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# THE EFFECT OF BLACK SEED OIL (*NIGELLA SATIVA*) ON *IN VITRO* DIGESTIBILITY, CHEMICAL AND FERMENTATION CHARACTERISTICS OF HUNGARIAN VETCH-WHEAT SILAGE

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**Abstract**

The aim of the study to determine some chemical, fermentation properties and *In vitro* organic matter digestibility, metabolisable energy and net energy lactation contents of silages obtained by adding black seed (*Nigella sativa*) oil to vetch-wheat mixtures at different levels. For this purpose, 0.00% (control), 0.02, 0.04, 0.08 and 1.60 levels of black seed oil were added to the vetch-wheat in laboratory conditions and the ensiling period was 75 days. According to the findings obtained from the study, dry matter and organic matter contents of silages were significantly increased by adding the black seed oil compared to the control group ($p<0.01$). It has been determined that the differences in crude protein, ether extract, crude ash, NDF, ADF, pH and propionic acid, *In vitro* organic digestibility, metabolisable energy and net energy lactation contents of silages are not statistically significant ($p>0.05$). However, the lactic acid content of the silages was increased with the addition of black seed oil by 1.60% ($p<0.05$). Ammonia nitrogen and butyric acid contents were not determined in the experiment silages. At the end of the research, it was concluded that silage can be obtained at good quality by adding 1.60% of black seed oil to Hungarian vetch-wheat silages.

**Key words:** Vetch, Wheat, Black seed oil, Silage, Quality, *In vitro* digestibility.

## Introduction

Black seed plant (*Nigella sativa*) is known throughout the world with different names such as Seed of blessing. Black seed oil is reported to be beneficial due to its content of over a hundred components such as aromatic oils, trace elements and vitamins (Ali & Blunden, 2003; Wu *et al.,* 2019; Altindal, 2019). Black cumin, used mainly for culinary and medicinal purposes was observed to possess anti-carcinogenic, anti-ulcer, anti-bacterial and anti-fungal activities (Petrovic *et al.*, 2019; Majeed *et al.,* 2019; El-Lateef *et al.,* 2019).

Many essential oils obtained recently are derived from edible medicinal and aromatic plants. Since these aromatic compounds are majorly volatile, they are generally extracted through steam distillation or solvent extraction (Simon, 1990). Since plant volatile oils are complex mixtures including different compounds, they also vary in terms of their biological effects. Many volatile oils whose effectiveness varies depending on the properties of the active agent they contain are known to have antimicrobial characteristics (Karasu & Öztürk, 2014). Antimicrobial activity depends on the type of the plant, from which part of the plant it is obtained, the geographical structure of the cultivation area, its composition and concentration, the type and load of the target microorganism. Proteins, lipids, salts, pH and temperature are the factors which affect the antimicrobial activities of phenolic matters (Sagdic, 2003; Evren & Tekgüler, 2011).

The ferment types closely related with aerobic dissolution are also inhibited by some essential oils in silages. For instance, it was determined that carvacrol inhibited the growth of some *Saccharomyces cerevisiae* strains (Knowles & Roller, 2001) and thymol inhibited the growth of *Debaryomyces hansenii* (Curtis *et al.*, 1996). Juglal *et al*., (2002) reported that essential oils had antifungal activities against fungi-producing mycotoxins. One of these medicinal and aromatic plants to be recommended as silage additive materials is the black seed oil derived from the black seed. Phytochemical analysis of black seed indicated the presence of essential oils and fatty acids responsible for the pharmacological effect (Khan, 1999; Ali & Blunden 2003; Ramadan, 2007; Waheed *et al*., 2018). It was reported that 99.5 % of the fatty acids contained eight fatty acids [four saturated (17.5 %), four unsaturated (82.5 %)] and 0,5 % of them contained volatile oils (Cheikh *et al*., 2007). Black seed contains many active compounds in itself. Especially 25 % of carbonyl and 2 % of phenol in its structure produce antiseptic and antibacterial effects of the black seed (Daba & Abdel, 1998). It is also reported that black seed oil can lower blood glucose and cure digestive and liver diseases besides its antibacterial properties (Bashandy, 1996).

In this study, the effects of black seed oil added to Hungarian vetch-wheat at different levels on some chemical, fermentation characteristics, *In vitro* organic matter digestibility, metabolizable energy and net energy lactation contents of silages were investigated.

## Materials and Methods

The Hungarian vetch-wheat mixture was chopped 5 cm in length and black seed oil was mixed homogeneously (four parallel) as 0.00, 0.02, 0.04, 0.08 and 1.60% by wet weight. Ensiling was performed in a 1 lt volume of anaerobic plastic jar and the fermentation process lasted for 75 days at room temperature. Analysis of dry matter, organic matter, crude protein, crude ash and ether extract of silages according to the methods described in AOAC (Anon., 1999) was made. ADF and

NDF contents of silage samples were analyzed by using Fiber Analyzer device described by Van Soest (1982). When the silages were opened, 100 ml of distilled water was added to 25 g of the silage and this extract was measured by a digital pH meter after it was shaken for 5 minutes (Polan *et al*., 1998).

While determining the organic acids contents. 40 g of fresh material was shaken for 5 minutes in 360 mL of water and then they were filtered through the filter-paper (Whatman No:1). The filtrate was centrifuged for 30 minutes at 14.000 rpm/min. The solution without any particles was taken to eppendorf tubes and kept at -18 ºC until the day of analysis. They were taken out of the deep freezer before the analysis and waited to be thawed. At the day of analysis, they were injected to HPLC device (Suzuki & Lund, 1980) under the conditions indicated below and organic acids (acetic acid, propionic acid, butyric acid and lactic acid) contents were determined (HPLC Conditions: Column: C18, 5 µm, 4,6 x 250-mm; Mobile Phase: Isocratic; 25-mM K-phosphate buffer; pH 2,4; Flow Rate: 1.5 mL/min.; Column Temperature: 30ºC; UV Detector: Wavelength: 210 nm; Injection Volume: 20 µL). Ammonia nitrogen ($NH_3$-N) of the silages was determined according to Kjeldahl distillation procedure by taking 100 ml of the same solution (Broderick & Kang, 1980).

*In vitro* gas production technique was used in determining the *In vitro* organic matter digestibility (IVODM), metabolisable energy (Menke & Steingass, 1988) and net energy lactation contents of the silages (Blümmel & Ørskov, 1993). 3 rumen cannulated Holstein breed infertile cows were used in the application of *In vitro* gas production technique. Incubation was started just after the rumen liquid was taken in the morning and gas production level of the feed was determined. These parameters were calculated with the help of NEWAY package programme (Ørskov & McDonald, 1979; Menke *et al*., 1979).

In the evaluation of the obtained findings after the experiment, ONE-WAY ANOVA procedure in SPSS program package and for the within-groups differences, Duncan Multiple Comparison Test were used (Anon., 2007).

## Results and Discussion

Chemical composition of the experiment silages obtained by adding black seed oil to vetch-wheat mixtures at different levels are presented in Table 1.

With the black seed oil additives to vetch-wheat silages, dry matter and organic matter contents remarkably increased as compared to control group ($p<0.01$). Dry matter content of feed is one of the most important criteria affecting silage quality. If the dry matter is high, the silage cannot be compressed well. However, if the dry matter content of silages is low, a significant amount of dehydration occurs during fermentation, resulting in loss of nutrients in the silages (Filya, 2001). According to the findings, it is understood that dry matter losses can be reduced by the addition of black seed oil to Hungarian vetch-wheat silages. Therefore, it can be said that there is sufficient amount of water-soluble carbohydrate content in the silage in ensiling period and the lactic acid bacteria which consume them grow and stop the growth of undesirable factors and reduce the loss of dry matter and organic matter. Keles & Demirci (2011) reported that dry matter content of the baled Hungarian vetch-wheat silages was 44.1%. The differences of silages in terms of crude protein, crude ash, NDF, ADF and ether extract contents were not statistically significant ($P>0.05$). Water soluble carbohydrates increase the degradability of cell wall compounds through producing numerous lactic acids in a very short period by accelerating the activities of lactic acid bacteria in silages (Filya, 2001). Also, it is known that ADF content is directly related with the digestibility of that roughage. Thus, ADF is taken as a basis for the relative evaluation of the roughages. As the ADF increases, digestibility of the roughages decreases. It can be said that the activities of lactic acid bacteria did not accelerate to increase the degradabilities of cell wall compounds in silages with black seed oil additives because there was no significant difference with the control group in terms of NDF and ADF. In essential oils ensiled with different fresh materials, Chaves *et al*., (2012) indicated that oregano oil additives to silages did not change the NDF and ADF. Crude protein content of the silages did not change in parallel with the lack of crude protein content of the black seed oil added to the Hungarian vetch-wheat silages. Similarly, Demirci *et al*., (2011) identified that crude protein content did not change with the inoculant additive to baled Hungarian vetch-triticale silages. However, Koç *et al*. (2010) stated that crude protein of vetch-wheat silages with organic acid at different temperatures (20°C, 30°C and 37°C) increased. It was also determined that although the black seed oil used as silage additive material within the experiment increased the ether extraction in percentage, in the groups especially black seed oil added at 1.60%, it did not have a significant effect. This may be due to the low level of black seed oil treatment. Chaves *et al*., (2012) indicated that crude fat contents increased with the carvacrol to barley silages; however, the ether extraction of the silages did not change with Swedish orange oil.

Fermentation characteristics of Hungarian vetch-wheat silages are presented in Table 2.

The differences in terms of pH values of vetch-wheat silages with black seed oil additives at different levels were not found as significant ($p>0.05$). It is reported that 4.5-4.6 of pH value in forages with dry matter content of 40-45 % is highly satisfactory (Weissbach, 1996) although there is no change in pH values of the silages. Similarly, with the lactic acid application, Weinberg *et al*., (1988) and with the formic acid additives, Pursiainen & Tuori (2008) and Keles & Demirci (2011) indicated in their studies that pH value of baled Hungarian vetch-triticale silages was 4.6 and this value did not change with homofermentative + heterofermentative lactic acid bacteria.

**Table 1. Chemical composition of silages.**

| Parameters | Black seed oil levels, % | | | | | P |
|---|---|---|---|---|---|---|
| | Control | 0.02 | 0.04 | 0.08 | 1.60 | |
| DM* | 38.46 ± 0.80[b] | 40.52 ± 1.91[a] | 41.49 ± 0.82[a] | 41.02 ± 1.39[a] | 40.41 ± 0.79[a] | 0.001 |
| CA** | 12.63 ± 0.81 | 12.58 ± 0.29 | 12.71 ± 0.46 | 12.49 ± 0.45 | 12.60 ± 0.26 | 0.94 |
| OM** | 26.10 ± 1.62[b] | 27.95 ± 1.88[a] | 28.78 ± 1.16[a] | 28.53 ± 1.33[a] | 28.16 ± 1.54[a] | 0.01 |
| CP** | 13.65 ± 0.41 | 12.54 ± 0.28 | 13.16 ± 0.29 | 13.41 ± 1.46 | 13.81 ± 0.43 | 0.42 |
| EE** | 1.57 ± 0.26 | 1.30 ± 0.14 | 1.63 ± 0.24 | 1.31 ± 0.24 | 1.82 ± 0.25 | 0.34 |
| NDF** | 36.78 ± 1.72 | 37.60 ± 0.63 | 37.75 ± 1.08 | 36.24 ± 0.84 | 37.34 ± 1.44 | 0.13 |
| ADF** | 24.20 ± 0.77 | 25.05 ± 0.36 | 25.49 ± 1.26 | 24.59 ± 0.54 | 24.85 ± 0.45 | 0.06 |

[a-b]: The differences between the averages in the same line are significant ($p<0.01$)

DM: Dry matter; CA: Crude ash; OM: Organic matter; CP: Crude protein; EE: Ether extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber

* %; ** % Dry matter

**Table 2. Fermantation characteristics of silages.**

| Parameters | Black seed oil levels, % | | | | | P |
|---|---|---|---|---|---|---|
| | Control | 0.02 | 0.04 | 0.08 | 1.60 | |
| pH | 4.71 ± 0.13 | 4.73 ± 0.12 | 4.80 ± 0.09 | 4.74 ± 0.11 | 4.77 ± 0.04 | 0.51 |
| LA, % | 0.72 ± 0.07[b] | 0.77 ± 0.0.23[b] | 0.77 ± 0.16[b] | 0.97 ± 0.14[ab] | 1.13 ± 0.14[a] | 0.03 |
| AA, % | 0.00[c] | 0.04 ± 0.07[c] | 0.17 ± 0.04[b] | 0.25 ± 0.05[a] | 0.00[c] | 0.001 |
| PA, % | 0.11 ± 0.08 | 0.15 ± 0.11 | 0.14 ± 0.03 | 0.14 ± 0.01 | 0.04 ± 0.09 | 0.27 |
| BA, % | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |
| $NH_3$-N | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | |

[a-c]: The differences between the averages in the same lines are significant ($p<0.05$)

LA: Lactic acid; AA: Acetic acid; PA: Propionic acid; BA: Butyric acid. $NH_3$-N: Ammonia nitrogen

When the experiment silages were analyzed in terms of lactic acid content, they were found remarkably higher with black seed oil additives of 1.60% than all the other groups except for the group including 0.08% black seed oil ($p<0.05$). However, it was determined that 0.08 % black seed oil includes the highest acetic acid content ($p<0.01$) while the acetic acid content was not observed in the control group and the groups with 1.60 % black seed oil. The differences in propionic acid contents of Hungarian vetch-wheat silages were not found as significant ($p>0.05$). After the end of respiration of the water-rich fresh material which was chopped and ensiled after the harvest, the nutrient materials such as protein, corbonhydrate and fat contained in the feeds are expose to some microorganisms depending on the conditions in the silage. Therefore, volatile fatty acids (acetic acid, propionic acid and butyric acid), lactic acids and some alcohols were generated in the silages. One of the most important elements that are needed in a good quality of silage is lactic acid. Ensiling is based on the transformation of water-soluble carbohydrates into lactic acid through fermentation by lactic acid bacteria in anaerobic conditions (Kung *et al*., 2008). Zhang *et al*., (2015) concluded that the lactic acid increased with the treatment of lactic acid bacteria and propionic acid.

Ammonia nitrogen and butyric acid contents were not identified in experiment silages. Fermentation quality of the silages varies according to the presence and proportion of lactic acid and butyric acid in silages. Although the lactic acid content is high in the silage of good quality, it should not be butyric acid or should produce very small amounts. The lack of butyric acid indicates that the required fermentation conditions were achieved in the silages with black seed oil additives. Demirci *et al*., (2011) identified that the butyric acid content of the vetch silages was 0.03% and it did not change with the inoculant supplement and Demirel *et al*., (2003) reported that the butyric acid of the silages composed of 50% of Hungarian vetch + 50% of Sudan grass was 1.62%. The ammonia concentration in the silage is an important criterion which indicates the degradation level of the proteins by butyric acid bacteria during the fermentation. It is reported that ammonia nitrogen should be lower than 80 g/kg of total N in good quality silage (Petterson, 1988). In addition, McDonald *et al*., (1991) stated that deamination occurring in aminoacids due to the presence of acetic acid with the high concentration in silages and the ammonia level increased at the end of this reaction. However, through the addition of additives especially to hardly ensiled forage herbages, it is aimed either to minimize or block the growth of unwanted butyric acid bacteria generation in silages and the ammonia nitrogen generation, which is end product of proteolisis, by developing the lactic acid fermentation in silages with a rapid pH decrease. Keles & Demirci (2011) identified that ammonia nitrogen content of Hungarian vetch-triticale silages was 113 g/kg and it did not change with innoculant.

When the experiment silages were analyzed in terms of fermentation properties at the end of the research, pH value was found between the required ranges in literature, the lactic acid was produced in sufficient amount for good quality silage (water soluble carbohydrate in silages pressurized the acetic acid by promoting the lactic acid production and even it prevented its generation in some groups), transformation of proteins into ammonia was prevented by providing the required fermentation conditions in the silage (ammonia nitrogen was not identified in experiment silages and the acedic acid was produced sufficient enough to prevent the deterioration of the silages during the fermentation process), butyric acid and ammonia nitrogen were not identified (it achieved the real proteins to be protected by blocking the activities of microorganisms and protease which fermentate the aminoacids and prevented the generation of ammonia nitrogen concentration in silages through a good fermentation process).

The values belonging to *In vitro* Organic Matter Digestibility (IVOMD), Metabolisable Energy (ME), and Net Energy Lactation (NEL) are shown in Table 3.

The differences identified in *In vitro* Organic Matter Digestibility, ME and NEL contents of Hungarian vetch-wheat silages with black seed oil were not found as significant ($p>0.05$). One of the methods commonly used in the evaluation of silages and referred in order to estimate the digestibility of organic matters and the energy contents of silages is *In vitro* gas production technique. This method is based on the measurement of $CO_2$ released as a result of the fermentation of forage haerbage. Kung *et al*., (1990) reported that *In vitro* Organic Matter Digestibility did not change with the inoculant to vetch silages harvested in different periods.

**Table 3. *In vitro* organic matter digestibility, metabolisable energy and net energy lactation of silages.**

| BSO levels, % | IVOMD, % | ME, MJ/kg DM | NEL, MJ/kg DM |
|---|---|---|---|
| Control | 70.76±4.59 | 9.08±0.63 | 5.55±0.44 |
| 0,02 | 70.21±6.98 | 9.44±0.70 | 5.53±0.79 |
| 0,04 | 69.22±2.04 | 8.70±0.19 | 5.34±0.28 |
| 0,08 | 72.18±4.09 | 9.67±0.62 | 5.89±0.44 |
| 1,60 | 71.40±3.23 | 9.17±0.51 | 5.66±0.39 |
| P | 0.96 | 0.22 | 0.70 |

BSO: Black seed oil; IVOMD: *In vitro* organic matter digestibility, ME: Metabolisable energy, NEL: Net Energy lactation

## Conclusion

Black seeds have a lot of rich nutrients, and these nutrients are much important for human and animal health. At the end of the study, it was determined that protection of Hungarian vetch-wheat mixture was easier and its nutrient value as silage was higher. From the obtained findings, it was concluded that Hungarian vetch-wheat silages can be ensiled without additives, if black seed oil is used, good quality silage can be made with 1.60 % black seed oil additives. Finally, it is considered appropriate to make studies by using higher levels of black seed oil in vetch-wheat silages without ignoring economic conditions.

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