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Determining the effect of natural inhibitors on sesame meal degradability using *in vitro* three step method

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ABSTRACT

The aim of this experiment was to investigate the beneficial effect of monensin, tannic acid and cinnamon essential oil addition on sesame meal degradability by the three-step *in vitro* method. The effect of experimental additives on the degradability of sesame meal in the rumen, after rumen and in the whole gastrointestinal tract was significant ($P<0.05$). The *in vitro* ruminal and intestinal digestibility of sesame meal crude protein with experimental additives was in the range of 76 to 84% and 49 to 60%, respectively. The intestinal degradability of crude protein increased with the addition of cinnamon essential oil (about 10%). Addition of monensin, tannic acid, and cinnamon essential oil significantly increased the degradability of Neutral Detergent Fiber (NDF) and Acid Detergent Fiber (ADF) in the rumen, intestines and the whole gastrointestinal tract. The results showed that cinnamon essential oil (125 mg/L) increased the degradability of dry matter (DM), organic matter (OM), crude protein (CP), ADF and NDF in the rumen, after rumen and the whole digestive tract compared to all treatments ($P<0.05$). The results showed that addition of tannic acid (100 mg/L) decreased the disappearance of crude protein in the rumen, while it increased crude protein's disappearance in the after rumen ($P<0.05$).

Key words: monensin; tannic acid; cinnamon essential oil; sesame meal; three step degradability

Introduction

Agricultural by-products such as sesame meal can have a major impact on reducing production costs (OBEIDAT et al., 2019; MISHRA et al., 2019). Sesame meal is a by-product of the sesame oil manufacturing, containing about 46% crude protein. Not only could this proteinaceous by-product replace soy as a protein supplement in animal nutrition but could also reduce the increasing cost of feed (GHORBANI et al., 2018). In ruminants, feed efficiency is low due to ruminal fermentation and gas production (OWENS and BASALAN, 2016; KARLSSON et al., 2019).

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Methane production is directly related to feed consumption, and about 2-12% of the feed's raw energy is lost as methane production (TAPIO et al., 2017). Although the use of antibiotics increases feed efficiency, due to the creation of drug-resistant bacteria this class of compounds have been banned in the EU as growth promoters. Today, other additives such as herbal essential oils are safe natural alternatives used for this purpose (HUANG et al., 2018; LILLEHOJ et al., 2018). Natural essential oils and tannic acid have successfully been used to reduce methane production, ruminal protein decomposition and ammonia production, and increase ruminal protein for further digestion in the gut, improving ruminal fermentation efficiency (CAROPRESE et al., 2020). In a study, researchers reported oxygen monoterpenes (especially alcohols) and monoterpene aldehydes among the chemical compounds of essential oils, as potent biomolecules manipulating the growth and metabolism of ruminal microbes. On the other hand, monoterpene hydrocarbons have fewer inhibitory effects and sometimes stimulate microbial activity (BENCHAAR et al., 2008). Overall, essential oils have a great effect on the activity of ruminal microorganisms and can improve energy and nitrogen consumption in the rumen (COBELLIS et al., 2016; RIBEIRO et al., 2020). Cinnamon is a plant whose stem extracts, young shoots and leaves are used medicinally. One of the major active ingredients in cinnamon essential oil is cinnamaldehyde. Cinnamaldehyde, a compound of the phenylpropanoid class with strong antimicrobial activity, is the most abundant compound (about 75%) of cinnamon essential oil (RIBEIRO-SANTOS et al., 2017; LEE et al., 2020). The aim of this experiment was to investigate the effect of monensin, tannic acid and cinnamon essential oil on sesame meal degradability using a three-step method (GARGALLO et al., 2006).

Material and methods

The chemical composition of sesame meal, including dry matter (DM), ether extract (EE), crude ash (CA), acid detergent insoluble fiber (ADF) and neutral detergent fiber (NDF), were determined according to the proposed AOAC (2005) methods. Crude protein was measured using a Kjeldahl analyzer (Foss Tecator AB analyzer, Hoganas, Swede Kjeltec 2300 Auto analyzer) according to the AOAC standard method (AOAC, 2005). The results are presented in Table 1.

Table 1. Chemical composition of sesame meal (%)

Chemical composition	%
DM	93.21
CA	9.93
OM	90.07
CF	14.30
CP	40.95
NDF	41.50
ADF	18.40
Hemicellulose	23.10

DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; CA: crude ash; OM: organic matter; CF: crude fat.

Additives used and chemical analyses of essential oil. Cinnamon essential oil extraction was performed according to the standard method by water distillation using Clevenger apparatus (JAHANI-AZIZABADI et al., 2011). For this purpose, cinnamon was ground to fine particles and then 70 g of the sample was placed into the balloon of the Clevenger apparatus and 750 mL of distilled water was added to each balloon. It was then boiled for 3.5 hours. After this period, the essential oil was collected at the appropriate location in sterile glass (JAHANI-AZIZABADI et al., 2011). The cinnamon essential oils were characterized by GC-MS. For GC-MS analysis, an Agilent 7890B gas chromatograph with a 30m to 0.25mm HP-5MS capillary column, coupled with an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA) operating in EI mode at 70 eV were used. The temperatures of the injector and detector ports were set at 250 and 150 ° C respectively. Initially, the column temperature was held at 60 °C for 3 min and then increased at a rate of 5 °C/min to 220 °C. The temperature of column was held at 220 °C for 10 min. The composition of cinnamon essential oil is shown in Table 2. 72.32% cinnamaldehyde content of cinnamon was obtained.

Table 2. Composition of Cinnamon essential oil

Composition		Percentage
Styrene	C_8H_8	0.55
Benzaldehyde	C_7H_6O	1.31
Cinnamaldehyde	C_9H_8O	72.32
Cinnamyl alcohol	$C_9H_{10}O$	0.10
1-Naphthol	$C_{10}H_8O$	0.13
Benzylideneacetone	$C_{10}H_{10}O$	3.20
Cuminaldehyde	$C_{10}H_{12}O$	0.69
Carveol	$C_{10}H_{16}O$	3.17
Borneol	$C_{10}H_{18}O$	0.16
Benzene 1,5-dimethyl	$C_{15}H_{22}$	1.21
Nonylphenol	$C_{15}H_{24}$	14.17

Samples of oven-dried sesame meal were ground with a mill so that the particle diameter was equal to 1 mm. Dacron bags (12 × 10 cm with a 50 ± 10 micron porosity) were dried in an oven at 65 °C and heated to a constant weight. The bags were then placed into a desiccator and the sesame meal samples were poured into the bags. They were completely sealed using a plastic sewing machine. The other additives used in this experiment were monensin (Monencivet®) and tannic acid (Merck GmbH, Darmstadt, Germany).

In vitro experimental design. In order to determine the effect of various additives on the degradability of sesame meal, GARGALO et al's (2006) method was used using a Daisy^{II} incubator

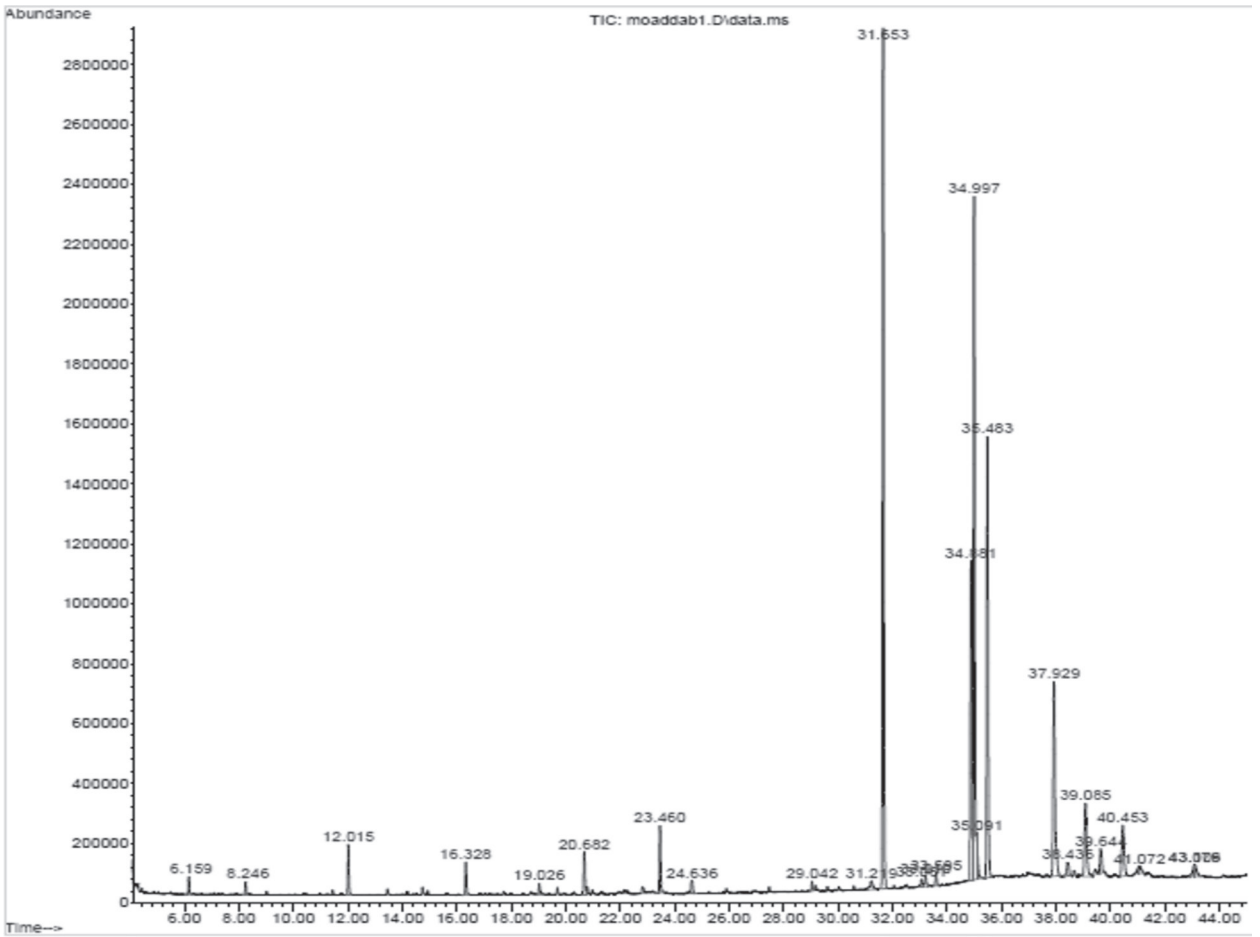

Fig 1. The graph of GC-mas of cinnamon essential oil

(Ankom, Fairport, NY). In this experiment, about 3 g of the sample were poured into Dacron bags and sewn with heat. Nine bags for each treatment were placed into a bottle containing a buffer prepared by BESHARATI et al. (2020) and rumen liquid (2:1). The buffer solution was flushed with CO_2 to remove the oxygen. The rumen liquid was prepared from 3 sheep from a slaughterhouse after slaughter. The samples were incubated inside the Daisy for 16 hours at 39°C. After incubation, they were removed from the bottle and rinsed with cold water for 15 minutes. They were placed in an oven at 55°C for 48 hours. After drying, DM, OM and CP digestibility were measured according to the AOAC (2005) methods.

The pepsin-pancreatin procedure of CALSAMIGLIA and STERN (1995) was conducted with 15 mL of a solution containing high enzymatic activity pepsin (P-7012, Sigma, St. Louis, MO). The 6 bags containing ruminal digested samples were placed inside each jar, and were kept for 1 hour at 39 °C. Then, the bags were kept in jars containing pancreatin solution for 24 hours at 39 °C. After incubation, the bags were taken out of the jars and washed. The bags were dried in the oven at 55 °C for 48 hours and then weighed.

Using the following equation, the percentage of sample digestibility was calculated:

$$\%Dig = \frac{(W1 - W2)}{Wm} \times 100$$

where,

W1: Bag and sample weight before digestion
W2: Weight of the bag and sample after digestion
Wm: Sample Weight (g)

Run 1:

Experimental treatments included: sesame meal (control), sesame meal+12 mg monensin/L medium, sesame meal+500 mg tannic acid/ L medium, sesame meal+150 mg cinnamon essential oil/ L medium.

Run 2:

Experimental treatments included: sesame meal (control), sesame meal+24 mg monensin/L medium, sesame meal+1000 mg tannic acid/ L medium, sesame meal+250 mg cinnamon essential oil/ L medium.

Statistical analysis. The data obtained were analyzed as a completely randomized design using a GLM procedure of SAS (2018), with Duncan's multiple range test used for comparison of means. Additives (cinnamon oil, monensin and tannic acid) were the only sources of variation considered (PALANGI and MACIT, 2019). The following mathematical model was used for statistical analysis.

$$Y_{ijk} = \mu + T_{i} + e_{ij}$$

where Y_{ij} represents dependent variable; μ the general mean; T_i is the effect of treatment (additives); e_{ij} is error.

Results and discussion

The chemical composition of sesame meal is given in Table 1. The tested sesame meal contained 40.95% CP, which was higher than the values reported by other researchers (GHORBANI et al., 2018; OMER et al., 2019).The effect of adding monensin (12 mg/L), tannic acid (500 mg/L) and cinnamon essential oil (125 mg/L) on the three-step degradability of sesame meal using GARGALLO et al. (2006) method is given in Table 3. The results showed that the values of digestibility of all parameters measured throughout the gastrointestinal tract, the rumen and intestines were different. In the present study, the *in vitro* ruminal, intestinal and total GI tract DM digestibility values were affected by experimental treatments, and the difference between the means was statistically significant.

According to the results of this study, the disappearance of *in vitro* ruminal and intestinal organic matter was affected by the experimental treatments, where the highest amount was related to the treatment containing cinnamon essential oil (125 mg/L). Organic matter degradability in the total tract in the treatments with monensin, tannic acid and cinnamon essential oil showed the highest rate of organic matter degradation, with a significant difference compared to the control ($P<0.05$). The digestibility of *in vitro* ruminal and intestinal sesame meal CP, with the addition of monensin, tannic acid and cinnamon essential oil, were in the range of 76 to 84% and 49 to 60%, respectively.

Table 3. Evaluation of the effect of adding low levels of monensin, tannic acid and cinnamon essential oil on the degradability kinetics of sesame meal in a three-step method

Treatments	1	2	3	4	SEM	P-Value
Ruminal degradability						
DM	67.94^{c}	75.71^{b}	82.09^{a}	84.56^{a}	1.610	<0.0001
OM	70.03^{c}	75.74^{b}	81.92^{a}	84.42^{a}	1.729	<0.0001
CP	76.69^{b}	84.69^{a}	87.10^{a}	85.56^{a}	1.234	<0.0001
ADF	73.27^{c}	80.74^{b}	88.50^{a}	90.13^{a}	2.014	<0.0001
NDF	82.27^{d}	86.00^{c}	91.17^{b}	94.02^{a}	0.957	<0.0001
Intestinal degradability						
DM	11.43^{c}	16.22^{b}	19.74^{b}	21.53^{a}	0.871	<0.0001
OM	12.86^{c}	16.95^{b}	21.80^{b}	24.13^{a}	0.861	<0.0001
CP	50.72^{b}	47.78^{c}	49.34^{b}	59.48^{a}	0.374	<0.0001
ADF	13.82^{c}	19.48^{b}	22.60^{a}	23.95^{a}	0.829	<0.0001
NDF	11.56^{c}	24.17^{a}	20.20^{b}	24.25^{a}	0.844	<0.0001
Total tract degradability						
DM	73.72^{b}	82.73^{a}	90.26^{a}	90.23^{a}	0.381	<0.0001
OM	72.75^{b}	82.90^{a}	87.48^{a}	90.46^{a}	0.275	<0.0001
CP	82.14^{b}	93.21^{a}	94.48^{a}	95.28^{a}	0.114	<0.0001
ADF	77.18^{c}	86.84^{b}	92.37^{ab}	93.95^{a}	0.047	<0.0001
NDF	75.58^{c}	91.00^{b}	93.46^{ab}	96.35^{a}	0.384	<0.0001

Differences between the averages indicated by different letters in the same row are important. Treatments: 1 - Sesame meal (control), 2 - Sesame meal + 24 mg / l Monensin, 3 - Sesame meal + 1000 mg /l tannic acid, 4 - Sesame meal + 250 mg/L cinnamon essential oil

The results indicate that addition of cinnamon essential oil increased the intestinal CP degradability by about 10%. The addition of monensin, tannic acid, and cinnamon essential oil significantly increased the degradability of ADF and NDF in the rumen, intestine, and total tract, which was further observed in the treatment containing cinnamon essential oil. In a study conducted by YANG et al. (2010) supplementing different levels of cinnamaldehyde (400, 800, 1600 mg/day) using an *in situ* method, they reported that at levels 400 and 800 mg, the ruminal biodegradability of OM increased compared to the control, but by increasing the cinnamaldehyde level to 1600 mg/day, the ruminal biodegradability of OM decreased, which was consistent with the results of our study. Also, an increase in the amount of cinnamaldehyde led to a decrease in ruminal NDF degradability. However, the degradability of organic matter and NDF in the post-rumen (intestinal) was not affected by the increase in cinnamaldehyde content. OSBORNE et al. (2004) examined 22 mg/kg DM of monensin supplementation using 2 fistulas of Holstein cows. They found that ruminal DM, CP, ADF, NDF degradability was not affected by the addition of monensin, but total tract degradability increased compared to the control, which is consistent with the results of the present experiment. In another study, BENCHAAR et al. (2008) evaluated monensin (16 mg / kg DM) and essential oil (a mixture of thymol, eugenol, and limonene at 2 g per day) supplementation on soybean meal using Breastfeeding Holstein cows, and found that DM, OM, and NDF digestibility throughout the gastrointestinal tract was not affected by experimental treatments, which does not match the results of the present experiment.

The effect of adding monensin (24 mg / L), tannic acid (1000 mg/L) and cinnamon essential oil (250 mg/L) on the three-step degradability of sesame meal using GARGALLO et al. (2006) method is shown in Table 3. In the present study, adding monensin, tannic acid and cinnamon essential oil to sesame meal significantly increased the DM, CP, OM, ADF and NDF *in vitro* ruminal, intestinal and total tract degradability, and this increase on degradability in the rumen was greater than in the intestine. The values of *in vitro* ruminal, intestinal, and total tract degradability tested parameters did not significantly differ between treatments containing monensin and cinnamon essential oil, but the differences were significant compared to tannic acid treatments. However, in the intestine, treatments containing tannic acid and cinnamon essential oil had a higher CP degradability compared to monensin treatments. Our results differed from those of BENCHAAR et al. (2008), who reported that adding thymol (200 mg/L), carvacrol (400 mg/L) and eugenol (800 mg/L) would reduce crude fiber. This may be due to the fact that they used active ingredients instead of using essential oils, which resulted in improved effects. An increase in DM degradability may be considered as a positive effect of cinnamon essential oil, because in most studies the use of essential oils has led to a decrease in DM degradability, which is an adverse effect of the use of herbal essential oils (COBELLIS et al., 2016; ELWAKEEL et al., 2019; DAVOODI et al., 2019). An increase in DM degradability is highly desirable in terms of nutrition, and an increase in CP degradability can be considered as one of the negative effects of using cinnamon essential oil, because it reduces the efficiency of nitrogen

Table 4. Evaluation of the effect of adding high levels of monensin, tannic acid and cinnamon essential oil on the degradability kinetics of sesame meal in a three-step method

Treatments	1	2	3	4	SEM	P-Value
Ruminal degradability						
DM	56.55^{c}	74.82^{a}	63.64^{b}	71.24^{a}	1.281	<0.0001
OM	57.90^{d}	78.29^{a}	64.37^{c}	71.72^{b}	1.245	<0.0001
CP	66.62^{d}	83.77^{a}	70.87^{c}	77.34^{b}	1.001	<0.0001
ADF	61.72^{c}	84.87^{a}	71.01^{b}	84.25^{a}	0.954	<0.0001
NDF	74.58^{c}	85.62^{ab}	86.38^{a}	83.89^{b}	0.604	<0.0001
Intestinal degradability						
DM	13.83^{c}	29.76^{ab}	26.78^{b}	33.54^{a}	1.479	<0.0001
OM	14.23^{c}	33.00^{a}	28.48^{b}	34.33^{a}	1.422	<0.0001
CP	50.94^{d}	54.44^{c}	61.99^{a}	57.73^{b}	0.625	<0.0001
ADF	14.21^{c}	33.20^{ab}	30.02^{b}	36.42^{a}	0.914	<0.0001
NDF	11.99^{c}	40.51^{a}	26.95^{b}	37.42^{a}	0.804	<0.0001
Total tract degradability						
DM	73.72^{b}	82.73^{a}	90.26^{a}	90.23^{a}	1.560	<0.0001
OM	72.75^{b}	82.90^{a}	87.48^{a}	90.46^{a}	1.492	<0.0001
CP	82.14^{b}	93.21^{a}	94.48^{a}	95.28^{a}	0.676	<0.0001
ADF	77.18^{c}	86.84^{b}	92.37^{ab}	93.95^{a}	1.041	<0.0001
NDF	75.58^{c}	91.00^{b}	93.46^{ab}	96.35^{a}	0.702	<0.0001

Differences between the averages indicated by different letters in the same row are important. Treatments: 1- Sesame meal (control), 2- Sesame meal + 24 mg / l Monensin, 3- Sesame meal + 1000 mg / l tannic acid, 4- Sesame meal + 250 mg / l cinnamon essential oil.

use, and reduces the amount of protein passing into the intestine. The essential oils and natural extracts of medicinal plants have antimicrobial properties (AUMEERUDDY-ELALFI et al., 2016; MOUSSAOUI and ALAOUI, 2016; SAKKAS and PAPADOPOULOU, 2017), therefore, they may be used as alternatives to ruminal fermentation enhancers due to their ability to improve energy efficiency and protein utilization. There is a direct relationship between the ruminal DM and OM disappearance with the VFA production, and increasing or decreasing the rate of disappearance have affected the ruminal VFA production (SARMADI et al., 2016; CHOWDHURY et al., 2018; SALAS et al., 2019).

Conclusion

Adding Cinnamon essential oil increased the disappearance of *in vitro* ruminal and intestinal organic matter. Overall, the additives used in this study increased the total tract degradability of sesame meal in comparison with the control.

Conflict of interest

The authors declare that they have no known compering financial interests or personal relationship that could appear to have influenced the work reported in this paper.

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BESHARATI, M., V. PALANGI, A. TAGHIZADEH, A. KAYA, S. ABACHI: Istraživanje učinka prirodnih inhibitora na razgradivost sezama primjenom *in vitro* metode u tri koraka. Vet. arhiv 91, 513-521, 2021.

SAŽETAK

Cilj ovog rada bio je istražiti pozitivni učinak dodatka monenzina, tanina i esencijalnog ulja cimeta na razgradivost sezama *in vitro* metodom u tri koraka. Pronađen je znakovit učinak pokusnih dodataka na razgradivost sezama u buragu, a zatim i u cijelom gastrointestinalnom traktu ($P < 0,05$). *In vitro* buražna i intestinalna probavljivost sirovih bjelančevina sezama s pokusnim dodacima bila je od 76 do 84 %, odnosno 49 do 60 %. Intestinalna razgradivost sirove bjelančevine povećala se s dodatkom esencijalnog ulja cimeta (oko 10 %). Dodatak monenzina, tanina i esencijalnog ulja cimeta znakovito je povećao razgradivost neutralnih vlakana deterdženta (NDF) i kiselih vlakana deterdženta (ADF) u buragu, crijevima i cijelom gastrointestinalnom traktu. Rezultati su pokazali da esencijalno ulje cimeta (125 mg/L) povećava razgradivost suhe tvari (DM), organske tvari (OM), sirovog proteina (CP), ADF-a i NDF-a u buragu, a zatim i u cijelom probavnom sustavu u usporedbi s drugim pokusnim postupcima ($P < 0,05$). Rezultati su pokazali da dodatak tanina (100 mg/L) smanjuje razgradnju sirovog proteina u buragu, a povećava njegovu razgradnju u dijelu probavnog trakta nakon buraga ($P < 0,05$).

Ključne riječi: monenzin; tanin; esencijalno ulje cimeta; sezamovo brašno; razgradnja u tri koraka