

Nutritional and Logarithmic Fungal Count of Brewers' Grain Under Different Conservation Techniques and Brewery Sources

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Abstract

This study was contained two trials. The objectives of the first and the second trials were to explore variations in nutritional merits and investigate the effect of local conservation practices on nutritional and fungal load dynamics of wet brewers' grain (WBG) received from different brewery sources, respectively. Fresh WBG samples for the first trial were obtained from four breweries (Meta Abo, Habesha, Dashen and Bedele) located in different districts of the country. Brewers' grain sample received from Meta Abo brewery factory had superiorly higher ($p < 0.05$) acid detergent fiber, lignin and digestible organic matter in the dry matter (DOMD), but comparable dry matter (DM), total ash, crude protein (CP) and neutral detergent fiber with the other breweries. In the second trial, an attempt was made to evaluate three locally available WBG conservation practices, preservation of WBG using an ensiling technique maintained feed quality slightly comparable to the control sample (freeze dried) but outperformed ($p < 0.05$) other locally available conservational practices "soaking" and "sun drying " with minimal loss on feed DM and other nutrients, lower total fungal, yeast and mold colony counts and above all superior CP digestion kinetics as measured through nylon bag degradability constants. If supply is not a constraint under local conditions, ensiling can fairly be recommended as a best bet WBG conservation practice for use at wider scale.

Key Points

Nutritional values of brewery grain are affected by the brewery factory.

Locally storage practices can be used as a means of conservation for brewery grain with slight feeding value losses.

Introduction

Fresh brewer's grain is a cheap, non-conventional protein source which is becoming increasingly available to urban and peri-urban dairy farms in Ethiopia with the boom in the beer industry in the last few decades. In the year 2016/17 alone the annual total brewery spent grain production from twelve factories in Ethiopia was estimated at 26, 723 tons DM (Getu et al., 2018). Wet brewery grain has the major constituents of hemicellulose cellulose, protein and lignin (Mussatto et al., 2006; Xiros and Christakopoulos, 2012). A number of factors, such as the cereal variety, type of hops added, the malting and mashing regime, and whether adjuncts were employed during brewing (Steiner et al., 2015). Fresh brewer's grain contains 70 – 80% water by weight (Kunze, 210). This high moisture level poses two major difficulties when using it as a feed for different classes of animals. Firstly, transport of wet WBG can be costly, this being a particular reason why supply to local farmers as cattle feed has primarily been the main outlet. Secondly, the rich polysaccharide and protein content and the high moisture content of WBG make it susceptible to microbial growth and spoilage, this being identified as a potential problem area which might restrict its successful exploitation as livestock feed. This being the case, improper storage of WBG results in a large loss of DM and nutrients, characterized by an unpleasant odor, and even

stimulates mold to produce mycotoxins (Asurmendi et al., 2013; Amézqueta et al., 2009). To this end a number of methods have been examined for their suitability to preserve WBG. These methods includes: acid solutions such as lactic, acetic, formic and benzoic acids have been used, however, use of such chemicals under local context can be at odds with the livestock producers who not only had access to the chemicals but also lack proper knowledge and skill to handle such chemicals. Several physical methods of preservation have also been examined elsewhere, including oven-drying, freeze-drying, freezing and use of superheated steam Bartolomé et al. (2002). Under local farmers conditions these too are not affordable associated to initial procurement costs and costs of energy maintainance. In Ethiopia, farmers traditionally depend on either fresh WBG or that treated and soaked with a brine solution (Getu et al., 2018). According to same source sun drying and ensiling are also seldom used. The efficiency of these local WBG conservation practices, particularly from perspectives of nutritional and fungal load dynamics have not so far been well documented by R & D efforts. This study was, therefore, aimed at investigating nutritional variations among WBG samples received from different brewery sources and also exploring best bet practices from among the most commonly used local WBG conservation practices based on nutritional merits and fungal load dynamics.

Materials And Methods

Ex p erimental location, sam p ling procedures and measurements

Two laboratory based experiments were conducted on-station at Holetta Agricultural Research Center (HARC), animal nutrition research and dairy microbiology research laboratories. For the first experiment about a kilogram of pooled fresh samples were received in an ice box over two days' samplings from Meta Abo, Dashen, Habesha and Bedele breweries. For each day about 500 g of pooled fresh sample was taken from three batches in order to make the samples more representative. Each sample was then labeled and kept in a separate sterile bag, stored in HARC animal nutrition laboratory under -20°C temperatures upon arrival to the lab. After thawing and remixing, the samples were then subjected to laboratory analysis. In the second laboratory trial, an attempt was made to evaluate prominently used three local WBG conservation practices. These were; soaking, sun drying and ensiling of fresh WBG were compared to a control, freeze-dried sample. After sub-sampling of some 40 kg of fresh sample brought from same brewery above in an ice box, 2 kg fresh WBG sample in five replication was immediately subjected to frozen inside the freeze dry system (Lyph Lock 4.5 L (77510-00)) between temperatures -50°C and -80°C , pressure lowered at $1,33 \times 10^{-3}$ mbar. Samples were then allowed to dry until the moisture in WBG reached 4% by removing the ice in the frozen WBG through the sublimation process (Freeze Dry Systems Catalog, 2004). The remaining 30 kg of the fresh sample was divided in to three equal parts and subjected to the first local storage treatment option called soaking in which 2 kg fresh WBG sample was uniformly treated with salt at roughly 3% on DM basis and placed in cold water submerged plastic container of 2 kg capacity, finally covered with a lead for partial aerobic storage. To mimic local farmers' practice, cold water re-filling was maintained for over fifteen storage days. In practicing the second locally used conservation technique, 2 kg fresh WBG sample was exposed to sun

drying for about three days, eight hours a day. Sun drying of the grain on a clean sterile plastic sheet was made following local farmers practice and recommendation for sun drying under tropical conditions by Boessinger et al. (2005) that roughly assumes 90% DM in the final dried grain sample. In practicing the last locally used conservation type, another 2 kg fresh WBG sample was ensiled in a plastic bottle of 2 kg capacity following farmers practice and WBG ensiling procedures outlined by Boessinger et al. (2005). In addition to WBG, molasses was added at the rate of 3% on DM basis to enhance proper fermentation and compaction inside the silo, sealed with caps and left to properly ferment under anaerobic conditions for about 42 days (Boessinger et al., 2005). Samples from ensiled WBG were subjected to pH reading upon opening each silo to ensure whether or no proper fermentation has taken place. All samples except those from sun drying and freeze drying were subjected to oven drying at 55°C for 72 hrs. About 150 g (DM basis) of the samples from all conservation practices were taken and placed in a sterile plastic bag and kept in a refrigerator at -20 °C for major feed chemical compositions (DM, ash, CP, NDF, ADF, permanganate lignin, DOMD), dry matter losses and, fungal count (yeast and mold colony). For the purpose of statistical calculation samples from the control treatment and that stored using the three local conservation practices were replicated five times.

In-situ DM and CP degradability characteristics of brewer's grain conserved using local practices

The DM degradability study was carried out according to Ørskov and McDonald (1979) procedure. Three F1 Boran-Friesian steers (550 ± 15 kg live weight) fitted with ruminal canola were used from HARC. The steers were fed with maintenance diet i.e., *ad libitum* natural pasture hay (6.8% CP) harvested from on-station grazing field and 2 kg concentrate head⁻¹ day⁻¹ on DM basis. The concentrate with 20% CP was formulated on-station from 63% wheat bran, 36% cottonseed cake, and 1% salt. Samples from freeze drying, soaking, ensiling and sun drying were ground to pass through 2 mm sieve size. Three-gram sample was placed into nylon bags of dimensions 6.5 × 14 cm, 50 µm pore size and incubated in the rumen using sequential addition at 0800 hours for 0 (bags washed with cold tap water without incubation), 6, 12, 24, 48, 72 and 96 hours of fermentation. The bags were all subjected to hand washing using tap water until clean water comes out of the washing bag. The bags with the residues were dried at 55°C for 72 hours in an air forced oven, hot weighed and finally the residues recovered for further CP analysis. Data on ruminal CP degradability characteristics was obtained as the difference of the DM and CP in the residues and original samples.

Laboratory analysis

Brewers' spent grain samples from the different brewery sources and local conservation practices were dried in a forced-ventilation oven (55°C for 72 h) and ground to pass through 1 and 2-mill Cyclotec sample mill screen (Tecator 1093, Tecator AB, Hoganas, Sweden). All samples including residues recovered from an in-situ trial were analyzed for DM, total ash and crude protein (CP) using the procedure of AOAC (1990). Neutral detergent fiber (NDFom-NDF), acid detergent fiber (ADFom-ADF) and lignin (pm-Lignin determined by oxidation of lignin with permanganate) were determined by the procedures of Van Soest and Robertson (1985). Tilley and Terry (1963) two-stage in-vitro digestibility technique was

employed to analyze and calculate the digestible organic matter in the dry matter of the samples. Metabolizable energy (ME) was estimated from the in-vitro organic matter in the dry matter digestibility (DOMD) as EME (MJ/kg) = 0.16 x DOMD (McDonald et al., 2002). Dry matter losses were calculated as a difference of DM for the fresh WBG sample (control) and the same samples that were subjected to local aerobic and anaerobic conservational practices. The pH during the ensiling of the fresh WBG was measured using a digital bench top pH meter (Hannan Instrument, pH 210, microprocessor pH meter) after 10 gram of the ensilage sample was allowed to uniformly mix in a 100 ml of distilled water (Cherney and Cherney, 2003), and was left to stand for an hour before reading. Yeasts and molds were direct plate counted by pour plating of 25g ground dried brewers' grain samples dissolved in 225 ml of peptone water onto Potato Dextrose agar medium injected with 1ppm per each 100 ml of agar with chloramphenicol and streptomycin to restrict bacterial growth (FAO, 1997). Plates were incubated aerobically at 28±1°C for 3 day and growing molds and yeast colonies were directly counted (MoH, 2010).

Statistical analyses

The model used for both lab trial was a completely randomized design with five replications: $Y_{ijk} = \mu + C_i + e_{ij}$, Where; μ = Overall mean; C_i = Effect of brewery source (experiment-1) and/or effect of local storage practices (experiment-2); e_{ij} = Random error.

Data from in situ CP degradability trial was fitted into the exponential models of Ørskov and McDonald (1979) as: $P = a + b(1 - e^{-ct})$; where; P = CP disappearance in rumen at time t; a = the washing loss fraction; b = the slowly but potentially degradable fraction; c = the rate at which the "b" fraction degraded (in % h⁻¹). Similarly, effective CP degradability was calculated applying the equation of Ørskov and McDonald (1979) as $ED = a + b[c / (c+k)]$, Where; k = the rumen outflow rate (3 % per h). Data from all trials were subjected to analysis of variance using the general linear model (GLM) procedures of statistical analysis system, version 9.3 (SAS, 2014). Mean separations were made using least significant differences (LSD) analysis at $p \leq 0.05$.

Results

Nutritional variations among brewers' grain samples collected from different breweries

The nutritional compositions of brewery spent grain from four breweries are presented in Table 1. Significant variations ($p < 0.05$) for all measured nutritional parameters were observed among fresh WBG samples collected from the four breweries. The sample from Meta Abo and Dashen had higher DM but lower NDF contents ($P < 0.05$) than the other two breweries. Ash content of the samples was in the order of Meta Abo = Habesha D/B > Dashen > Bedele; while the CP content was in the order of Meta Abo = Habesha D/B > Dashen > Bedele ($p < 0.05$). The ADF content was highest for Habesha followed by Bedele and was lowest for Meta Abo ($p < 0.05$) while the lignin content was highest for Habesha and Bedele, intermediate for Meta Abo and lowest for Dashen. The DOMD values differ ($p < 0.05$) among breweries being in the order of Meta Abo > Dashen > Bedele, while the value for Habesha differ only with Meta Abo.

Table 1
Chemical composition and in-vitro digestibility of fresh brewery's spent grain samples from four breweries

Brewery name	Chemical compositions and in-vitro digestibility (g/kg DM)						
	DM (g/kg)	Ash	CP	NDF	ADF	Lignin	DOMD
Meta Abo	242 ^a	46 ^a	265 ^a	630 ^b	252 ^d	66 ^b	699 ^a
Habesha D/B	208 ^b	44 ^a	263 ^a	673 ^a	315 ^a	76 ^a	655 ^{bc}
Dashen D/B	240 ^a	37 ^b	235 ^c	636 ^b	271 ^c	51 ^c	668 ^b
Bedele	219 ^b	33 ^c	236 ^b	677 ^a	293 ^b	72 ^a	630 ^c
SEM	9.6	4.5	6.3	8.5	11.9	6.7	15.3
P-value	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0006
^{a,b,c} Means within a column with different superscripts differ ($p < 0.05$); DM=Dry matter; CP=Crude protein; NDF=Neutral detergent fibre; ADF=Acid detergent fibre; DOMD=Digestible organic matter in the dry matter; D/B= Debre Birhan; SEM = Standard error of the mean							

Chemical composition and IVDOM of brewer's grain stored using local conservation practices

The effects of locale WBG conservation practices on the chemical composition, IVDOMD, and logarithmic fungal colony count are given in Table 2. All parameters measured differed significantly ($p < 0.05$) among local storage practices. The DM content was in the order of control > ensiled > sundried > soaked, while the CP content was in the order of control = ensiled > sundried > soaked ($p < 0.05$). The in-vitro DOMD values were similar between the control and ensiling treatments ($p > 0.05$), with the values being higher than the other two treatments. Dry matter loss was the lowest for ensiling and highest for soaking ($p < 0.05$). The fiber content was maintained similar to the control group in the case of ensiling but was increased by sun drying. The current results indicated that ensiling preserves fresh WBG better than soaking and sun drying.

Table 2

Effect of different local brewer's grain conservation practices on the chemical compositions and IVDOMD

Parameter	Control	Soaking	Ensiling	Sun drying	SEM	P-value
Nutritive values (g/kg DM)						
DM (g/kg)	247 ^a	217 ^d	240 ^b	226 ^c	7.5	<0.0001
Ash	45.3 ^c	55.6 ^b	63.0 ^a	38.8 ^d	1.20	<0.0001
CP	260 ^a	216 ^c	265 ^a	231 ^b	3.5	<0.0001
NDF	635 ^b	587 ^c	610 ^{bc}	719 ^a	13.8	<0.0001
ADF	246 ^c	267 ^b	245 ^c	297 ^a	5.4	<0.0001
Lignin	62.3 ^b	65.0 ^{ab}	62.0 ^b	66.9 ^a	1.02	<0.0180
DOMD	687 ^a	630 ^b	693 ^a	637 ^b	4.6	<0.0001
DML (%)	-	12.4 ^a	2.9 ^c	8.5 ^b	0.62	<0.0001
<i>a-d Means with in a row with different superscripts differ (p<0.05); Control= freeze dried; DM= Dry matter; CP=Crude protein; NDF=Neutral detergent fiber; ADF= Acid detergent fiber; DOMD=Digestible organic matter in the dry matter; DML=Dry matter loss; SEM =standard error of the mean</i>						

Fungal load dynamics of brewery spent grain conserved using local storage practices

Yeast count was in the order of soaking > sun drying > ensiling = control ($p < 0.05$) Table 3. The mold count was lowest for the control and highest for soaking, while the other two treatments had intermediate values. The total fungal count was lowest for the control followed by ensiling and was the highest for soaking ($p < 0.05$). This result also suggests that ensiling to be a better storage practice than sun drying and soaking.

Table 3

Effect of different local brewer's grain conservation practices on fungal load dynamics

Parameter	Control	Soaking	Ensiling	Sun drying	SEM	P-value
Mold and yeast count (log CFU/g DM)						
Yeast	2.7 ^c	4.9 ^a	3.0 ^c	3.8 ^b	0.22	<0.0001
Mold	2.1 ^c	3.7 ^a	2.8 ^b	3.1 ^b	0.16	<0.0001
TFC	4.8 ^d	8.6 ^a	5.8 ^c	6.9 ^b	0.29	<0.0001
<i>a-d Means with in a row with different superscripts differ (p<0.05); Control= freeze dried; WBG = Brewers' grain; cfu= Colony forming unit; DM= Dry matter; TFC=Total fungal count; SEM =standard error of the mean</i>						

In Situ Crude protein degradability characteristics of brewer's grain conserved using local storage practices

In-situ CP degradability characteristics of WBG sample stored using different local conservation practices are presented in Table 4. Soaking and sun drying increased and that of ensiling decreased the "a" fraction of CP ($p < 0.05$). However, the "b" fraction for CP was lower for ensiling than soaking and sun drying but was comparable to the control. The rate at which the "b" fraction degraded (i.e., c value) was higher ($p < 0.05$) for soaking, intermediate for the Control and was lower for the ensiled and sun dried WBG samples. The sample conserved using the ensiling method had the least Effective CP degradability (ED) followed by sun drying, control and soaking treatments in that order ($p < 0.05$). Calculated ED for soaking was 10.1, 26.73 and 18.42% higher ($p < 0.05$) than that recorded for the control, ensiling and sun-dried samples, respectively. Potential degradability (PD) fraction of WBG sample differed ($p < 0.05$) among conservation methods. Accordingly, the PD for CP was higher for the Soaked as compared to the other preservation methods ($p < 0.05$). On the other hand, *in situ* ruminal CP disappearance for WBG stored using the different conservation practices along the incubation hour is shown in Figure below. Although CP percentage disappearance differed among treatments the trend along the incubation hours appeared to be similar. At the early phase of ruminal incubation (≤ 48 hours), CP disappearance rates of all treatments were higher, and the rate slowed down afterward. The trend of ruminal CP disappearance at any given incubation hour was in the order of soaking > control (fresh) > sun drying > ensiling.

Table 4
Effect of local conservation practices on the *in-situ* CP degradability characteristics

Degradability constants	Conservation practices					
	Control	Soaking	Ensiling	Sun drying	SEM	P-value
Soluble fraction, a (%)	11.1 ^c	17.8 ^a	8.1 ^d	14.2 ^b	0.72	<0.0001
Slowly degradable fraction, b (%)	55 ^{ab}	58.1 ^a	51.8 ^b	55.8 ^a	0.99	<0.0087
The rate of degradation of b fraction, c (% h ⁻¹)	0.05 ^b	0.07 ^a	0.04 ^{bc}	0.04 ^c	0.006	<0.0002
Effectively degradable fraction, ED (%)	46.2 ^b	56.3 ^a	41.8 ^d	43.6 ^c	0.84	<0.0001
Potentially degradable fraction, PD (%) = a + b	66.1 ^b	73.1 ^a	66.25 ^b	66 ^b	0.9	<0.0002
<i>a-d</i> Means within a row with different superscripts differ ($p < 0.05$); Control= fresh sample subjected to freeze-drying; CP=Crude protein; SEM =standard error of the mean						

Discussion

Nutritional variations among brewers' grain samples collected from different brewery sources

In general, all chemical compositions and *in vitro* digestibility values with exception for some nutritional parameters from the present trial were found within ranges of summary of recent review findings for fresh WBG samples (Heuzé et al., 2017). However, slight deviations were noted against NRC (2001) for all measured parameters; NDF and ADF by Westendorf and Wohlt (2002) and DM on as fed basis to that reported by Senthilkumar et al. (2010). The reasons for observed variations both among local breweries and/or when these were compared to findings reported elsewhere maybe speculated to a variety of factors that include: grain and/or varietal differences among the malt grain used as foundation grain, harvesting time and the conditions under which it was cultivated; the conditions used for malting and mashing and the amount and type of the adjuncts added in mixture with the barley malt during the process of wort production. Apart from the above mentioned reasons, period of fermentation, processing techniques and analytical procedures followed may be partly responsible for the observed variations.

In agreement to the present finding, Mussatto et al. (2006) and Waters et al. (2012) found nutritional variations among WBG samples derived from brewing processes without addition of adjuncts (i.e. using

100% barley malt), but the former used Brazilian barley malt while the latter used barley malt from Ireland. Differences can also be observed when comparing the results of these authors with those reported by Meneses et al. (2013) and Carneiro et al. (2006) who used WBG derived from a process using barley malt with adjuncts obtained from two different Portuguese breweries. The variations observed in the results of these authors suggest that the differences in the source of malt grain and the brewing process conditions affected the composition of the residual WBG material. In fact, the conditions used for the brewing process (i.e., heat applied during the malting and mashing process, and the type of procedure followed for starch extraction during the wort filtration process etc.) were not reported in any of the studies reported and this is probably another factor with significant influence on the results indicated in Table 2 above.

Chemical composition and IVDOMD of brewer's grain stored using local conservation practices

Locally used storage practices conserved the BG with slight feeding value losses. Soaking is the most prominently used WBG conservation practice under on-farm conditions despite extensive losses in both feed and microbial quality compared to the other local storage methods evaluated in the current study. Sodium chloride is reported to have good anti-microbial property and often regarded as "fermentation inhibitor" in feed preservation process (McDonald et al., 1991). However, sufficient scientific literature was not found to substantiate this with the result obtained from the soaking method in the present study. In line to this, the level of salt used in the soaking process and the storage duration that optimizes proper storage of fresh WBG need further research. Lower DM loss for the ensiled sample was a good sign of the absence of any significant degradation of nutrients during the ensiling process as compared to the other two local WBG conservation practices. Higher DM loss of the soaked and sun-dried samples compared to the control (freeze-dried) and ensiled samples could probably be associated to the relatively higher fungal colony count that arise from the slow sun drying process mainly attributed to the lower solar energy and to the frequent opening of the storage container for feed removal that often induces aerobic deterioration in the soaked sample. In general, during sun drying and soaking process, residual soluble carbohydrates in WBG possibly have been converted into ethanol, CO₂ and water in the presence of large colonies of yeast and mold (McDonald et al., 1991) thereby leading to excessive loss of DM and other nutrients from the WBG. Moreover, in agreement to same author, the lower CP observed for WBG samples conserved using these two preservation practices could probably be associated to the extensive proteolysis that might have occurred during the open air storage conditions. Higher values for NDF, ADF and permanganate lignin across all the preservation practices except ensiling indicated that there were undesirable microbial activities which can be witnessed from the larger fungal colony counts in the present study, as soluble nutrients have been degraded, the proportion of fibre components tended to have proportionally increased. Likewise, lower DOMD values for the sun-dried and soaked sample, can be possibly linked to the loss in DM and other soluble nutrients and a sharp increase in cell wall fractions. In a related study Baskett et al. (2009) also reported losses in DM equivalent to 8.6% and 9.6% for wet distiller grains stored in aerobic and anaerobic storage conditions in bunker silos.

Fungal load dynamics of brewery spent grain conserved using local storage practices

Higher fungal count in the soaked sample compared to the other two local conservation practices and the control sample can be speculated to the aerobic exposure of WBG during feed removal for routine feeding on the one hand and the proportion of salt to water that might not have optimized proper fermentation partly due to lack of research recommendations for the soaking technique. Longer storage durations may also hold responsible for the larger fungal contamination seen in the soaking process. Nutrient loss from sun drying in the present study was also relatively high since drying was done under a low temperature that increased risks of mold growth and mycotoxin production (Chulze, 2010). The drying process also needs frequent turnings to ensure uniform drying and avoid secondary fermentations due to fungal contaminations. According to McDonald et al. (2002), a pH reduced to approximately 3.8-4.2 is optimum for good preservation during ensiling. The pH measurement values for the ensiled sample from the present trial ranged 3.9 to 4.1, indicating the silage from the current trial was aerobically stable. Thus, such pH value coupled with the little change in feed nutritional merits in the ensiled sample can lead to wider on-farm recommendation under local conditions. The efficiency of ensiling in WBG conservation has also been noted by some other authors (Heuzé, et al. 2017; Geron et al., 2008). In addition to ensiling, under the present context of Ethiopian farmers, sun drying offers a viable option and thus may have practical field significance provided that efficient solar driers are developed; adequate drying space and labor are available. According to the recommendation by Woolford (1990) and Dairy one (2017) the presence of yeasts and mold in a stored feed greater than 5.00 CFU/g DM, is considered undesirable leading to higher losses of DM and other essential nutrients. Hence, based on these recommendations both the yeast and mold colony counts from the present study were considerably lower implying such feeds can safely be fed to local dairy cattle. On the other hand, the average yeast and mold forming colony units (log CFU/g DM) obtained from the current study were slightly higher than the values of 2.7 and 1.8 reported earlier for aerobically and anaerobically conserved WBG using organic acids (selected lactic acid strains) (Marston et al., 2009), the variation being attributed to the strong inhibitory effect of the organic acid used in the latter case.

In Situ DM and CP degradability characteristics of brewer's grain conserved using local storage practices

Degradability constants excluding the highly variable water-soluble CP fraction from the current study lie within the ranges of values reported for fresh, dry and ensiled WBG samples (NRC, 2001; Kazemi et al., 2014; Heuzé et al., 2017). The reason for the differences in the rapidly soluble CP fraction among conservation practices in the current study is not apparent. The variation with in and across global literature in the "a" fraction can however speculated to differences in the type of WBG conservational practices employed and differences in the type of washing methods (hand Vs machine) used in each cases. In agreement to the present finding, Gao et al. (2015) also noted low repeatability for rapidly soluble ruminal and post-ruminal DM, nitrogen and amino acids fractions of three supplemental protein sources. The "c" and "ED" values of CP fraction varied greatly among the conservation practices and the control sample. This variation could in part be due to differences in the modes of such as dry, fresh or ensilage (Nocek, 1985), in which the WBG appears to have been available. The variation could have also emanated from differences in the major cell wall constituents. In agreement to the present finding, Kamalak et al. (2005) noted that in situ DM disappearance after 48 and 96 h to be negatively correlated

with NDF of the WBG samples stored using the different preservation practices. Likewise, lower NDF and ADF contents in the soaked and higher values of same fractions for the sun-dried sample might explain the reason for higher 'c' and 'ED' value for the soaked than the sun-dried sample. On the other hand, lower 'ED' and rate constant 'c' value for CP of the ensiled sample relative to the control, soaked and the dried samples in the present study provided clear evidence that the ensiled samples have had relatively lower rumen fermentable protein. Similar observations have also been reported earlier by Armentano et al. (1986) and NRC (2001). In line to this, the proportion of rumen undegradable protein (RUP) according to Goa et al. (2015) is given by $100\% - ED\%$. Thus, estimated RUP percentage in this study was 53.8, 43.7, 58.2 and 56.4% for the control, soaked, ensiled and sun-dried samples, respectively. This estimated RUP percentage for the fresh and the remaining local storage techniques from the present study was slightly higher than the mean values reported by NRC (1989) for cottonseed meal (41%) and sunflower meal (26%) but was lower than the content of RUP for dried distillers' grain (67.1%) (Kelzer et al., 2010). The higher RUP in alcoholic fermentation byproduct feeds such as brewers' grain and distillers' dry grain according to Gao et al. (2015) was attributed to heat-damaged protein usually indicated by low soluble protein contents and high acid detergent insoluble crude protein contents of the feeds obtained after the beer and/or ethanol production process.

Lower potential CP degradability (a + b) values was found for the control, ensiled and sun-dried samples compared to WBG samples preserved using the soaking method. Similar results were noted from related research work by Promkot et al. (2007). The reason could be attributed to the higher 'a' and 'ED' fraction for the soaked sample. But, in general, WBG reportedly have had both lower ruminal protein solubility and degradability (Armentano et al., 1986) although in-situ degradability results could have been affected by factors such as feed particle and sample size, bag material pore size; test animal diet and washing procedures (Nocek, 1985; Gao et al., 2015). Owing to its higher RUP and minimal feed safety issues, under local conditions ensiling can be preferred to the other local WBG conservation practices. However, further evaluation of local conservation practices for their mycotoxicological effects using biochemical tests; storage durations and level of salt in WBG soaking that optimizes both feed and microbial quality, and other efficient solar drying techniques that would enhance speedy drying process for WBG need to be explored further under local conditions. Sample from Meta Abo seem to have had superiorly higher nutritional values for major parameters over samples from remaining beer factories. Dairy producers located nearby to the breweries could possibly salvage this opportunity given that they had two or more breweries in their locality. In a second lab based study where an attempt was made to evaluate commonly used local conservation practices using major proximate compositions, digestible organic matter in the dry matter, in-situ crude protein degradability characteristics, and fungal load dynamics the ensiling technique outperformed the remaining local conservation practices of soaking and sun drying techniques. This implies that, if the current scenario for brewers' grain in the demand and supply line could be improved, the technique can somehow be scaled up for beneficiaries in the wider livestock farming community under on-farm conditions.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable

Data availability

The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Author contribution

GK, GT, and MF conceived and designed research. GK conducted experiments and analysed the data. GK and MF wrote the manuscript, with comments and revisions by GT. All authors read and approved the manuscript.

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Figures

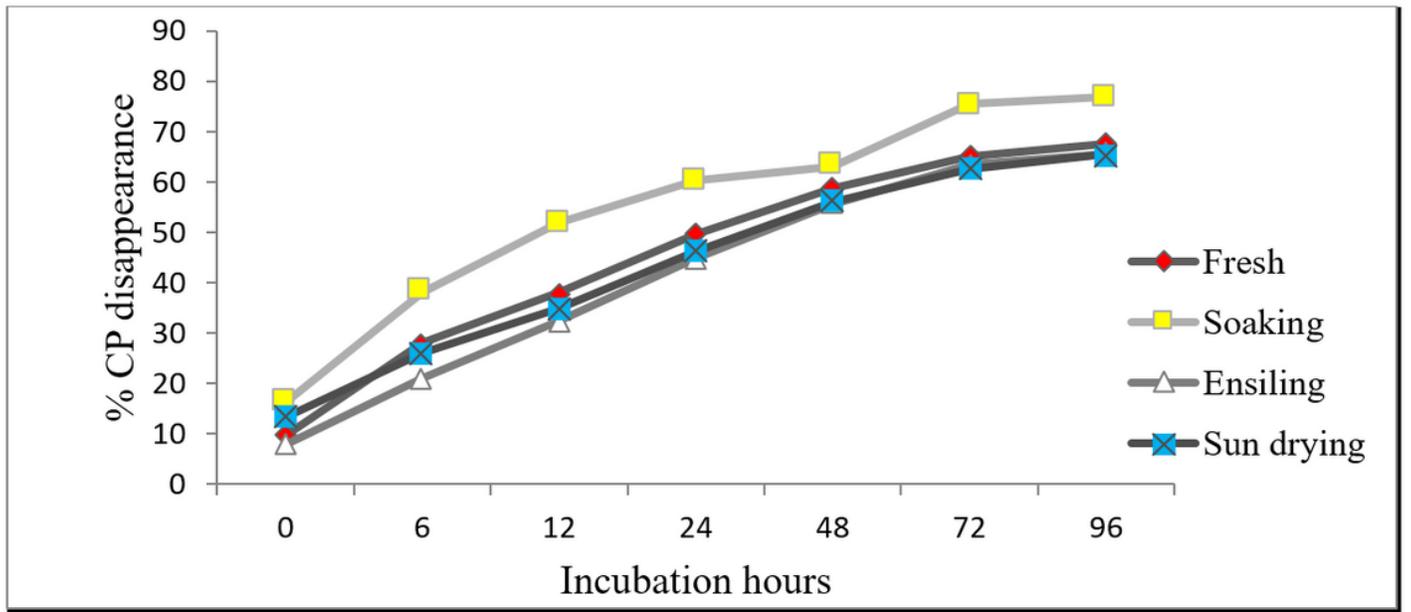


Figure 1

In situ CP disappearance characteristics of fresh Vs conserved WBG