Turkish Journal of Agriculture - Food Science and Technology, 7(sp3): 1-4, 2019
DOI: https://doi.org/10.24925/turjaf.v7isp3.1-4.3085

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology

Effect of Solid-state Fermentation on the Nutritional Composition of Nettle (*Urtica dioica* L.)[#]

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ARTICLE INFO

[#]This study was presented as an oral presentation at the 1st International Congress of the Turkish Journal of Agriculture - Food Science and Technology (Antalya, TURJAF 2019)

Research Article

Received : 17/11/2019
Accepted : 01/12/2019

Keywords:
Aspergillus niger
Nettle
Nutritional enrichment
Solid-state fermentation
Utrica dioica L.

ABSTRACT

The study was aimed to improve the nutritional composition of nettle (*Utrica dioica* L.) having positive effects on animals by solid-state fermentation. *Aspergillus niger* was used as a inoculant in fermentation of nettle. Before and after fermentation, crude protein (CP), ash, ether extract (EE), nitrogen-free extract (NFE), crude fiber (CF), hemicellulose (HC), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) contents of nettle were determined. The ash, NFE and HC content of nettle were increased by fermentation. However, *A. niger* decreased the CF and EE in nettle. The results showed that solid-state fermentation with *A. niger* can be used to improve the nutritional composition of nettle.

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Introduction

Herbs and herbal products have been used as food with medicinal purposes for centuries. Nettle (*Utrica dioica* L.) is widely grown in different parts of the world and has been used to improve human health. Nettle contains some phenolic compounds such as carvacrol and thymol (Bakır, 2018). These phenolic compounds have immune-stimulatory, anti-carcinogenic, anti-inflammatory, antioxidant, antimicrobial and antiallergenic properties (Safamehr et al., 2012).

Nettle contains 21-23% crude protein and 9-21% crude fiber (Zehraw et al., 2019). Researchers have focused on nettle as a feed additive in generally poultry nutrition. Nettle increased body weight and feed conversion ratio in broiler chickens (Safamehr et al., 2012) and has positive effects on egg production of laying hens (Mansoub, 2011). It also reduced serum triglyceride and cholesterol in broilers (Safamehr et al., 2012) and laying hens (Mansoub, 2011). Moreover, nettle improved the yellowness of the skin, liver (Loetscher et al., 2013a) and egg yolk (Loetscher et al., 2013b). Although nettle has positive effects on poultry, it can be used limited levels in poultry diets due to its high crude fiber (CF) content, which can be up to 21%.

Fermentation is one of the useful methods for recycling the agricultural by-products by enriching its nutritional composition. Fermentation is generally divided into liquid-state and solid-state fermentation. Solid-state fermentation is the preferred method for liquid-state fermentation because of being economical, using abundant and cheap substrates in and having relatively less risk of contamination. Solid-state fermentation refers to microbial growth in moistened solid substances without free water (Güngör et al., 2017). Agricultural residues can be enriched as nutritional content by solid-state fermentation (Gungor and Erener, 2019). Wang et al. (2018) reported increased crude protein (CP) and decreased CF in *Moringa oleifera* leaves by fermentation. Similarly, fermentation improved the amino acid composition of olive leaves (Altop et al., 2018a). *Aspergillus niger* is a recommended microorganism for solid-state fermentation because it can grow rapidly in low-water environments. It is also used as a probiotic in animal nutrition and is accepted as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration (FDA, Güngör et al., 2017). This study aimed to improve the nutritional composition of nettle by *A. niger* solid-state fermentation.

Materials and Methods

Solid-State Fermentation

The study was conducted in the Laboratory of Feeds and Animal Nutrition in the Department of Animal Science at Ondokuz Mayis University, Turkey. Nettle was harvested and dried at room temperature. *Aspergillus niger* was obtained from American Type Culture Collection (ATCC) and left in incubation in Potato-Dextrose-Agar on 28°C for 7 days according to agar plate technique. After incubation, *A. niger* spores were harvested by turning the plate upside down and gently hitting the top. Spore counting was conducted according to Fuch Rosenthal technique using a hematocytometer. Dried nettle was ground to a size of 1 mm and sterilized at 121°C for 15 minutes. Samples were enriched by Potato Dextrose Broth (150 ml for each 100 gram sample). *Aspergillus niger* spores (10^6 spores/ml) were inoculated the samples in the sterile cabin and left incubation for 7 days at 28-30 °C according to Gungor and Erener (2019).

Chemical Composition and Nutrient Values

The CP, ether extract (EE), ash, and CF contents of CK were determined according to AOAC (2000). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed as reported by Van Soest et al. (1991). Hemicellulose (HC) 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

Experiments were conducted in triplicate and the results were given as means and pooled standard error of mean (SEM). Data were analyzed with Student's t-test (SPSS 21.0 Statistics, IBM, 2012). Results were considered significantly different at $P < 0.05$.

Results and Discussion

Aspergillus niger increased the ash ($P<0.001$), NFE ($P<0.001$) and HC ($P<0.05$) content of nettle but decreased the CF ($P<0.001$) and EE ($P<0.05$) content (Table 1). The CP, NDF, ADF and ADL content were not changed ($P>0.05$) by the solid-state fermentation.

Altop (2019) reported increased ash content in olive leaves similar to the findings of the present study. Fermentation also increased the ash content of pomegranate peel and *Larrea tridentate* leaves (Aguilar et al., 2008), cassava peels (Okpako et al., 2008) and pineapple waste (Omwango et al., 2013). The increase in ash content possibly was relative due to decrease in other nutrients rather than actual increase.

Microorganisms preferred soluble carbohydrates to other nutrients for using a carbon source (Papagianni, 2007). Vandenberghe et al. (2000) noted that the NFE content of cassava bagasse was decreased by solid-state fermentation. Similar findings were reported by the studies on olive leaves (Altop et al., 2018a) cassava peels (Aro, 2008; Okpako et al., 2008) and grape seed (Altop et al., 2018b). However, Altop (2019) reported no effect of solid-state fermentation on the nutritional composition of olive leaves. *Aspergillus niger* increased the NFE content of nettle in this study. Similarly, Apata (2011) showed increased NFE in *Terminalia catappa* fruit meal by *A. niger*.

Güngör et al. (2017) reported decreased EE content by *A. niger* in sour cherry kernel. In the present study, nettle showed lower EE content after fermentation. Similar findings were reported in the studies on *T. catappa* (Apata, 2011), mango kernel (Kayode and Sani, 2008), grape seed (Altop et al., 2018b) and sour cherry kernel (Güngör et al., 2017). Decreasing of the ether extract content may be due to the lipase enzymes which can be produced by *A. niger* in solid-state fermentation (Kumar and Kanwar, 2012).

Aspergillus niger can increase the CP content of the substrates may be owing to its mycelia and/or producing enzymes (Güngör et al., 2017). Enrichment in CP content by fermentation was reported in the studies on *M. oleifera* leaves (Wang et al., 2018), pomegranate peels and *L.tridentata* leaves (Aguilar et al., 2008), *Ginkgo biloba* leaves (Zhang et al., 2013; Zhao et al., 2013) and sour cherry kernel (Gungor and Erener, 2019). However, there is no change in CP content between of unfermented and fermented nettle. This may be due to changes in the ratios of the other nutrients in the nettle. Similarly, Altop et al. (2019) reported no changed CP level in cottonseed meal by *A. niger* solid-state fermentation.

Table 1 Nutritional composition of unfermented nettle (N) and fermented nettle (FN)

Composition (% DM)	N	FN	SEM	P
Crude protein	23.19	24.27	0.339	NS
Ether extract	2.46	1.40	0.260	*
Ash	20.24	24.12	0.878	***
NFE	21.19	30.75	2.153	***
Crude fiber	32.93	19.45	3.018	***
NDF	38.85	37.99	0.956	NS
ADF	29.15	25.53	1.288	NS
ADL	9.40	10.73	0.909	NS
Hemicellulose	9.71	12.46	0.702	*

*: $P<0.05$, ***: $P<0.001$, NS: Not significant, NFE: nitrogen-free extract, SEM: standard error of means

Structural carbohydrates such as cellulose, hemicellulose and lignin reduce the nutrient digestibility in feeds because they are difficult to digest by animals (Graminha et al., 2008). Therefore, the structural carbohydrate level of the feed is an important indicator for the estimation of feed digestibility. *Aspergillus niger* decreased the CF content of nettle by solid-state fermentation in this study. Similar results were taken from the studies on *M. oleifera* leaves (Wang et al., 2018), olive leaves (Altop, 2019), pineapple waste (Omwango et al., 2013) and cassava peel (Aro, 2008). Xie et al. (2016) showed that *A. niger* can produce the cellulase enzyme during fermentation. Reduction in the CF of nettle by fermentation can be attributed to the cellulose enzymes which is possibly produced by *A. niger* during fermentation processes.

Conclusions

The results of the present study showed that *A. niger* changed the nutritional composition of nettle by increasing ash, NFE and HC content and decreasing EE and CF content. Fermentation can be used to increase the inclusion level of nettle to poultry diets. Further studies are needed to confirm the usability of fermented nettle in poultry nutrition.

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