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RESEARCH ARTICLE

Potential Nutritive Value and Condensed Tannin Contents of Acorns from Different Oak Species [1]

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Summary

The potential nutritive values of acorns of *Quercus suber, Quercus branti, Quercus coccifera, Quercus cerris* and *Quercus infectoria* were estimated by chemical composition and *in vitro* gas production technique. Acorns collected at least 10 different trees in three experimental plots. There were significant (P<0.001) differences in the chemical composition among acorns obtained from different oak species. Crude protein (CP) contents of acorns ranged from 25.48 to 61.94 g/kg dry matter (DM). Neutral detergent fiber (NDF) contents ranged from 231.4 to 326.3 g/kg DM. Acid detergent fiber (ADF) contents ranged from 155.9 to 215.4 g/kg DM. Condensed tannin (CT) contents ranged from 7.2 to 26.7 g/kg DM. Starch contents ranged from 600.0 to 681.5 g/kg DM. Polyethylene glycol (PEG) addition significantly (P<0.001) increased the gas production and some estimated parameters of oak acorns. Although there is no significant (P>0.5) differences in the potential gas production of acorn among oak species when incubated in the absence of PEG, there is significant (P<0.001) differences in the potential gas production of acorn among oak species when incubated in the presence of PEG. Although the estimated organic matter (OMD) and metabolisable (ME) contents of acorn for *Q. suber* and *Q. infectoria* were significantly (P<0.001) higher than those for *Q. branti, Q. coccifera* and *Q. cerris* when incubated in the absence of PEG, the estimated OMD and ME contents of acorn for *Quercus cerris* and *Q. infectoria* were significantly lower than those for *Q. suber, Q. branti* and *Q. coccifera* when incubated in the presence of PEG. The improvement in gas production, OMD and ME in the presence of PEG emphasizes the negative effect of tannins on digestibility. As a conclusion, oak acorns have potential nutritive values for ruminant animals such as sheep and goat since acorns have high starch, OMD and ME but low level of CT contents. However these results obtained in the current study should be supported by *in vivo* feeding experiments.

Keywords: *Oak acorn, Condensed tannin, In vitro gas production Digestibility, Metabolizable energy, Polyethylene glycol*

Farklı Meşe Türlerinden Elde Edilen Palamutlarının Potansiyel Besleme Değeri ve Kondense Tanen İçerikleri

Özet

Quercus suber, Quercus branti, Quercus coccifera, Quercus cerris ve *Quercus infectoria* meşe palamutlarının potansiyel besleme değerleri, kimyasal kompozisyon ve *in vitro* gaz üretim tekniği kullanılarak tahmin edilmiştir. Meşe palamutları üç farklı deneme ünitesinden en az 10 ağaçtan toplanmıştır. Farklı meşe türünden elde edilen palamutlar arasında önemli farklılar olup, ham protein içeriği bir kg'da 25.58 ile 61.94 g arasında, NDF içeriği 231.4 ile 326.3 g arasında, ADF içeriği 155.9 ile 215.4 g arasında, kondense tanen içeriği 7.2 ile 26.7 g arasında, nişasta içeriği 600.0 ile 681.5 g arasında değişmiştir. Polyethylene glycol (PEG) eklenmesi meşe palamutlarından üretilen gaz ve tahmin edilen parametreleri önemli derecede artırmıştır. PEG eklenmeden elde edilen potansiyel gaz üretimi bakımından meşe palamutları arasında önemli farklar olmamasına rağmen, PEG eklenmesiyle birlikte potansiyel gaz üretimleri bakımından meşe palamutları arasında önemli farklar bulunmuştur. PEG eklenmeden yapılan hesaplamada, *Q. suber* ve *Q. infectoria* palamudunun organik madde sindirim derecesi (OMSD) ve metabolik enerji (ME) değerleri *Q. branti, Q. coccifera* ve *Q. cerris* palamutlarının OMSD ve ME değerlerinden önemli derecede yüksek bulunmasına rağmen, PEG ekleyerek yapılan hesaplamada, *Q. cerris* ve *Q. infectoria* palamudunun OMSD ve ME değerleri, *Q. suber, Q. branti* ve *Q. coccifera* palamutlarından daha düşük bulunmuştur. PEG katılmasıyla gaz üretimi, OMSD ve ME değerlerindeki meydana gelen iyileşmeler kondense tanenin sindirim üzerindeki negatif etkisini göstermektedir. Sonuç olarak yüksek nişasta, OMSD ve ME fakat düşük miktarda kondense tanen içermesinden dolayı meşe palamutları koyun keçi gibi ruminant hayvanlar için besleme potansiyeline sahiptir. Bunula birlikte bu çalışmada elde edilen sonuçlar yedirme denemeleriyle desteklenmelidir.

Anahtar sözcükler: *Meşe palamudu, Kondense tanen, İn vitro gaz üretimi, Sindirim derecesi, Metabolik enerji, Polyethylene glycol*

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INTRODUCTION

Leaves, pods and acorns of shrub and tree are used to meet the requirements of ruminant animals in the most parts of world where few or no alternatives are available [1-4]. The oak trees from different species produce considerable amount of acorn in the southern of Turkey although exact quantities are very difficult to estimate. There is limited information about the potential nutritive value of acorn from different oak species, although the acorns produced from different oak species were used in ruminant diets on farms where there are no alternative cheap feedstuffs available in the Southern of Turkey. Although the starch contents of acorns are high, the crude protein contents of acorns are considerably low. The starch contents of acorns for several oak species ranged from 56.60 to 59. 90% [5]. The crude protein contents of acorn ranged from 3.7 to 7.9% depending on the oak species and maturity stage [6-7]. Recently *in vitro* gas production technique has been approved to be as a powerful technique to determine the biological effect of tannin since current analytical techniques do not reflect the biological effects of tannin [8-12]. PEG, a non-nutritive synthetic polymer, has a high affinity to tannins and makes tannins inert by forming tannin PEG complexes [13]. PEG also can also liberate protein from the preformed tannin-protein complexes [14]. The aim of PEG addition was to determine the adverse effect of CT on the gas production and estimated parameters. In addition, *in vitro* gas production technique, in combination with chemical composition, has been widely used to determine the potential nutritive value of forages which are previously limited or uninvestigated [15-17]. The aim of the current study was to screen acorns from five oak species grown in the Southern of Turkey to (1) quantify chemical compositions and level of condensed tannin contents of acorn, (2) assess the effect of tannin activity on feed digestibility and nutrient availability *in vitro* using PEG tannin bio-assay.

MATERIAL and METHODS

Acorns of *Q. suber, Q. branti, Q. coccifera, Q. cerris* and *Q. infectoria* from at least 10 different trees in three experimental plots in 2009 in the Southern of Turkey were collected and dried at 50-52°C using a forced air oven. Dried acorn samples were ground to pass through 1 mm sieve for subsequent analysis. This experiment was approved by the Animal Experimentation Ethics Committee of University of Kahramanmaras Sutcu Imam, Faculty of Agriculture (Protocol No: 2011/01)

Dry matter (DM) of acorn was determined by drying the samples at 105°C overnight and ash by igniting the acorn samples in muffle furnace at 525°C for 8 h. Nitrogen (N) content of acorn was measured by the Kjeldahl method [18]. Crude protein of acorn was calculated as N X 6.25. Cell wall contents (NDF and ADF) of acorn were determined using the method described by Van Soest [19]. Total condensed tannin of acorn was determined by butanol-HCl method as described by Makkar et al.[13]. Starch content of acorn was determined by the Ewers polarimetric method [20]. All chemical analyses were carried out in triplicate except starch content which is determined in duplicate.

In the absence and presence of PEG (1 g, MW, 6000; Sigma, UK), the ground acorn samples (0.200 g DM) were incubated *in vitro* with diluted rumen fluid (10 ml rumen fluid + 20 ml culture medium) in calibrated glass syringes of 100 ml following the procedures of Menke and Steingass [21]. The aim of PEG addition was to determine the adverse effect of CT on the gas production and estimated parameters. All incubations were carried out in triplicate. Rumen fluid was obtained from two fistulated sheep fed a daily ration of 800 g alfalfa hay and 250 g concentrates dived into two equal meals at 8:00 and 16:00 h daily. The sheep had free access to water throughout the experiment. Rumen samples was collected before the morning meal in the thermos flaks and taken immediately to the laboratory where it was strained through various layers of cheesecloth and kept at 39°C.

Gas production of acorn samples was determined at 3, 6, 12, 24, 48, 72 and 96 h after incubation. Total gas values were corrected for blank gas production. Cumulative gas production data were fitted to non-linear exponential model as: $Y=A(1-\exp^{-ct})$

Where Y is gas production at time 't', A is the potential gas production (ml/200 mg DM), c is the gas production rate constant (h^{-1}) and t is the incubation time (h).

Where,

Metabolizable energy (MJ/kg DM) and OMD (%) values of oak acorn samples were calculated using equations suggested by Menke et al.[22] as follows:

$$\text{ME (MJ/kg DM)} = 2.20 + 0.136\,\text{GP} + 0.057\,\text{CP} \quad R^2=0.94$$

$$\text{OMD (\%)} = 14.88 + 0.889\,\text{GP} + 0.45\,\text{CP} + 0.00651\,\text{XA} \quad R^2=0.92$$

Where GP is 24 h net gas production (ml/200 mg DM)

CP: Crude protein

XA: Ash content (%)

Data on chemical composition of acorn from oak species was subjected to the one way of ANOVA using GLM of SPSS for windows [23] and were analyzed based on the statistical model: $Y_{ij} = \mu_{ij} + S_i + e_i$. Where, Y_{ij} = the general mean common for each parameter under investigation. S_i the ith effect of oak species on the observed parameters, e_i the standard error term. Data on the *in vitro* gas production kinetics, OMD and ME contents of oak acorn were subjected to the two way of ANOVA using GLM of SPSS for windows [20] and were analyzed based on the statistical model: $Y_{ij}: = \mu_{ij} + S_i + P_j + (S \times P)_{ij} + e_i$. Where, Y_{ij} is the general observation on *in vitro* gas production kinetics, OMD and ME contents, S_i the ith effect of oak species on the observed parameters and P_j the jth effect of PEG on the observed parameters. The $(S \times P)_{ij}$ term represents ith and jth interaction effects of species and PEG on gas production and *in vitro* digestibility, and *ei* the standard error term common for all observations. Significance between individual means was identified using the Tukey test. Mean differences were considered significant at P<0.05. Standard errors of means were calculated from the residual mean square in the analysis of variance.

RESULTS

The chemical compositions of acorns of *Q. suber, Q. branti, Q. coccifera, Q. cerris* and *Q. infectoria* are given in *Table 1*. There were significant (P<0.001) differences in the chemical composition among acorn obtained from different oak species.

The CP contents of acorn ranged from 25.48 to 61.94 g/kg DM. NDF contents ranged from 231.4 to 326.3 g/kg DM. ADF contents ranged from 155.9 to 215.4 g/kg DM. CT contents ranged from 7.2 to 26.7 g/kg DM. Starch contents ranged from 600.0 to 681.5 g/kg DM.

The CP contents of acorn from *Q. suber* was significantly (P<0.001) higher than those of acorns from *Q. branti*, *Q. coccifera, Q. cerris* and *Q. infectoria* whereas NDF and ADF contents of acorn from *Q. coccifera* were significantly (P<0.001) higher than those of acorn from *Q. suber, Q. branti, Q. cerris* and *Q. infectoria*. The starch content of acorn from *Q. infectoria* was significantly (P<0.001) higher than those for *Q. suber, Q. branti,* and *Q. cerris*. Condensed tannin content of acorn from *Q. coccifera* was significantly (P<0.001) higher than that for *Q. suber*.

The *in vitro* gas production of oak acorn in the absence and presence of PEG are given in *Fig. 1*. Gas production in the presence of PEG was considerably higher than those obtained in the absence of PEG irrespective of oak species at all incubation times. However oak species showed variable responses on increase in gas production. Acorn of *Q. cerris* and *Q. infectoria* had the highest increase in the gas production at 96 h incubation times.

The gas production, organic matter digestibility (OMD) and metabolisable energy (ME) of acorn from different oak species incubated in the presence and absence of PEG are given in *Table 2*.

Although there is no significant (P>0.5) differences in the gas production rate and gas production among oak species when incubated in the absence of PEG, there is significant (P<0.001) differences in the gas production rate (c) among oak species when incubated in the presence of PEG. The PEG supplementation significantly increased the gas production rate and gas production. The gas production rate (c) for *Q. branti* was significantly (P<0.001) higher than those for *Q. suber, Q. cerris* and *Q. infectoria* whereas potential gas productions for *Q. cerris* and *Q. infectoria* were significantly (P<0.001) higher than those for *Q. suber, Q. cerris* and *Q. coccifera*.

There were significant (P<0.001) differences in the estimated OMD and ME contents of acorn incubated in the presence and absence of PEG among oak species. The species and PEG supplementation had a significant (P<0.001) effect on the estimated OMD and ME contents.

Although the estimated OMD and ME contents of acorn for *Q. suber* and *Q. infectoria* were significantly higher than those for *Q. branti, Q. coccifera* and *Q. cerris* when incubated in the absence of PEG, the estimated OMD and ME contents for *Q. cerris* and *Q. infectoria* were significantly lower than those for *Q. suber, Q. branti* and *Q. coccifera* when incubated in the presence of PEG.

Two-way anova revealed significant interaction between PEG treatment and oak species for estimated parameters such as gas production rate, potential gas productions, OMD and ME contents.

DISCUSSION

The species had a significant (P<0.001) effect on the chemical composition of acorn. There is significant (P<0.001) variation in the chemical composition of acorn. The CP and

Table 1. *Chemical composition of acorns from different oak species*
Tablo 1. *Farklı meşe türlerinden elde edilen palamutların kompozisyonu*

Constituent	Oak Species					SEM	Sig.
	Q. suber	***Q. branti***	***Q. coccifera***	***Q. cerris***	***Q. infectoria***		
DM	916.1^{b}	912.6^{b}	935.1^{a}	916.2^{b}	928.7^{a}	3.16	***
CA	21.2^{a}	20.9^{a}	18.1^{b}	17.5^{b}	19.5^{ab}	0.78	***
CP	61.9^{a}	33.7^{c}	25.4^{d}	43.0^{b}	43.7^{b}	0.42	***
NDF	236.5^{c}	231.4^{c}	326.3^{a}	236.2^{c}	316.8^{b}	1.68	***
ADF	155.9^{d}	190.7^{b}	215.4^{a}	180.9^{c}	179.4^{c}	1.26	***
Starch	600.0^{d}	630.0^{c}	662.0^{ab}	645.5^{bc}	681.5^{a}	5.74	***
CT	20.9^{ab}	7.2^{b}	26.7^{a}	10.5^{ab}	15.5^{ab}	5.56	*

*Row means with common superscript did not differ (P>0.05), **SEM:** Standard error of mean, **DM:** Dry matter, **CA:** Crude ash(g/kg DM), **CP:** Crude protein (g/kg DM), **NDF:** Neutral detergent fibre (g/kg DM), **ADF:** Acid detergent fibre (g/kg DM), **CT:** Condensed tannin (g/kg DM), **Sig:** significance level, * P<0.05, *** P<0.001*

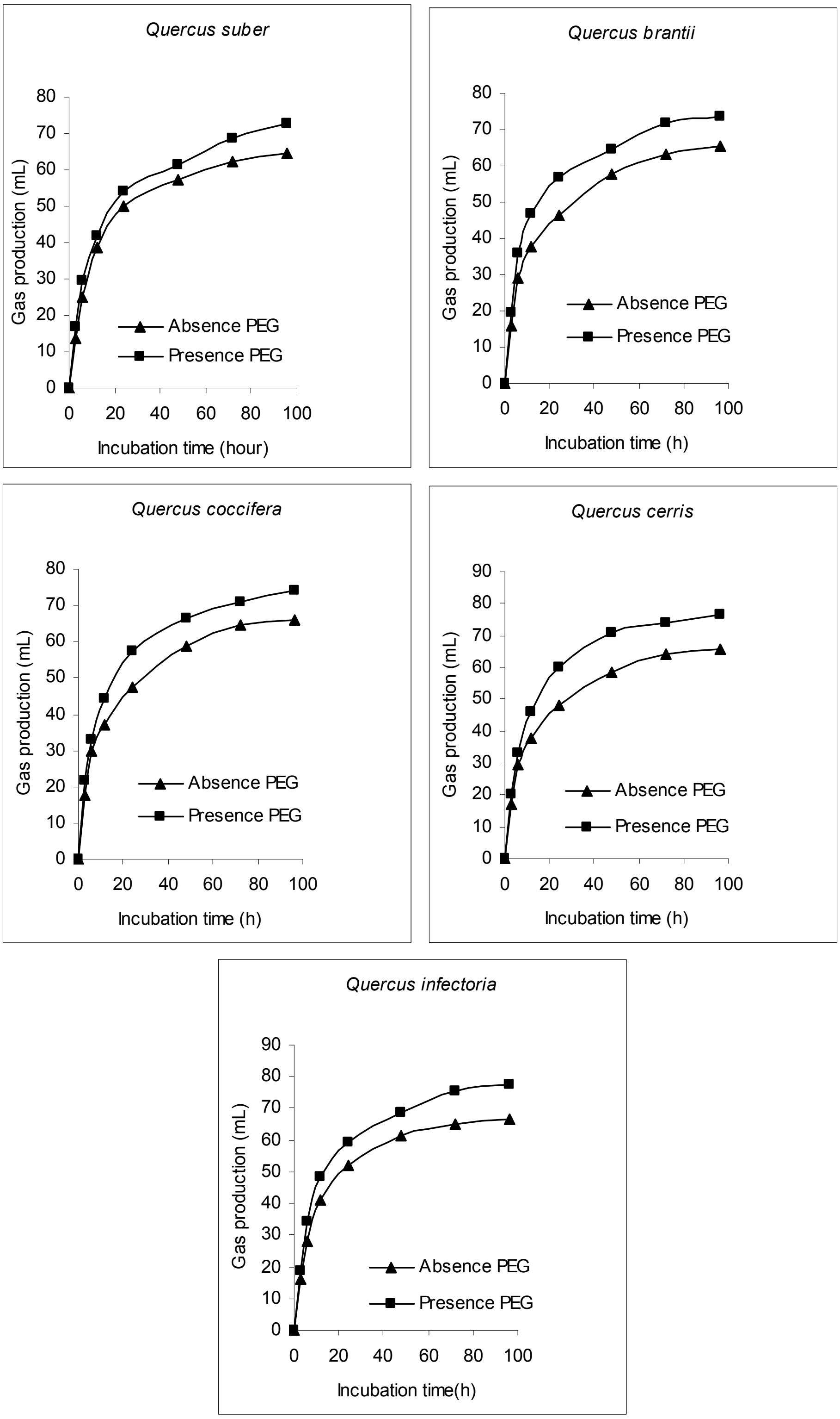

Fig 1. The effect of polyethylene glycol (PEG) on the gas production of oak acorn from different species

Şekil 1. Polyethylene glycol'ün farklı meşe türlerinden elde edilen palamutların gaz üretimine etkisi

NDF contents of acorn from *Q. coccifera* obtained in the current study were considerably lower than those obtained by Moujahed et al.[6], whereas ADF content of acorn from *Q. coccifera* was higher than that reported by Moujahed et al.[6]. The CP, NDF, ADF and CT contents of *Q. suber* were considerably lower than those reported by Baubaker et al.[22]. The differences among studies are possibly associated with differences in production site and stage of maturity of acorn. Baubaker et al.[7] suggested that some variation in the chemical composition of acorn can be expected due to variation in production site, *Quercus* species and stage of maturity. On the other hand, Maujahed et al.[24] showed that the chemical composition of acorn from *Q. coccifera* was significantly changed with maturity. Especially cell wall (NDF and ADF) contents of acorn decreased with increasing maturity.

Table 2. *The effect of polyethylene glycol (PEG) and species on the gas production, organic matter digestibility (OMD) and metabolisable energy (ME) of acorns*
Tablo 2. *Polyethylene glycol'ün ve türün meşe palamutların gaz üretimine, organik madde sindirim derecesine ve metabolik enerji değerine etkisi*

Species	c		A		ME		OMD	
	PEG		PEG		PEG		PEG	
	Absence	Presence	Absence	Presence	Absence	Presence	Absence	Presence
Q. suber	7.81	8.14^{c}	61.73	67.56^{c}	9.34^{a}	9.89^{c}	62.16^{a}	65.81^{b}
Q. branti	8.09	10.04^{a}	61.50	68.93^{c}	8.71^{b}	10.12^{bc}	57.87^{b}	67.06^{b}
Q. coccifera	8.01	9.24^{ab}	62.60	69.87^{b}	8.80^{b}	10.16^{b}	58.37^{b}	67.26^{b}
Q. cerris	8.34	8.71^{bc}	62.13	73.48^{a}	8.99^{b}	10.59^{a}	59.75^{b}	70.27^{a}
Q. infectoria	8.53	8.97^{bc}	64.18	73.30^{a}	9.50^{a}	10.54^{a}	63.08^{a}	69.90^{a}
SEM	0.264	0.252	0.855	0.627	0.096	0.068	0.638	0.457
Sig.	NS	***	NS	***	***	***	***	***
Species	***		***		***		***	
PEG	***		***		***		***	
Species X PEG	***		***		***		***	

Column means with common superscript did not differ (P>0.05), ***A:*** *potential gas production (mL)* ***c:*** *the gas production rate (ml/h),* ***SEM:*** *Standard error of mean,* ***Sig:*** *significance level, *** P<0.001,* ***NS:*** *Non-significant*

It seems to be likely that oak acorn studied in this experiment will not meet the CP requirements of ewes for maintenance and lactation since the CP content of oak acorn studied in this experiment lower than those requested for maintenance and lactation of sheep. El-Shatnawi and Mohawesh [25] reported that ewes require 7-9% CP for maintenance and 10-12% for lactation. On the other hand, it is well known that tannins may form a less digestible complex with dietary proteins and may bind and inhibit action of the endogenous protein, such as digestive enzymes [26]. Tannin can also adversely affect the microbial and enzyme activities [27-32]. However in ruminants, dietary CT (20-30 g/kg DM) has been shown to have beneficial effects because they reduce the protein degradation in the rumen by the formation of a protein-tannin complex [33]. Oak acorn selected in this study had low CT contents which are ranged from 7.2 to 26.7 g/kg DM. Therefore optimal utilization of CP in oak acorn could not be limited by low levels of condensed tannin due to tannin activity through the chemical binding with dietary nutrients. Low level of CP contents of oak acorns should be taken into consideration when oak acorn is included into ruminant diets as an alternative source of concentrate.

The potential gas production (A) and gas production rate(c) of *Q. coccifera* were comparable with findings of Moujahed et al.[6] who reported that potential gas production (A) and gas production rate(c) of *Q. coccifera* were 66.5 ml and 6.3% respectively.

On the other hand, Moujahed et al.[24] also showed that the *in vitro* potential gas production and gas production rate (c) of acorn from *Q. coccifera* was significantly increased with maturity, possibly due to reduction in cell wall contents (NDF and ADF) with increasing maturity.

Although oak acorn selected in this study had low CT contents, PEG supplementation resulted in increase in gas production and some estimated parameters. These results obtained in current study are consistent with findings of Rubanza et al.[34] and Karabulut et al.[35] who found that PEG supplementation increased the gas production and estimated parameters such as ME and OMD values. The increase in the gas production and estimated parameters in the presence of PEG emphasizes the negative effect of tannins on digestibility of oak acorn. The increase in the gas production, their kinetics, OMD and ME in the presence of PEG is possibly due to an increase in the available nutrients to rumen micro-organisms, especially carbohydrates and nitrogen contents of oak acorn. Makkar et al.[13] suggested that PEG, a non-nutritive synthetic polymer, has a high affinity to tannins and makes tannins inert by forming tannin PEG complexes. It was also reported that PEG is also able to liberate protein from the preformed tannin-protein complexes [14].

Significant interaction between species and PEG supplementation for the estimated gas production kinetics, OMD and ME values *(Table 2)*, indicating that acorns did not show similar response to PEG supplementation during *in vitro* incubation study. The increase for gas production rate of acorn of *Q. branti* in the presence of PEG was approximately 24.1% whereas the increase for gas production rate of acorn of *Q. suber* in the presence of PEG was approximately 4.2%. On the other hand, the increase for potential gas production of acorn of *Q. cerris* in the presence of PEG was approximately 18.26% whereas the increase for the increase for potential gas production of acorn of *Q. suber* in the presence of PEG was approximately 9.44%. This possibly due to differences in the amount or chemical composition of the tannins acorns contained.

The acorns from different oak species seem to be very energetic alternative sources due to high starch contents and would be used to some extent in sheep and goats diets where there is food shortage. However the substitution rate of acorn for barley is very important. Jassim et al.[4] suggested that substitution of acorn for barley at a maximum level of 25% would be economically advantageous. High substation rate of acorn for barley resulted in decreased daily gain due to the decreased digestibility of nutrients [4,7].

As a conclusion, oak acorns have potential nutritive values for ruminant animals such as sheep and goat since acorns have high starch, OMD and ME but low level of CT contents. However these results obtained in the current study should be supported by *in vivo* feeding experiments.

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