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

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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<sub>GP</sub> 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, Bartin, 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

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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 ANKOMRF 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<sub>2</sub>. Then modules transferred to incubator at temperature about 39 °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]:

Gas (Y)= b (1-
$$e^{-ct}$$
) (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):

$$T_{1/2} = Ln2/c$$
 (2)

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

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

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

OMD (%) = 
$$57.2+0.365$$
 GP+ $0.304$  CP- $1.98$  ADL (5)

GP (mL/200 mg DM)

$$ME_{OMD} (MJ/kg DM) = 0.16 OMD$$
 (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):

Inhibition % = [(blank absorbance - sample absorbance)/blank absorbance] x 100 (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<sub>ADF</sub> 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	$3.34\pm0.02$	$3.30\pm0.04$	$3.29\pm0.01$	
OM	$87.83 \pm 0.02$	87.81±0.03	$87.78\pm0.03$	
CP	5.97±0.04	$5.93\pm0.03$	$5.89\pm0.02$	
CF	21.16±0.08a	$20.32\pm0.06b$	17.15±0.03c	
NDF	$30.30\pm0.05$	$30.29 \pm 0.03$	$30.29 \pm 0.05$	
ADF	$48.68 \pm 0.05$	$48.67 \pm 0.03$	$48.66 \pm 0.02$	
ADL	$25.43\pm0.08$	$25.43\pm0.07$	$25.39\pm0.08$	
$ME_{ADF}$ , $MJ/kg~KM$	$8.27 \pm 0.03$	$8.27 \pm 0.04$	$8.27 \pm 0.02$	

 $^{a,b,c}$  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<sub>ADF</sub>= 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,  $GP_{MI}/200MG$  DM) of RH, PH and AH are shown in Table II. Changes of gas production volume with *in* 

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*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 <sub>ML</sub> /200MG DM) OF RH, PH AND AH				
In vitro Gas Production Parameters	RH(n=16)	PH(n=16)	AH(n=16)	
	(Mean±SE)	(Mean±SE)	(Mean±SE)	
$P_{psi}$	3.23±0.15c	3.42±0.17b	3.54±0.14a	
$GP_{mL}$	8.0±0.30c	$8.47 \pm 0.32b$	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	$3.64\pm0.02a$	
$ME_{GP}$	$3.69\pm0.02c$	$3.75\pm0.02b$	$3.79\pm0.04a$	
b	8.82±0.35c	9.31±0.41b	9.69±0.36a	
c	$0.28\pm0.032c$	0.35±0.021a	$0.30\pm0.028b$	
$T_{1/2}$	2.52±0.23a	$1.98\pm0.32c$	2.31±0.18b	

a,b,c Mean within a row with different superscripts differ (P< 0.05). ME<sub>OMD</sub>=Metobolisable energy estimated from OMD, ME<sub>GF</sub>= 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.

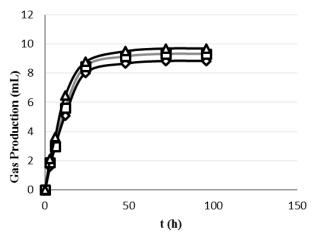


Fig. 1 Changes of gas production volume (mL) with *in vitro* incubation times (h) for round ( $\Diamond$ ), pointed ( $\square$ ) and almond ( $\Delta$ ) hazelnuts

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#### REFERENCES

- D. O. Gursoy. Findik ve mamulleri sektoru. Ordu'da Gıda Guvenligi. Ordu Gıda Tarım ve Hayvancılık Mudurlugu Dergisi. 20:45-47, 2013.
- [2] S. Kuleyin. OMU Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases. Master Thesis. Samsun, Turkey, 2016
- [3] AOAC. Official Methods of Analysis, 18th edn. Association of Official Analytical Chemists, Inc., Arlington, VA. 2006.
- [4] K. H. Menke, H. Steingass. Estimation of the energetic feed value obtained from chemical analysis and *in-vitro* gas production using rumen fluid. Anim. Res. Dev. 28:7-55, 1988.
- [5] S.M.A., Sallam, Nutritive value assessment of alternative feed resources by gas production and rumen fermentation *in-vitro*. Res. J. Agric. Biol. Sci. 1: 200, 2005.
- [6] S. Polyorach, M. Wanapat, A. Cherdthong. Influence of yeast fermented cassava chip protein (YEFECAP) and roughage to concentrate ratio on ruminal fermentation and microorganisms using *in-vitro* gas production technique. Asian-Aust. J. Anim. Sci. 27: 36-45, 2014.
- [7] S.R. Hernandez, J.O. Perez, M.M.M.Y. Elghandour, M. Cipriano-Salazar, B. Avila-Morales, L. M. Camacho-Diaz, A. Z. M. Salem, M. A. Cerrillo Soto. Effect of polyethylene glycol on in vitro gas production of some non-leguminous forage trees in tropical region of the south of Mexico. Agroforest Syst. 89: 735–742, 2015.
- [8] I.A. Muraina, M. M. Suleiman, J. N. Eloff. Can MTT be used to quantify the antioxidant activity of plant extracts? Phytomed. 16: 665–668, 2009.
- [9] J. Dai, R. J. Mumper. Plant Phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules.15: 7313-7352, 2010.
- [10] N. Swapana, N. Lokendrajit, S. Warjeet, C. Laitonjam, B. Singh. Antioxidant activities of the rhizomes of different Zingiberaceae plants of north-east India. Asian J. Biol. Life Sci. 2: 19–22, 2013.
- [11] T. Noipa, S. Srijaranai, T. Tuntulani, W. Ngeontae. New approach for evaluation of the antioxidant capacity based on scavenging DPPH free radical in micelle systems. Food Res. Int. 44:798–806, 2011.
- [12] R. Haigh. Safety and necessity of antioxidants: EEC approach. Food and Chem. I Toxicol. 24: 1031-1036,1986.
- [13] F. Tozoğlu. Erzincan kirazı (*Cerasus erzincanica*; Ş. Yıldırımlı) sap ve tohum kısımlarının antioksidan aktivitelerinin belirlenmesi. Erzincan Üniv., Fen Bil. Enst. Yüksek Lisans Tezi, 2011.
- [14] S. Ogut. Dogal antioksidanların onemi. J. of Adnan Menderes Univ. Agric. Fac. 11(1): 25 – 30. 2014.
- [15] P.J. Van Soest, J.D. Robertson, B.A. Lewis. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597, 1991.

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- [16] E. R. Ørskov, I. McDonald. The estimation of protein degradability in the rumen from incubation measurement weighed according to rate of passage. J. Agric. Sci. 92: 499-503,1979.
- [17] K. H. Menke, L.A. Raab Salewski, H. Steingass, D. Fritz, W. Schneider W. The estimation of the digestibility and metabolisable energy content of ruminant feding stuffs from the gas production when they are incubated with rumen liquor. J. Agric. Sci. 93: 217-222,1979.
- [18] A. Meda, C.E. Lamien, M. Romito, J. Millogo, O. G. Nacoulma. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well at their radical scavenging activity. Food Chemistry. 91: 571–77,2005.
- [19] F. Dimins, P. Kuka, I. Augspole. Characterisation of honey antioxidative properties. International Conference of Food Innova. 28-29- Oct. Latvia, 2010
- [20] SAS. SAS statistic software, SAS campus DRİVE. Cary NC, USA. 2007.
- [21] Z. Acar, M. Ozturk, G. Keles. Bugday, Misir ve karabugday samanlari iceren rasyonlarla beslenen disi tokluların performanslarinin belirlenmesi. Turk Tarim-Gida Bilim ve Teknoloji Dergisi. 3(2):59-62, 2015.
- [22] T. Gungor, M. Basalan, I. Aydogan. Kirikkale yoresinde uretilen bazi kaba yemlerde besin madde miktarlari ve metabolize olabilir enerji duzeylerinin belirlenmesi. Ankara. Univ. Vet. Fak. Derg. 55: 111-115, 2008
- [23] N. Cetinkaya, F. Erdem. Effects of Different Juncus acutus: Maise Silage Ratios on Digestibility and Rumen Cellulolytic Bacteria. Kafkas Univ Vet Fak Derg. 21(4)499-505.2015.
- [24] H. Kalkan, I. Filya. Sellüulaz enziminin bugday samanininn besleme degeri, *in vitro* sindirimi ve mikrobiyal protein uretimi üzerine etkileri. Kafkas Univ. Vet. Fak. Derg. 17 (4): 585-594, 2011.
  [25] I. Mujić, E. Šertović, S. Jokić, Z. Sarić, V. Alibabić, S. Vidović, J.
- [25] I. Mujić, E. Šertović, S. Jokić, Z. Sarić, V. Alibabić, S. Vidović, J. Živković. Isoflavone content and antioxidant properties of soybean seeds. Croat. J. Food Sci. Technol. 3 (1):16-20, 2011.
- [26] E. Karimi, P. Mehrabanjoubani, M. Keshavarzian, E. Oskoueian, H. Z. E. Jaafara, A. Abdolzadeh. Identification and quantification of phenolic and flavonoid components in straw and seed husk of some rice varieties (Oryza sativa L.) and their antioxidant properties. J Sci Food Agric. 94: 2324–2330. 2014.
- [27] F. Erdem, N. Cetinkaya, C. Nisbet, E. Altin. Estimation organic matter digestibility, metabolisable energy, phenolic compounds and antioxidant activity of stem and seed of Juneus acutus plant for ruminants. South African J. Anim. Sci. 45 (No.5) 502-509, 2015.