How to cite: *Cömert* Acar, M., Özelçam, H., Şayan, Y., Önenç S., The Accuracy of Pepsin-Cellulase Technique for Estimating the In Vivo Metabolizable Energy Values of Maize Silage and Dry Forages. J. Anim. Prod., 2018, 59 (2):49-53, DOI: 10.29185/ hayuretim.465306

Research Article
(Araştırma Makalesi)

J. Anim. Prod., 2018, 59 (2):49-53
DOI: 10.29185/ hayuretim.465306

The Accuracy of Pepsin-Cellulase Technique for Estimating the In Vivo Metabolizable Energy Values of Maize Silage and Dry Forages

Mısır Silajı ve Kuru Kaba Yemlerin in vivo Metabolik Enerji Değerlerinin Tahminlenmesinde Pepsin-Sellülaz Tekniğinin Kullanımı

Muazzez CÖMERT ACAR[1*]
Hülya ÖZELÇAM[1]
Yılmaz ŞAYAN[2]
Sibel SOYCAN ÖNENÇ[3]
[1] Department of Animal Science, Faculty of Agriculture, Ege University, TR-35100 Bornova, İzmir – Turkey
[2] Faculty of Agriculture and Natural Sciences, Düzce University, Düzce-Turkey
[3] Department of Animal Science, Faculty of Agriculture, Namık Kemal University, 59030 Tekirdağ –Turkey
*Correspondence: muazzez.comert@ege.edu.tr

Alınış (Received): 12.10.2018 Kabul tarihi (Accepted): 27.11.2018

Key Words:

Forages, in vivo metabolizable energy, enzymatic technique, determination coefficient

ABSTRACT

Objective: The aim of this study was to examine the validity of chemical composition and pepsin-cellulase solubility in predicting in vivo metabolizable energy (ME) values of maize silages and dry forages to develop prediction equations for routine use.

Materials and Methods: Forty samples of maize silage (n=10) and dry forages (n=30) of known in vivo ME were used to compare an in vitro method with pepsin followed by cellulase technique. Dry forages were alfalfa hay, grass hay and wheat straw. For this reason, simple or multiple linear regression analysis with pepsin-cellulase solubility (ELOS) or insolubility (EULOS) and the chemical composition was used to establish equations for the prediction of ME.

Results: The pearson correlation coefficients (r) were found significantly important between in vitro and in vivo ME for maize silages (r = 77.6, % and p= 0.008) and dry forages (r = 81.8, % and p= 0.000). While the highest determination coefficient (R2) value (72.3, %) using single chemical composition with EULOS were obtained with crude fiber, the combination ash and ether extract gave best R2 value (88.3,%) with EULOS for maize silages. Adding more than four chemical compositions with ELOS did not improve the R2 values (80.4, %) for dry forages.

Conclusion: In conclusion, a reasonably acceptable prediction equation of ME values of maize silage could be made by using ash, crude protein, ether extract and crude fiber or nitrogen free extract with EULOS. Adding more than four chemical compositions with ELOS did not improve the R2 of ME values of dry forages. Further work is needed to found the causes of variability in predictive equations and significance of environmental and other factors such as the use of different forage sources.

Anahtar Kelimeler:

Kaba yem, in vivo metabolik enerji, enzimatik teknik, belirleme katsayısı

ÖZ

Amaç: Bu çalışmanın amacı, mısır silajı ve kuru kaba yemlerin in vivo metabolik enerji (ME) değerlerini tahminleme de rutin kullanılacak tahminleme eşitliği geliştirmek için kimyasal kompozisyon ve pepsin-selülaz çözünürlüğü tekniğinin kullanımını incelemektir.

Materyal ve Metot: In vivo ME değeri bilinen toplam kırk adet mısır silajı (n=10) ve kuru kaba yem (n=30), pepsin takiben selülaz tekniği olan in vitro bir metodu karşılaştırmak için kullanıldı. Kuru kaba yemler yonca kuruotu, çayır kuruotu ve buğday samanıdır. Bu amaçla, ME değerini tahminleme eşitliği geliştirmek için, pepsin-selülaz çözünebilirliği (ELOS) veya çözünmeyen kısmı (EULOS) ve kimyasal kompozisyonlarla birlikte tekli ve çoklu linear regresyon analizleri yapıldı.

Bulgular: In vitro ve in vivo ME değerleri arasındaki pearson korelasyon katsayısı mısır silajında (r = 77.6, % ve p= 0.008) ve kuru kaba yemlerde (r = 81.8, % ve p= 0.000) önemli bulundu (p<0.01). Mısır silajında en yüksek belirleme katsayısı (R2 %, 72.3) EULOS ve tekli kimyasal kompozisyon kullanıldığında ham selüloz ile, ikili kombinasyonlarda da ham kül ve ham yağ ile en iyi R2 (% 88) değerini verdi. Kuru kaba yemlerde R2 değerleri (% 80.4) ELOS'la dörtten fazla kimyasal kompozisyon kullanıldığında geliştirilemedi.

Sonuç: Sonuç olarak, mısır silajının ME değerlerini tahminleyici kabul edilebilir eşitlik EULOS ile kül, ham protein, ham yağ ve ham selüloz veya nitrojensiz öz maddeler kullanıldığında oluşturuldu. Kuru kaba yemlerin ME değerlerinin belirleme katsayısı ELOS ile dörtten fazla kimyasal kompozisyon kullanıldığında geliştirilemedi. Bundan sonraki çalışmalarda tahminleyici eşitlikleri etkileyen varyasyonların nedenleri, çevresel ve farklı kaba yem kaynakları kullanımı gibi diğer önemli faktörler incelenmelidir.

INTRODUCTION

The evaluation of the nutritive value of feedstuffs especially forages requires high demands for ration formulation (Kowalski et al. 2014). Feed evaluation methods involves the determination of chemical composition and digestibility, followed by calculation of energy values (Keleş and Çıbık 2014; Kılıç and Gülboy 2015). In vivo digestion trials gives reliable results however, this method is expensive, time consuming and laborious. Therefore, in vitro digestibility techniques have been developed using small quantities of feed (<1g) to simulate in vivo digestion (Tilley and Terry 1963; Iantcheva et al. 1999). Several cellulose-based techniques from the in vitro digestibility methods found wide application to estimate forage digestibility (De Boever et al. 1996). Compared to rumen fluid-based methods, such methods are generally simpler, less time-consuming, more convenient and reproducible and don't require rumen fistulated animals. The main problem with such techniques is the variability in the activity of the enzyme preparations due to the batch and source of the enzyme (De Boever et al. 1988). Some results indicate that enzyme-based predictions of in vivo digestibility and energy are more accurate than others (Adesogan, 2002). However, Barber et al. (1989) and Givens et al. (1995) said that such predictive relationships developed have limited application because of vary with forage species, population and season of harvest. In addition, Kirilov et al. (2001) said that the results from the enzymatic degradability can be successfully used for prediction of metabolizable energy (ME) content of fresh and preserved forages because enzymatic procedures were correlated with in vivo dry matter digestibility (DMD) and ME. Coello et al. (1988) found that DMD had the highest (r2 = 0.88) and lowest (r2 = 0.69) coefficients of determination with pepsin-cellulase+ hemicellulase, and pepsin+cellulase solubility techniques for the forages such as alfalfa, mature ryegrass, common bermudagrass respectively. Jones and Theodorou (2000) found that pepsin-cellulase solubility was highly correlated (r=0.87) with in vivo methods, but different regression equations were required for grasses and legumes.

The aim of this study was to examine the validity of chemical composition and pepsin-cellulose solubility in predicting in vivo ME values of maize silages and dry forages to develop prediction equations for routine use.

MATERIALS and METHODS

Forage samples

In the study, the 10 samples of maize silage (MS) and 30 samples of dry forages (alfalfa hay:AH, grass hay:GH and wheat straw:WS) chemical compositions, pepsin-cellulose solubility parameters and in vivo ME values that determined by Sayan et al. (2004) were used. The feed materials were collected from the Western Anatolia livestock farms of Turkey.

Method

The classical 2-stage technique described by Tilley and Terry (1963) was used to determine the enzyme-soluble organic matter (ELOS) and enzyme-insoluble organic matter (EULOS). First: Pre-treatment with pepsin in 0.1 N HCL at 40 °C for 24 hours; Second: incubation with cellulase (trichoderma viride, onazuka R-10, 1 U/mg) in an acetate-acetic acid buffer at 40 °C 24 hours. For this reason, approximately 300 mg of feed sample ground to pass a 1 mm sieve were weighed to the glass crozier (800 °C heat resistant, por. 1, 50 ml Gooch crucible). All determinations were carried out in three replicates. For the first step, 30 ml pepsin HCL solution was added crucibles and then the samples were shaken before the incubation. For the second step, 30 ml cellulose buffer solution was added to the crucibles and incubated. At the end of incubation, the samples were filtered by moderate vacuum. After filtration, the crucibles were dried at 105 °C for at least 3 hours (dry weight) and then burned at 550 °C (burned weight) and weighed. By using the obtained weighing feed samples, the ELOS and EULOS were calculated by the following equations:

ELOS, % = (DM, %-CA, %-G*, %); EULOS,%=100-ELOS.

* G, % = (dry weight, g–burn weight, g)/ samples weight x 100). Dry matter (DM) and crude ash (CA) values are based on fresh on the equations.

Pepsin-HCl solution: 2 g pepsin (2000 FIP/g) + 0.1 N HCl; Acetate buffer solution 5.9 ml Acetic acid +1 lt distilled water (solution A) and 13.6 g sodium acetate + 1 lt distilled water (solution B). (400 ml solution A + 600 ml solution B were mixed for Pepsin-HCl solution): cellulose buffer solution: 3.3 g cellulose enzyme (trichoderma viride, onazuka R-10, 1 U/mg)+ 1 lt Acetate buffer solution.

The calculation ME values of maize silage and dry forages

The ME values of the feed samples were calculated by using the ELOS (or EULOS) values obtained by the in vitro pepsin-cellulase technique and some chemical compositions (CA: ash, CP: crude protein, EE: ether extract, CF: crude fibre, NFE: nitrogen free extract) (GFE, 1998) and these values were shown in Table 1. For maize silage; ME (MJ/kg DM)= + 14.27-(0.0120x EULOS)+(0.0023xCrude Protein)-(0.0147xCrude Ash). For dry forages (for hays and straw); ME, MJ/kg DM= -1.04 + (0.00001611xELOSxELOS)-(0.0003674xELOSxEther Extract) + (0.3724xEther Extract)-(0.0004919xEther Extract x Crude Fiber)+

(0.01548 x Crude Fiber). All values are expressed as g/kg DM on the equations.

Table 1. The *in vitro* and *in vivo* ME values (MJ/kg DM) of maize silages and dry forages

Çizelge 1. *Mısır silajı ve kuru kaba yemlerin in vitro ve in vivo metabolik enerj değerleri (MJ/kg KM)*

Feed samples (n=40)	ME *in vitro* (EULOS/ELOS + CC)	ME (*in vivo*)
MS	8.66	9.71
AH	8.85	8.93
GH	7.92	8.33
WS	7.26	7.07

MS (Maize Silage), AH (Alfalfa Hay), GH (Grass Hay), WS (Wheat Straw), Metabolizable energy (ME), pepsin-cellulase enzyme soluble organic substance (ELOS), pepsin-cellulase enzyme insoluble organic matter (EULOS), chemical composition (CC)

Statistical analysis

The relationships between the in vitro pepsin-cellulase solubility and in vivo ME values of feed samples were determined by simple and multiple correlation and regression analysis (SPSS, 2006).

Results and Discussion

In the study, first the pearson correlation coefficients were calculated between in vitro pepsin-cellulase technique ME and in vivo ME values, then initially regression equations to using different combinations of EULOS or ELOS with chemical compositions were developed to predict in vivo ME values of feed samples (Table 1 and Table 2). Only those equations were withheld, in which each variable explained a significant ($p<0.05$) part of the variation in energy value. The p values were also given in Table 1 and 2.

The pearson correlation coefficients were found significantly important between in vitro and in vivo ME for maize silages (r = 77.6, % and p= 0.008) and dry forages (r = 81.8, % and p= 0.000). This results were agreed that the solubility of dried grass in Trichoderma cellulase to be highly correlated (r=92,% $P<0.001$, residual standard deviation 2.5) with in vivo digestibility (Jones and Hayward, 1973). The effectiveness of Trichoderma cellulase is confirmed by other studies, showing simple solubility in cellulase to be well-related in vivo digestibility and/or ME values (McLoed and Minson 1978; De Boever et al. 1988). Because of this high relation, the regression equations were required to relate pepsin-cellulase solubility to predict in vivo digestibility and/or ME. Instead of in vivo digestibility, we preferred the ME values of forage samples especially used for ration formulation in ruminants.

Table 2 shows the regression equations which are developed to predict in vivo ME (MJ/kg DM) values by using the chemical composition (g/kg DM) and EULOS (g/kg DM) values of maize silages (n=10).

Table 2. Regression equations to evaluate *in vivo* ME (MJ/kg DM) values of maize silage (n=10)

Çizelge 2. *Mısır silajlarının in vivo ME (MJ/kg KM) değerlerini tahminlemek için geliştirilen regresyon eşitlikleri (n=10)*

Regression Equations	**R^2,%**	**SEE**	**p**
ME = 13.112 - 0.009 EULOS	44.5	0.51	0.035
ME = 13.326 - 0.004 EULOS – 0.026 CA	63.3	0.44	0.030
ME = 13.025 - 0.020 EULOS + 0.020 CF	72.3	0.38	0.011
ME = 9.556 + 0.004 EULOS - 0.046 CA + 0.084 EE	88.3	0.27	0.003
ME = 13.148 - 0.015 EULOS - 0.013 CA + 0.016 CF	75.5	0.39	0.029
ME = 21.466 - 0.011 EULOS - 0.027 CA - 0.009 NFE	75.6	0.39	0.029
ME = 13.142 - 0.021 EULOS - 0.005 CP + 0.022 CF	72.7	0.41	0.040
ME = 12.424 - 0.019 EULOS + 0.013 EE + 0.020 CF	73.1	0.41	0.038
ME = 9.197 - 0.020 EULOS + 0.025 CS + 0.004 NFE	73.4	0.41	0.037
ME = 9.276 + 0.006 EULOS - 0.052 CA - 0.007 CP + 0.095 EE	89.3	0.28	0.012
ME = 31.317 - 0.014 EULOS - 0.036 CA - 0.027 CP - 0.019 NFE	80.9	0.38	0.048
ME = 9.705 + 0.002 EULOS - 0.046 CA - 0.010 CP + 0.086 EE + 0.006 CS	90.0	0.31	0.040
ME = 15.248 + 0.002 EULOS - 0.051 CA - 0.016 CP + 0.080 EE - 0.006 NFE	90.0	0.31	0.040
ME = -36.074 + 0.002 EULOS - 0.036 CP - 0.132 EE + 0.051 CF + 0.046 NFE	89.9	0.31	0.040

R^2 Determination coefficient, SEE, standard error of estimate, CA: ash, CP: crude protein, EE: ether extract, CF: crude fibre, NFE: nitrogen free extract, EULOS: pepsin-cellulase insolubility

In our study, the EULOS value as a single parameter had 44.5, % R2 value with ME (Table 2). This EULOS was worse than the R2 (77.1,%, coefficient of variations, 2.6%) value of De Boever et al. (1988) found for maize silage (n=50) in relation with in vivo digestibility. However, we found as good as combinations crude fiber or ash better than the single EULOS value. While the highest R2 value (72.3,%) using single nutrient with EULOS were obtained with crude fiber, the combination ash and ether extract gave best R2 value (88.3,%) with EULOS. This result were agreed with De Boever et al. (1988) that the R2 value was 73.0,%, (coefficient of variations, 2.9 %) and the best R2 value were found with the combination ash and ether extract (Table 2). The R2 value findings about three and four nutrients with EULOS were reasonably high in our study from 89.3, % to 90.0 %. It was seen that every parameter included to the equation from different combinations of chemical composition with EULOS increased R2 values. This finding in accordance with other studies (Mcleod and Minson, 1978; De Boever et al. 1988) claimed that there were high correlations between in vivo ME values and some the chemical compositions. Based on R2 values (90.0, %, standard error of estimate, 0.31), best predictions were made when EULOS values were used with ash, crude protein, ether extract and nitrogen free extract or crude fiber together (Table 2).

Table 3 shows the regression equations which are developed to predict in vivo ME (MJ/kg DM) values by using the chemical composition(g/kg DM) and ELOS (g/kg DM) values of dry forages (n=30).

Table 3. Regression equations to evaluate *in vivo* ME (MJ/kg DM) values of dry forages (n=30)

Çizelge 3. *Kuru kaba yemlerin in vivo ME (MJ/kg KM) değerlerini tahminlemek için geliştirilen regresyon eşitlikleri (n=30)*

Regression Equations	R^2,%	SEE	p
ME = 4.948 + 0.007 ELOS	76.9	0.48	0.000
ME = 5.086 + 0.007 ELOS - 0.002 CA	77.3	0.48	0.000
ME = 4.501 + 0.009 ELOS - 0.004 CP	78.0	0.47	0.000
ME = 4.796 + 0.006 ELOS - 0.038 EE	78.0	0.47	0.000
ME = 5.943 + 0.006 ELOS - 0.002 CS	77.2	0.48	0.000
ME = 2.396 + 0.008 ELOS + 0.005 NFE	79.7	0.45	0.000
ME = 4.625 - 0.009 ELOS - 0.001 CA - 0.000 CP	78.2	0.47	0.000
ME = 4.938+ 0.006 ELOS - 0.002 CA + 0.039 EE	78.5	0.48	0.000
ME = 9.119 + 0.005 ELOS - 0.008 CA - 0.007 CF	79.3	0.47	0.000
ME = 2.321 + 0.008 ELOS - 0.000 CA - 0.005 NFE	79.8	0.46	0.000
ME = 4.583 + 0.008 ELOS - 0.002 CP + 0.023 EE	78.3	0.48	0.000
ME = 5.876 + 0.021 ELOS - 0.005 CP + 0.022 CF	78.6	0.47	0.000
ME = 1.824 + 0.007 ELOS + 0.003 CP - 0.007 NFE	79.9	0.46	0.000
ME = 5.652 + 0.006 ELOS + 0.037 EE - 0.002 CF	78.3	0.48	0.000
ME = 2.601 + 0.007 ELOS + 0.013 EE + 0.004 NFE	79.9	0.46	0.000
ME = 3.139 + 0.007 ELOS - 0.001 CF + 0.00 5NFE	79.9	0.46	0.000
ME = 4.798 + 0.007 ELOS - 0.002 CA - 0.001 CP + 0.031 EE	78.6	0.48	0.000
ME = 8.661 + 0.006 ELOS - 0.007 CA - 0.004 CP - 0.007 CF	80.1	0.47	0.000
ME = 1.686 + 0.007 ELOS + 0.001 CA + 0.003 CP + 0.007 NFE	80.0	0.47	0.000
ME = 5.772 + 0.007 ELOS - 0.003 CP + 0.015 EE - 0.002 CF	78.7	0.48	0.000
ME = 1.796 + 0.005 ELOS + 0.005 CP + 0.030 EE + 0.007 NFE	80.4	0.46	0.000
ME = 3.316 + 0.007 ELOS + 0.013 EE - 0.001 CF + 0.004 NFE	80.0	0.47	0.000
ME = 8.659 + 0.005 ELOS - 0.007 CA - 0.002 CP + 0.024 EE - 0.007CS	80.4	0.47	0.000
ME = 1.849 + 0.005 ELOS + 0.000 CA + 0.005 CP + 0.031 EE + 0.007 NFE	80.4	0.47	0.000
ME = 1.649 + 0.005 ELOS + 0.005 CP + 0.031 EE + 0.000 CS + 0.007 NFE	80.4	0.47	0.000
ME = 6.904 + 0.005 ELOS - 0.005 CA + 0.026 EE + 0.005 CS - 0.002 NFE	80.4	0.47	0.000

R^2 Determination coefficient, SEE, standard error of estimate, CA: ash, CP: crude protein, EE: ether extract, CF: crude fibre, NFE: nitrogen free extract, ELOS: pepsin-cellulose solubility

R2 values increased related to the increasing number of chemical compositions in the equation (Table 3). This finding were in agreement with the reports that adding chemical composition and especially crude fiber to ELOS improve in vivo digestibility of forages in the multiple regression equations (Jones and Theodorou, 2000). However, adding more than four chemical compositions with ELOS did not improve the R2 values (80.4,%) in our study (Table 3). The highest R2 value (79.7, %) using single nutrient with ELOS were obtained with nitrogen free extract. The combinations nitrogen free extract and crude protein or ether extract or gave best R2 value (79.9,%) with ELOS. Similarly, De Boever et al. (1988) found the highest R2 value (91.0, %, coefficient of variations, 3.6 %) using crude fiber and exter extract combinations with ELOS for grass silage (n=50) to predict in vivo ME.

In conclusion, a reasonably acceptable prediction equation of ME values of maize silage could be made by using ash, crude protein, ether extract and crude fiber or nitrogen free extract with EULOS. Adding more than four chemical compositions with ELOS did not improve the R2 of ME values of dry forages. Further work is needed to found the causes of variability in predictive equations and significance of environmental and other factors such as the use of different forage sources.

ACKNOWLEDGEMENTS

The authors thank to the Technical Research Council of Turkey (TUBİRTAK, Project No: VHAG 1491) and Ege University, Science and Technology Centre (EBILTEM) for its financial support.

REFERENCES

Adesogan T. 2002. What are feeds worth?: A critical evaluation of selected nutritive value methods Proceedings 13th Annual Florida Ruminant Nutrition Symposium, pp 33-47.

Barber GD, Givens DI, Kridis MS, Offer NW, Murray I. 1990. Prediction of the organic matter digestibility of grass silage. Anim. Feed Sci. and Tech. 28: 115-128

Coello M, Hembry FG, Barton FE, Saxton AM. 1988. A comparison of microbial, enzymatic, chemical and near-infrared reflectance spectroscopy methods in forage evaluation. Anim. Feed Sci. and Tech., 20:3, 219-231.

De Boever JL, Cottoyn BG, Andries JI, Buysse FX, Vanacker JM. 1988. The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. Anim. Feed Sci. and Tech. 19 (3):247-260

De Boever JL, Cottoyn BG, De Brabander DL, Vanacker JM, Boucque ChV. 1996. Prediction of the feeding value grass silages by chemical parameters, in vitro digestibility and NIRS. Anim. Feed Sci. and Tech., 60: 103-115.

Gesellschaft für Ernährungsphysiologie (GfE). 1998. Ausschuss für Bedarfsnormen der Gesellschaft für Ernährungsphysiologie. Proc. Soc. Nutr. Physiol., 7:141-149.

Givens DI, Cottyn BG, Dewey PJS, Steg A. 1995. A comparison of the neutral detergent-cellulase method with other laboratory methods for predicting the digestibility in vivo of maize silages from three European countries. Anim. Feed Sci. and Tech. 54:55-64.

Iantcheva N, Steingass H, Todorov N, Pavlov, D. 1999. A comparison of in vitro rumen fluid and enzymatic methods to predict digestibility and energy value of grass and alfalfa hay. Anim. Feed Sci. and Tech. 81: 333-344.

Jones DIH, MV Hayward. 1973. A cellulase digestion technique for predicting the dry matter digestibility of grasses. Journal of the Sci. of Food and Agric. 24(11):1419-1426.

Jones DIH, Theodorou MK. 2000. Enzyme Technique for estimating digestibility. Chapter 8. Forage Evaluation in Ruminants (book chapter). Edited by D.I. Givens ADAS Nutritional Sciences Research Unit Alcester Road Stratford-upon-Avon, UK E. Owen Department of Agriculture University of Reading Reading, UK R.F.E. Axford School of Agricultural and Forest Sciences University of Wales Bangor, UK and H.M. Omed School of Agricultural and Forest Sciences University of Wales Bangor, UK CABI Publishing. 258p

Keleş G, Çıbık, M. 2014. Mısır silajının besin ve besleme değerini etkileyen faktörler. Hayvansal Üretim 55: 27-37.

Kılıç Ü, Gülboy Ö. 2015. Evaluation of Some Equations Developed for Determining Metabolizable Energy Values in İn Vitro Gas Production Technique (İn Vitro Gaz Üretim Tekniği Çalışmalarında Metabolize Edilebilir Enerji Değerinin Belirlenmesi İçin Geliştirilen Bazı Eşitliklerin Değerlendirilmesi) Anim. Prod. (Hayvansal Üretim) 56 (2): 35-37.

Kowalski ZM, Ludwin J, Goka P, Rinne M, Weisbjerg MR, Jagusiak W. 2014. The use of cellulase and filter bag technique to predict digestibility of forages. Anim. Feed Sci. and Tech. 198: 49-56.

McLoed MN, Minson MJ. 1978. The accuracy of the Pepsin cellulase technique for estimating the dry matter digestibility in vivo of grasses and legumes. Anim. Feed Sci. and Tech. 3(4):277–287.

SPSS for Windows. 2006. Released 150 version SPPS IncChicago IL.

Sayan Y, Ozkul H, Alcicek A, Coskuntuna L, Onenc S, Polat M. 2004. Comparison of the parameters using for determination of metabolizable energy value of the roughages. J. Agric. Fac. Ege Univ., 41 (2): 167-175.

Tilley JMA, Terry RA. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassl. Soc., 18: 104-111.