Kafkas Univ Vet Fak Derg
22 (5): 785-792, 2016
DOI: 10.9775/kvfd.2016.15403

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Comparison of Alfalfa *(Medicago sativa)* Energy Values Estimated by Using the NRC-2001, Hohenheim and UC Davis Equations [1]

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[1] *This study was financially supported by Selcuk University (Project Number: BAP 14401115)*

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Article Code: KVFD-2016-15403 Received: 18.02.2016 Accepted: 29.04.2016 Published Online: 29.04.2016

Abstract

In this study, our aim was to compare estimated metabolizable energy (ME) and net energy lactation (NEL) contents in dried alfalfa samples in their different growth stages, using models such as NRC-2001, $Hohenheim_{Menke}$ and University of California at Davis (UC Davis). A total of 73 alfalfa hay samples obtained during three different growth stages (Vegetative, Bud and Bloom) were used. Chemical analyses were performed for each sample. Energy values of the alfalfa hay samples were calculated with NRC-2001 equations from chemical analysis results, and with $Hohenheim_{Menke}$ and UC Davis equations from *in vitro* gas production volumes. Gas production in alfalfa samples in vegetative period (S1) was higher than other periods (P<0.001). Again, ME values calculated with all three methods in the alfalfa hay samples of this period were significantly higher than the samples in bud and bloom periods (P<0.001). In addition, energy values obtained with $Hohenheim_{Menke}$ equation in all periods were found to be higher than the averages obtained with NRC-2001 and UC Davis equations. It is concluded that, for the alfalfas in vegetative period, a correlation of 85.6% between energy values obtained with NRC-2001 and $Hohenheim_{Menke}$ equations, a correlation of 81.8% between energy values obtained with UC Davis and NRC-2001 equations, and over 99% correlation between energy values obtained with $Hohenheim_{Menke}$ and UC Davis equations were determined (P<0.000).

Keywords: *Alfalfa, NRC-2001, UC Davis, In vitro gas production*

Yoncanın *(Medicago sativa)* NRC-2001, Hohenheim ve UC Davis Eşitlikleri Kullanılarak Tahmin Edilen Enerji Değerlerinin Karşılaştırılması

Özet

Çalışmada farklı büyüme dönemlerinde biçilerek kurutulmuş yonca örneklerinde, NRC-2001, $Hohenheim_{Menke}$ ve Davis Kaliforniya Üniversitesi (UC Davis) gibi modeller kullanılarak tahmin edilen metabolize olabilir enerji (ME) ve net enerji laktasyon (NEL) içeriklerinin kıyaslanması amaçlandı. Üç farklı büyüme (vejetatif, tomurcuklanma ve çiçeklenme) döneminde elde edilmiş toplam 73 yonca kuru otu örneği kullanıldı. Her bir numunenin kimyasal analizleri yapıldı. Yoncaların enerji değerleri kimyasal analiz sonuçlarından NRC-2001 denklemleri ile ve *in vitro* gaz üretim miktarlarından $Hohenheim_{Menke}$ ve UC Davis eşitlikleri kullanılarak hesaplandı. Yonca örneklerinde vegetative dönemde (S1) gaz üretimi diğer dönemlerdekinden daha yüksek idi (P<0.001). Yine bu dönemdeki yoncalarda her üç yöntemle hesaplanan ME değerleri tomurcuklanma ve çiçeklenme dönemlerindeki yoncalarınkinden belirgin bir şekilde yüksekti (P<0.001). Ayrıca bütün dönemlerde $Hohenheim_{Menke}$ eşitliğinden elde edilen enerji değerleri NRC-2001 ve UC Davis eşitliklerinden elde edilen ortalamalardan yüksek bulundu. Sonuç olarak vejetatif dönemdeki yoncaların NRC-2001 ve $Hohenheim_{Menke}$ eşitliğinden elde edilen enerji değerleri arasında %85.6; UC Davis ve NRC-2001 eşitliklerinden elde edilen enerji değerleri arasında %81.8; $Hohenheim_{Menke}$ ve UC Davis eşitliklerinden elde edilen enerji değerleri arasında ise %99'un üzerinde bir korelasyon belirlendi (P<0.000).

Anahtar sözcükler: *Yonca, NRC-2001, UC Davis, In vitro gaz üretimi*

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INTRODUCTION

It is very important to know energy and nutrient contents of feeds to be used during the ration formulation for ruminants. Nutrients can be determined by chemical analyses. However, it is difficult to determine the energy amount. While metabolizable energy (ME) contents of ruminant feeds can be estimated often by looking at chemical compositions of the feeds [1], alternatively, they can be evaluated with *in situ* or *in vitro* methods. For example, energy contents of feed materials can be learned by looking at amount of *in vitro* gas production [2-4]. Currently, the results obtained by providing real rumen environment with ruminal *in situ* methods are more reliable than the results obtained with *in vitro* methods. However, the limitations of *in situ* methods are that the laboratory and analysis costs are quite expensive, rumen cannulated animals are required and that these methods cannot measure the actual fermented amount of the feeds and can only measure the amount of the lost feed [5,6].

In calculation of energy values of ruminant feeds, *in vitro* methods based on gas production are more commonly used due to high costs of *in situ* methods. With Hohenheim method, metabolizable energy of the feeds can be evaluated with various chemical components and by calculating total gas amount formed by incubating them for 24 h in syringes using an equation [2,7]. This method has more advantages compared to *in vivo* tests, not only due to being more economical, but also due to providing more sustainable experimental conditions. In addition, *in vitro* gas production technique is often not considered ethical because of the fistulated animal requirement [8]. The difficulty in provision of sample rumen liquor required for gas production technique is also among the disadvantages of the method [9].

Apart from these methods, there are NRC-2001 equations that calculate TDN from nutrients with chemical formulas. By means of this model, accurate results can be obtained in a very practical way and free of charge, without any need for animal experiments and rumen liquor [10]. With analysis results of feeds and formulas including some coefficients, tdNFC, tdCP, tdFA, tdNDF and TDN are calculated, and digestible energy (DE), metabolizable energy (ME) and net energy lactation (NEL) calculations can be performed from TDN [11,12]. Although it was argued that TDN values calculated with NRC-2001 equations are not accurate for feeds in the tropic regions [13], Kishore and Parthasarathy [10] obtained very similar results when they compared their study where they estimated TDN values of various tropical herbs and leaves, using NRC-2001 equations, to *in vivo* tests. Similarly, in their study Şayan et al.[14] suggest that ME values can be calculated by means of regression equations developed for roughage and determination of 24 h gas production volumes of feeds with CP, EE, NDF and ADF should be used primarily as reliable parameters.

The UC Davis equation is a modification of the method of Menke and Steingass [2] that predicts ME from gas produced *in vitro* at 24 h of incubation, as well as estimates of the feed's levels of CP and fat.

In this study, from nutritional values of alfalfa hays in different vegetation periods, determined with chemical analyses, metabolizable energy (ME_{NRC}) and net energy lactation (NEL_{NRC}) values were calculated by using models in NRC-2001. It was also determined *in vitro* gas production of the same alfalfa hays with calculated energy values (ME_{NRC} and NEL_{NRC}), it was aimed to compare and interpret energy values (ME_{Menke}, NEL_{Menke} and ME_{UCD}) obtained from Hohenheim [2] and UC Davis [3].

MATERIAL and METHODS

Sample Collection

Alfalfa samples (Elçi, Bilensoy 80, Kayseri, Prosementi and Local variety) were obtained from fields of private producers in different districts of Konya, from 2-3. Cuttings using a quadrate of 50x50 cm from at least 4 different parts. A total of 73 alfalfa samples of different maturity stages were collected. The alfalfa samples were divided into 3 groups according to their maturity stages;

Stage 1: Vegetative (S1);

Stage 2: Bud (S2);

Stage 3: Bloom (Midbloom) (S3).

Numbers of alfalfa hays for each maturity stage were 27, 23, 23 respectively. The harvested alfalfa samples were chopped up in big pieces and dried in fan drying-ovens (VWR, Dry-Line) at 65°C to a constant weight. All dried samples for chemical analysis were ground to pass a 1 mm screen in a Retsch mill (Retsch GmbH, Haan, Germany).

Chemical Analysis

Milled samples were analyzed for DM, crude ash, crude protein (CP) and ether extract (EE) by AOAC [15]. Neutral detergent fiber (aNDF) and acid detergent fiber (ADF) were assayed according to the methods prescribed by Van Soest et al.[16] using Ancom^{200} Fiber Analyzer (Ancom Technology, Fairport, NY, USA). Neutral detergent fibre was determined with the inclusion of heat stable α-amylase and express inclusive of residual ash [16]. Sodium sulfite was used prior to neutral detergent extraction. The NDFn was also adjusted by: NDF-NDICP. Acid detergent lignin (ADL) was determined in ADF samples by soaking in 72% sulfuric acid for 3 h in beakers. The Neutral (NDICP) and acid (ADICP) detergent-insoluble CP was determined on the samples obtained from NDF and ADF residues [17]. Acid detergent insoluble protein (ADICP) and neutral detergent insoluble protein (NDICP) were calculated as ADICP = 6.25×ADIN and NIDCP = 6.25 ×NDIN, respectively. All samples were analyzed in duplicate and repeated if chemical analysis error was in excess of 5%.

In Vitro Gas Production Technique

In vitro gas production of 73 samples was analyzed. Rumen fluid was obtained from a steer beef fed with a diet containing alfalfa hay (40%) and concentrates (60%). Rumen fluid was collected into a glass bottle (Isolab, Germany). The bottle was transported to laboratory in a sealed thermos container at 39±1°C and filtered through four layers of cheesecloth under CO_2 gas. The samples were incubated in rumen fluid and buffer mixture (in 100 mL glass syringes (Model Fortuna, Germany) following the procedures of Menke and Steingass [2]. About 200±10 mg dry samples were weighed in triplicate into glass syringes. The syringes were prewarmed at 39°C in a thermostatically cabinet (Lovibond, Switzerland), before 10 mL of rumen fluid and 20 mL of prewarmed buffer mixture were dispensed anaerobically in each syringe using an automatic bottle top dispenser (Isolab, Germany). Syringes were closed using one position polypropylene clamps and incubated at 39±0.5°C for 24 h. In addition, three blank syringes (no template; rumen fluid + buffer mixture) were used to calculate the total gas production. After 24 h of incubation, the total gas volume (mL) was recorded from the calibrated scale on the syringe.

Energy Values

Estimated energy contents for metabolizable energy (ME) and net energy lactation (NEL) were calculated separately using the three different equations as follows:

- Using NRC predictive equations: The NRC-2001 chemical formula is one method to estimate energy values for feeds for dairy cattle. This method is a chemical approach that uses analytical results to estimate the values of truly digestible nutrients (tdNFC, tdCP, tdFA, tdNDF, TDN). Metabolizable energy at production level of intake (ME_{NRC}) and net energy for lactation at production level of intake (NEL_{NRC}) were determined using a summative approach [18] from NRC [11]. The values of proximate analysis and fiber analysis were used in the following equations of NRC-2001 to predict the truly digestible (td) nutrients of the test feeds and then the digestibility values were summed up to arrive at the TDN content of the feeds.

$$NFC = 100 - [CP + EE + ash + (NDF - NDICP)]$$

$$tdNFC = 0.98 \times \{100 - [CP + EE + ash + (NDF - NDICP)]\} \times PAF$$

where;

PAF = Processing adjustment factor, 1 for alfalfa

$$tdCP_{forage} = CP \times \exp[-1.2 \times (ADICP\ CP)]$$

$$tdCP_{concentrate} = [1 - (0.4 \times (ADICP\ CP))] \times CP$$

tdFA (fatty acid) = FA (FA = EE -1)

$$tdNDF = 0.75 \times (NDFn - L) \times [1 - (L/NDFn)^{0.667}]$$

where;

L = Acid detergent lignin and NDFn= NDF - NDICP

$$TDN = tdNFC + tdCP + (tdFA \times 2.25) + tdNDF - 7$$

The energy values of digestible energy (DE) and metabolizable energy (ME) and net energy lactation (NEL) were estimated using the following equations of NRC [11].

$$DE\ (MJ/kg) = 0.04409 \times TDN(\%)/4.184$$

$$ME_{NRC}\ (MJ/kg) = 1.01 \times DE\ (Mcal/kg) - 0.45/4.184$$

$$NEL_{NRC}\ (MJ/kg) = (0.0245 \times TDN(\%) - 0.12)/4.184$$

- Using UC Davis predictive equations: ME_{UCD} content was also estimated using the UC Davis equation proposed by Robinson et al.[3] and Tagliapietra et al.[4] resulting from a modification of that proposed by Menke and Steingass [2] as:

$$ME_{UCD}\ (MJ/kg\ DM) = 1.25 + 0.0292GP + (0.000143(CP - ADICP)) + 0.0246EE$$

(GP is 24 h *in vitro* gas production in ml/g of DM, and EE, CP and ADICP are as g/kg of DM).

- Using Hohenheim predictive equations: The metabolizable energy (ME_{Menke}) and net energy lactation (NEL_{Menke}) contents of alfalfa were calculated using equations of Menke and Steingas [2] as follows:

$$ME_{Menke}\ (MJ/kg\ DM) = 2.20 + 0.1357GP + 0.0057CP + 0.0002859EE^2$$

$$NEL_{Menke}\ (MJ/kg\ DM) = 0.54 + 0.0959GP + 0.0038CP + 0.0001733EE^2$$

(GP is 24 h net gas production in ml/200 mg of DM, and CP, EE are as g/kg of DM)

Statistical Analysis

Relationships between the ME values estimated by each of the predictive approaches were tested using standard ANOVA procedures within SPSS [19]. The paired t test procedure and Pearson correlation analysis were performed to establish the relationship between values of alfalfa by using NRC 2001, $Hohenheim_{Menke}$ and UC Davis predictive equations. The methods were compared by linear regression of the mean values of ME_{NRC}, ME_{Menke} and ME_{UCD} data obtained for each alfalfa hays. Treatment means were compared using the Duncan multiple range test.

RESULTS

Chemical analysis results of alfalfa hays in different maturity stages are shown in *Table 1*. According to the results, harvesting of alfalfa in different maturity stages had a great impact on chemical composition and *in vitro* gas production of alfalfa. A great difference was observed especially in CP, Crude ash, ADF, NDF, ADL (P<0.000) in terms of maturity stages.

Minimum, maximum and average values of ME and NEL values of alfalfa hays in different maturation periods determined with NRC-2001, $Hohenheim_{Menke}$ and UC Davis equations are shown in *Table 2*. In all maturity stages, the energy value averages obtained from $Hohenheim_{Menke}$ technique were found to be higher than the averages obtained from NRC-2001 and UC Davis equations.

Although there is a correlation of 85.6% between ME and NEL values obtained with $Hohenheim_{Menke}$ and NRC-2001 methods in S1 alfalfa samples (P<0.000), a statistically significant difference between the average values was observed (P<0.000). The same difference was determined also in other maturity stages. However, as vegetation progressed, it was observed that this relationship reduced *(Table 3)*.

Equations and relations obtained after regression analysis between ME and NEL values of all alfalfa samples in the study, calculated with NRC-2001, $Hohenheim_{Menke}$ and UC Davis equations are shown in *Table 4*. The fact that

Table 1. *The chemical composition (g/kg DM) and gas production (mL/200 mg DM) of alfalfa hays*
Tablo 1. *Yonca kuru otlarının kimyasal kompozisyonları (g/kg DM) ve gaz üretimleri (mL/200 mg KM)*

Items	**Maturity Stages**			**SEM**	**P**
	S1	**S2**	**S3**		
DM	937.32^{b}	943.57^{a}	937.39^{b}	0.9	0.005
CP*	253.47^{a}	200.84^{b}	198.87^{b}	4.66	0.000
EE*	40.23^{a}	38.04^{ab}	34.98^{b}	1.03	0.108
NFC*	272.75	274.08	285.24	4.16	0.417
Ash*	93.44^{a}	79.74^{b}	80.32^{b}	1.7	0.000
NDF*	340.10^{b}	407.30^{a}	400.58^{a}	7.25	0.000
ADF*	259.60^{b}	313.09^{a}	309.66^{a}	6.14	0.000
Lignin*	67.56^{b}	97.61^{a}	99.08^{a}	3.72	0.000
NDICP*	20.83	19.72	22.42	0.68	0.287
ADICP*	12.83^{ab}	11.55^{b}	13.60^{a}	0.33	0.040
Gas Production**	54.20^{a}	44.72^{b}	45.32^{b}	1.24	0.001

*^{a,b} Means between different maturity stages of alfalfa having different letters are significantly different; * (in g/kg on DM basis; ** Net gas production (ml/200 mg DM)*

Table 2. *Comparison of ME and NEL obtained from NRC-2001, $Hohenheim_{Menke}$ and UCD equations in alfalfa different maturity stages*
Tablo 2. *Yoncanın farklı olgunlaşma dönemlerinde NRC-2001, $Hohenheim_{Menke}$ ve UCD eşitliklerinden elde edilen ME ve NEL'in kıyaslanması*

Method		**ME,MJ/kg DM**			**Between Groups**	**NEL,MJ/kg DM**			**Between Groups**
Maturity Stage		**S1**	**S2**	**S3**	**P**	**S1**	**S2**	**S3**	**P**
Menke	**Mean**	11.50^{aA}	9.84^{bA}	9.84^{bA}	0.000	7.00^{aA}	5.85^{b}	5.86^{bA}	0.000
	Min	8.61	7.87	8.05		5.01	4.46	4.6	
	Max	14.71	12.67	12.87		9.24	7.86	7.99	
	SEM		0.2				0.14		
NRC-2001	**Mean**	9.95^{aB}	8.99^{bB}	8.88^{bB}	0.000	6.22^{aB}	5.54^{b}	5.45^{bB}	0.000
	Min	8.09	7.82	7.66		4.89	4.71	4.59	
	Max	12.24	10.94	9.72		7.86	6.95	6.05	
	SEM		0.11				0.08		
UCD	**Mean**	10.19^{aB}	8.74^{bB}	8.75^{bB}	0.001	-	-	-	
	Min	7.17	6.54	6.95		-	-	-	
	Max	13.1	11.9	11.77		-	-	-	
	SEM		0.2				-		
Between Methods	P	0.001	0.003	0.005		0.000	0.086	0.034	

^{a,b} Means from different maturity stages of alfalfa having different letters are significantly different
^{A,B} Means obtained different methods having different letters are significantly different

determination coefficient is close to 1, and slope value being also close to 1 means ME_{Menke} and ME_{UCD} methods show similar change.

DISCUSSION

In Stage 1, which is the early growth period of alfalfas, CP, EE, Ash and gas production volume were higher than other periods *(Table 1)*. As plant matures, it was reported a decrease, similar to the literature [20-25], especially in CP, ash and EE volumes. While it was reported that cell wall elements (ADF and NDF) increased with maturation [20,21,26,27], in this study it increased during transition from vegetative period to budding [28], fiber content was affected very little after transition to flowering stage and no statistically significant difference was observed [25] *(Table 1)*.

As a reason for the decline in gas production, with the growth of alfalfa and extension of the stem, decreased digestibility with increased amount of fiber can be considered [29], *(Table 1)*. In the study they conducted by Getachew et al.[30] reported that gas production of many feeds evaluated in 7 different laboratories were 43.6 to 53.6 mL/200 mg, DM and ME values were 9.92 to 11.37 MJ/kg DM, and there were substantial differences between laboratories. Because animal which rumen liquor is received from, diet, time of receiving the liquor

Table 3. *Comparison and correlation analysis between NRC-2001, Hohenheim$_{Menke}$ and UCD equations in the determination energy values of alfalfa hays with different stages*

Tablo 3. *Farklı dönemlerdeki yonca kuru otlarının enerji değerlerini belirlemede NRC-2001, Hohenheim$_{Menke}$ ve UCD eşitlikleri arasındaki ilişki ve kıyaslanma*

Comparison NRC-2001, Hohenheim$_{Menke}$ and UCD Equations				Correlation Analysis NRC-2001, Hohenheim$_{Menke}$ and UCD Equations	
Types of Energy	x - y	Paired Differences		r_{xy}	P
		SEM	P (2-tailed)		
ME	$S1_{Menke}$ - $S1_{NRC}$	0.209	0.000	0.856	0.000
	$S2_{Menke}$ - $S2_{NRC}$	0.246	0.002	0.347	0.105
	$S3_{Menke}$ - $S3_{NRC}$	0.253	0.001	0.512	0.012
	$S1_{UCD}$ - $S1_{NRC}$	0.216	0.287	0.818	0.000
	$S2_{UCD}$ - $S2_{NRC}$	0.269	0.368	0.286	0.186
	$S3_{UCD}$ - $S3_{NRC}$	0.257	0.622	0.490	0.018
	$S1_{Menke}$ - $S1_{UCD}$	0.035	0.000	0.995	0.000
	$S2_{Menke}$ - $S2_{UCD}$	0.029	0.000	0.996	0.000
	$S3_{Menke}$ - $S3_{UCD}$	0.018	0.000	0.998	0.000
NEL	$S1_{Menke}$ - $S1_{NRC}$	0.147	0.000	0.856	0.000
	$S2_{Menke}$ - $S2_{NRC}$	0.175	0.087	0.332	0.121
	$S3_{Menke}$ - $S3_{NRC}$	0.177	0.034	0.516	0.012

Table 4. *Relationships between ME values (MJ/kg DM) of alfalfa hays (n=73) estimated according to different equations*

Tablo 4. *Yonca kuru otlarının farklı eşitlikler aracılığıyla belirlenen ME değerleri arasındaki ilişkiler*

Methods		Equations	SE (P)		R^2
(y)	(x)		Intercept	Slope	
ME_{Menke}	ME_{NRC}	y=-1.981+1.335x	0.141 (0.000)	1.320 (0.138)	0.558
ME_{Menke}	ME_{UCD}	y=0.982+1.021x	0.118 (0.000)	0.013 (0.000)	0.989
NEL_{Menke}	NEL_{NRC}	y=-1.146+1.289x	0.800 (0.156)	0.138 (0.000)	0.551
NEL_{NRC}	NEL_{Menke}	y=3.075+0.428x	0.293 (0.000)	0.046 (0.000)	0.551
ME_{NRC}	ME_{Menke}	y=4.943+0.418x	0.467 (0.000)	0.044 (0.000)	0.558
ME_{NRC}	ME_{UCD}	y=5.591+0.401x	0.460 (0.000)	0.049 (0.000)	0.488
ME_{UCD}	ME_{NRC}	y=-2.049+1.217x	1.385 (0.143)	0.148 (0.000)	0.488
ME_{UCD}	ME_{Menke}	y=-0.853+0.969x	0.126 (0.000)	0.012 (0.000)	0.989

ME_{NRC}: *ME estimated from NRC-2001 equations;* ***ME_{Menke}:*** *ME estimated from the equation in Menke and Steingass [2];* ***ME_{UCD}:*** *ME estimated from UCD equation* and even the place where the liquor is received affect *in vitro* gas production volume obtained from Hohenheim technique, hence the obtained energy level [9]. The reason for the differences between the energy values obtained in different laboratories may be due to these factors. Low repeatability can also be considered as one of the problems of gas production technique.

Chemical differences between feeds affect *in vitro* gas production significantly [31]. In addition, gas production decreases as plant matures [32], *(Table 1)*. Although it was reported that gas production occurs as a result of fermentation of primary carbohydrates, and protein fermentation has a minor affect [33,34], in this study, gas production in the 1st stage of alfalfa is higher (P<0.001), yet NFC levels are similar in this stage, and NDF and ADF levels are higher in other stages. This result is in line with findings of Zinash et al.[35], Lee et al.[36], Kamalak et al.[37], Canbolat et al.[38], Karabulut et al.[39]. High protein levels, especially NPN and soluble protein (SP) rates can be considered as the reason for higher gas production during the first stage. On the contrary, however, there are also views suggesting that ammonia in the media binds to carbon dioxide and reduces the gas production [40]. Karabulut et al.[39], on the other hand, demonstrated a significant positive correlation between gas production and crude protein in alfalfa hay. Coblentz et al.[41], reported that in oat hays with high NDF, *in vitro* gas production is negatively correlated with NDF, lignin, HK; and positively correlated with water soluble carbohydrates (WSC) and non-fiber carbohydrates (NFC), however there is no such correlation in hay with low NDF.

While Canbolat and Karaman [42] found gas production in alfalfa hay in 24-h incubation as 52.9 mL/200 mg DM, similar to our study, Iantcheva et al.[43] found in 20 samples, Özkul et al.[44] found in 10 samples of alfalfa hay as 25.8-39.0 mL, which is lower.

As a result of analyses and calculations, energy values of alfalfa samples harvested in the S1 obtained with each of three equations were found to be higher than other periods (P<0.001) *(Table 2)*. With maturation of alfalfa hays, a decrease was observed in ME and NEL values calculated from all equations with reduction of digestible nutritional values, which is compatible with the results reported by some researchers [4,25,28,45].

In the study, ME and NEL values of the samples calculated with NRC-2001 equation were similar to the values determined by different researchers [4,25,28]. When some studies, where vegetation period of alfalfa hays are uncertain, were examined; ME_{NRC} and NEL_{NRC} values calculated in this study were found to be lower than ME values [46] calculated using CP and Ash data as well as ME and NEL levels [47] determined with *in vitro* gas production technique. However, in this study, average ME_{UCD} values determined with UC Davis equation of the alfalfas were found to be lower than or similar to [4] the values of different researchers [3].

While ME_{NRC} value averages were lower than ME_{Menke} with a rate of 5.69%, NEL_{NRC} values were found to be lower than NEL_{Menke} with a rate of 4.18%. In the study conducted by Tagliapietra et al.[4], on the other hand, the energy values obtained from NRC-2001 equation were found to be higher than energy values obtained from $Hohenheim_{Menke}$ equation. The reason for this may be that Tagliapietra et al.[4] have used tdNDF48 instead of tdNDF calculated from lignin in NRC-2001 equation. Because Robinson et al.[3] detected that the energy value calculated with tdNDF48 was higher than the energy value calculated with lignin.

Because of lignin level increases with maturation of alfalfa and NDF digestion based on lignin is used in calculation in NRC-2001, it depends on taking the 24-h gas production as basis in gas production method, an incubation 24-h might not be sufficient. A correlation of 81.8% was found between ME values obtained with UC Davis and NRC 2001 method (P<0.000) and results was not found to be statistically significant (P<0.287). Again relationship between the two methods in the subsequent stages decreased *(Table 3)*. Similarly over 99% correlation was observed between ME values of alfalfa samples in all maturity stages, calculated with $Hohenheim_{Menke}$ and UC Davis equations (P<0.000) and the average values were found to be statistically different from each other (P<0.000). The high correlation originates from the fact that 24-h *in vitro* gas production amounts were present in both methods *(Table 4)*.

In this study, when ME values of all alfalfa samples calculated with each of the three equations, regardless of development stages, were compared, the following sequence was obtained; $ME_{UCD}<ME_{NRC}<ME_{Menke}$ (9.23<9.27 <10.39 MJ/kg KM). When it is considered that the result obtained with NRC-2001 model was between the results obtained with $Hohenheim_{Menke}$ and UC Davis models, and also the difficulties in the implementation of *in vitro* gas production, it may give the impression that it is more advantageous for determination of energy content of alfalfa. However Tagliapietra et al.[4] obtained alfalfa hay ME content as 8.5<9.5<10.6 MJ/kg KM from $ME_{Menke}<ME_{UCD}<ME_{NRC}$ equations. While data obtained after 48-h incubation was used in ME_{NRC} calculation, in this study, lignin method in NDF digestibility was used. In studies where different equations were used, Robinson et al.[3] also obtained the lowest ME level from UC Davis equation, similarly to our study. Nuez-Ortín and Yu [12] reported no difference in energy levels in corn and wheat DDGSs obtained with NRC-2001 equations and *in situ* method, there was a strong relation between TDN and energy values obtained with both methods (P<0.05), and there were significant differences in tdNDF, tdCP, tdFA, tdNFC values. Yu et al.[48] reported energy values determined in alfalfa and timothy according to the methods as $ME_{NRC}<ME_{Invitro}<ME_{Insitu}$.

In NRC-2001 equation, used to estimate energy value of ruminant feeds, it is suggested that the obtained from 48-h *in vitro* incubation should be used instead of calculating NDF digestibility according to lignin content [4]. However, there are also opinions suggesting that more accurate results can be obtained by decreasing incubation period [3,4,49,50]. In this study, only alfalfa hay was used as feed material and Robinson et al.[3] also found energy contents of alfalfa hay determined with *in vivo* and NRC-2001 model very similar. Therefore, it seems that NRC-2001 equations can be safely used to determine energy contents of alfalfa. On the other hand, Das et al.[8] found in their study that the energy contents of 14 different concentrated feeds, calculated with NRC-2001 equations were similar to those *in vitro* results, and reported that NRC-2001 equations were suitable to estimate energy values of tropical ruminant feeds. Magalhães et al.[51] also reported that use of 48-h *in vitro* NDF digestion, instead of 24-h gas production, in UC Davis for determination of energy values of feeds in tropical regions, and also NCR-2001 and Detmann equations allow more accurate estimates.

In ruminant feed, it is very important to know the energy values of roughages such as alfalfa hay, which will be used throughout the year. Due to difficulties in implementation of *in vivo* and *in vitro* methods in determining energy values, estimations obtained from equations based on nutrients is preferred. In this study, energy values of alfalfa hay were determined using NRC-2001, $Hohenheim_{Menke}$ and UC Davis equations and values obtained with NRC-2001 equations were found to be between the other two methods' values. It suggested more comprehensive studies with different feeds and different equations are needed.

Acknowledgements

We would like to acknowledge the financial support of Selcuk University. (Project Number: BAP 14401115).

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