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Improvement of Nutritional Quality of Some Oilseed Meals Through Solid-State Fermentation Using *Aspergillus niger*

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ABSTRACT

This study aimed to investigate the effect of solid-state fermentation on the nutritional composition of cottonseed meal (CSM), sunflower meal (SFM) and hazelnut kernel meal (HKM). In this study, each feedstuff was divided into two treatment groups (unfermented and fermented) with five replicates. Nutritional changes in the feedstuffs were determined by analyzing crude protein, ether extract, ash, crude fiber, neutral detergent fiber, acid detergent fiber contents. Solid-state fermentation had effects on the nutritional composition in all feedstuffs. The best improvement in nutritional quality was obtained from the HKM with increasing the crude protein and decreasing the structural carbohydrates content. Fermented HKM can be considered as an alternative protein feed for soybean meal considering the nutritional composition.

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Introduction

Deficiency in production fields and unsustainable of feedstuff supply have threaten the development of the livestock industry (Ergin and Kızıl Aydemir, 2018). The threat can be eliminated if the alternatives new feed sources in animal nutrition instead of protein and energy sources used in human nutrition are found. Cottonseed meal (CSM), sunflower meal (SFM), hazelnut kernel meal (HKM) are among the major protein sources not to compete with human nutrition and are cheaper than soybean meal. However, they cannot be used efficiently in animal nutrition due to nutritional deficiencies or having antinutritional factors or both (Özen et al., 2005).

Cottonseed was produced in Turkey approximately 2.7-2.8 million tons in 2018 and its 1.5 million tons was used in the oil industry (TUIK, 2018). Also, CSM was produced about 750 thousand tons in 2018 in Turkey. The CSM is one of the important protein sources for animal nutrition and contains nearly 35 % crude protein (Wang et al., 2012). However, some anti-nutritional factors may be posed a risk to animal health. Polyphenol compounds, which is known as free gossypol, decreased growth performance and mortality in poultry (Henry et al., 2001; Jazi et al., 2017). Although a number of methods can be used to eliminate these anti-nutritional factors, these methods are not preferred because of negative effects on the nutrient content of CSM or the animals consumed them (Kanyinji and Sichangwa, 2014).

In Turkey, sunflower production was 7.3 million tons in 2018. As considering the average oil yield of 45-50% in sunflower seed, SFM production was 2.5-3 million tons in 2018 (TUIK, 2018). The nutrient composition of SFM varies depending on whether it contains crust or not. In general, shelled SFM contains 20-25% crude protein (CP), 30-40% crude fiber (CF), and unshelled SFM contains 50-55% CP and 28% CF (Farran et al., 2010; Yenice et al., 2018). Although it is an important source of plant protein, its high cellulose content and low amount or digestibility of amino acids such as lysine and methionine cause problems, especially for non-ruminant animals (Tasan et al., 2011). Therefore, it was suggested that SFM could be used only up to 10-15% in poultry diets (Calıslar and Kustimur, 2017).

The hazelnut was produced approximately, 550 thousand tons per year in Turkey, which constituted 42% of the world's total hazelnut production (TUIK, 2018). The HKM is a by-product produced after obtaining the oil from the nuts. The protein content of HKM varies between 39-43% depending on the extraction process of oil (Erener and Altop, 2008). Although HKM has the potential plant protein sources in animal nutrition, it contains higher cellulose or tannin and insufficient in terms of amino acids such as lysine, methionine, and threonine. These factors limits to usage of HKM in animal nutrition (Erener et al., 2003).

In recent years, solid-state fermentation has been considered as a useful and inexpensive method to improve the nutritional composition of feedstuffs and to eliminate its antinutritional factors. Previous studies reported that improvement of both protein quantity and quality (Mathivanan et al., 2006; Zhang et al., 2012), degradation of non-starch polysaccharides to monosaccharides such as glucose and eliminating of possible antinutritional factors (Zhao et al., 2013) in feedstuffs and agricultural residues by solid-state fermentation. Given all this information, it may be possible to improve the nutritional composition of CSM, SFM, and HKM. Therefore, the effects of solid-state fermentation on the nutritional composition of CSM, SFM, and HKM were examined in this study.

Materials and Methods

Microorganism and Feedstuffs

CSM, SFM, and HKM were obtained from commercial sources and ground to a size of 2 mm. *Aspergillus niger* (ATCC 9142) obtained from American Type Culture Collection (ATCC) was used as the inoculant. *A. niger* was incubated at 24°C for 7 days according to agar plate technique. Spores counted in a hemacytometer cell with Fuchs-Rosenthal ruling. Prepared cultures were inoculated (10^5 per 100 g) into the substrates on the same day.

Solid-state Fermentation

After the autoclaving process (121°C, 10 min), each CSM, SFM and HKM sample was divided into two treatment group (unfermented and fermented) with five replicates. Fermentation media was prepared by 100 g solid substrate (CSM, SFM or HKM) and 160 g PDA broth (Merck). *A. niger* inoculated to the substrates at 10^6 spores for each 100 g solid media. After gentle mixing, the substrates were incubated at 28-30°C for 7 days. At the end of this period, fermented feeds were dried in polythene sheets under the room temperature and milled to pass through a 2 mm sieve.

Main Nutritional Composition

The CSM, SFM, and HKM samples were analyzed for dry matter (DM), crude protein (CP), ether extract (EE), ash according to AOAC (2000) and for crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) by fiber analyzer (A2000, Ankom) according to Van Soest et al. (1991) before and after fermentation. Hemicellulose was calculated as NDF minus ADF. Nitrogen-free extract (NFE) was estimated on a dry weight basis by subtracting the percentages of CP, EE, CF and ash from 100%.

Statistical Analysis

Data from unfermented and fermented feedstuffs were separately analyzed with the Student t test (SPSS 21.0 Statistics). Normality of distribution was tested with Shapiro-Wilk's test. Differences were considered significant at P<0.05.

Results

Solid state fermentation affected the nutritional composition of CSM, SFM, and HKM (Table 1, Table 2 and Table 3, respectively). The crude protein content of HKM and SFM were increased (P<0.001) although The CP of CSM was not affected (P>0.05). After fermentation, the EE content of CSM and SFM were decreased (P<0.05 and P<0.001, respectively) but not the EE content of HKM (P>0.05). Ash content in all groups was increased (P<0.01). Although a decrease in the NFE content of SFM and HKM was observed (P<0.001), the NFE content of CSM was increased (P<0.01) after fermentation.

After fermentation, although crude fiber of CSM content did not affect (P>0.05), its NDF, ADF and ADL contents (P<0.01, P<0.001 and P<0.05, respectively) were increased. There was an increase (P<0.001, P<0.01 and P<0.05, respectively) in crude fiber, ADF and ADL content of SFM, but not to affect (P>0.05) its NDF content. Crude fiber, NDF and ADF content of HKM were decreased (P<0.001, P<0.001 and P<0.01, respectively) by fermentation while there was no significant (P>0.05) effect of the fermentation on the ADL content of HKM. Hemicellulose content of all oilseed meals was decreased (P<0.05) by fermentation.

Table 1 Chemical composition of unfermented and fermented CSM (% DM)

Composition (% DM)	CSM	FCSM	SEM	P
Crude Protein	20.39	19.66	0.359	NS
Ether Extract	8.56	5.18	0.773	***
Ash	8.39	9.70	0.312	**
NFE	31.99	34.89	0.679	**
Crude Fiber	30.67	30.57	0.210	NS
NDF	51.65	53.29	0.380	**
ADF	39.70	43.13	0.782	***
ADL	10.79	13.84	0.778	*
Hemicellulose	11.95	10.16	0.405	***

** = P<0.01, *** = P<0.001, NS = not significant, CSM = cottonseed meal, FCSM = fermented cottonseed meal, SEM = standard error of means.

Table 2 Chemical composition of unfermented and fermented SFM

Composition (% DM)	SFM	FSFM	SEM	P
Crude Protein	33.00	41.53	1.912	***
Ether Extract	1.47	0.86	0.150	*
Ash	7.38	9.87	0.558	***
NFE	37.72	24.69	2.923	***
Crude Fiber	20.43	23.05	0.594	***
NDF	34.67	36.07	0.484	NS
ADF	23.82	26.15	0.580	*
ADL	8.53	10.71	0.525	**
Hemicellulose	10.85	9.92	0.238	*

*=P<0.05, **=P<0.01, ***=P<0.001, NS = not significant, SFM = sunflower meal, FSFM = fermented sunflower meal, SEM = standard error of means.

Table 3 Chemical composition of unfermented and fermented HKM (% DM)

Composition (% DM)	HKM	FHKM	SEM	P
Crude Protein	44.66	58.40	3.088	***
Ether Extract	1.00	1.62	0.235	NS
Ash	8.60	10.88	0.516	***
NFE	31.97	20.51	2.577	***
Crude Fiber	13.78	8.60	1.159	***
NDF	32.43	25.27	1.617	***
ADF	22.44	19.36	0.719	**
ADL	12.46	14.52	0.603	NS
Hemicellulose	9.99	5.91	0.915	***

** = P<0.01, *** = P<0.001, NS = not significant, HKM = hazelnut kernel meal, FHKM = fermented hazelnut kernel meal, NFE = nitrogen-free extract, SEM = standart error of means.

Discussion

The study showed that the nutritional quality of CSM, SFM and HKM through solid-state fermentation using *Aspergillus niger* were improved. These results were similar to studies which was reported that Fermented CSM (Zhang et al., 2007), SFM (Jannathulla et al., 2018), palm kernel cake (Iluyemi et al., 2006; Lawal et al., 2010), sour cherry kernel (Güngör et al., 2017) and grape seed (Altop et al., 2018) were higher nutrient value as compared to their unfermented.

In general, CP contents of the oil seed meal were increased after fermentation in the study. This increase may be due to *A. niger* spore counts on the substrates because it is a microbial protein source and has highly nutrient value. In the study, increased *A. niger* spores during to fermentation could be increased CP content of the meals (Altop et al., 2018). Therefore, it need to detailed studies on protein quality and quantity of the meals.

The NFE indicates the carbohydrates, sugars, starches and other easily degradable non-nitrogenous substances in the feed. Microorganisms prefer to utilize the soluble carbohydrates rather than other nutrients to meet its carbon requirements (Papagianni, 2007). Jannathulla et al. (2018) reported a decrease in the NFE content of SFM and soybean meal by fermentation. In this study, fermentation decreased the amount of NFE in SFM, as well as in HKM. Similar findings were also reported in the studies on shea nut meal (Dei et al., 2008), sour cherry kernel (Güngör et al., 2017) and grape seed (Altop et al., 2018). However, fermentation increased the NFE content of CSM in this study in contrast to the results of the studies mentioned above. This may indicate that *A. niger* did not choose the NFE for a carbon source or could not grow properly in CSM or both. Indeed, fermentation did not change the nutritional composition of CSM efficiently compared with the SFM and HKM in this study. Similarly, Apata (2011) reported increasing NFE content of the Indian almond meal after fermentation.

Jannathulla et al. (2018) showed that *A. niger* decreased the ether extract content of SFM and soybean meal. Similarly, ether extract was decreased by fermentation in CSM and SFM in the present study. *A. niger* can produce lipase enzymes in solid-state fermentation (Kumar and Kanwar, 2012), which can be the reason of the diminishing of ether extract content. Similar results were noted in the studies on the Indian almond meal (Apata, 2011) and mango kernel (Kayode and Sani, 2008).

Cellulolytic enzymes such as cellulase (Xie et al., 2016) and hemicellulase (Mathivanan et al., 2006) can be produced by *A. niger* in solid-state fermentation. In the present study, hemicellulose content was decreased by fermentation in all feeds. Similarly, crude fiber, NDF, and ADF content decreased in HKM after fermentation. Jannathulla et al. (2018) noted that crude fiber, NDF and ADF content of SFM and soybean meal were decreased through fermentation. Similar findings were reported in previous studies on palm kernel cake (Iluyemi et al., 2006; Lawal et al., 2010), Indian almond meal (Apata, 2011), grape seed (Altop et al., 2018). However, solid-state fermentation increased the crude fiber, ADF, ADL content of SFM and NDF, ADF and ADL content of CSM in this study. Similar results were reported in the studies on sour cherry kernel (Güngör et al., 2017), mango kernel (Kayode and Sani, 2008). Cellulosic components increased by fermentation because of the fact that the cell wall of *A. niger* contains chitin, a cellulose-like component (Beauvais et al., 2014). Moreover, raising of structural carbohydrates contents may be due to the reduction of the other nutrients.

Fermentation increased ash contents in all groups. This increase may be due to the decreased nutrients by fermentation instead of the actual increase. Similar results were obtained from the studies on shea nut meal (Dei et al., 2008), sour cherry kernel (Güngör et al., 2017), mango kernel (Kayode and Sani, 2008), grape seed (Altop et al., 2018).

Conclusion

In conclusion, *A. niger* solid-state fermentation improved the nutritional quality of SFM and HKM although desired effects were not observed in CSM. Nutritional quality of HKM had the highest improvement by fermentation with raising crude protein and reducing structural carbohydrates by fermentation. Fermented HKM can be recommended as a protein source for animal nutrition instead of soybean meal.

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