Partial Replacement of Noug Seed Cake with Brewery Grain and Linseed Cake to Mitigate Aflatoxin in Dairy Cattle Concentrate Feed

Rehrahie Mesfin[1]*, Ulfina Galmesa[1], and Getenesh Teshome[1]

[1]Holetta Agricultural Research Center, Dairy Cattle Research, Telephone: 0112370006, Fax: 01123703

Abstract: The study was undertaken to minimize aflatoxin level of dairy cow concentrate feed by blending aflatoxin more susceptible feed ingredient (noug seed cake) with aflatoxin less susceptible feed ingredient (brewery grain and linseed cake) to reduce the aflatoxin levels in feeds and milk. The study was carried out at Holetta dairy research station using 15 mid lactating F1 Boran x Friesian crossbred dairy cows having parities of 1-5 and average weight of 450 kg. Cows were fed three diets: Treatment 1 = native hay + noug seed cake (40%) + wheat bran (58%) + salt (2%); Treatment 2 = native hay + wheat bran (42%) + brewery grain (36%) + noug seed cake (20%) + salt (2%); Treatment 3 = native hay + wheat bran (58%) + linseed cake (20%) + noug seed cake (20%) + salt (2) using Randomized Completely Blocked Design (RCBD. The feed ingredients and compound concentrate mixtures were examined for aflatoxin groups vis: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2). Milk samples were analyzed for aflatoxin M1 (AFM1), aflatoxin M2 (AFM2), aflatoxin G1 (AFG1) and aflatoxin G2 (AFG2) following the standard procedures described for analysis of aflatoxin using High Liquid Chromatography (HPLC). The results revealed that, the aflatoxin level of feeds (AFB1) of native hay and dried brewery grain was found to be zero (0 μg/kg AFB1). Wheat bran, noug cake, noug cake based concentrate mixture, brewery grain based concentrate mixture had moderate AFB1 levels of 5.25μg/kg, 3.45μg/kg, 1.12μg/kg and 4.09μg/kg respectively. Cows supplemented brewery grain based concentrate feed had the lowest level of AFM1(0.31μg/L) as compared to milk of cows supplemented noug seed cake based concentrate feed (0.43μg/L) and that of milk of cows supplemented linseed cake based concentrate feed (4.58μg/L). Cows that fed brewery grain based concentrate feed supplement had relatively higher feed intake (14.7 ± 0.05 kg), higher daily milk yield (9.1 ± 0.09 L), better milk fat (3.91 ± 0.18), higher milk total solids (12.62 ± 0.17) and better daily weight gains (0.34 ± 0.01), as compared to the conventional feed supplement which resulted feed intake (13.8 ± 0.05kg); daily milk yield (8.1 ± 0.09L); milk fat (3.87 ± 0.18%); milk total solids (12.33 ± 0.19%); and daily weight gain (0.24 ± 0.01kg) and linseed cake based concentrate feed supplement which resulted in feed intake (14.1 ± 0.07kg); daily milk yield (7.7 ± 0.1L); milk fat (3.71 ± 0.16%)and milk total solids (12.61 ± 0.18%). Therefore, dried brewery grain based concentrate feed supplement should be considered and be utilized as an important dairy feed supplement, as it provides dual benefits of minimizing aflatoxin levels and contributes to better milk production in dairy cows. Further studies should focus on aflatoxin investigation of linseed cake that is prepared from freshly harvested grain.

Keywords: *Boran x Fresian, Feed intake, Hay, Holetta, Milk composition, Milk yield, Wheat bran*

Introduction

Agriculture in Ethiopia is indispensable component of rural livelihoods. The country has good potential for dairy production and there is an increasing demand for utilization of quality and safe milk and milk products (Gian, 2006). However, dairy production is constrained by various factors like poor Genetic potential of animals, disease and poor quality of feeds (Mesfin *et al*, 2009). Feed is the most important input for sustainable livestock production (Adugna *et al*., 2012). Dairy feeds and milk in Ethiopia are contaminated with aflatoxins (Dawit *et al*., 2016; Mulugeta, 2017; Rehrahie *et al*., 2018; Lamesgn, 2020; Ftalew *et al*., 2021). The existence of aflatoxin in milk is due to feeding of dairy animals with aflatoxin contaminated feeds. Although aflatoxin appears in all commodities, there is variation among grains to aflatoxin contamination. Peanuts (0-1600 μg/kg) (Chauhan *et al*., 2010), oilseeds (0.1-399.9) (Keliani *et al*., 2014) and maize 0.7-35 μg/kg (Bhat *et al*., 2017) are the most susceptible grains to aflatoxin contamination. Cereals and pulses are moderately susceptible to aflatoxin contamination. Whereas, linseed grain containing 2.5 μg/kg AFB1 (Bhat *et al*., 2017) and sunflower seeds having 3-4 μg/kg AFB1 (Al-Musawi, 2017) were reported to be less susceptible to aflatoxin contamination (Bankole *et al*., 2010). Cristina and Gebriel (2005) also witnessed for the variation in aflatoxin levels among different grains. To mention some, soybean meal, sun flower meal and rape seed meal had aflatoxin level of 21.3-48μg/kg, 6.6-24.4

*Corresponding Author. E-mail: rehrahiemesfin@gmail.com

Published by Haramaya University, 2021

ISSN: 2616-8804 (Print); 2959-0531 (Online)

µg/kg and 6.3-12.8 µg/kg respectively. Research results in Ethiopia also indicated noug seed cake is more susceptible (362µ/kg) to aflatoxin contamination than cereal by-products/ brewery grain ((15µ/kg) (Dawit *et al*., 2016; Mulugeta, 2017). Brewery grain is important dairy feed (Getu *et al*., 2019) as it is one of the protein source feeds containing 25 - 34% CP (Chanie & Fievez, 2017), having moderate ME of 8.8 MJ/kg (Fekede *et al*., 2015) and containing lower rumen degradable protein of 35% (NRC, 2001). It is relatively cheap (Chanie & Fievez, 2017) and abundant accounting 85% of total by-products in the brewing process (Alyu and Bala, 2011).

Some pre-harvest aflatoxin prevention practices (biological control in field including good agricultural practice), post-harvest aflatoxin prevention practices (good storage practice, good feed processing practice, sorting of contaminated grains, detoxification using radiation and light treatments, pressure treatments, use of chemicals have been practicing as aflatoxin mitigating strategies across the globe (Sipose *et al*., 2021). However, for developing countries like Ethiopia, some of these practices are not technically and economically feasible to implement among smallholder livestock producers. Therefore, there is a need to generate feasible and cost-effective aflatoxin mitigating alternative interventions through research. Blending of aflatoxin less susceptible feed ingredients with aflatoxin more susceptible feeds could be one dimension of aflatoxin mitigating strategies (Genet *et al*., 2019). Therefore, the objective of this study was to determine the reduction level of aflatoxin in feeds and milk when noug seed cake is partially replaced by brewery grain and linseed cake in concentrate dairy feed supplements.

Materials and Methods

Study Area

The study was undertaken at Holetta dairy research station. The station is located 45 kms Western of Addis Ababa, the capital city of Ethiopia and situated at 93^{o} N latitude and 38^{o} 30' E longitude at an altitude of 2400 masl. The mean annual rainfall is 1000 mm and the mean minimum and maximum temperatures are 6^{o}C and 22^{o}C, respectively (metrology data of Holetta Agricultural Research Centre).

Selection and Management of Experimental Cows

Cows were selected based on their breed, parity and stage of lactation so that they can stay on milking for 120 days. A total of 15 lactating F1 Boran x Friesian crossbred cows with different parities (1-5) targeted to exclude aged cows having parity of greater than six. The cows were almost in similar stage of lactation (3 in early lactation and 12 in mid lactation) having average weight of 450 kg (400-500 kg). Cows were managed on install bases with daily exercises for 15 minutes. The experimental cows were dewormed for internal parasites with Albendazole one week before the start of the experiment.

Experimental Diets/Treatments

The experimental diets are mentioned below and each treatment was allocated randomly to five experimental cows.

1. Treatment 1 = *Ad libitum* native hay + noug seed cake (40%) + wheat bran (58%) + salt (2%)
2. Treatment 2 = *Ad libitum* native hay + wheat bran (42%) + brewery grain (36%) + noug seed cake (20%) + salt (2%)

Treatment 3 = *Ad libitum* native hay + wheat bran (58%) + linseed cake (20%) + noug seed cake (20%) + salt (2%)

Experimental Design and Statistical Model

The experiment was handled in a Randomized Completely Block Design (RCBD) by blocking the variations coming from parity difference and the statistical model was:

$$Yij = \mu + Ti + Pj + eij$$

Where; Yij = the observation for ith treatment and Pth parity

µ = Overall mean
Ti = Effect of the experimental feeds
Pj = Effect of parity
eij = error term

Preparation of Experimental Feeds and Feeding

The experimental feeds were formulated based on daily nutrient requirements of the experimental cows. The requirements of total feed intake, crude protein (CP), metabolizable energy (ME) was taken from the standard table indicated in Kearl (1982). Nutrient composition of the feed ingredients was taken from laboratory investigation of Holetta Feeds and nutrition research program (Table 1). "Double Pearson's Square method" was used to formulate the experimental diets. The experimental feeds were formulated to have similar content of metabolizable energy (123 MJ/d) and crude protein (CP) content of 1534 g/d and were calculated for the requirements of an average body weight of 450 kg (400 kg-500 kg) milking cow (Kearl, 1982). Fresh and wet brewery grain from Sebeta Meta Abo brewery factory was collected and delivered to Holetta research center. Just upon arrival, wet brewery grain was drained to remove the water and followed by sun drying. During the drying process salt was added to retard growth of fungus in the feed and proper follow-up and checking was imposed to ensure complete drying before subjecting to feed formulation. The dried brewery grain was thoroughly mixed with wheat bran and noug seed cake based on the planned proportion. The daily concentrate supplement allowance to each experimental cow was divided in to two equal portions and offered during milking at 5:00 AM and 5:00 PM in the morning and in the afternoon. Hay prepared from natural pasture was offered daily at ad-libitum bases and drinking water was available at all times in their barn.

Table 1. Nutrient composition of feed ingredients used for formulation of experimental diets.

Feed ingredients	CP (g/kg feed)	ME (MJ/kg feed)
Hay	64	75
Wheat bran	177	114
Noug seed cake	308	102
Brewery grain	226	81
Linseed cake	280	81

CP= Crude protein; ME= Metabolizable energy.

Table 2. Proportion (%) of feed ingredients in 100 kg concentrate mixture**.**

Feed ingredients	Conventional concentrate mix	Brewery based concentrate mix	Linseed cake based concentrate mix
Wheat bran	58	42	58
Noug seed cake	40	20	20
Brewery grain	-	36	-
Linseed cake	-	-	20
Salt	2	2	2
	100	100	100

Measurements and Recording

The feed offer (hay), refusal and concentrate were weighed and provided and recorded every day in the morning. Milk yield of the experimental cows were measured and recorded in the morning and in the afternoon and total milk yield was recorded daily. Milk samples for laboratory analysis was collected from each experimental cow ones in a month using 100 ml capacity polyethylene plastic bottles and were analyzed for milk fat, protein, lactose, total solids and Ash. The experimental cows have been weighed during the start of the experiment and every 15 days thereafter.

Sample Collection and Aflatoxin Analysis

To make samples representative, sub-samples from different parts of the bulk of brewery grain was taken randomly and the sub-samples were properly mixed. One kg composite feed sample was taken in a clean paper bag and sealed immediately to avoid cross contamination. The samples were kept in a cool and dry place at room temperature in order to minimize growth of fungi. Similar trend of sampling procedures (AOAC, 2016) was employed for sampling the compounded concentrate mixture (table 2) belonging to each experimental feed. Samples were grinded and passed through a 0.25-mm mesh PVC sieve and submitted for laboratory analysis. Then, samples were investigated for AFB1, AFB2, and AFG1and AFG2. Aflatoxin in feeds was investigated in Bless Agro-processing laboratory following standard procedures described for analysis of aflatoxin in feeds using High Liquid chromatography (HPLC). With regard to milk, samples from morning and afternoon milking of each experimental cow were collected for one week and finally polled based on treatment bases. Then 100 ml of milk was sampled from the polled milk and taken in to polyethylene plastic bottles and submitted for analysis AFM1, AFM2, AFG1 and AFG2 following the standard procedures of HPLC (McMaster, 2007) described for analysis of raw whole milk in the laboratory of animal products, veterinary drugs and feed quality assessment center located at Kaliti. The USA standards for aflatoxin maximum limits in feed (i.e. 20μg/kg) and in milk (i.e. 5μg/lt) were used as references for comparison.

Statistical Analysis

To control the variations in milk yield coming due to differences in lactation stage of experimental cows, initial daily milk of experimental cows was subjected in to covariate analysis. Data of feed intake, milk yield and milk compositions were analyzed using General Linear Model (GLM) procedures in SAS, 2002 software. With regard to results of aflatoxin in feeds and in milk, data generated from the laboratory investigation were described in tables as they are received from the laboratory.

Results and Discussion

Level of Aflatoxin in Feeds

The level of aflatoxin in feeds (AFB1) is indicated in table 3. Though results of aflatoxin examination are reported in terms of four common aflatoxin groups vis: Aflatoxin B1, Aflatoxin B2, Aflatoxin G1 and Aflatoxin G2, the discussions are lying on Aflatoxin B1. The reason is, aflatoxin B1 is common and dominant and was recognized by the International Agency for Research on Cancer as one of the most naturally occurring toxic and carcinogenic substances (Feddern *et al.*, 2013). The AFB1 level of native hay and brewery grain in this study was 0μg/kg (free of aflatoxin) which was lower than the AFB1 level of hay examined in samples collected from Hawassa which had very low level of aflatoxin of 0.13 ± 1.7μg/kg (Rehrahie *et al.*, 2018). The main reasons could be due to proper drying, good storage conditions and short storage duration of less than 3 months employed to this feed resource. Dried brewery grain in this study was also found to be free of aflatoxin (0μg/kg), which was lower than the AFB1 level of dried brewery yeast (15.4μg/kg) reported by Dawit *et al.* (2016). This is because, wet brewery grain just after receiving from the brewing factory was carefully drained and then properly dried with full follow up of attendants. The other reason is after drying, brewery grain was stored for shorter duration of time. The inclusion of salt in the drying process of brewery grain also contributed to retardation of mold growth. Wheat bran in this study contained AFB1 level of 5.25µg/kg which was lower than the AFB1 level reported by Dawit *et al.* (2016) in samples collected from Sendafa, Sululuta, Debrezeit and Sebeta which was 15.6µg/kg. This is because; wheat bran before purchase was not stored for longer period of time in the flour factory. In addition, wheat bran after purchase was not stored in the dairy farm for longer period of time and was utilized for feeding. It is known that the time length of storage matters for formation of aflatoxin in agricultural products (grain or feeds). This is evidenced by the findings of Wantanwa *et al.* (2017), in which the AFB1 level of commercial dairy concentrate feeds and corn by-products before storage was 5.1, 4.1, 4.0, 4.2, 5.5 and 5.5 µg/kg, respectively, and was 9.7, 6.5, 9.8, 12.3, 11.4 and 20.0 µg/kg, respectively after storage for one month. The AFB1 content of noug cake in this study (3.45µg/kg) was lower than the AFB1 level of noug seed cake reported by Dawit *et al.* (2016) in samples collected from Sendafa, Sululuta, Debrezeit and Sebeta which was 362 ± 3.8µg/kg.

Table 3. Level of Aflatoxin B1 (AFB1) (µg/kg DM) in feeds.

Feed ingredient/experimental diet	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Total aflatoxin
Native hay	0	0	0	0	0
Wheat bran	5.25	0	1.19	0	6.44
Noug cake	3.45	0	2.1	0	5.55
Linseed cake	146.5	3.23	53.85	0	203.58
Brewery grain	0	0	0	0	0
Noug seed cake based concentrate supplement	1.12	0	0.57	0	1.69
Brewery grain based concentrate supplement	4.09	0	2.77	0	6.86
Linseed cake based concentrate supplement	211.08	7.98	27.13	0.51	246.7

Except the AFB1 levels in hay, brewery grain, and noug seed cake based concentrate feed supplement, the AFB1 levels of the other feed ingredients used in this study (table 3), had AFB1 levels higher than the one reported in maize by-product (1.21 µg/kg) in Kenya (Oduho *et al.*, 2013). Except for linseed cake, the other feed ingredients used in this study, contained lower levels of AFB1 (table 3) which was lower than the AFB1 level of dairy feed concentrate (by-products of maize) reported in Kenya by Senerwa *et al.* (2016) which was 107.34 µg/kg. Linseed cake and linseed cake based concentrate compounded feed supplement used in this study contained higher levels of AFB1 of 146.5µg/kg and 211.08µg/kg respectively. The reason could be partially due to having longer storage duration of 1 year kept in feed retailers before purchase. Though, no reports are available on aflatoxin content of linseed in Ethiopia, the aflatoxin level of linseed cake (146.5µg/kg) and linseed cake based concentrate mixture (211.08 µg/kg) in this study is lower than the aflatoxin level of flax seed reported by Ting *et al.* (2020) in United States and 120- 810 µg/kg reported by Sahay *et al.* (2006). The level of AFB1 in wheat bran in this study (5.25 µg/kg) was lower than the level reported by Mulugeta *et al.*, 2017 (21.55µg/kg) studied in Sululuta, Debrezeit and Debrebrhan. It was also lower than the level of wheat bran reported by Dawit *et al.*, 2016 (15.6µg/kg) studied in Sendafa, Sululuta, Debrezeit and Sebeta.

Previous reports indicated that, noug-seed cake is susceptible to aflatoxin contaminations and cows that fed noug seed based diet has produced milk which is contaminated by aflatoxin (Dawit *et al.*, 2016). Unfortunately, the level of AFB1 in noug cake in this study was found to be lower (3.45 µg/kg) than the levels reported by Mulugeta *et al.*, 2017 which was 293.99µg/kg in Debreberhan, 302.96 µg/kg in Debrezeit and 408.63 µg/kg in Sululuta), Ethiopia.

With the exception of linseed cake, the level of AFB1 in dairy feeds vis: wheat bran=5.25µg/kg, noug cake=3.45µg/kg, brewery grain=0µg/kg in this study (table 3) was lower than the level of AFB1 examined in sorghum in North Showa Zone, Kiwet=3.95 - 153.72µg/kg (Geremew *et al.*, 2015) and in sorghum in South, East and Northwest Ethiopia=29.5µg/kg (Alemayehu *et al.*, 2014). With regard linseed cake, although information on aflatoxin level of linseed cake is limited; Bhat *et al.* (2017) reported low level of aflatoxin of 2.5µg/kg in linseed after 8 months of storage.

Level of Aflatoxin in Raw Whole Milk

The level of aflatoxin in raw whole milk (AFM1) is indicated in table 4. The range of AFM1in milk in this study (0.31-4.58 µg/L) was similar to the AFM1 level of milk (0.028-4.98 µg/L) reported by Dawit *et al.* (2016). The AFM1 level of milk of cows that fed brewery grain based concentrate feed supplement in this study was relatively lower (0.31µg/L) than the milk of cows that fed noug seed cake (0.43µg/L) and linseed cake based concentrate feed supplements (4.58 µg/L). The AFM1 level of milk of cows that fed the three experimental feeds supplements vis: brewery grain based concentrate feed (0.31µg/L), noug seed cake based concentrate feed (0.43µg/L) and linseed cake based concentrate feed (4.58µg/L) was lower than the

AFM1 level of milk of cows that fed by products of maize, rice, wheat, sorghum, cotton seed and sunflower reported by Senerwa *et al.* (2016) in Kenya in which the AFM1 level was 30.94 μg/L. It was also lower than the AFM1 level of milk of cows that fed by-products of maize reported by Kang'ethe & Lang'a (2009) in Kenya in which the AFM1 level was 74.95 ± 81.9μg/L.

Unlike to this, the AFM1 level of milk in this study was higher than that reported earlier by Jalel *et al.* (2022) from Oromiya, Finfinne special Zone (0.02-0.08μg/L) and Yohannes *et al.* (2018) from Guragie Zone (0.0.2-0.31 μg/L). The average AFM1 level of milk of cows fed the three experimental diets in this study (1.77μg/L) was also higher than the average AFM1 level of milk (0.55μg/L) reported by Ftalew *et al.* (2021) in South Gondar.

Although the AFB1 level of feeds in linseed and linseed cake based diet in this study was high (above the permitted level AFB1 for feeds recommended by USA, 20μg/kg), the AFM1 level of milk of cows fed linseed cake based diet did not pass the limit of AFM1 recommended by USA (0.5μg/L). This happened because of the fact that ruminant animals can possess detoxification ability through their rumen microorganisms, and these microbes are able to adapt and utilize such toxic secondary metabolites like aflatoxins (Loh *et al.*, 2020).

Effects of Experimental Diets on Feed Intake and Daily Milk Yield

The Effects of experimental diets on feed intake and milk yield is presented in table 5. Dietary feed treatments did not brought significance difference (P > 0.05) on daily feed intake and milk yield. Though experimental feeds did not show significance difference (P > 0.05) on daily milk yield, cows fed brewery grain based concentrate feed supplement produced the highest amount of milk yield (9.1 ± 0.09 L) as compared to cows fed the conventional concentrate feed supplement (8.4 ± 0.09 L) and milk of cows that fed linseed cake based concentrate fed cows (7.7 ± 0.1L). The state of being brewery grain to be better contributor to higher milk production has similarity with the findings of Getu (2019) in that with increasing proportion of brewery grain in the concentrate mixture from 33%, 76% and 100% replacements to cottonseed cake, the milk yield has increased from 13.8 L, to 14.3 L and 14.5 L, respectively. Getu (2019) also indicated that the inclusion of brewery grain in the concentrate mixture has contributed to the increment of DM and nutrient digestibility of dairy diets. Results of this study regarding brewery grain have agreement with results of Al-Talib *et al.* (2014) in that inclusion of brewer grain in the dairy cow diet contributes to increased milk yield. This is because brewery grain has lower level of rumen degradable protein (35%), which contributed to increased amount of microbial protein and amino acids in the intestine which lead to increased level of feed utilization and results to increased level of milk production. Fekede *et al.* (2015) also reported the importance of brewer grain as appropriate dairy feed as it maintains the recommended nutritional supply (17-19% CP) to dairy cattle. The dietary feed treatments had resulted to significance differences (P< 0.05) on daily weight gain, in which cows fed brewery grain based concentrate feed showed (0.34 ± 0.01kg) the highest weight gain as compared to cows fed the conventional feed supplement (0.24 ± 0.01kg) and linseed cake based concentrate feed supplement (-0.02 ± 0.01kg). Though the feed intake of cows that fed linseed cake based concentrate feed supplement was almost similar (14.1 ± 0.07kg/d) to those cows that fed the conventional feed supplement (13.8 ± 0.05kg/d) and brewery grain based concentrate feed supplement (14.7± 0.05kg/d), the corresponding milk yield and daily weight gain respectively of cows that fed linseed cake based concentrate feed supplement was lower (7.7 ± 0.1L and 0.02 ± 0.01kg) than cows that fed the conventional feed supplement (8.1 ± 0.09L and 0.24 ± 0.01kg) and cows that fed brewery grain based concentrate feed supplement (9.1 ± 0.09 and 0.34 ± 0.01kg). The reason that cows that fed linseed cake based concentrate feed supplement producing lower milk yield and daily weight gain (7.7 ± 0.1L and 0.02 ± 0.01kg) could be related to the fact that linseed cake based concentrate feed had contained higher aflatoxin level than the conventional and brewery grain based concentrate supplement (table 2) and also because of the fact that aflatoxin contaminated feeds can affect productivity of milk (Bennett and Klich, 2003; Bhat and Vasanthi 2003; Dhanasekaren *et al.*, 2011. This is because, aflatoxin can reduce the metabolizable energy and crude protein content of feeds and justified by the report that, as compared to non-moldy maize (ME 3.41 Kcal/kg, CP 89 g/kg and fat 40 g/kg), the ME, CP and fat content of moldy maize respectively was 3.25 Kcal/kg, 83 g/kg) & 15 g/kg) and the loss in nutritive value was 4.6%, 6.7% & 62.5% for ME, CP and fat content respectively (Akande *et al.*, 2006).

Table 4. Level of Aflatoxin M1 (AFM1) (μg/lt) in milk.

Experimental diet	Aflatoxin M1 (μg/lt)
Noug seed cake based concentrate supplement	0.43
Brewery grain based concentrate supplement	0.31
Linseed cake based concentrate supplement	4.58

Table 5. Effect of experimental diets on feed intake and daily milk yield (DMY) (LS-means and SE).

Daily feed intake (Kg) & milk yield (L)	Experimental diets			P-value
	Conventional concentrate	Brewery based concentrate	Linseed based concentrate	
Native hay	9.5 ± 0.05	10.1 ± 0.04	9.9 ± 0.05	0.2712
Concentrate	4.4 ± 0.02	4.6 ± 0.02	4.3 ± 0.03	0.5727
Total feed	13.8 ± 0.05	14.7± 0.05	14.1 ± 0.07	0.3478
Milk yield	8.1 ± 0.09	9.1 ± 0.09	7.7 ± 0.1	0.6769
Daily weight gain (kg)	0.24 ± 0.01	0.34 ± 0.01	-0.02 ± 0.01	0.0418

Brewery grain based concentrate feed supplementation in this study brought dual benefit of minimizing aflatoxin level in feeds and milk and also availed alternative and highly nutritive feed supplementation to dairy cows. Result of this study is similar with the report of Tesfaye *et al.* (2015) in which case supplementing basal diets with breweries dried grain based concentrate mixture could be regarded as an alternative dairy feed supplements to provide adequate and required amount of CP and ME to dairy cows. Although different literatures mention various aflatoxin mitigation strategies such as Good Agricultural Practices (GAP) (Codex alimentations, 2014), use of organic acids as fungal growth inhibitors (Lee *et al.*, 2015; Rastegar *et al*, 2017), use of afla-binders during feeding (Feddern *et al.*, 2013), use of biocontrol agents like lactic acid bacteria (Tsige and Alemayehu, 2017) and "AflaSafe" (Food Business Africa, 2017). This study, however, exceptionally tested the use of different feed ingredients in dairy feed to reduce aflatoxin level in formulated feeds and consequently in milk, that generates another alternative of overcoming aflatoxin problems and adds up to the already existing aflatoxin mitigation strategies.

Effects of Experimental Diets on Milk Composition

The Effects of experimental diets on milk composition is presented in table 6. Though experimental feeds did not show significant difference ($P > 0.05$) on milk composition, the fat content of milk of cows that fed breweries dried grain based concentrate mixture had shown a tendency of increased fat percent (3.91 ± 0.18) as compared to cows fed the conventional concentrate feed (3.87 ± 0.18) and linseed cake based concentrate feed supplements (3.71 ± 0.16). This trend has similarity with the results of Getu (2019) in that, with the replacement of 33% brewery grain in the concentrate mixture, milk fat percentage was slightly higher (4.1%) than that of cows that fed the conventional concentrate supplement (4.07%). Similarly, the total solids content of milk of cows that fed breweries dried grain based concentrate mixture had shown an increasing trend (12.62 ± 0.17) as compared to cows fed the conventional concentrate feed supplement (12.33 ± 0.19). A similar trend was observed in Kliem *et al.* (2016) that reported a little bit increment in milk fat from 3.71% to 3.74% was observed when cows were fed concentrate supplementation without linseed to linseed fortified concentrate feed supplementation respectively. A similar trend was also observed in Egger *et al.* (2007) in that, little increment in milk fat from 4.09% to 4.15% was observed when cows were supplemented concentrate feed without linseed meal to concentrate supplementation containing linseed meal respectively. With regard to milk protein, Jahani-Moghadam *et al.* (2015) reported that Holstein dairy cows supplemented with extruded linseed meal has produced relatively higher milk protein (3.28%) than cows supplemented with palm oil based concentrate feed containing no linseed meal (3.22%). Contrary to this, the addition of 1.7% fat from oilseeds to dairy cow diet (containing alfalfa hay, alfalfa silage and barley) resulted in reduced percent of milk fat and protein in dairy cow milk (Johnson *et al.*, 2002).

Table 6. Effect of experimental diets on milk composition (LS-means and SE).

Milk composition (%)	Conventional concentrate	Brewery based concentrate	Linseed based concentrate	P-value
Fat	3.87 ± 0.18	3.91 ± 0.18	3.71 ± 0.16	0.8207
Protein	3.07 ± 0.03	3.20 ± 0.03	3.28 ± 0.04	0.1348
Ash	0.70 ± 0.01	0.72 ± 0.01	0.72 ± 0.01	0.3102
Lactose	4.63± 0.04	4.77± 0.06	4.89± 0.05	0.2482
Solid non-fat	8.46 ± 0.07	8.72 ± 0.07	8.90 ± 0.09	0.2595
Total solids	12.33 ± 0.19	12.62 ± 0.17	12.61± 0.18	0.7040

Conclusion

Native hay and dried brewery grain contained zero level of aflatoxin (0μg/kg). Wheat bran, brewery grain, brewery grain based concentrates, noug cake and noug cake based concentrate feed supplements contained lower level of aflatoxin. Linseed cake and linseed cake based concentrate mixture contained the highest level of aflatoxin. Despite the presence of aflatoxin at a level above the USA recommended limit in the experimental cows feed, the level of aflatoxin in their milk was not above the USA recommended maximum limit. Among the experimental concentrate feeds, brewery grain based diet had the least level of aflatoxin, the highest feed intake, highest milk yield, and daily weight gain. It can be concluded that dried brewery grain based concentrate mixture become one alternative source of dairy feed supplements comprising dual benefits of minimizing aflatoxin level and contributes to production of better milk yield. Further studies should focus on aflatoxin investigation of linseed cake that is prepared from newly harvested linseed grain.

Acknowledgments

The authors are grateful to the Ethiopian Institute of Agricultural Research (EIAR) for budgeting finance to undertake the study and the farm workers for handling the experiment. We are grateful for Veterinary Drug and Animal Feed Administration and Control Authority (VDFACA) especially Dr. Belachew Tefera, for allowing our milk samples investigated for aflatoxin in their center and the laboratory technicians for doing the laboratory work. We also thank Bless Agro-processing laboratory for careful investigation of the feed samples for aflatoxin contaminations.

Conflict of Interests

The authors declare that they have no competing interests.

References

Adugna Tolera, Alemu Yami, Alemayehu Mengistu, Dawit Alemu, Diriba Geleti, Getnet Aseffa, Lema Gizachew, Seyoum Bediye & Yirdaw Weldesemayat (2012). Livestock feed resources in Ethiopia: Challenges, opportunities and the need for transformation. In: Adugna Tolera, Alemu Yami and Dawit Alemu (Eds.), Ethiopian Animal Feed Association (EAFA).

Akande, K.E., Abubakar, M.M., Adegbola, T.A. & Bogoro, S.E. (2006). Nutritional and health implications of mycotoxins in animal feeds: A review. *Pakistan Journal of Nutrition*, 5: 398-403.

Alemayehu Chala, Wondimeneh Taye, Amare Ayalew, Krska, R., Sulyok, M. & Logrieco, A. (2014). Multimycotoxin analysis of sorghum (*Sorghum bicolor* L. Moench) and finger millet (*Eleusine coracana* L. Garten) from Ethiopia. *Food Control,* 45: 29-35.

Al-Musawi, A.T. (2017). Detection of mycotoxins in sunflower seed intended or edible for human consumption flower. *International Journal of Advanced Biological Research (IJABR).* 7:278-281.

AL-Talib, A.A., AL-Khashab, A.T. & Almahdawi, M.K. (2014). Effect of introducing dried brewers grains in diets of dairy cows on milk production and its composition. *Euphrates Journal of Agriculture Science,* 6: 50-64.

Alyu, S. & Bala, M. (2011). Brewer's spent grain: A review of its potentials and applications. *African Journal of Biotechnology*, 10: 324-331. DOI: 10.5897/AJBx10.006.

AOAC (2016). Official methods of analysis, Association of Official Analytical Chemists International, 18th ed.

Bankole, S. A., Adenusi, A. A., Lawal, O. S. & Adesanya, O. O. (2010). Occurrence of aflatoxin B1 in food products derivable from 'egusi' melon seeds consumed in southwestern Nigeria. *Food Control*, 21: 974-976.

Bennett, J.W. & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews,* 16: 497-516.

Bhat, R.V. & Vasanthi, S. (2003). Mycotoxin food safety risk in developing for food, agriculture and environment. Food safety in food security and food trade, International Food Policy Research Institute (IFPRI).

Bhat, R., Ravindra, K. & Reddy, N. (2017). Challenges and issues concerning mycotoxins contamination in oil seeds and their edible oils: Updates from last decade. *Food chemistry,* 215: 425-437.

Chanie, D. & Fievez, V. (2017). Review on preservation and utilization of wet brewery spent grain as concentrate replacement feed for lactating dairy cows. *Journal of Animal Health and Production*, 5: 10-13.

Chauhan, Y.S., Wright, G.C., Rachaputi, R.C.N., Holzworth, D., Krosch, S. & Robertson, M.J. (2010). Application of a model to assess aflatoxin risk in peanuts. *Journal of Agricultural Science*, 148:341-351.

Codex Alimentarius (2014). Code of practice for the prevention and reduction of mycotoxin contamination in cereals, CAC/RCP51-2003. Codex Alimentarius, Rome, Italy.

Cristina, T. & Gebriel, S. (2005). Assessment of mycologic and mycotoxicologic contamination of soybean, sunflower and rape seeds and meals during 2002 – 2004. *Archiva Zootechnica*, 8: 5156.

Dawit Gizachew, Barbara, S., Azage Tgegne, Hanson, J. & Grace, D. (2016). Aflatoxin analysis of dairy feeds in the greater Addis Ababa milk shed, Ethiopia. *Food Control*, 59:773-779.

Dhanasekaren, D., Shanmugapiriya, S., Thajuddin, N. & Panneerselvam, A. (2011). Aflatoxins and aflatoxicosis in humans and animals. In: Ramon G. Gueivara-Gonzalez (Ed.), Aflatoxins-biochemistry and molecular biology, InTech. *Available at*: http://dx.doi.org/10.5772/896.

Egger, P., Holzer, G., Segato, S., Werth, E., Schwienbacher, F., Peratoner, G., Andrighetto, I. & Kasal, A. (2007). Effects of oilseed supplements on milk production and quality in dairy cows fed a hay-based diet. *Italian Journal of Animal Science*, 6: 395-405.

Fekede Feyisa, Getu Ktaw & Getnet Assefa (2015). Nutritional qualities of agro-Industrial by-products and local supplementary feeds for dairy cattle feeding. *Ethiopian Journal of Agricultural Sciences*, 26: 13-26.

Feddern, V., Dors, C., Tavernari, F.C., Mazzuco, H., Cunha Jr. A., Krabbe, E.L. & Scheuermann, G.N. (2013). Aflatoxins-recent advances and future prospects. In: R-A. Mehdi (Ed.), Aflatoxins importance on animal nutrition, InTech, Iran, pp: 408. *Available at*: http://dx.doi.org/10.5772/51952.

Fekede Feyissa, Getu Kitaw & Getnet Assefa (2015). Nutritional qualities of agro-industrial by-products and local supplementary feeds for dairy cattle feeding. *Ethiopian Journal of Agricultural Sciences,* 26 (1): 13-26.

Food Business Africa (2017). Africa food and beverage industry Megazine. Posted in industry special reports, Nairobi, Kenya.

Ftalew Tadele, Addisu Melak, Bruk Demissie, Chalachew Yenew, Megebu Legesse, Tgst Tefera, Teka Obsa, Ermyas Sisay, Markeshaw Truneh, Tabarak Malik & Tadsse Asamnew (2021). Occurrence and associated factors of aflatoxin M1 in raw cow milk in South Gondar Zone, north west Ethiopia, 2020. *Food Science and Nutrition,* 9: 6286–6293.

Genet Mengesha, Tilahun Bekele & Ashagrie Zewdu (2019). Level of aflatoxin in dairy feed, poultry feeds and feed ingredients produced by feed factories in Addis Ababa, Ethiopia, MSc Thesis, Addis Ababa University, Ethiopia.

Geremew Tassew, Tilahun Bekele & Ashagrie Zewedu (2015). Study on *Aspergillus* species and Aflatoxin levels in sorghum (*Sorghum bicolor* L.) stored at different period and storage system in Kewet Districts, Northern Shewa, Ethiopia, MSc Thesis, Addis Ababa University, Ethiopia.

Getu Kitaw, Birhan Tamir, Getnet Assefa & Getachew Animut (2019). Production, preservation, and utilization patterns of brewery grain, *Ethiopian Journal of Agricultural Sciences*, 28:1-17.

Gian, N. F. (2006). Promoting milk quality in smallholders' cooperatives: Evidence from Ethiopia. In: Tadelle Dessie (Ed.), Institutional arrangements and challenges in market-oriented livestock agriculture in Ethiopia, Part I: Plenary Session, Proceedings of the 14th Annual conference of the Ethiopian Society of Animal Production (ESAP), held in Addis Ababa, Ethiopia, pp: 69-82.

Jahani-Moghadam, M., Mahjoubi, E. & Dirandeh, E. (2015). Effect of linseed feeding on blood metabolites, incidence of cystic follicles, and productive and reproductive performance in fresh Holstein dairy cows. *Journal of Dairy Science*, 98:1828–1835.

Jalel Fikadu, Berhan Tamir, Ulfina Galmessa & Kefena Effa (2022). Feed quality, prevalence of aflatoxin contamination in dairy feed and raw milk in Oromia Special Zone surrounding Finfinne, Ethiopia. *Asian Journal of Dairy and Food Research*, 41: 8-14.

Johnson, K. A., Kincaid, R. L., Westberg, H. H., Gaskins, C. T., Lamb, B. K. & Cronrath, J. D. (2002). The effect of oilseeds in diets of lactating cows on milk production and methane emissions. *Journal of Dairy Science,* 85:1509–1515.

Kang'ethe, E.K. & Lang'a, K.A. (2009). Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya. *African Health Sciences*, 9 (4): 218-226.

Kearl, L.C. (1982). Nutrient requirement of ruminants in developing countries, International Feed Stuffs Institute, Utah Agricultural Experiment Station, Utah State University, Logan, Utah 84322, USA.

Kliem, K. E., Humphries, D. J., Reynolds, C. K., Morgan, R. & Givens, D. I. (2016). Effect of oilseed type on milk fatty acid composition of individual cows, and also bulk tank milk fatty acid composition from commercial farms. *Animal*, 11:354–364. https://doi.org/10.1017/S1751731116001403.

Lamesgen, Y. (2020). Aflatoxines contaminations levels in animal feeds and milk around Addis Ababa, Ethiopia. *Academic Research Journal of Agricultural Science and Research,* 8: 174-188.

Lee, J., Her, J.Y. & Lee, K.G. (2015). Reduction of aflatoxins (B_1, B_2, G_1, and G_2) in soybean-based model systems. *Food Chemistry*, 189: 45-51. DOI: 10.1016/j.foodchem.2015.02.013.

Loh, Z.H., Ouwerkerk, D., Klieve, A.V., Hungerford, N.L. & Fletcher, M.T. (2020). Toxin degradation by rumen microorganisms: A review. *Toxins*, 12, 664. doi:10.3390/toxins12100664.

Mesfin Dejene, Seyoum Bediye, Getu Kitaw & Aemiro Kehaliw (2009). On-farm evaluation of lactating local (*Bos indicus*) dairy cows fed urea-treated wheat (*Triticum aestivum*) straw supplemented with soluble protein source-based concentrate mixture during the dry season in crop-livestock production system of south west shoa, Ethiopia. In: Zelalem Yilma and Aynalem Haile (Eds.), Climate change, livestock and people: Challenges, opportunities, and the way forward, Proceedings of the 17th Annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, pp: 239-252.

Mulugeta Fikre, Emana Getu & Tilahun Bekele (2017). Study on level of aflatoxin in dairy cattle feeds and asses knowledge, attitude and practice of food producers, dairy farmers and feed traders around Addis Ababa, Ethiopia, MSc Thesis, Addis Ababa University, Ethiopia.

NRC (National Research Council) (2001). Nutrient requirements of dairy cattle 7th.rev.ed., Washington, D.C. National. Academy Sciences Press.

Oduho, G.W., Masinde, J.W., Lusweti, F.N., Kitilit, J.K. & Nagaira, V.M. (2013). Assessment of aflatoxin in dairy feeds, milk, blood and evaluation of mitigation measures in north of Rift Valley counties. *African Journal of Education, Science and Technology*, 1: 1-7.

Rastegar, H., Shoeibi, S., Yazdanpanah, H., Amirahmadi, M., Khaneghah, A.M., Campagnollo, F.B. & Sant Ana, A. S. (2017). Removal of aflatoxin B1 by roasting with lemon juice and/or citric acid in contaminated pistachio nuts. *Food Control*, 71:279-284. DOI: 10.1016/j.foodcont.2016.06.045.

Rehrahie Mesfin, Getnet Assefa & Fassil Assefa (2018). Determination of aflatoxin in dairy feeds and milk in some selected areas of Ethiopia. *Journal of Food* and *Environment safety*, 17: 286-299.

Sahay, S.S., Parasad, T. & Sinha, K.K. (2006). Post-harvest incidence of aflatoxins in linum usitatissimum seeds. *Journal of the Science of Food and Agriculture*, 53: 169-174.

SAS (Statistical Analysis Systems) (2002). SAS Institute Version 9.0. SAS Inc., Cary, North Carolina, USA.

Senerwa, D.M., Sirma, A.J., Mtimet, N., Kang'ethe, E.K., Grace, D. & Lindah, J.F. (2016). Prevalence of aflatoxin in feeds and cow milk from five counties in Kenya. *African Journal of Food, Agriculture, Nutrition and Development*, 16: 311004–11021.

Sipos, P., Peles, F., Brassó, D.L., Béri, B., Pusztahelyi, T., Pócsi, I. & Gy˝ori, Z. (2021). Physical and chemical methods for reduction in aflatoxin content of feed and food. *Toxins,* 13 (3): 204. https://doi.org/10.3390/toxins13030204.

Ting, W. T. E., Chang, C., Szonyi, B. & Dawit Gizachew (2020). Growth and aflatoxin B1, B2, G1, and G2 production by *Aspergillus flavus* and *Aspergillus parasiticus* on ground flax seeds (*Linum usitatissimum*). *Journal of Food Protection*, 83: 975-983.

Tesfaye, F., Getnet Aseffa & Mengistu Urge (2015). Effect of supplementation with different proportions of breweries dried grain and maize bran mixtures on the performance of crossbred dairy cows. *International Journal Technology Enhancements and Emerging Engineering Research*, 3: 1-7.

Tsige Shigute & Alemayehu P. Washe (2017). Reduction of aflatoxin M1 Levels during Ethiopian traditional fermented milk *(Ergo)* production. *Hindawi: Journal of Food Quality*, 2018. Doi: /10.1155/2018/4570238.

Wantanwa, M., Yoshiko, S., Wasana, C. & Witaya, S. (2017). Aflatoxin B1 contamination of dairy feeds after storage in farm practice in tropical environment. *Biocontrol Science*, 22: 41-45.

McMaster, M. C. (2007). HPLC: A practical user's guide, 2nd ed., John Wiley & Sons, Inc., Hoboken, New Jersey.

Yohannes Besufekad, Wondwesen Ayalew & Anteneh Getachew (2018). Analysis to ascertain the determination for aflatoxin contamination of milk and feeds from Guragie Zone, Ethiopia. *International Journal of Agricultural Research,* 13:1-11.