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Evaluation of Hazelnut Hulls as an Alternative Forage Resource for Ruminant Animals

N. Cetinkaya, Y. S. Kuleyin

***Abstract*—**The aim of this study was to estimate the digestibility of the fruit internal skin of different varieties of hazelnuts to propose hazelnut fruit skin as an alternative feed source as roughage in ruminant nutrition. In 2015, the fruit internal skins of three different varieties of round hazelnuts (RH), pointed hazelnuts (PH) and almond hazelnuts (AH) were obtained from hazelnut processing factory then their crude nutrients analysis were carried out. Organic matter digestibility (OMD) and metabolisable energy (ME) values of hazelnut fruit skins were estimated from gas measured by *in vitro* gas production method. Their antioxidant activities were determined by spectrophotometric method. Crude nutrient values of three different varieties were; organic matter (OM): 87.83, 87.81 and 87.78%), crude protein (CP): 5.97, 5.93 and 5.89%, neutral detergent fiber (NDF): 30.30, 30.29 and 30.29%, acid detergent fiber (ADF): 48.68, 48.67 and 48.66% and acid detergent lignin (ADL): 25.43, 25.43 and 25.39% respectively. OMD from 24 h incubation time of RH, PH and AH were 22.04, 22.46 and 22.74%; ME_{GP} values were 3.69, 3.75 and 3.79 MJ/kg DM; and antioxidant activity values were 94.60, 94.54 and 94.52 IC 50 mg/mL respectively. The fruit internal skin of different varieties of hazelnuts may be considered as an alternative roughage for ruminant nutrition regarding to their crude and digestible nutritive values. Moreover, hazelnut fruit skin has a rich antioxidant content so it may be used as a feed additive for both ruminant and non-ruminant animals.

***Keywords*—**Antioxidant activity, hazelnut fruit skin, metabolizable energy, organic matter digestibility.

I. Introduction

THE world hazelnut production shows fluctuations depending on climatic conditions. Turkey is a leading country in hazelnut production; an average production is around 650.000 t/year which covers approximately 75-80% of total world production. The remaining 20% of hazelnut production is shared by Italy, USA, Azerbaijan, Georgia and Spain [1]. Turkey is producing 16 different hazelnut varieties in Giresun, Ordu, Trabzon, Rize, Artvin, Sinop, Samsun, Kastomonu, Bartın, Kocaeli, Duzce, Sakarya and Zonguldak provinces which are located in Black Sea Region of Turkey [1].

Hazelnut produced in Turkey is generally classified in three main groups according to fruits shape and features: RH, PH and AH. Hazelnut hull or hazelnut fruit internal skin is a by-product or waste obtained during hazelnut processing in factories [1]. Hazelnut fruit internal skin is obtained as waste in the amount of 4-5% of the total processed hazelnuts according to data received from the hazelnut processing factory. The amount of this waste is around 26.000-32.500 t/year. Hazelnut has also been consumed by people without removing internal skin of hazelnut which indicates that internal skin of fruit is edible [2].

The crude nutritive value of a ruminant feedstuffs is determined by chemical analysis [3]. *In vitro* gas production technique is useful to evaluate the nutritive value of feedstuffs in which produced gas is regarded as an indicator of carbohydrates degradation [4]. Sallam suggested that gas volume is a good parameter from which to predict digestibility and microbial protein synthesis of the substrate by rumen microorganisms in the *in vitro* system [5]. OMD and ME values of feedstuffs have mostly been determined by using *in vitro* gas production method [4], [6], [7].

Nowadays, natural antioxidant sources as health promoting nutrients are gaining great importance in human nutrition [8]. There are several extraction procedures and determination methods for evaluation of the total antioxidant activity of plants [9], [10]. 2,2 diphenyl-1-picrylhydrazyl radical (DPPH) method has widely been used due to its simplicity and its simple reaction system which involves only direct reaction between radical and antioxidant [11].

Since synthetic antioxidants may be toxic and carcinogenic which they have also been well demonstrated with many studies, limitations or prohibitions on their use have been put in the application [12]-[14]. These consequences are directed animal nutrition scientists to search safe and natural resources.

The objective of the present study was to estimate the digestibility and antioxidant activity of the fruit internal skin of different varieties of hazelnut to propose hazelnut fruit skin as an alternative feed source as roughage in ruminant nutrition.

II. Material and Methods

A. Animal Material

The rumen fluid was collected from slaughtered cattle in Florya Meat Joint-Stock Company, Samsun, Turkey. Collected rumen fluids were immediately transferred from Florya slaughterhouse to the laboratory approximately in 5 minutes.

B. Feed Material

In 2015, the fruit internal skins of three different varieties of RH, PH and AH were obtained four times from hazelnut processing factories.

C. Experimental Procedure

Chemical analysis, *in vitro* gas production experiment and total antioxidant activity analysis were carried out with quartet

N. Cetinkaya is with Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Ondokuz Mayis University, 55139, Samsun, Turkey (phone: +905065816351; fax: + 903624576922; e-mail: nurcanc@omu.edu.tr).

Y.S. Kuleyin is with Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Ondokuz Mayis University, 55139, Samsun, Turkey (e-mail: serhatkuleyin@gmail.com).

four samples in the Ruminant Feed Evaluation Laboratory of Department of Animal Nutrition and Nutritional Diseases and in Laboratory of Department of Biochemistry, Faculty of Veterinary Medicine, OMU, Samsun, Turkey.

D. Chemical Analysis

Collected fruit internal skin samples were milled through a 1 mm sieve for total antioxidant activity, chemical analysis and *in vitro* gas production method. Dry mater (DM), ash, ether extract (EE) and nitrogen (N) contents were determined according to AOAC procedure [3]. CP was calculated as N x 6.25. NDF, ADF and ADL were determined by using ANKOM fiber analyzer [15].

E. In vitro Gas Production Method

The ANKOM ^{RF} gas production system which consists of incubator, 12 glass jars named modules, each one having of 250 mL capacity was connected to computer. Gas accumulating in the headspace of module was automatically released when the pressure inside the units reached to 1.5 kPa above ambient pressure. The produced gas pressure was recorded at 10 minute intervals by using ANKOM^{RF} gas production system program.

Approximately 1 g of each grounded sample was weighted and put into module. The prepared artificial salivia solution [4] was mixed with rumen fluid 4:1. A mixture of 100 mL of this solution was added to preheated sample containing modules under anaerobic conditions by continuously flushing CO_2. Then modules transferred to incubator at temperature about 39 ^{0}C and pH about 6.5 to 6.8 and *in vitro* gas production system was started. After 96 hours, system was stopped.

The average cumulative pressure recorded at 0, 3, 6, 12, 24, 48, 72 and 96 hours were converted to mL of gas at standard temperature and pressure. Cumulative gas production data at 24 h was fitted to the model (1) of Ørskov and McDonald [16]:

$$\text{Gas (Y)} = b\,(1-e^{-ct}) \quad (1)$$

where b: The gas production from the insoluble fraction (mL), c: The gas production rate constant for the insoluble fraction (mL/h), t: Incubation time (h). $T_{1/2}$: The time taken to produce the half of the gas volume was calculated [17], [16], using (2) and (3):

$$T_{1/2} = \text{Ln}2/c \quad (2)$$

$$T_{1/2} = 0.693/c \quad (3)$$

OMD %, ME_{GP}, and ME_{OMD} (MJ/ kg DM) values of samples were estimated by using [5]:

$$ME_{GP}\,(\text{MJ/kg DM}) = 2.2+0.136\,GP+0.057CP+0.0029\,EE \quad (4)$$

$$\text{OMD (\%)} = 57.2+0.365\,GP+0.304\,CP-1.98\,ADL \quad (5)$$

GP (mL/200 mg DM)

$$ME_{OMD}\,(\text{MJ/kg DM}) = 0.16\,OMD \quad (6)$$

ME_{GP}: ME calculated from gas production; ME_{OMD}: ME calculated from OMD.

F. Determination of Total Antioxidant Activity

Total antioxidant activity and free radical scavenging activity of fruit internal skin of different varieties of hazelnut samples were determined by DPPH method [17], [18]. The absorbances were measured at 520 nm. Quercetin (0–50 mg/L) and ascorbic acid (0–40 mg/L) were used as positive controls.

The radical scavenging activity was calculated by (7):

$$\text{Inhibition \%} = [(\text{blank absorbance} - \text{sample absorbance})/\text{blank absorbance}] \times 100 \quad (7)$$

The mean concentrations of samples were calculated from three readings causing 50% inhibition values (IC50).

G. Statistical Analysis

The data obtained from the chemical analysis, antioxidant and *in vitro* gas production experiments were analyzed by the procedure of the software package SAS [18]. Differences between mean values of fruit internal skin of different varieties of hazelnut samples were performed by t-test.

III. Results and Discussion

Chemical composition of fruit internal skin of three different varieties RH, PH and AH of hazelnut is shown in Table I. DM % in air dried of RH, PH and AH were calculated as 91.17, 91.11 and 91.07% respectively. The statistically significant differences were not observed between chemical composition parameters and ME estimated from ADF values of hazelnut varieties RH, PH and AH at 24 h of incubation with the exception of CF or ether extract. Mean ME_{ADF} values of RH, PH and AH were not significantly different and they were higher than the reported values for wheat straw, maize straw and black wheat straw [21]; however, they were close to marc, chick pea straw [22] and *Juncus acutus* [23].

TABLE I
Chemical Composition of Fruit Internal Skin of Three Different Varieties of Hazelnut

Crude Nutrients (%)	RH (n=16)	PH (n=16)	AH (n=16)
	Mean±SE	Mean±SE	Mean±SE
DM	91.17±0.01	91.11±0.03	91.07±0.04
CA	3.34±0.02	3.30±0.04	3.29±0.01
OM	87.83±0.02	87.81±0.03	87.78±0.03
CP	5.97±0.04	5.93±0.03	5.89±0.02
CF	21.16±0.08a	20.32±0.06b	17.15±0.03c
NDF	30.30±0.05	30.29±0.03	30.29±0.05
ADF	48.68±0.05	48.67±0.03	48.66±0.02
ADL	25.43±0.08	25.43±0.07	25.39±0.08
ME_{ADF}, MJ/kg KM	8.27±0.03	8.27±0.04	8.27±0.02

[a, b, c] Mean in the same row with different letters in their superscripts differ ($P<0.05$). DM=Dry Matter, CA=Crude Ash, CF=Crude Fat, ME_{ADF}= ME Calculated from ADF.

Estimated OMD %, ME_{OMD} (MJ/KG DM), ME_{GP} (MJ/KG DM) values based on at 24 hour *in vitro* gas production volume (P_{PSI}/1 G DM, GP_{ML}/200MG DM) of RH, PH and AH are shown in Table II. Changes of gas production volume with *in*

vitro incubation times for RH, PH and AH is shown in Fig. 1. The mean ME_{GP} values of internal skin of three different fruits of RH, PH and AH were found significantly different ($P<0.05$). These differences may be originated from different gas production of RH hulls as seen in Table II. ME_{GP} values of three different hazelnut varities were found similar to wheat straw [24], *M. indica, L. arborea ve S mexicana* tree leaves [6]. Estimated OMD % and ME_{OMD} as well as c, b and $T_{1/2}$ values of internal skin of three different fruits of RH, PH and AH at 24 h incubations were significantly different ($P<0.05$). The reason may be originated from low gas production at 24 h incubation of RH besides high ADL values of hazelnut fruit hulls. The mean OMD % values changed between 22.04-22.74% which are similar to reported values of M. *indica, L. arborea* and *S mexicana* tree leaves [7].

TABLE II
ESTIMATED OMD %, ME_{OMD} (MJ/KG DM), ME_{GP} (MJ/KG DM) VALUES BASED ON AT 24 HOUR *IN VITRO* GAS PRODUCTION VOLUME (P_{PSI}/1 G DM, GP_{ML}/200MG DM) OF RH, PH AND AH

In vitro Gas Production Parameters	RH(n=16) (Mean±SE)	PH(n=16) (Mean±SE)	AH(n=16) (Mean±SE)
P_{psi}	3.23±0.15c	3.42±0.17b	3.54±0.14a
GP_{mL}	8.0±0.30c	8.47±0.32b	8.77±0.23a
OMD	22.04±0.04c	22.46±0.08b	22.74±0.05a
ME_{OMD}	3.53±0.04c	3.60±0.03b	3.64±0.02a
ME_{GP}	3.69±0.02c	3.75±0.02b	3.79±0.04a
b	8.82±0.35c	9.31±0.41b	9.69±0.36a
c	0.28±0.032c	0.35±0.021a	0.30±0.028b
$T_{1/2}$	2.52±0.23a	1.98±0.32c	2.31±0.18b

[a,b,c] Mean within a row with different superscripts differ ($P< 0.05$). ME_{OMD}=Metobolisable energy estimated from OMD, ME_{GP}= Metobolisable energy estimated from *in-vitro* gas production, b=Potential gas production, c= The gas production rate constant for the insoluble fraction (mL/h), $T_{1/2}$= The time taken to produce the half of the total gas pool (h).

The cumulative volume of gas production increased with increasing incubation time as seen in Fig. 1.

Total antioxidant activity values of RH, PH and AH were 94.60, 94.54 and 94.52 IC 50 mg/mL respectively. There was no significant difference between studied varieties. The mean total antioxidant values were higher than the reported values of different varieties of soybean [25] and rice straw [26] but similar to *Juncus acutus* [27].

In conclusion, the obtained nutritive values of fruit internal skin of different varieties of hazelnut showed similar profiles when compared with common crop residues like wheat or barley straw, therefore, it can be proposed as an alternative roughage source in ruminant feeding. Furthermore, it may also be considered as food additive because of its high antioxidant content in animal even in human nutrition.

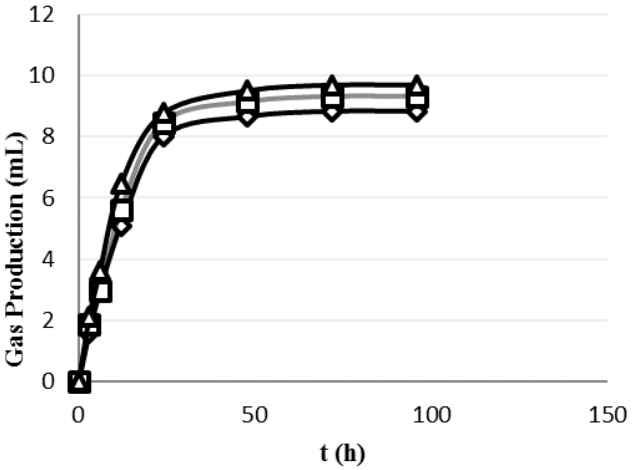

Fig. 1 Changes of gas production volume (mL) with *in vitro* incubation times (h) for round (◊), pointed (□) and almond (Δ) hazelnuts

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