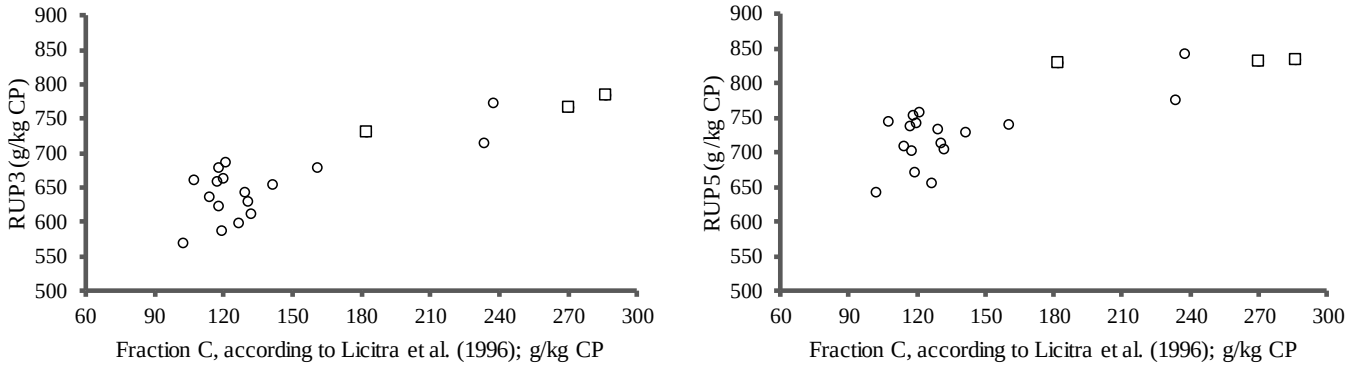

Figure 4.1. Relationship between ruminally undegraded crude protein at passage rate 0.03/h (RUP3; r=0.86; *left*), at 0.05/h (RUP5; r=0.80; *right*), and fraction C of brewers grains (0) + tella-atella (□)

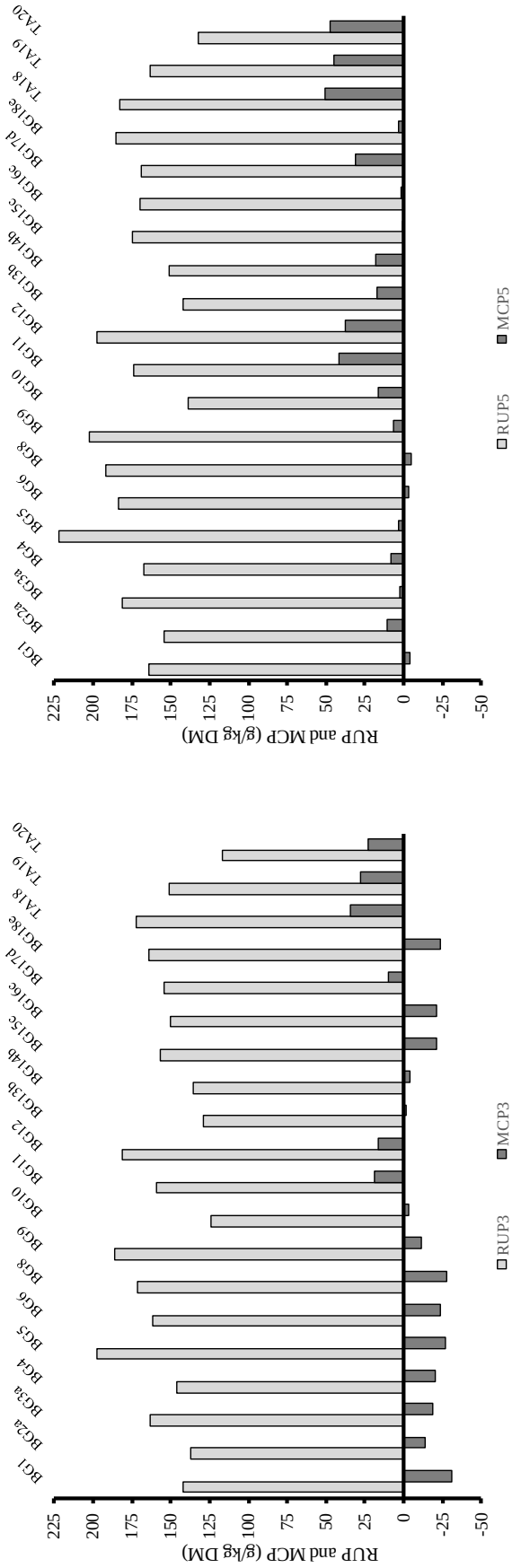

Figure 4.2. Ruminally undegraded crude protein (RUP) and microbial crude protein (MCP) of brewers grains (BG) and tella-atella (TA).

[a]Samples obtained from the same beer type in different location (different brewery); [b]samples obtained from the same brewery but at different time point; [c]samples obtained from the same brewery but at different time point; [d]sample obtained from micro-brewery; [e]Sample obtained from German brewery.

Table 4.5. *In vitro* estimations of effective ruminally undegraded crude protein (RUP) and effective utilizable crude protein at the duodenum (uCP) of brewers grains and traditional brewing by-products (tella-atella).

Brewery by-products	(g/kg CP)		(g/kg DM)	
	RUP3	RUP5	uCP3	uCP5
Brewers grains				
BG1	611	705	111	160
BG2[a]	569	642	123	165
BG3[a]	637	709	144	184
BG4	587	671	126	175
BG5	662	744	171	226
BG6	643	733	138	181
BG8	658	739	143	187
BG9	679	740	175	209
BG10	664	743	121	155
BG11	772	843	178	215
BG12	715	777	198	235
BG13[b]	689	758	127	159
BG14[b]	679	754	132	169
BG15[c]	655	730	135	175
BG16[c]	631	713	129	171
BG17[d]	598	656	164	200
Mean	653	729	145	185
SD	50	48	25	25
Min	569	642	111	155
Max	772	843	198	235
BG18[e]	623	703	141	189
Traditional brewing by-products (tella-atella)				
TA1	785	834	206	233
TA2	766	831	179	209
TA3	731	829	140	180
Mean	761	831	175	207

RUP3 and RUP5, RUP corresponding to a ruminal passage rate of 0.03/h and 0.05/h, respectively; uCP3 and uCP5, uCP corresponding/representing to a ruminal passage rate of 0.03/h and 0.05/h, respectively; CP, crude protein; DM, dry matter.

[a]Samples obtained from the same beer type in different location (different brewery).

[b]Samples obtained from the same brewery but at different time point.

[c]Samples obtained from the same brewery but at different time point.

[d]Sample obtained from micro-brewery.

[e]Sample obtained from German brewery.

Results on *in vitro* gas production (GP2 and GP24), parameters of GP kinetics, OMD and estimated energy values for BG and TA derived from experiment 2 are shown in Table 6. Gas volumes (GP2 and GP24) and energy concentrations (ME and NEL) varied considerably among BG samples as well as when compared to TA; both gas volumes and energy concentrations exceeding in TA samples. Apart from two samples, GP24 in BG was at least 8.1 ml/200 mg DM lower than in TA samples. Both GP2 and maximum GP were higher for non-pure barley malt BG; and in general, the mean values were lower for BG than TA. The GP2, and GP24 values showed a positive correlation (r=0.80, and r=0.98, respectively) with starch concentration (Fig. 3). *In vitro* gas production volume, gas production rate, and OMD varied among BG samples, and in relation to TA samples (fig. 4, and table 6). Estimated OMD ranged from 48.6 to 71.6% for BG and 64.7 to 71.2 for TA; samples of TA exceeded BG, on average, by nearly 50% in terms of gas production rate.

Table 4.6. *In vitro* gas production after 2h (GP2) and 24h incubation (GP24), maximum GP (a + b), gas production rate (C), digestibility of organic matter (OMD); metabolizable energy (ME) and net energy for lactation (NEL) of brewers grains and traditional brewing by-products (tella-atella)

Brewery by-products	(ml/ 200 mg DM)				(%)	(MJ/kg DM)	
	GP2	GP24	A+B	c	OMD	ME	NEL
Brewers grains							
BG1	2.2	26.2	41.5	0.038	49.8	9.3	5.3
BG2[a]	5.2	32.6	43.4	0.047	56.8	10.1	5.8
BG3[a]	2.3	26.2	40.5	0.040	51.2	9.6	5.5
BG4	4.7	29.5	42.9	0.041	54.0	9.8	5.6
BG5	6.3	30.9	45.8	0.038	58.4	10.4	6.0
BG6	2.6	26.8	39.9	0.041	51.5	9.6	5.4
BG8	2.5	26.7	42.2	0.038	51.9	9.5	5.4
BG9	2.6	25.3	42.5	0.034	51.4	9.1	5.1
BG10	5.7	30.8	45.3	0.040	51.7	9.1	5.2
BG11	9.9	49.8	59.0	0.057	71.6	12.1	7.4
BG12	10.5	46.2	56.5	0.053	70.9	12.8	7.8
BG13[b]	6.0	27.8	42.5	0.036	48.6	8.2	4.6
BG14[b]	6.2	30.8	42.9	0.043	52.4	9.5	5.5
BG15[c]	2.6	26.7	40.1	0.041	50.6	9.5	5.4
BG16[c]	2.2	25.4	38.7	0.040	49.2	9.2	5.2
BG17[d]	7.3	35.3	48.0	0.045	60.3	10.4	6.1
Mean	4.9	31.1	44.5	0.042	55.0	9.9	5.7
SD	2.7	7.2	5.7	0.006	7.1	1.1	0.8
Min	2.2	25.3	38.7	0.034	48.6	8.2	4.6
Max	10.5	49.8	59.0	0.057	71.6	12.8	7.8
BG18[e]	5.1	32.2	44.8	0.045	57.6	10.3	6.0
Traditional brewing by-products (tella-atella)							
TA1	8.4	43.7	53.7	0.059	66.5	11.7	7.0
TA2	6.5	43.4	54.2	0.057	64.7	11.4	6.8
TA3	6.2	52.2	62.7	0.067	71.2	12.3	7.5
Mean	7.0	46.4	56.9	0.061	67.4	11.8	7.1

ME, NEL, and OMD were estimated from GP24 using equations of Menke and Steingass (1988): ME = 1.06 + 0.157 GP24 + 0.0084 CP + 0.022 CL − 0.0081 ash, NEL = −0.36 + 0.1149 GP24 + 0.0054 CP + 0.0139 CL − 0.0054 ash; OMD = 9.00 + 09991 GP24 + 0.0595 CP + 0.0181 ash. Fermentation parameters a, b, and c were estimated from Ørskov and McDonald (1979) equation: $y = a + b\ (1 - e^{-ct})$; GP24 is a value corrected for the overall gas production factor of hay and concentrate

[a]Samples obtained from the same beer type in different locations (different breweries).

[b]Samples obtained from the same brewery but at different time point.

[c]Samples obtained from the same brewery but at different time point.

[d]Sample obtained from micro-brewery.

[e]Sample obtained from German brewery.

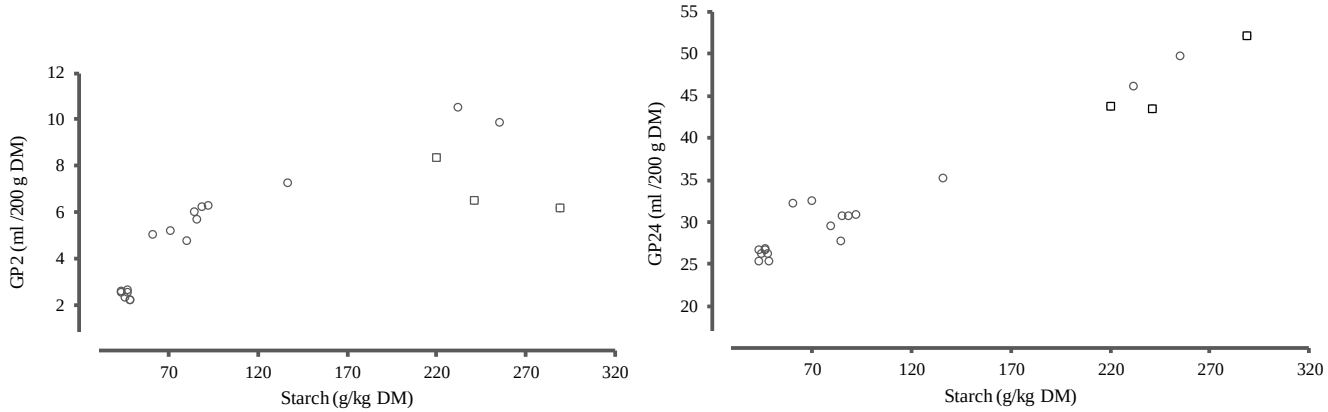

Figure 4.3. Relationship between starch content and gas production after 2h (GP2; *left*), and after 24h (GP24; *right*) *in vitro* incubation of brewers grains (0) + Tella-atella (□)

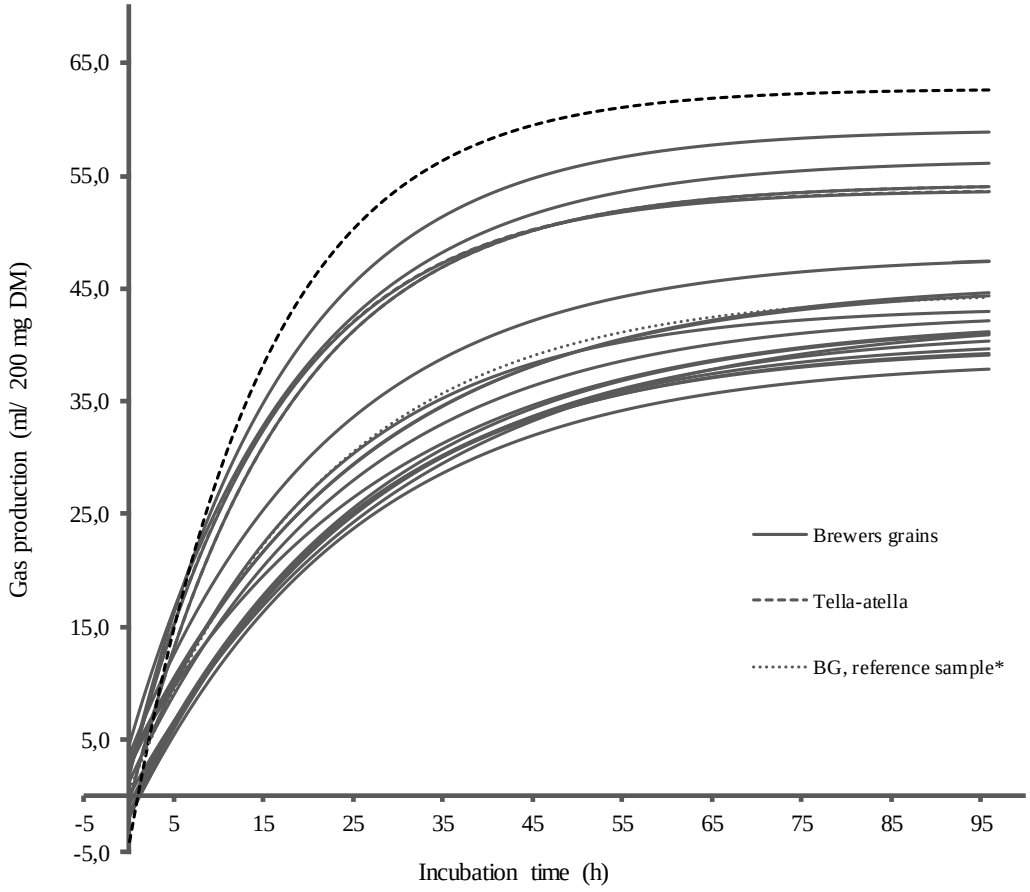

Figure 4.4. Fermentation pattern of BG and TA samples over 96 h of incubation; the curves are plotted from calculated values based on the fermentation parameters estimated from Ørskov and

McDonald (1979) equation $y = a + b(1 - e^{-ct})$); *A sample obtained from one Germen brewery as a reference prepared in the same manner.

Discussion

General Chemical composition

The DM content in fresh BG moderately varied and the mean value from the present study is in line with previous studies (Allen and Stevenson, 1975; Dhiman *et al.*, 2003). High moisture content of BG limits its preservation to not more than a week (Thomas *et al.*, 2010). The variability of DM concentration might particularly be related to the mashing technology – BG which are obtained from a mash filter exhibit higher DM concentration (≥350 g/kg) than BG obtained from mash or lauter tun (≤220 g/kg) (O'Rourke, 2003). In the present study, BG samples were obtained directly after separation from wort in the lauter tun or mash filter; and hence the effect of yeast is not taken in to an account. It is noted that some breweries, in Ethiopian case, simply mix it into BG as a feed (Mohammed *et al.,* 2019), and, more than half of the breweries in Ethiopia dispose away a yeast to the sewerages after it is killed through self-rupturing of the yeast cell known as autolysis (Kitaw *et al.*, 2018). Moreover, the current samples were taken fresh, and the nutritional quality changes that may occur during prolonged shipping and storage were not considered. In the latter case, feed value may decrease related to DM losses.

The results on chemical composition suggest that both BG and TA can be a source of fiber and CP in ruminant feed, with considerable differences generally between breweries and for BG compared to TA. The chemical composition and nutritive value of BG highly depend on the raw materials used and brewing process (Santos *et al.,* 2003; Robertson *et al.,* 2010). In the current study, basic information on malt grains used and type of cereal grains added to the malt could be obtained, although information was limited to what was revealed by breweries and further identification was not possible. Hence, linking this information to chemical composition in terms of data analysis might not be possible.

Concentration of CP generally varied between BG samples (range of 112 g/kg DM). However, CP concentration of non-pure barley malt BG, unlike the starch concentration, did not show notable variation compared to other BG samples. Most studies on BG also reported varying CP content of 150 to 340 g/kg DM (Santos *et al.*, 2003; Xu *et al.*, 2007; Connolly *et al.*, 2013; Westendorf *et al.*,

2014; Moriel *et al.*, 2015; Moriel *et al.*, 2016). The variations in CP concentration could be due to type and varieties of malt grains, brewing processes (malting, mashing, and lautering conditions) or technologies (Santos *et al.*, 2003; Robertson *et al.*, 2010)

Substantial concentrations of aNDFom, starch, sugar and EE contribute to energy value of BG, with a considerable variability between breweries. Previous studies reported partially comparable starch concentration of BG, ranged from 21 to 130 g/kg DM (Robertson *et al.*, 2010; Westendorf *et al.*, 2014). Brewers grains originating from sorghum and rice grains added to the malts showed up to three-fold higher starch when compared to the average starch concentration of BG samples, indicating that added cereal grains affect starch concentration in BG. Addition of cereal grains is usually performed at the mashing step and the grains are added unmalted. During the mashing process, enzymes extensively degrade much of the starch in the barley malt (Allen and Stevenson, 1975) – only 1% of total starch content of the malt remains in BG (Hennemann *et al.*, 2019). However, considerable amount of starch remained in BG originating from added unmalted cereals at mashing in the present study. The use of alternative carbohydrate source cereals or addition of other cereals certainly require appropriate enzymes and optimum gelatinization temperatures for wort extraction during mashing process (Meussdoerffer and Zarnkow, 2009). Sorghum malts, for instance, require relatively higher gelatinisation temperature and exhibit lower enzymatic activity than barley malts (Meussdoerffer and Zarnkow, 2009). Enzymes are required to make kernels soft and water-soluble during malting, and to break down the starch in the grain in to fermentable sugar during mashing processes of brewing (Gupta *et al.*, 2010). In this regard, starch in added cereal grains (unmalted) might not be degraded as efficiently as barley malt during the brewing processes and more starch remains in non-pure barley malt BG. Similarly, the high starch content in the BG sample that was obtained from micro-brewery could be due to inefficient conversion of starch during mashing. This has, indeed, a positive indication from animal nutritional point of view.

Higher starch concentration in TA again might be explained in two ways. The first justification relates to brewing process of tella (Berhanu, 2014; Lee *et al.*, 2015), where the brewing steps are limited and differ from industrial beer brewing to various extent. Traditional tella brewing lacks a typical malting process of cereal grains except that only a small proportion of barley malt (usually about 3% of other grains) is mixed to *Rhamnus prinoides (*locally known as Gesho) leaves and stems, act similarly as hops in beer, and water to be used as a starter of fermentation. In addition, the mashing step of tella involves mixing of fermented solution (ground barley malt, leaves and

water) obtained from the previous step and pieces of unleavened bread (*kita*) baked from various cereal flours. Hence, both steps largely differ from typical brewing process where the efficiency of starch conversion to fermentable sugars can be reduced, so that starch remains in BG. The other reason for higher starch in tella could be related to raw material used where tella brewing mainly based on proportion of nearly half of unmalted sorghum cereals particularly in the current study. This could link to the high starch of non-pure barley malt BG in which non-malted sorghum, rice and barley grains were added. However, with the fact that breweries targets to extract as efficiently as till almost no starch remain in BG (Hennemann *et al.*, 2019), starch concentration in the BG might be insignificant when the target is efficiently achieved.

Result of fiber fractions indicate that the fiber in BG is more degradable/usable by ruminants with only less than a mean value of 100 g/kg DM of ADL content except for one sample. A sample (BG9) obtained from a barley malt with dark color particularly showed higher ADL concentration that was considered as an outlier, accompanied with considerable content of crude protein fraction C. This might result due to Maillard reaction of high temperature during kilning of Malt. Similarly, though TA fell behind BG in terms of aNDFom content, ADL concentration for TA was either comparable or surpassed most of BG samples. In this regard, considering brewing process of tella which involves baking, to make unleavened bread (*kita*), using very high temperature could be important (Lee *et al.*, 2015). On the other hand, the lowest ADL concentration of the three BG samples with non-malted sorghum and rice grains added to the malts could be associated to comparative abundance of starch concentrations in the samples.

Mineral concentrations

Dietary minerals requirement is usually well below the detrimental level. However, toxicity from several of essential minerals occur in practical feeding (NRC, 2001). For instance, excretion of excess P should be considered from animal health as wells as environmental point of view. From the present study, BG contains acceptable concentration of P. NRC (2001) stated that the maximum tolerable concentration of P in diet as 1% in dry basis. The present results suggest that TA samples exhibit high concentration of both Ca and P, while BG appears relatively low in Ca concentration. However, both BG and TA are either used as supplements or mixed with the rations, and not as a sole feedstuff; hence, Ca can be complemented from other feed sources. For instance, teff straw

and grass pea haulm, the common feedstuffs used in Ethiopia, were reported approximately twice (1.7 times) and more than six times higher concentration of Ca (in g/kg DM), respectively, than BG (Gizachew and Smit, 2005). The mean concentration for most minerals but K and Fe was in line with typical composition of BG stated by NRC (2001). Concentration of K in the present study was five times less than typical value of BG indicated in NRC (2001), and five to sixteen times less than the value reported for both teff straw and grass pea haulm, respectively (Gizachew and Smit, 2005); but still in line with several other studies (Westendorf *et al.*, 2014; Thomas *et al.*, 2010). In contrast to the present study, Feyissa *et al.* (2015) reported comparatively higher value of Ca (8.1 g/kg DM) than P (6.0 g/kg DM) for the BG obtained from Ethiopian brewery.

The contrary relationship of Ca and P in BG and TA might require further investigation. However, the leaves of *Rhamnus prinoides* (African shrub or small tree in the family *Rhamnaceae*) traditionally used as hops in tella brewing may contain high concentrations of Ca (up to 22.2 g/kg DM) and other minerals (Gebre and Chandravanshi, 2012). Unlike BG, TA remains at the end point of brewing process where *Rhamnus prinoides* and all other ingredients are included. Hence, the high level of minerals such as Ca, in TA could originate from *Rhamnus prinoides*. In general, mineral concentration in by-products such as brewery by-products like in forage feeds are variable and are influenced by method of processing (NRC, 2001), and probably the raw materials used. It can be concluded that both type of brewery by-products, but particularly TA contained considerable mineral concentrations except for K and Mn.

Composition of crude protein and protein value

Ruminal feed CP degradation depends on the feed protein properties and ruminal passage rate; ruminal passage rate is in-turn affected by the physical characteristics of feed and DM intake. Taking DM intake as low as 76.1–138 g/day/ $BW^{0.75}$ (Hussien *et al.*, 2013; Tekeba *et al.*, 2013) and accordingly low digesta passage of Ethiopian local cattle into account, effective RUP and uCP were estimated for moderate assumed passage rates of 0.03 and 0.05/h. Accordingly, only a mean value of 355 g/kg CP in BG and 239 g/kg CP in TA were degraded in the rumen at the lower assumed passage rate of 0.03/h, indicating the majority of the protein is ruminally undegraded protein. Previous studies also reported high RUP value of BG (Armentano *et al.*, 1986; Dhiman *et al.*, 2003; Thomas *et al.*, 2010).

High RUP in BG accompanied by low proportions of soluble protein (CP fraction A + B) and higher values of cell wall associated fractions (B3 + C) might be related to several factors. Malting and mashing enhance the solubility of protein into wort and thereby less soluble protein remains in BG. Soluble proteins in the wort are utilised mainly as nutrients for yeast growth in the subsequent steps of brewing and enhance beer quality (Steiner *et al.*, 2011). Moreover, the use of increased heat in the brewing processes (drying and mashing) might affect the protein structure in such a way that protein resists rumen degradation and increase in RUP. Protein might bind to cellulosic material during the heating of mashing and drying (Crowe *et al.*, 1985).

Compared to the current findings, Seifried *et al.* (2012) reported a lower mean value of effective RUP5 both from the same *in vitro* method (542 vs. 729 g/kg CP) and in situ (498 vs. 729 g/kg CP) studies. However, nearly the same effective uCP5 concentration (189 g/kg DM) was reported from the same *in vitro* study (Seifried *et al.*, 2012). Utilisable crude protein at the duodenum is sum of RUP and MCP. However, at lower passage rate of 0.03/h and partly at 0.05/h, the estimated RUP value exceeded the estimated uCP value. Consequently, the proportion of MCP in uCP was very low with a maximum of 0.19 for sample with highest starch concentration. Negative calculated value of MCP that actually cannot exist theoretically at an assumed passage rate of 0.03/h could be related to the method used to estimate RUP; because it is not a measured value, rather it is a calculated value. Moreover, the non-viable result of estimated MCP may arise because RUP and uCP do not stem from the same calculation and assumptions even though obtained from the same HGT incubation procedure. Nevertheless, generally very low MCP estimated from the current study could indicate a deficiency of energy for rumen microbes. Under certain feeding conditions, for instance, when animals are fed on highly fermentable energy feeds, and protein supplements that are slowly degraded, ammonia or N may become more deficient for microbes (Clark *et al.*, 1987). Results of CP fractionation and estimated RUP values indicate that availability of rapidly degraded N could have been limited also for the current BG and TA samples. However, it is unlikely that low MCP resulted from N deficiency since the ruminally degradable protein is by far higher than MCP (Wild *et al.*, 2019). Hence, it seems the deficiency of energy for the use of rumen microbes could limit the MCP synthesis, should BG would have been considered as a solely feedstuff. Positive correlations (r=0.86, 0.92) of MCP with starch and sugar concentrations in both passage rates is a good indication for the possible improvement of MCP synthesis from BG. Moreover, in the sense of actual animal feeding terms, the NDF in BG is

degraded slowly by rumen microbes, and can be utilised as a source of energy for microbes. On the other hand, BG is practically fed to animals either as a supplement or mixed in ration, but not as solely feedstuff. Hence, energy requirement of microbes should be considered from other ingredients of the ration where there exist energy rich feedstuffs. Merchen *et al.* (1979) also reported slightly lower bacterial protein in the rumen for dried BG (DBG) supplemented feed than urea supplemented. In fact, further investigation on MCP synthesis from BG and BG supplemented diet might be important. Comparatively higher MCP of TA indicate that microbes utilise more energy from TA than from BG at the assumed passage rates. Both effective uCP and RUP of specific feed might decrease at lower passage rate in practical terms like in Ethiopia where animals encounter lower DM intake and longer awaiting time in the rumen (low rumen outflow rate).

Results on CP fractions also suggest that CP of BG is mostly resistant to rumen degradation. Low concentration of buffer soluble protein fractions (A + B1) and high content of cell wall associated fractions (B3 + C) indicate the presence of high RUP in both BG and TA. Moreover, effective RUP showed strong positive correlation with fraction C at both passage rates. Krishnamoorthy *et al.* (1982) found similar proportion of CP fractions of DBG using different method based on solubility in bi-carbonate phosphate buffer. In addition, Armentano *et al.* (1986) reported consistently lower results of the rapidly degraded CP fraction A, and higher fraction B for WBG, from *in vitro* study where CP fractions classified in to only three fractions: fraction of feed CP degraded rapidly (A), fraction of feed CP degraded slowly (B) and fraction of feed CP undegraded (C). Peculiar higher value of fraction A of sample BG7 (data not shown) that was estimated as about four fold higher than the mean value, thereby, leading in to a TP as low as 724 g/kg DM can be possibly linked to protein denaturation/degradation during sample collection before drying. This was somewhat apparent by an ammonia or fish smell (amines). Hence, the results of this particular sample from all analyses was not considered in all the data shown in the present study.

The present result was in line with the result reported by Xu *et al.* (2007) in terms of both content of ADICP (37 vs 33 g/kg DM), and NDICP (95 vs 110 g/kg DM). On the other side, higher contents of ADICP (45 g/kg DM), and lower NDICP (54 g/kg DM) was reported by Pereira *et al.* (1998) where the samples were dried in similar temperature of 50 °C in the drying oven. Relatively higher content of ADIN and fraction C in TA than in BG in the present study might relate to higher ADL concentration and bound N in TA. High ADL in TA is again related to heat influence and

formation of Maillard products due to high temperature used during *kita* making in tella brewing process. Drying by-product feeds like BG at various high temperatures significantly minimises rumen degradation and increases both DM and N ruminal escape (Armentano *et al.*, 1986). In addition to this, the characteristics of raw ingredients used for tella brewing could also determine degradation characteristics of TA.

By-product feeds such as dried distillers grains with solubles (Waters *et al.*, 1992) and BG (in the present case) outweigh the foundation grains from which they were derived in terms of absolute ADIN content. The higher ADIN content of BG when compared to, for example, ADIN content (26 g/kg N) of the foundation grain barley (Marx *et al.*, 2000) would suggest that there is 'added' ADIN in BG. This could include either lignin-bound N, and/ or Maillard reaction products. The 'added' ADIN that formed from the Maillard reaction has finite degradability in the rumen and digestibility in small intestine (Waters *et al.*, 1992). Hence, the assumption that ADIN is completely undegradble and indigestible has been criticized when applied to by-product feeds which have high ADIN due to Maillard reaction (Waters *et al.*, 1992).

Ruminally undegraded feed CP is an important factor in dairy cow nutrition. Nevertheless, the high CP and RUP of feed sometimes may not guarantee the high milk production in dairy cow; rather protein quality is important. Ruminally undegraded CP should be digestible and have an appropriate amino acid pattern in the small intestine for efficient use (Armentano *et al.*, 1986). Microbial CP as a portion of uCP should also be considered as an important protein source, delivering over half of the amino acids absorbed by ruminants (Dewhurst *et al.*, 2000). Synchronising availability of fermentable energy and degradable N in the rumen without compromising other several factors and complexity of the rumen system would necessarily maximize MCP synthesis (Sinclair *et al*., 1995). This suggests that for maximum performance of dairy cows, utilizing diets with high RUP requires also considering the balance of fermentable energy sources in the diet. Nevertheless, determining amino acids pattern and availability in the duodenum could more describe the effect of BG on the milk yield of dairy cows.

In vitro gas production, digestibility of organic matter, and energy value

The deficiency of energy for microbes in the rumen has been considered as a reason for low MCP production at the assumed passage rates in experiment 1. However, the extent of degradation of

BG and TA over the long hours of incubation that might have supplied more energy for the microbes was not well understood. Hence, to determine the extent and rate of fermentation over long hours in the rumen, gas production kinetics over 96 h was performed (experiment 2). The mean degradation rate from present study was in line with degradation rate 0.049/h that was reported for DBG from in situ study (Batajoo and Shaver, 1998). The degradation is faster at the first 24 h of incubation indicating that sugars and significant amount of starch are degraded rapidly. However, the gas production continued at a considerable rate indicating that a significant portion of OM was left undegraded after 24 h of incubation to be degraded slowly. The gas production after 24 h incubation is attributed to mainly fiber and probably some starch of BG. Starch from ensiled and processed grains are degraded rapidly; but starch from dried grains might be partly insoluble and is degraded slowly (Sniffen *et al.*, 1992).

However, to understand the contribution of fermentable carbohydrates and the degradability of NDF portion of the carbohydrates, further information on the kinetics of NDF fraction of the samples might also be important. From literatures, it has been reported that about 76% of the starch of DBG is ruminally available at the assumed passage rate of 0.07/h; and about 59.1 % of the starch of BDG is rapidly disappearing DM fraction. Moreover, only about 33.3% NDF from DBG has been disappeared after 24 h of ruminal incubation, and approximately half of the NDF content of DBG is ruminally degraded (Stern *et al.*, 1995), suggesting the possible energy supply in the rumen. The rate of NDF digestion in situ for DBG has been estimated at 0.037−0.071/ h (Firkins, 1997). Generally, the rate and volume of the gas production varied among BG samples. All gas production parameters for non-pure barley malt BG exceed the other samples that might partly be related to relatively higher content of starch and sugar. Relative higher OMD and gas production rate (degradation rate) of TA samples when in comparison to BG samples could be related to the higher starch concentration of TA samples.

Energy value of BG and TA were estimated by calculating ME and NEL from *in vitro* gas production using equations of (Menke and Steingass, 1988). High positive correlation of starch to both GP and ME indicate that starch content in BG, in addition to NDF and EE, considerably contributes to energy value of BG. Getachew *et al.* (2002) also conducted a Hohenheim gas test study for various feedstuffs including BG at seven different laboratories to see the inter-laboratory variations and reported a comparable gas production (35.3–41.5 ml/200mg DM), and ME (10.35–11.30 MJ/kg DM). However, the result on the mean ME of BG in the present study was slightly

lower than 10.8 MJ/kg DM estimated from analysed composition (Rooke *et al.*, 2016). A comparable ME value of 12.2 MJ/kg DM was reported for TA (Nurfeta and Abdu, 2014). In general, a considerable concentration of energy and high CP concentrations in TA indicate the feeding value for both dairy and fattening animals.

Conclusions

Brewers grains can be considered as a potential protein and energy supplement for ruminants regardless of considerable variability in chemical composition and feeding value between breweries. In Ethiopian context, unmalted cereal grains seem to be considerably in use and, hence, contribute to the variation in chemical composition, most importantly to starch concentration increment of non-pure barley malt BG compared to BG obtained from pure-barley malt. Due to overall high CP, uCP and RUP concentration BG could be used in dairy ration formulation to promote milk production. However, RUP digestibility and the amino acid patterns in the small intestine requires further investigation. Relatively high MCP concentrations of TA suggest that microbes of the rumen can more efficiently utilise its protein due to its high starch concentration. Based on the variability observed in the current study, periodic evaluation of feeding value is vital for efficient utilisation of brewery by-products from Ethiopian breweries due to inconsistent use of raw ingredients and unmalted cereal grains.

References

Allen WR and Stevenson KR (1975) Influence of additives on the ensiling process of wet brewers' grains. *Canadian Journal of Animal Science* **55**, 391–402.

Aranguiz AA, Berhanu T and Vernooij A (2019) Utilization and management of spent brewers grain in Ethiopian dairy farms: Report 1207. Wageningen, Netherlands: Wageningen Livestock Research. https://doi.org/10.18174/504103.

Armentano LE, Herrington TA, Polan CE, Moe AJ, Herbein JH and Umstadt P (1986) Ruminal degradation of dried brewers grains, wet brewers grains, and soybean meal. *Journal of Dairy Science* **69**, 2124–2133.

Batajoo KK and Shaver RD (1998) In situ dry matter, crude protein, and starch degradabilities of selected grains and by-product feeds. *Animal Feed Science and Technology* **71**, 165–176.

Berhanu A (2014) Microbial profile of Tella and the role of gesho (Rhamnus prinoides) as bittering and antimicrobial agent in traditional Tella (Beer) production. *International Food Research Journal* **21**, 357–365.

Bogale S, Melaku S and Yami A (2008) Potential use of crop residues as livestock feed resources under smallholder farmers conditions in Bale highlands of Ethiopia. *Tropical and Subtropical Agroecosystems* **8**, 107–114.

Böttger C and Südekum K-H (2017) European distillers dried grains with solubles (DDGS): Chemical composition and *in vitro* evaluation of feeding value for ruminants. *Animal Feed Science and Technology* **224**, 66–77.

Clark JH, Murphy and Crooker BA (1987) Supplying the protein needs of dairy cattle from by-product feeds. *Journal of Dairy Science* **70**, 1092–1109.

Connolly A, Piggott CO and FitzGerald RJ (2013) Characterisation of protein-rich isolates and antioxidative phenolic extracts from pale and black brewers' spent grain. *International Journal of Food Science & Technology* **48**, 1670–1681.

Crowe NL, Alli I and Baker BE (1985) Solubilisation of nitrogenous constituents of brewers' spent grain. *Journal of the Institute of Brewing* **91**, 148–150.

Demeke S (2007) Comparative nutritive value of Atella and industrial brewers grains in chicken starter ration in Ethiopia. *Livestock Research for Rural Development* **19**.

Dewhurst RJ, Davies and Merry RJ (2000) Microbial protein supply from the rumen. *Animal Feed Science and Technology* **85**, 1–21.

Dhiman TR, Bingham HR and Radloff HD (2003) Production response of lactating cows fed dried versus wet brewers' grain in diets with similar dry matter content. *Journal of Dairy Science* **86**, 2914–2921.

Edmunds B, Südekum K-H, Spiekers H, Schuster M and Schwarz FJ (2012) Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for ruminants, from forages using a modified gas test. *Animal Feed Science and Technology* **175**, 106–113.

FAO (2019*a) Ethiopia: Availability and utilization of agroindustrial by-products as animal feed 2018*. Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO (2019*b) The future of livestock in Ethiopia: Opportunities and challenges in the face of uncertainty*. Rome, Italy: Food and Agriculture Organization of the United Nations.

Feyissa F, Assefa G, Kebede G and Prasad S (2015) Mineral profiles of agro-industrial by-products and locally available supplementary feeds and their implications for dairy cattle nutrition in Ethiopia. *Ethiopian Journal of Animal Production* **15**, 17–30.

Firkins JL (1997) Effects of feeding nonforage fiber sources on site of fiber digestion. *Journal of Dairy Science* **80**, 1426–1437.

Gebre A and Chandravanshi BS (2012) Levels of essential and non-essential metals in *Rhamnus prinoides* (Gesho) cultivated in Ethiopia. *Bulletin of the Chemical Society of Ethiopia* **26**, 329–342.

Getachew G, Crovetto GM, Fondevila M, Krishnamoorthy U, Singh B, Spanghero M, Steingass H, Robinson PH and Kailas MM (2002) Laboratory variation of 24 h *in vitro* gas production and estimated metabolizable energy values of ruminant feeds. *Animal Feed Science and Technology* **102**, 169–180.

Gizachew L and Smit GN (2005) Crude protein and mineral composition of major crop residues and supplemental feeds produced on Vertisols of the Ethiopian highland. *Animal Feed Science and Technology* **119**, 143–153.

Gupta M, Abu-Ghannam N and 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**, 318–328.

Hennemann M, Gastl M and Becker T (2019) Inhomogeneity in the lauter tun. *European Food Research and Technology* **245**, 521–533.

Hussien R, Tegegne F, Yilma Z, Mekuriaw Z and Taye M (2013) Feed intake, milk yield and milk composition of Fogera cows supplemented with different feeds. *World's Veterinary Journal* **3**, 41–45.

Kitaw G, Tamir B, Assefa G and Animut G (2018) Production, preservation, and utilization patterns of brewery spent grain in Ethiopia. *Ethiopian Journal of Agricultural Sciences* **28**, 1–17.

Krishnamoorthy U, Muscato TV, Sniffen CJ and van Soest PJ (1982) Nitrogen fractions in selected feedstuffs. *Journal of Dairy Science* **65**, 217–225.

Lee M, Regu M and Seleshe S (2015) Uniqueness of Ethiopian traditional alcoholic beverage of plant origin, tella. *Journal of Ethnic Foods* **2**, 110–114.

Licitra G, Hernandez TM and van Soest PJ (1996) Standardization of procedures for nitrogen fractionation of ruminant feeds. *Animal Feed Science and Technology* **57**, 347–358.

Marx TA, McKinnon JJ, Mustafa AF, Christensen DA and Racz VJ (2000) The feeding value of grain screenings for ruminants. *Canadian Journal of Animal Science* **80**, 673–680.

Mekasha Y, Tegegne A, Yami A and Umunna NN (2002) Evaluation of non-conventional agro-industrial by-products as supplementary feeds for ruminants. *Small Ruminant Research* **44**, 25–35.

Menke KH and Steingass H (1988) Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Animal Research and Development* **28**, 7–55.

Merchen N, Hanson T and Klopfenstein T (1979) Ruminal bypass of brewers dried grains protein. *Journal of Animal Science* **49**, 192–198.

Meussdoerffer F and Zarnkow M (2009) Starchy raw materials. In HM Esslinger (ed.). *Handbook of brewing: Processes, technology, markets*, pp. 43–83, Weinheim, Germany: Wiley-VCH.

Mohammed D, Addis D, Berhanu T and Vernooij A (2019) Mapping of spent brewers' grain supply chain in Ethiopia: Report 1210. Wageningen, Netherlands: Wageningen Livestock Research. https://library.wur.nl/WebQuery/wurpubs/fulltext/506451.

Moriel P, Artioli LFA, Piccolo MB, Marques RS, Poore MH and Cooke RF (2016) Frequency of wet brewers grains supplementation during late gestation of beef cows and its effects on offspring postnatal growth and immunity. *Journal of Animal Science* **94**, 2553–2563.

Moriel P, Artioli LFA, Poore MH and Ferraretto LF (2015) Dry matter loss and nutritional composition of wet brewers grains ensiled with or without covering and with or without soybean hulls and propionic acid. *The Professional Animal Scientist* **31**, 559–567.

Mussatto SI, Dragone G and Roberto IC (2006) Brewers' spent grain: generation, characteristics and potential applications. *Journal of Cereal Science* **43**, 1–14.

Negesse T, Makkar HPS and Becker K (2009) Nutritive value of some non-conventional feed resources of Ethiopia determined by chemical analyses and an *in vitro* gas method. *Animal Feed Science and Technology* **154**, 204–217.

NRC (2001) *Nutrient requirements of dairy cattle, 7th ed*. Washington, D.C., USA: National Academies Press.

Nurfeta A (2010) Feed intake, digestibility, nitrogen utilization, and body weight change of sheep consuming wheat straw supplemented with local agricultural and agro-industrial by-products. *Tropical Animal Health and Production* **42**, 815–824.

Nurfeta A and Abdu Y (2014) Feeding value of different levels of malt sprout and katikala atella on nutrient utilization and growth performance of sheep fed basal diet of Rhodes grass hay. *Tropical Animal Health and Production* **46**, 541–547.

O'Rourke T (2003) Mash separation systems. *Brewer International* **3**, 57–59.

Ørskov ER and McDonald I (1979) The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *The Journal of Agricultural Science* **92**, 499–503.

Pereira JC, Carro MD, González J, Alvir MR and Rodríguez CA (1998) Rumen degradability and intestinal digestibility of brewers' grains as affected by origin and heat treatment and of barley rootlets. *Animal Feed Science and Technology* **74**, 107–121.

Raab L, Cafantaris B, Jilg T and Menke KH (1983) Rumen protein degradation and biosynthesis. *British Journal of Nutrition* **50**, 569–582.

Robertson JA, I'Anson KJA, Treimo J, Faulds CB, Brocklehurst TF, Eijsink VGH and Waldron KW (2010) Profiling brewers' spent grain for composition and microbial ecology at the site of production. *LWT - Food Science and Technology* **43**, 890–896.

Rooke JA, Duthie C-A, Hyslop JJ, Morgan CA and Waterhouse T (2016) The effects on cow performance and calf birth and weaning weight of replacing grass silage with brewers grains in a barley straw diet offered to pregnant beef cows of two different breeds. *Journal of Animal Physiology and Animal Nutrition* **100**, 629–636.

Santos M, Jiménez JJ, Bartolomé B, Gómez-Cordovés C and Nozal MJd (2003) Variability of brewer's spent grain within a brewery. *Food Chemistry* **80**, 17–21.

Seifried N, Steingass H, Leberl P and Rodehutscord M (2012) Feeding value of brewers´ grains for ruminants: 21th International Scientific Symposium on Nutrition of Farm Animals. Murska Sobota, Slovenia: Kmetijsko gozdarska zbornica Slovenije, 17–21.

Sinclair, LA, Garnsworthy PC, Newbold, JR and Buttery PJ (1995) Effects of synchronizing the rate of dietary energy and nitrogen release in diets with a similar carbohydrate composition

on rumen fermentation and microbial protein synthesis in sheep. *The Journal of Agricultural Science* **124**, 463–472.

Sniffen CJ, O'Connor JD, van Soest PJ, Fox DG and Russell JB (1992) A net carbohydrate and protein system for evaluating cattle diets. *Journal of Animal Science* **70**, 3562–3577.

Steiner E, Gastl M and Becker T (2011) Protein changes during malting and brewing with focus on haze and foam formation. *European Food Research and Technology* **232**, 191–204.

Steingaß H and Südekum KH (2013) Proteinbewertung beim Wiederkäuer–Grundlagen, analytische Entwicklungen und Perspektiven. *Übersichten zur Tierernährung* **41**, 51–73.

Stern MD, Ziemer CJ, Getz WR, Simms RH and Glenn BP (1995) By-product feeds as energy sources for ruminants. *The Professional Animal Scientist* **11**, 51–66.

Tadesse A and Yayneshet T (2011) Comparative chemical composition evaluation of local brewery and liquor by-products made from different ingredients. *Journal of Drylands* **4**, 307–309.

Tekeba E, Wurzinger M, Baldinger L and Zollitsch WJ (2013) Effects of dietary supplementation with urea molasses multi-nutrient block on performance of mid lactating local Ethiopian and crossbred dairy cows. *Livestock Research for Rural Development* **25**.

Thomas M, Hersom M, Thrift T and Yelich J (2010) Wet Brewers' Grains for Beef Cattle: EDIS (Electronic Data Information Source), Extension Publication Series, AN241. Gainesville, FL, USA: University of Florida, Institute of Food and Agricultural Sciences. https://journals.flvc.org/edis/article/view/118590.

Tolera A, Berg T and Sundstøl F (1999) The effect of variety on maize grain and crop residue yield and nutritive value of the stover. *Animal Feed Science and Technology* **79**, 165–177.

VDLUFA (2012) *Verband Deutscher Landwirtschaftlicher Untersuchungs-und Forschungsanstalten. Handbuch der Landwirtschaftlichen Versuchs-und Untersuchungsmethodik (VDLUFA‐Methodenbuch), Bd. III. Die Chemische Untersuchung von Futtermitteln*. Darmstadt, Germany:

Waters CJ, Kitcherside MA and Webster AJF (1992) Problems associated with estimating the digestibility of undegraded dietary nitrogen from acid-detergent insoluble nitrogen. *Animal Feed Science and Technology* **39**, 279–291.

Westendorf ML and Wohlt JE (2002) Brewing by-products: their use as animal feeds. *The Veterinary Clinics of North America: Food Animal Practice* **18**, 233–252.

Westendorf ML, Wohlt JE, Sniffen CJ and Ward RT (2014) Nutrient content of brewers grains produced at a commercial brewery. *The Professional Animal Scientist* **30**, 400–406.

Wild KJ, Steingaß H and Rodehutscord M (2019) Variability of *in vitro* ruminal fermentation and nutritional value of cell-disrupted and nondisrupted microalgae for ruminants. *Global change biology. Bioenergy* **11**, 345–359.

Xu C, Cai Y, Moriya N and Ogawa M (2007) Nutritive value for ruminants of green tea grounds as a replacement of brewers' grains in totally mixed ration silage. *Animal Feed Science and Technology* **138**, 228–238.

CHAPTER 5

General discussion

The feeding value and factors affecting feeding value, and utilisation of brewers grains (BG) in ruminant feeding are concisely discussed in Chapter 3 of this thesis. Chapter 4 provides results on chemical composition, protein value, and energy value of BG and tella-atella (TA) obtained from several breweries in Ethiopia, and discusses the results considering the literature. Both BG and TA are mainly used as protein sources; thus, protein value in terms of rumen degradability and associated factors are primarily discussed in the current Chapter. The energy value in relation to starch content and organic matter digestibility (OMD) are also discussed. Moreover, the preservation, safe storage for extended periods, and utilisation of BG are usually a challenge in livestock farming, and particularly for Ethiopian dairy producers; this is discussed from the perspective of Ethiopian livestock production and feeding systems.

5.1. Protein value of brewery by-products

Fermentation by-product feeds such as distillers (dried) grains, BG, and TA contain high concentrations of crude protein (CP) and ruminally undegradable feed CP (RUP). The CP contents in BG from this study generally are in the range of literature data (Westendorf and Wohlt, 2002), and significantly vary among samples with a range of 11 percentage. Content of CP within the same category of by-product feeds may vary generally by 3 to 10 percentage units depending mainly on the raw ingredients they are derived from (Clark *et al.*, 1987; Arosemena *et al.*, 1995; Adewole *et al.*, 2016; Broderick *et al.*, 2016; Böttger and Südekum, 2017). In Chapter 4, the highest CP value was observed in a sample (BG5) derived from barley malt and added rice grain (at a proportion of 20%). On the other hand, the samples with added sorghum did not show noticeable differences in terms of CP content, but exhibited higher starch and energy contents. However, further explanation on variation in CP concentrations of brewery by-products in relation to raw ingredients seems inadequate due to lack of further identification of raw grains beyond the information obtained from the breweries.

Understanding the extent of degradation of CP and protein in the rumen is a central theme of most feed protein evaluation systems for ruminants (Krishnamoorthy *et al.*, 1983; Edmunds *et al.*, 2012). Nevertheless, there is no simple technique and quantifying CP degradation accurately is a continuous interest and challenge for ruminant nutritionists. The estimation of

RUP supply to the small intestine of dairy cows is an essential approach for feed protein evaluation (Edmunds *et al.*, 2012). Higher contents of RUP helps to improve the flow of protein to the duodenum of ruminants. In the current work, the estimated CP content of BG degraded in the rumen (ruminally degradable crude protein, RDP) at the assumed passage rate of 3% per hour align with the range of 280–430 g/kg CP reported by Thomas *et al.* (2010). Comparatively, lower values for RDP were reported by Westendorf *et al.* (2014) ranging from 245 to 353 g/kg CP. The result of these studies indicate that a significant portion of BG protein would be RUP or 'bypass protein'.

The physico-chemical nature of the protein and the methods by which the by-product feeds are processed are two main factors that affect rumen resistance of the feed protein (Clark *et al.*, 1987). In barley grain, the largest portion of protein is composed of prolamins and glutelins, and only 3 to 4% of albumins and 10 to 20% of globulins (Clark *et al.*, 1987). Because of higher molecular weight, prolamins and glutelins are of low solubility, and resistant to microbial degradation in the rumen. Therefore, the CP in barley per se is of low degradablity in the rumen, which, however, was not always reflected when barley was compared with rye and triticale (Krieg *et al*., 2018). Moreover, BG are obtained after several physio-chemical treatments during the malting and mashing processes (Chapter 3); far more undegraded CP in BG (Chapter 4) than in the original barley grains could be the result of several heat treatments of barley grain and the malt during malting and brewing (Celus *et al.*, 2006).

The RUP that passes to the duodenum should be digested and provide a balanced AA profile to enhance milk production (Armentano *et al.*, 1986; Vaga *et al.*, 2017). In the current thesis, amino acid profiles and intestinal digestibility of BG and TA samples were not evaluated. Existing research data on the intestinal digestibility of RUP of BG are limited (Pereira *et al.*, 1998). However, a considerable fraction of acid detergent insoluble CP (ADICP) of samples in the present work indicates that the RUP may not be digested entirely in the small intestine. Some portions might be rather inaccessible. Acid detergent insoluble CP includes lignin-bound N, heat-damaged Maillard products, and tannin-protein complexes that are highly resistant to mammalian and microbial enzymes (Krishnamoorthy *et al.*, 1983).

The CP concentrations of TA in this thesis are in general agreement with previous studies reporting CP concentrations from 185 to 218 g/kg DM (Mekasha *et al.*, 2002; Nurfeta, 2010; Nurfeta and Abdu, 2014). The content of CP in TA samples appears to be lower than in BG, yet high enough to serve as supplement for low-quality forages and crop residues. The literature

on the protein value of TA in terms of RUP and utilisable crude protein at the duodenum (uCP) is scarce. However, the data of the present thesis indicates that TA is a valuable protein source next to BG although the impact of high ADICP concentrations in TA samples shown in Chapter 4 requires further investigation. In addition to the protein characteristics of the feed per se, various factors of feeding situations affecting the degradability of protein in the rumen should be considered in the feeding of ruminants. For instance, feed intake, feed passage rate from the rumen and possible associative effects of other feeds such as the ratio of forage to concentrate might affect rumen CP degradability in ruminants (Clark *et al.*, 1987).

To understand small intestinal digestibility of RUP of BG and TA fully, however, further research might be required. Because estimated uCP contents of both by-products were higher than for most other local feedstuffs used in Ethiopia (Aredo and Musimba, 2003; Geleti *et al.*, 2013), efficient use of the brewery by-products would inevitably enhance the protein value of ruminant rations.

5.2. Energy value and organic matter digestibility of brewery by-products

Brewery by-products are usually categorized as a concentrate feed, mainly as a protein supplement. However, they also have a considerable energy content. Concentration of metabolisable energy (ME) of BG, ranging from 8.2 to 12.1 MJ/kg DM (Chapter 4) has to be considered comparatively high particularly in the Ethiopian situation where most feedstuffs have very low energy concentrations. The ME of common crop residues such as maize stover, wheat straw, teff straw, haricot bean haulms, and grass hay in Ethiopia has been reported in the range of as low as 4.0 to 8.7 MJ/kg DM (Aredo and Musimba, 2003; Geleti *et al.*, 2013). Moreover, most of the commonly used browse and herbaceous legumes, cereal straws, and native grass hays contain comparable or less ME than BG (Geleti *et al.*, 2013). Hence, brewery by-products as a supplement have potential to enhance not only the protein value but also the energy value of ruminant feed.

Variation in energy concentrations has been observed among the samples as shown in Chapter 4; this is likely associated with variations in starch content and resulting OMD. The starch content in BG is expected to be very low; most starch is degraded to glucose in the wort for further utilization during beer production (Langenaeken *et al.*, 2020). The mean starch value of all BG samples is within the range of values reported in studies listed in Chapter 3. However, as much as 255 g/kg DM of starch was found in BG samples that were derived from unmalted

sorghum grains, which were added to barley malt (Chapter 4). In this regard, the local brewing by-product TA that was mainly produced from sorghum and/or maize exhibited noticeable energy value as it has been discussed in detail in Chapter 4. Conditions of the process of malting, brewing, and the type of raw cereals used obviously determine the starch and eventually the energy content of brewery by-products (Wenwen *et al.*, 2019). However, the information on grain sources of BG is mostly not given by the breweries and usually missing in published studies. Nevertheless, from the attempt done in this thesis to relate the ingredients to the compositional variation, the variability of grain source contributed to the variability of energy content of the resultant BG.

The result on maximum (potential) degradability (A+B) and degradation rate (c) of BG in this study was slightly lower than the values of 54% and 6.6%, respectively, reported by Seifried *et al.,* (2012). In general, the result on estimated degradation parameters indicate low effective DM degradability at specified passage rate but higher potential degradability of BG (Chapter 4). The gas kinetics (Chapter 4) shows that more than one third of the degradation took place after more than 24 h where the role of glucose and starch is low. These components should actually be digested mainly, if not entirely, during the first 20 h of incubation (Sniffen *et al.*, 1992; Schofield and Pell, 1995). Neutral detergent solubles play an important role in degradation only at the early hours but becomes less significant in later hours of incubation (Schofield and Pell, 1995). Thus, the kinetics of gas development indicates that a significant portion of organic matter, mainly from neutral detergent fibre (NDF) was left undegraded after 24 h of incubation and was degraded thereafter. Overall, the OMD of the BG is variable with a range of 23 percentage units between samples. The variability of NDF degradation might influence the energy value and intake of the brewery by-products by ruminants (Oba and Allen, 1999). In general, this thesis suggests considerable variabilitiy between BG samples in terms of both protein and energy values. Interestingly, BG derived from unmalted cereal grains in addition to barley malt, and TA tended to exceed the BG derived from malted barley in terms of energy value. These variations in brewery by-products should be considered, and might require further studies.

5.3. Utilisation of brewery by-products from the perspective of Ethiopian livestock production and feeding systems

Brewery by-products are particularly important in the case of Ethiopia where the contributions of extremely low-quality feedstuffs such as crop residues and straws, and the total grazing feed (as DM) comprise 52, and 57 million tonnes, respectively, out of 114.4 million tonnes of total available livestock feeds (FAO, 2018). In this regard, increasing awareness and assuring efficient use of BG and TA as energy and protein supplements is vital. Particularly, BG are commonly used in the vicinity of breweries; the demand for BG currently remains higher than the supply in the adapted areas (Aranguiz *et al.*, 2019; FAO, 2019*a*). From the personal observation during the sample collection, however, it was evident that both the livestock experts and farmers have limited knowledge and experiences on how BG are efficiently preserved, stored, and included in the diets. In utilisation of BG, preservation for longer storage is usually challenging for livestock farmers in Ethiopia, as it might be elsewhere (Kitaw *et al.*, 2018). In Chapter 3, the possible methods of preservation and storage are discussed. Hence, from the technological point of view in the Ethiopian situation, ensiling of BG with dry forages as a total ensiled mixed ration (Ferraretto *et al.*, 2018; Wang *et al.*, 2020) seems feasible instead of drying using various sources of heat energy. Moreover, more research-based trainings on the storage and preservation of BG are vital at the agricultural expert and farmer levels.

Some of the breweries supply BG through market chains that delay time of use by animals, and others provide BG directly to the dairy farm owners (Kitaw *et al.*, 2018; Mohammed *et al.*, 2019). Appropriateness of these long market chains need to be evaluated from a feed quality perspective and from the nutritional point of view. During the present study, though it was not part of deliberate experimental design, a sample (BG7) that has been dried several hours (approximately 16 h) later than the other samples, exhibited a fishy smell and was of inferior nutritional value with the lowest concentration of starch and CP. This indicates that in case of poor storage conditions, deterioration may occur within a short period of time that could result in nutrient loss, and even a possibility of proliferation of mycotoxin-producing fungal species (personal discussion with experts). Hence, using fresh BG in long supply chains requires a strict quality control and a reduction in trading time to ensure the product reaches end users promptly and safely.

The other challenge that appears, in addition to storage and transportation, is the question of efficient use of these by-products in terms of inclusion level and ration formulation with

other feedstuffs. Dairy ration formulation in general and/or optimum inclusion level for specific ingredients largely consider the type and amount of locally available feeds, their cost, nutrient composition, nutrient requirements of the animal, physiological status of the animal, feed ingredient limit, and nutrient limit (Suresh, 2016). Either a lower limit and/or an upper limit for each nutrient and ingredient requires to be set. The fact that most Ethiopian rations are mainly based on crop residues and straws with extremely high fibre content, the inclusion level of brewery by-products should consider this to improve and potentially maximize the DM intake. As it has been discussed in several studies and indicated in Chapter 3, the recommended maximum inclusion level of wet BG in the dairy ration is 20–30% on DM basis (Murdock *et al.*, 1981; Davis *et al.*, 1983; West *et al.*, 1994). Depending on the environmental temperature, the inclusion of up to 30% DM of the diet could be suggested to increase dry matter intake and milk yield. For Zebu breeds, however, practical studies based on the experimental trials may be required to set the limits.

In Ethiopia, cattle production can be classified into several production systems based on integration of livestock with crop production, level of input and intensity of production, agro-ecology, and market orientation. Mixed crop livestock production (MCLP), pastoral and agro-pastoral livestock production, urban and peri-urban livestock production, and specialized intensive (dairy and beef) production systems are common ones (FAO, 2019*b*). Generally, utilising brewery by-products like BG and TA could be effectively used in all four livestock production systems except in pastoral and agro-pastoral systems where access to the by-products and to the breweries could be difficult or even impossible.

The MCLP system makes up more than three-fourth of the cattle production systems (FAO, 2019*b*), where cattle rely mainly on agricultural and agro-industrial by-products. In this production system, cattle feed includes mainly crop residues, straw, hay, and seasonal green forages (Gizaw *et al.*, 2017). The use of concentrate feed is generally limited to the industrial areas mostly in urban and peri-urban productions systems due to high cost, low availability, lack of transportation, and lack of storage facilities (Duguma and Janssens, 2016). Hence, efficient integration of brewery by-products in the feeding of MCLP system might result in significant improvement in ruminant productivity.

The urban and peri-urban medium scale livestock production that shares only 7% of livestock production systems currently could become increasingly important in the future (FAO, 2019*b;* Tadesse *et al.*, 2019). These livestock operations include commercial to

smallholder dairy farms. Brewers grains and TA are mainly in use in urban and peri-urban livestock system and play an important role as supplements (FAO, 2019*a*). The use of BG and TA in specialized intensive production systems that include both dairy and beef is viable in terms of accessibility and capability to transport and store the by-products. Particularly, due to its protein quality, use of BG in the diets of the dairy cows promotes the milk yield. Generally, in consideration of low standards of feed processing technologies and capacities in the country, BG appear to be a noble feedstuff in Ethiopian livestock productions systems as a concentrate feed supplement.

Conclusions and outlook

The basic brewing processes and products in Ethiopia are similar to other regions and are similarly affected by recent technological innovations in brewing. This may influence the efficiencies of specific brewing processes that in turn affect the beer production and the variability of nutrient concentrationsin the by-products. Indeed, the cereal grains used for malting and brewing appear particularly inconsistent in Ethiopian breweries, and addition of unmalted grains have been and are still common practices of the breweries, related to the various factors highlighted in this thesis.

The varaible and inconsistent use of different cereal grains and addition of unmalted grains contribute not only to the variability of chemical composition but, also, to the variability of protein quality in terms of RUP and uCP. Similarly, the addition of unmalted cereal grains contributed to notable increases of starch and energy contents in the BG. The variability in chemical composition might not affect their utilisation as a protein and energy supplemently; rather it recalls routine evaluation of nutritive value of the by-products for the efficient use and formulation of rations with other feeds.

As previously recognized and further supported by the the current findings, BG and TA serve as valuable protein and energy supplements for ruminants, with significant yet underutilized potential, particularly in Ethiopia, to enhance feed intake and animal performance. Major fractions of the CP in BG and TA appear to be ruminally undegradable which is particularly beneficial for dairy animals. However, a notable fraction of CP in both by-products and particularly for TA is present as indigestible CP, reducing their overall protein quality and feeding value.

Therefore, further studies on the fate of RUP in the small intestine – the accessibility of the protein and absorption of amino acids by the animal – are vital. In addition, research on the appropriate and efficient ways of transportation and storage of fresh TA and BG are recommended to utilise the by-products more efficiently. Despite the growing number of breweries and anticipated future expansion, the current limited supply of these feedstuffs highlights the need to explore and incorporate additional agro-industrial by-products as feed supplements to support Ethiopia's large livestock population.

REFERENCES

Adewole DI, Rogiewicz A, Dyck B and Slominski BA (2016) Chemical and nutritive characteristics of canola meal from Canadian processing facilities. *Animal Feed Science and Technology* **222**, 17–30.

Aranguiz AA, Berhanu T and Vernooij A (2019) Utilization and management of spent brewers grain in Ethiopian dairy farms: Report 1207. Wageningen, Netherlands: Wageningen Livestock Research, https://doi.org/10.18174/504103.

Aredo TA and Musimba NKR (2003) Study on the chemical composition, intake and digestibility of maize stover, tef straw and haricot bean haulms in Adami Tulu District, Ethiopia. *Agriculture and Natural Resources* **37**, 401–407.

Armentano LE, Herrington TA, Polan CE, Moe AJ, Herbein JH and Umstadt P (1986) Ruminal degradation of dried brewers grains, wet brewers grains, and soybean meal. *Journal of Dairy Science* **69**, 2124–2133.

Arosemena A, DePeters EJ and Fadel JG (1995) Extent of variability in nutrient composition within selected by-product feedstuffs. *Animal Feed Science and Technology* **54**, 103–120.

Böttger C and Südekum K-H (2017) European distillers dried grains with solubles (DDGS): Chemical composition and *in vitro* evaluation of feeding value for ruminants. *Animal Feed Science and Technology* **224**, 66–77.

Broderick GA, Colombini S, Costa S, Karsli MA and Faciola AP (2016) Chemical and ruminal *in vitro* evaluation of Canadian canola meals produced over 4 years. *Journal of Dairy Science* **99**, 7956–7970.

Celus I, Brijs K and Delcour JA (2006) The effects of malting and mashing on barley protein extractability. *Journal of Cereal Science* **44**, 203–211.

Clark JH, Murphy and Crooker BA (1987) Supplying the protein needs of dairy cattle from by-product feeds. *Journal of Dairy Science* **70**, 1092–1109.

Davis CL, Grenawalt DA and McCoy GC (1983) Feeding value of pressed brewers' grains for lactating dairy cows. *Journal of Dairy Science* **66**, 73–79.

Duguma B and Janssens GPJ (2016) Assessment of feed resources, feeding practices and coping strategies to feed scarcity by smallholder urban dairy producers in Jimma town, Ethiopia. *SpringerPlus* **5**.

Edmunds B, Südekum KH, Spiekers H, Schuster M and Schwarz FJ (2012) Estimating utilisable crude protein at the duodenum, a precursor to metabolisable protein for

ruminants, from forages using a modified gas test. *Animal Feed Science and Technology* **175**, 106–113.

FAO (2018) *Ethiopia: Report on feed inventory and feed balance 2018*. Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO (2019*a) Ethiopia: Availability and utilization of agroindustrial by-products as animal feed 2018*. Rome, Italy: Food and Agriculture Organization of the United Nations.

FAO (2019*b) The future of livestock in Ethiopia: Opportunities and challenges in the face of uncertainty*. Rome, Italy: Food and Agriculture Organization of the United Nations.

Ferraretto LF, Fernandes T, Silva Filho WI, Sultana H and Moriel P (2018) Dry matter loss, fermentation profile, and aerobic stability of wet brewers grains ensiled with various amounts of dry ground corn. *The Professional Animal Scientist* **34**, 642–648.

Geleti D, Hailemariam M, Mengistu A and Tolera A (2013) Nutritive value of selected browse and herbaceous forage legumes adapted to medium altitude subhumid areas of western Oromia, Ethiopia. *Global Veterinaria* **11**, 809–816.

Gizaw S, Ebro A, Tesfaye Y, Mekuriaw Z, Mekasha Y, Hoekstra D, Gebremedhin B and Tegegne A (2017) Feed resources in the highlands of Ethiopia: A value chain assessment and intervention options: LIVES Working Paper 27. Nairobi, Kenya: International Livestock Research Institute (ILRI), https://cgspace.cgiar.org/handle/10568/82636.

Kitaw G, Tamir B, Assefa G and Animut G (2018) Production, preservation, and utilization patterns of brewery spent grain in Ethiopia. *Ethiopian Journal of Agricultural Sciences* **28**, 1–17.

Krieg J, Seifried N, Steingass H Rodehutscord M (2018) In situ and *in vitro* evaluation of crude protein degradation and utilizable crude protein content of barley, rye and triticale grains for ruminants. *Journal of Animal Physiology and Animal Nutrition* **102**, 452-461

Krishnamoorthy U, Sniffen CJ, Stern MD and van Soest PJ (1983) Evaluation of a mathematical model of rumen digestion and an *in vitro* simulation of rumen proteolysis to estimate the rumen-undegraded nitrogen content of feedstuffs. *British Journal of Nutrition* **50**, 555–568.

Langenaeken NA, Schepper CF de, **Schutter DP** de and **Courtin CM** (2020) Carbohydrate content and structure during malting and brewing. *Journal of the Institute of Brewing* **126**, 253–262.

Mekasha Y, Tegegne A, Yami A and Umunna NN (2002) Evaluation of non-conventional agro-industrial by-products as supplementary feeds for ruminants. *Small Ruminant Research* **44**, 25–35.

Mohammed D, Addis D, Berhanu T and Vernooij A (2019) Mapping of spent brewers' grain supply chain in Ethiopia: Report 1210. Wageningen, Netherlands: Wageningen Livestock Research, https://library.wur.nl/WebQuery/wurpubs/fulltext/506451.

Murdock FR, Hodgson AS and Riley Jr RE (1981) Nutritive value of wet brewers grains for lactating dairy cows. *Journal of Dairy Science* **64**, 1826–1832.

Nurfeta A (2010) Feed intake, digestibility, nitrogen utilization, and body weight change of sheep consuming wheat straw supplemented with local agricultural and agro-industrial by-products. *Tropical Animal Health and Production* **42**, 815–824.

Nurfeta A and Abdu Y (2014) Feeding value of different levels of malt sprout and katikala atella on nutrient utilization and growth performance of sheep fed basal diet of Rhodes grass hay. *Tropical Animal Health and Production* **46**, 541–547.

Oba M and Allen MS (1999) Evaluation of the importance of the digestibility of neutral detergent fiber from forage. *Journal of Dairy Science* **82**, 589–596.

Pereira JC, Carro MD, González J, Alvir MR and Rodríguez CA (1998) Rumen degradability and intestinal digestibility of brewers' grains as affected by origin and heat treatment and of barley rootlets. *Animal Feed Science and Technology* **74**, 107–121.

Schofield P and Pell AN (1995) Measurement and kinetic analysis of the neutral detergent-soluble carbohydrate fraction of legumes and grasses. *Journal of Animal Science* **73**, 3455–3463.

Seifried N, Steingass H, Leberl P and Rodehutscord M (2012) Feeding value of brewers´ grains for ruminants. In . *21. International Scientific Symposium on Nutrition of Farm Animals (Zadravec-Erjavec Days 2012)*, Radenci, Slowenien: Kmetijsko gozdarska zbornica Slovenije.

Sniffen CJ, O'connor JD, van Soest PJ, Fox DG and Russell JB (1992) A net carbohydrate and protein system for evaluating cattle diets. *Journal of Animal Science* **70**, 3562–3577.

Suresh AV (2016) Feed formulation software. In SF Nates (ed.). *Aquafeed Formulation*, pp. 21–31, San Diego, USA: Academic Press.

Tadesse ST, Oenema O, van Beek C and Ocho FL (2019) Nitrogen allocation and recycling in peri-urban mixed crop–livestock farms in Ethiopia. *Nutrient Cycling in Agroecosystems* **115**, 281–294.

Thomas M, Hersom M, Thrift T and Yelich J (2010) Wet Brewers' Grains for Beef Cattle: EDIS (Electronic Data Information Source), Extension Publication Series, AN241. Gainesville, FL, USA: University of Florida, Institute of Food and Agricultural Sciences, https://journals.flvc.org/edis/article/view/118590.

Vaga M, Hetta M and Huhtanen P (2017) Effects of heat treatment on protein feeds evaluated *in vitro* by the method of estimating utilisable crude protein at the duodenum. *Journal of Animal Physiology and Animal Nutrition* **101**, 1259–1272.

Wang S, Zhao J, Yu C, Li J, Tao X, Chen S and Shao T (2020) Nutritional evaluation of wet brewers' grains as substitute for common vetch in ensiled total mixed ration. *Italian Journal of Animal Science* **19**, 1015–1025.

Wenwen Y, Tao K, Gidley MJ, Fox GP and Gilbert RG (2019) Molecular brewing: molecular structural effects involved in barley malting and mashing. *Carbohydrate Polymers* **206**, 583–592.

West JW, Lo Ely and Martin SA (1994) Wet brewers grains for lactating dairy cows during hot, humid weather. *Journal of Dairy Science* **77**, 196–204.

Westendorf ML and Wohlt JE (2002) Brewing by-products: their use as animal feeds. *The Veterinary Clinics of North America: Food Animal Practice* **18**, 233–252.

Westendorf ML, Wohlt JE, Sniffen CJ and Ward RT (2014) Nutrient content of brewers grains produced at a commercial brewery. *The Professional Animal Scientist* **30**, 400–406.

Appendix[1]: Supplementay information on Ethiopian traditional 'tella' brewing processes

Tella is a homemade brew (Ethiopian traditional alcoholic beverage) based on substrates such as barley, wheat, maize, millet, sorghum and teff (Tadesse and Yayneshet, 2011; Berhanu, 2014; Lee *et al.*, 2015). Almost every household of Ethiopia brews tella in a very significant amount, especially in Christian communities. Nevertheless, the compiled data on production at national level is limited. The raw materials and brewing procedures vary in different societies as well as slightly from brewer to brewer (Lee *et al.*, 2015). Only the basic brewing steps remain similar.

Tella brewing processes include four fermentation phases (Lee *et al.*, 2015). The first phase is called 'Tijet'. Leaves and stems of *Rhamnus prinoides*, the shiny-leaf buckthorn (locally known as Gesho), are sun dried and ground with mortar and pestle into flour. Leaves and stems of Gesho play a similar role in tella as hops in conventional beer, providing a bitter flavour and stabilizing the tella brew for longer shelf-life (Berhanu, 2014). Barley malt, called bikil, is prepared, then sun-dried and ground. Malt can be processed preferably from barley, but also from wheat or maize. At this phase, the ground Gesho leaves and stems are soaked in water for about 3-5 days depending on the environmental conditions (Lee *et al.*, 2015). Some tella-brewers also mix a small amount of the milled malt into Gesho to facilitate fermentation (Berhanu, 2014). Addition of traditional hops or Gesho in tella brewing, therefore, starts at an early step of the process and before addition of the main carbohydrate substrate.

The second phase is called 'tenses' and slightly resembles mashing in beer making. Cereal grain flour (mostly finger millet, sorghum and maize) are processed to a dough and baked to an unleavened bread ('kita'), then cut into pieces. In this step, malt (bikil) and unleavened bread pieces (kita) are mixed into the fermented solution (gesho leaf-soaked water) of the first step

[1] **References used in this note include:**

Berhanu A (2014) Microbial profile of Tella and the role of gesho (*Rhamnus prinoides*) as bittering and antimicrobial agent in traditional Tella (Beer) production. *International Food Research Journal* **21**, 357–365.

Lee M, Regu M and Seleshe S (2015) Uniqueness of Ethiopian traditional alcoholic beverage of plant origin, tella. *Journal of Ethnic Foods* **2**, 110–114.

Tadesse A and Yayneshet T (2011) Comparative chemical composition evaluation of local brewery and liquor by-products made from different ingredients. *Journal of Drylands* **4**, 307–309

with additional powder of gesho leaves and stems. The proportion of ingredients likely varies from brewer to the brewer, and the whole mixture is kept to ferment for about two or more days depending on the environmental temperature.

The third phase of fermentation is called ‘difdef’ – the crushed gesho leaves and stems and roasted barley flour (‘asharo’ or ‘enkuro’) are mixed into a thick slurry and kept to ferment for another two days or more. The more the roasting intensity of the flour and baking of kita increases, the darker becomes tella and probably affects the nutritional quality of tella-atella (TA), a by-product from tella brewing, indirectly due to Maillard reactions.

At the final phase, water is filled in to the slurry to reach at the optimum consistency, and stirred thoroughly. The container is then sealed airtight to create an anaerobic condition and left for two days or more. Finally, tella can be either filtered or poured directly from the container and consumed as a local drink. Hence, TA remains as a solidfiltrate or as a slurry at the bottom of the container depending the separation ways used by the brewer.

ACKNOWLEDGEMENT

I would like to extend my heartfelt gratitude to my supervisor, Professor Dr. Karl-Heinz Südekum, who showed trust in me by accepting me into his research group for my Ph.D. journey. I am profoundly thankful for your unwavering support, invaluable guidance, and genuine compassion from the very beginning. Your comprehensive support, including the financial assistance for field research sample collection in challenging conditions in Ethiopia, played a pivotal role in the successful completion of this research. In line with this, I would also like to extend my sincere appreciation to the Bonner Förderkreis Tierernährung e.V. group and Gesellschaft der Freunde der Agrar- und Ernährungswissenschaftlichen Fakultät e.V. for their generous financial support, especially towards the end of my stipend.

It has been a privilege to have Dr. Christian Böttger as my mentor, who not only imparted essential laboratory procedures but also supervised my day-to-day work, assisted in data analysis, and provided invaluable feedback to shape my thesis. I extend my heartfelt gratitude to you for your patience, curiosity, and critical analytical skills that you generously shared with me.

I would like to acknowledge the Institute of Animal Science working group and all my colleagues for fostering an excellent working environment and offering unwavering support and encouragement throughout my research journey. Special thanks to Dr. Thomas Hartinger (currently at the University of Veterinary Medicine Vienna, Austria) and Jan-Helge Deitmers for their assistance in facilitating the transportation of rumen fluid from the Frankenforst research station to the institute over several days. I am also deeply grateful to Klara Schmitz, Valérie Schumacher, Bernd Hilgers, and Beatrix Bonhoff for their enriching discussions, motivation, companionship, and inspiration. Your efforts and motivation to integrate me into the team and the university community, as well as your encouragement to improve my German language skills, were instrumental in my success during my stay. Thank you all for your kindness, motivation, and unwavering support in promoting my social integration.

I would like to express my sincere thanks to the technical staff of the Institute of Animal Sciences, Mrs. Nadine Leyer and Mrs. Petra Jacquemien, for their invaluable support during laboratory sample analysis. I extend my heartfelt appreciation to Stephan Knauf for his unwavering technical assistance in maintaining of laboratory equipment in times of need throughout the laboratory

routines. Additionally, I am deeply grateful to Mrs. Renate Kicker for her dedicated administrative support and assistance during my time at the institute.

I feel immensely grateful and honored to have received a scholarship grant from the Katholischer Akademischer Ausländer-Dienst (KAAD) for four years, without which pursuing my studies would not have been possible. In this regard, I extend my sincere appreciation to Dr. Marko Kuhn and Mrs. Miriam Rossmerkel, the KAAD Africa department team, for their unwavering and continuous support in arranging travel for research purposes and my family visits.

Lastly, but by no means least, my heartfelt gratitude goes to my spouse, Nigist Sebhat, and my children, Amen and Maria. I cannot thank you enough for your unwavering courage and patience throughout my six years of study leave. Your support has been my pillar of strength.