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Research Article

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Determination of Feed Value, İn Vitro Digestibility and Enteric Methane Production of Black Seed (Nigella sativa) Meal

Mahmoud O. A. Elfaki, Unal KILIC*

Ondokuz Mayis University, Faculty of Agriculture, Department of Animal Science, Samsun-Turkiye
*Corresponding author: unalk@omu.edu.tr

Abstract This study was carried out to determine the feed value, in vitro gas production, and enteric methane production of black seed (Nigella sativa) meal. Black seed meal was used as a feed material in this study. Hohenheim gas production technique and the Daisy incubator were employed to determine the feed's gas production and the in vitro true digestibility (IVTD), respectively. The obtained data were evaluated to show the current situation clearly. According to the study's findings, black seed meal was considered to be rich in crude protein (25.09% DM) and crude fat (18.39% DM). After 24 hours of incubation, black seed meal was observed to have 30.61 ml/200 mg DM of total gas production and 5.28 ml of methane production. It has also been determined that black seed meal has low methane production and is found to be highly digestible, which will be effective in reducing greenhouse gas emissions that found to be highly digestible. As a result, black seed meal can be evaluated as an alternative protein and energy source in ruminant and poultry nutrition.

Keywords Black seed meal, digestibility, in vitro gas production, methane.

1. Introduction

Black cumin (Nigella sativa) is an annual plant widely distributed in Asia and with minimal quantities in Southern Europe; known as a source of healing plant that has been used as a natural medicine for many years. Black cumin contains a high percentage of crude fat (32-40%) and 15-20.2% crude protein, while phenolic components (saponin, tannin, etc.) take an important place in its structure. Among the secondary components of black cumin comes thymoquinone, dithymoquinone, thymohydroquinone, and thymol first, then rochebrune and africaine components come in the second place [1-3].

It has been reported that black seed has an immune system strengthening effect, as well as antioxidant, antibacterial, and antimicrobial effects [4], along with improving respiratory diseases resistance, not to mention the diuretic, antiperspirant, blood pressure lowering, carminative, and milk yield-increasing effects [3].

Guler et al. [5] concluded that the use of 1% black cumin in the diets of poultry would positively affect growth. Denli et al. [6] suggested that using 1 g/kg of black cumin extract positively affects egg quality and production in quails.

Ozcelik and Bayram [3] reported that the use of black cumin seeds within the diet of lambs at a rate of 4% in concentrated feed does not cause problems in terms of fattening performance and some blood parameters of the lambs and even has advantages in terms of preventing acidosis (increasing rumen pH).

It has been reported that the different results of black cumin seeds in different studies are due to the different species, variety, locations, climate, harvest or maturation period differences, and storage conditions of the black cumin seeds used [7]. However, many studies have been conducted on different black seed meal usages in poultry [8] and buffaloes feed [9] to determine the suitability of black seed meal addition in animal nutrition.

This study aims to determine the nutrient content, feed value, digestibility, in vitro gas production, in vitro digestibility and methane production of the bagasse remaining from the black cumin seed oil and its potential use in animal nutrition. This study hypothesizes that black cumin seeds have a carminative effect, thus reducing in vitro gas and methane production.

2. Materials and Methods

2.1 Feed Material and Rumen Fluid Supply

In this study, the residue remaining after the oil extracted from the black seeds obtained from an herbalist, namely black seed meal, was used. Rumen fluid used for in vitro gas production technique and determination of in vitro true digestibility was obtained from the rumen of one healthy animal of Holstein×Simmental hybrid with a body weight of 700 kg, two years old from a slaughterhouse operating in Atakum, Samsun province. It was filtered with strainers into a thermos at 38-40°C, and a handful of solid rumen content was added for the purpose of being used in in vitro digestibility and transportation to the laboratory within 20-25 minutes. The pH value of the rumen fluid used was measured as 5.61-6.18 in the Hanna model digital pH meter.

2.2 Chemical analyses

The feed samples were ground to pass through a 1 mm sieve. The ground samples were analyzed for dry matter (DM), crude protein (CP), and ash, according to AOAC [10]. The acid detergent fiber (ADF), neutral detergent fiber (NDF), acid detergent lignin (ADL), and crude fiber (CF) analysis were run according to the method of Van Soest [11] using ANKOM 2000 semi-automated fiber analyzer (ANKOM Technology). The ether extract (EE) was determined using the ANKOM XT15 extraction system device as specified by AOCS [12]. Organic matter (OM), cellulose (CEL), and hemicellulose (HCEL) values were determined by calculation. Condensed tannins (CT) analysis was performed according to the method reported by Makkar et al. [13]. All chemical analyses were carried out in 3 parallels.

2.3 In Vitro Gas Production Technique

Hohenheim gas production test was used to determine the in vitro gas production of black seed meal [14-16]. Samples were ground to pass through a 1 mm sieve, and approximately 250 mg of dry material in the air (200 mg DM) was weighed. Gas productions were determined for 3, 6, 9, 12, 24, 48, 72 and 96 hours incubations, and gas production at the end of the study was determined as mL/200mg DM. The gas production was standardized according to the blank (rumen fluid + buffer mixture) and standard alfalfa hay.

2.4 Methane Measurement

An infrared methane analyzer (Europa GmbH, Erkrath, Germany model) was used to determine the methane production of the black seed meal [17]. After reading the 24-hour gas production through in vitro gas production technique, the remaining gases in the injectors were taken to the methane analyzer, and the methane production was calculated as follows.

$$\text{Methane production (mL)} = \text{Total gas production (mL)} \times \text{Percent of methane (\%)}$$

2.5 Determining True Nutrient Digestibility

In vitro true digestibility (IVTD) of black seed meal was determined by Ankom DaisyII Incubator D220 [18]. The rumen fluids used in the in vitro gas production technique were used in the digestibility study. The Daisy incubator consists of four independent jars, which served as artificial rumen in the in vitro study. In the Daisy incubator, bags were placed in a digestibility jar and placed for 48 hours. Feeds were tested in 5 parallels in the Daisy incubator. NDF analyses were performed on the samples remaining in the bags after incubation, and the in vitro true digestibility of black seed meal was calculated by applying the following formula.

$$\%\text{IVTD} = \frac{100 - ((\text{W3} - (\text{W1} \times \text{C1})) \times 100}{\text{W2}}$$

W1: Tare of F57 bags, W2: Amount of NDF in the dry sample, W3: Amount of NDF remaining in the bag after incubation, C1: Blank weight (weight of empty bag/original bag after removing from the incubator and drying in an oven).

2.6 Statistical Analysis

The means and standard deviations of the data obtained in the study were determined, and the similarities and differences between the results obtained in similar studies and these results were evaluated by comparison.

3. Results and Discussion

The nutrient contents of black seed meal are presented in Table 1. The CP content of black seed meal was determined as 25.09% DM, similar to the 29.8% DM value reported by Tekeli [8]. The Ash (4.95% DM) and CF (8.97% DM) values reported by Tekeli were also similar to the data obtained in this study. However, the EE content (19.38% DM) determined in the current study was found to be much higher than the EE content (4.31%) reported by Tekeli [8]. This shows that the oil extraction process is not done sufficiently in the seeds used in the current study, and it is not preferred economically because there is too much oil in the seeds. The CP content also shows a lower value. Mahmoud and Ghoneem [19] found the CP content to be 33.13% DM for black seed meal, while the EE content was determined as 12.72% DM. These values were quite different from those obtained in the present study due to the abovementioned reasons.

Abd-El Rahman et al. [9] stated that black seed meal has suitable nutrient contents for feeding buffaloes in lactation. The authors determined the CP content of black seed meal to be 28.85%, and the CF content was 13.31%. These values seem to be partially compatible with the data obtained in the current study.

Table 1. Nutrient contents and cell wall structural elements of black seed meal, % (as DM)

	DM*	**OM**	**Ash**	**CP**	**EE**	**CF**	**NFE**	**NDF**	**ADF**	**ADL**	**HCEL**	**CEL**
Mean	91.61	93.87	6.13	25.09	19.38	9.86	39.54	25.52	13.83	8.68	11.67	5.15
SD	0.10	0.02	0.02	0.84	0.17	1.62	2.23	0.67	0.20	0.48	0.48	0.29

DM*: dry matter (natural form), OM: organic matter, CP: crude protein, EE: ether extract, CF: crude fibre, NFE: nitrogen-free extracts, NDF: neutral detergent fibre, ADF: acid detergent fibre ADL: acid detergent lignin, SD: standard deviation.

El Ayek et al. [20] reported that black seed meal could be added to the rations to meet half of the protein requirement of lambs without any side effects.

It is known that if the condensed tannin content in the feeds is at the level of 5-10%, it can reduce the feed consumption of the animals, negatively affect their performance, and cause toxic effects [21]. In this study, it can be stated that black seed meal will not adversely affect feed consumption, even if added to the ration in large amounts, since the tannin contents (0.47%) determined in black seed meal are well below the specified limits. When adding black seed meal at low doses, it can be seen that using black seed meal will not negatively affect the rations.

Black seed meal has the potential to be used as a concentrated feed source in both poultry and ruminant rations in terms of high crude protein (25.09% DM), high crude fat content (19.38% DM), low values of ADF and NDF contents, and it has a very high digestibility value (IVTD: 85.93% DM).

Time-dependent in vitro gas production values of black seed meal are given in Table 2. In vitro gas production parameters (c, a+b), methane amounts measured after 24 hours incubation, organic matter digestibility, energy values (ME and NEL), in vitro true digestibility, and condensed tannin contents are given in Table 3.

The in vitro gas production of black seed meal after 24 hours of incubation was determined as 60.61 ml/200 mg DM, while the total gas production after 96 hours of incubation was determined as 47.75 ml/200 mg DM (Table 2).

Kilic et al. [22] added black seed essential oil to barley, soybean meal, and wheat straw at 50, 100, and 150 ppm doses; their 24-hour gas production parameters were measured as s 57.7, 58.0 and 55.6 ml/200 mg DM; 38.2, 36.6 and 36.1 ml/200mg DM and 25.6, 25.8 and 24.4 ml/200mg DM respectively. The values of gas production determined by Kilic et al. [22] were not compared to the data in this study because black seed meal was used.

Table 2. *In vitro* gas production (ml/200mg DM) of black cumin seeds and pH after 96 hours of incubation

	Incubation time (hours)								
	3	**6**	**9**	**12**	**24**	**48**	**72**	**96**	**pH**
Mean	9.35	12.99	13.71	23.40	30.61	37.82	42.73	47.75	6.71
SD	0.63	4.20	3.56	5.00	5.54	6.59	6.78	6.75	0.04

The pH value determined after 96 hours of incubation in the experiment was 6.71, which indicates that the buffer used in the present study was not exhausted. In this respect, this indicates that the study was conducted safely.

Table 3. Gas production parameters, methane production, OMD, ME and NE_L contents, IVTD, and Condensed tannin contents of black cumin seeds

	c, ml/h	**a+b, ml**	**Methane, ml**	**OMD, %**	**ME, Kcal/kg DM**	**NE_L, Kcal/kg DM**	**IVTD, %**	**CT % DM**
Mean	0.04	47.48	5.28	60.62	11.55	7.18	85.93	0.47
SD	0.01	5.62	0.53	4.21	0.87	0.42	3.77	0.06

c: gas production rate, a+b: total gas production, OMD: organic matter digestibility, ME: metabolizable energy. NE_L: net energy lactation, IVTD: *in vitro* true digestibility, CT: condensed tannen, SD: standard deviation.

The *in vitro* gas production parameters a+b values were determined as 47.48 ml and methane production was detected as 5.28 ml (Table 3). Accordingly, it can be stated that black seed meal causes low total gas and methane production due to the effect of reducing the enteric methane production of the crude fat content.

4. Conclusion

According to the data obtained in this study, it was determined that black seed meal had a high nutritional value and digestibility with a reducing effect on methane production. It was found that phenolic components, which restrict their use as feed in animal nutrition, are insignificant due to the low condensed tannin content.

In particular, as long as many studies have focused on reducing the production of greenhouse gases, which is on the agenda as an important problem today. Apparently, adding the black seed meal to the rations of ruminants will have positive effects in reducing greenhouse gas emissions in terms of reducing enteric methane production from ruminants, which is affirmed to cause 16% of methane production worldwide, and also preventing the waste of feed energy as methane gas. In vivo, studies are recommended to determine enteric methane production with diets using black seed meal.

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