



## The Different Between Methods and Determining of Metabolisable Energy Levels with Enzyme and Gas Techniques in Concentrate Feeds

Pınar Tatlı SEVEN  
İbrahim Halil ÇERÇİ  
Mehmet Ali AZMAN

Fırat Üniversitesi  
Veteriner Fakültesi,  
Hayvan Besleme ve  
Beslenme Hastalıkları  
Anabilim Dalı  
Elazığ-TÜRKİYE

This study was conducted to determine the metabolisable energy (ME) levels of some concentrates and to investigate the differences between enzyme and gas techniques.

This investigation was carried out with totally 35 concentrate feed samples; oat (5), barley (5), corn(5), wheat (5) soybean meal (5), cottonseed meal (5), sun flower meal (5). All the 35 samples were collected from different regions of Turkey. Enzyme and gas techniques were used to identify the levels of metabolisable energy (ME) of the feeds. ME levels of oat, barley, corn, wheat, soybean meal, cottonseed meal, sunflower meal with the enzyme technique were determined as 2083.10, 2394.68, 2399.00, 2391.68, 1882.37, 1748.50 and 1847.24 kcal/ kg DM, respectively. ME levels of oat, barley, corn, wheat, soybean meal, cottonseed meal, sunflower meal with gas technique for 24 h incubations were found as 2880, 3060, 2940, 2450, 2250, 2090 and 2130 kcal/kg DM, respectively. The ME values of 48 h incubations of feedstuffs in gas techniques were found as 3370, 3580, 3460, 3060, 2740, 2690, 2320 kcal/kg DM, respectively.

According to the results of this study, gas technique was found more utilizable than enzyme technique to determine metabolizable energy levels of ruminant feeds.

**Key Words:** *In vitro methods, metabolizable energy, ruminant*

### Konsantre Yemlerde Enzim ve Gaz Teknikleri ile Metabolize Olabilir Enerji Düzeylerinin Belirlenmesi ve Metotlar Arasındaki Fark

Bu çalışma bazı konsantre yem örneklerinin metabolize olabilir enerji (ME) düzeylerini belirlemek, enzim ve gaz teknikleri arasındaki farkı araştırmak amacıyla yürütüldü.

Araştırma; yulaf (5), arpa (5), mısır (5), buğday (5), soya fasülyesi küspesi (5), pamuk tohumu küspesi (5), ayçiçeği tohumu küspesi (5)'nden oluşan toplam 35 konsantre yem örneği ile yapıldı. 35 örneğin hepsi Türkiye'nin farklı bölgelerinden toplandı. Yemlerin ME düzeylerini belirlemek için enzim ve gaz teknikleri kullanıldı. Enzim tekniği ile yulaf, arpa, mısır, buğday, soya fasülyesi küspesi, pamuk tohumu küspesi ve ayçiçeği tohumu küspesinin ME düzeyleri sırasıyla 2083.10, 2394.68, 2399.00, 2391.68, 1882.37, 1748.50 ve 1847.24 kkal/ kg KM olarak belirlendi. Gaz tekniği ile yulaf, arpa, mısır, buğday, soya fasülyesi küspesi, pamuk tohumu küspesi ve ayçiçeği tohumu küspesinin 24 saatlik inkubasyonda ME düzeyleri sırasıyla 2880, 3060, 2940, 2450, 2250, 2090 ve 2130 kkal/kg KM olarak bulundu. Gaz tekniği ile yulaf, arpa, mısır, buğday, soya fasülyesi küspesi pamuk tohumu küspesi ve ayçiçeği tohumu küspesinin 48 saatlik inkubasyonda ME düzeyleri sırasıyla 3370, 3580, 3460, 3060, 2740, 2690 ve 2320 kkal/kg KM bulundu.

Bu araştırmanın sonuçlarına göre, ruminant yemlerinin ME düzeylerini belirlemede enzim tekniğine kıyasla gaz tekniği daha kullanılabilir bulundu.

**Anahtar Kelimeler:** *In vitro yöntemler,metabolize olabilir enerji, ruminant*

Geliş Tarihi : 20.02.2007  
Kabul Tarihi : 30.05.2007

### Introduction

The evaluation quality of feeds is important for the prediction of animal performance. In vivo measurements are expensive and there are difficulties associated with the procedures (1). For these reasons, different corresponding techniques have been developed. The energy value of feeds can be determined by enzymatic methods which do not require rumen fluid, and gas technique (2,3). The digestibility and metabolizable energy (ME) value of feedstuffs are affected by many chemical factors. The loss of potentially digestible material depends on animal species, physical form of the feed, and the ability of the feed and animal supply of elements required by the rumen microflora for maximum digestion. However, this loss is relatively small, it is unlikely to invalidate any conclusions regarding the effect of chemical composition on the digestibility and ME value of feedstuffs (4). For many purposes quick and inexpensive laboratory methods of predicting digestibility are required. In vitro methods usually predict in vivo digestibility with a lower error than any other chemical method (5,6).

### Yazışma Adresi Correspondence

Pınar Tatlı SEVEN  
Fırat Üniversitesi  
Veteriner Fakültesi,  
Hayvan Besleme ve  
Beslenme Hastalıkları  
Anabilim Dalı  
Elazığ-TÜRKİYE

ptatli@firat.edu.tr

This study was conducted so as to determine the ME levels of some concentrates and to investigate the difference between enzyme and gas methods.

## Materials and Methods

### Feed Samples and Chemical Analysis

The investigation was carried out with 35 concentrate feeds - oat (5), barley (5), corn (5) wheat (5), soybean meal (5), cottonseed meal (5), sunflower meal (5) collected from different regions of Turkey. Chemical composition of the feeds is shown in Table 1.

Feed samples were analyzed for dry matter (DM), crude protein (CP), ether extracts (EE) and ash following the procedures described by A.O.A.C.(7). Crude fibre (CF) analysis was performed as described by Crampton and Maynard (8).

### In vitro enzyme technique

In this study, the cellulose (*Trichoderma viride*, Sigma C-9422), hemicellulases (*Aspergillus niger*, Sigma H-2125), amylase (extracted porcine pancreas, Sigma A-3176) and pepsin (Merck, 7190-2000 FIP-U/g) were used. All procedures were carried out according to D'Orleans et al., (9), Aufrère (10) and Sauvart et al. (11). The results were expressed as the digestibility of 24 h incubation.

### In vitro gas technique

To take the rumen liquid, three ruminally fistulated sheep (two years old and with body weight of average 60kg, fed daily with a 900g good quality alfalfa hay and 300g concentrate diet) were used. Water and trace mineralized salt were available at all times. Gas production levels of feeds samples were estimated at incubation times 2, 4, 8, 16, 24 and 48 hours. Gas technique was used Menke and Steingass (12)'s method.

### Statistical analysis

Means and standard deviations of feed's chemical compositions and ME values of feeds, and t test values used to determine the differences between ME values of feeds were determined at SPSS packet program (13). t test results were considered as significant when p values were less than 0.05.

## Results

ME levels of oat, barley, corn, wheat, soybean meal, cottonseed meal and sunflower meal with the enzyme technique were determined as 2083.10, 2394.68, 2399.00, 2391.68, 1882.37, 1748.50 and 1847.24 kcal/kg DM, respectively. ME levels of oat, barley, corn, wheat, soybean meal, cottonseed meal, sunflower meal with gas technique were found as 2880, 3060, 2940, 2450, 2250, 2090 and 2130 kcal/kg DM, respectively (Table 2). The metabolisable energy values of 48 h incubations of feedstuffs in gas techniques were shown in Table 3.

**Table 1. The chemical composition of the feeds (DM, %).**

|                 | Dry Matter | Ash       | Organic Matter | Crude Protein | Crude Fiber | Ether Extracts | Nitrogen Free Extract |
|-----------------|------------|-----------|----------------|---------------|-------------|----------------|-----------------------|
| Oat             | 91.33±1.42 | 3.89±0.74 | 96.11±0.73     | 10.39±3.64    | 11.52±1.75  | 5.42±1.41      | 68.78±4.15            |
| Barley          | 90.32±0.68 | 2.57±0.40 | 97.43±0.40     | 11.22±0.99    | 4.70±1.53   | 2.88±1.06      | 78.63±1.33            |
| Corn            | 89.71±0.35 | 1.73±0.36 | 98.27±0.39     | 10.38±2.97    | 5.67±1.51   | 3.96 ±1.02     | 78.26±0.95            |
| Wheat           | 90.03±0.97 | 4.49±0.96 | 95.50±1.10     | 9.97±1.51     | 3.20±1.22   | 2.37±0.94      | 79.96±1.54            |
| Soybean Meal    | 90.68±1.17 | 6.28±1.85 | 93.71±1.18     | 52.75±2.10    | 8.13±1.11   | 4.43±1.23      | 28.40±2.40            |
| Cottonseed Meal | 94.36±1.41 | 5.28±1.52 | 94.72±1.25     | 30.79±1.32    | 22.02 ±1.55 | 6.34±1.41      | 35.65±1.22            |
| Sunflower Meal  | 93.31±0.89 | 6.36±0.96 | 93.64±1.02     | 24.94±1.20    | 23.60 ±0.94 | 3.20±0.85      | 41.90±1.63            |

**Table 2. The metabolisable energy values of 24 h incubations of feedstuffs in enzyme and gas techniques.**

|                 | Metabolisable Energy (kcal/kg DM) |               |  | t- value | * |
|-----------------|-----------------------------------|---------------|--|----------|---|
|                 | Enzyme technique                  | Gas technique |  |          |   |
| Oat             | 2083.10±60.20                     | 2880±21.10    |  | 42.35    | * |
| Barley          | 2394.68± 86.23                    | 3060±87.41    |  | 43.21    | * |
| Corn            | 2399.00±72.52                     | 2940±27.33    |  | 39.51    | * |
| Wheat           | 2391.68±82.47                     | 2450±17.20    |  | 5.29     | * |
| Soybean Meal    | 1882.37±98.47                     | 2250±49.30    |  | 35.83    | * |
| Cottonseed Meal | 1748.50±100.23                    | 2090±16.80    |  | 32.26    | * |
| Sunflower Meal  | 1847.24±110.70                    | 2130±78.00    |  | 43.82    | * |

\*: P<0.01

**Table 3. The metabolisable energy values of 48 h incubations of feedstuffs in gas technique.**

| Metabolisable Energy (kcal/kg DM) |               |
|-----------------------------------|---------------|
|                                   | Gas technique |
| Oat                               | 3370±20.00    |
| Barley                            | 3580±108.40   |
| Corn                              | 3460±41.22    |
| Wheat                             | 3060±17.05    |
| Soybean Meal                      | 2740±84.14    |
| Cottonseed Meal                   | 2690±85.04    |
| Sunflower Meal                    | 2320±78.22    |

### Discussion

ME values determined with especially enzyme technique were found lower than National Research Council (NRC) (14) values in this study. This may be due to different feed sources and enzymes used in studies which could change the research results (5). Stern et al. (15) reported that in the enzyme technique, enzyme activities in medium might be insufficient in comparison to those in ruminal medium. ME values determined by gas technique in incubations of 24 h were found generally higher than enzyme technique's. Çerçi et al. (16) reported that generally significant relations between ME and feed components with enzyme and gas technique were found, but grain feeds were not effective in determining of the prediction of the quality of feeds. In addition, they informed that positive high relationships between enzyme and gas techniques in the forages and protein sources were often found. Sileshi et al. (17) reported that nutrient components of feeds affected ME

### References

1. Tatlı Seven P., Çerçi, İH. Relationships between nutrient composition and feed digestibility determined with enzyme and nylon bag (in situ) techniques in feed sources. *Bulgarian Journal of Veterinary Medicine* 2006; 9: 107-103.
2. Yalçın S. Hayvan Besleme ve Beslenme Hastalıkları. In: Ergün A, Tuncer ŞD (Editors). *Yemlerin Sindirilme Derecelerinin Tespiti, Özkan Matbaacılık Ltd., Ankara: 2001: 97-106.*
3. Öğretmen, T., Kılıç A. Geviş getirenlerin beslenmesinde kullanılan önemli bazı yemlerin NEL içeriklerinin in vivo ve in vitro yöntemleri ile saptanması. *Doktora Tezi, İzmir: E.Ü. Fen Bilimleri Enstitüsü, 1991.*
4. Minson DJ. Effect of chemical composition on feed digestibility and metabolizable energy. *Nutrition Abstracts and Reviews, Series B* 1982; 52:591-615.
5. Brown VE, Rymer C, Agnew RE, et al. Relationship between in vitro gas production profiles of forages and in vivo rumen fermentation patterns in beef steers fed those forages. *Animal Feed Sci and Technol* 2002;98:13-24.
6. Tilley JMA, Terry RA. A two-stage technique for the in vitro digestion of forage crops. *The Journal of British Grassland Society* 1963;18:104-109.
7. A.O.A.C. Official Methods of Analysis Association of Agricultural Academy Press. Ninth Revised Edition, Washington, DC, 1990.
8. Crampton EW, Maynard L. The relation of cellulose and lignin content to nutritive value of animal feeds. *J Nutr* 1983; 15: 383-395.
9. D'orleans M, Giger S, Sauvant D. *Mise Au Point D'une Methode Enzymatique de Pre'vision de la Digestibilite de la Matiere Organique Des Aliments Concentres.* Institut National Agronomique.Paris Grignon, 1980.
10. Aufrere J. Etude de prevision de la digestibilite des fourrages par une methode enzymatique. *Ann Zootech* 1982; 31: 111-130.
11. Sauvant D, Aufrere J, Michalet-Doreau B, et al. Nutritive des aliments concentres simples tables et prevision. *Bull Tech CRZV Theix, I.N.R.A. 1987;70: 75-89.*
12. Menke KH, Steingass H. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. *Animal Research and Develop* 1988; 28:7-55.
13. SPSS for Windows, Released 6.0 June 17, 1993 Copy Right (c. Spss inc. 1989-1993), 1993.
14. National Research Council: Nutrient requirements of dairy cattle. National Academy Press. Washington, DC, 1988.
15. Stern MD, Bach A, Calsamiglia S. Alternative techniques for measuring nutrient digestion in ruminants. *J Anim Sci* 1977;75: 2256-2276.

levels determined with gas technique. Nevertheless, Cone (18) has reported that a lower correlation has been found between the techniques when different enzymes were used in the same study. In this study, of which reasons are found different both enzyme technique and gas technique's of ME values than NRC (14) values may be due to nutrient components, used enzyme and incubation time (16,17). ME values determined by gas technique in incubations of 48 h were found closer to those of NRC (Table 3)(14). Incubation time were affected ME values of concentrates. In gas technique, rumen liquid's activity has an important role. It was announced that (12) different results might be found in same samples in gas technique, because it is needed many good conditions such as suited animal election, given feed amount to the animal and transported of rumen liquid in aneorobic conditions. In an another study (19), it was found to be 2.96, 3.09, 3.08, 2.58, 2.77, 2.13 and 1.87 Mcal/kg DM, respectively of barley, wheat, corn, oat, soybean meal, cottonseed meal and sunflower meal in incubations of 24 h in gas test. These results are agreed on partly with our study results.

### Conclusion

ME values between enzyme and gas techniques were found significant statistically in concentrates. According to the results of this study, gas technique was found more utilizable than enzyme technique to determine ME levels of ruminant feeds.

### Acknowledgments

This study was funded by TUBITAK.

16. Çerçi İH, Tatlı Seven, P, Azman M et al. Relationships between nutrient composition and metabolic energy determined with enzyme and gas technique in feed sources. *Folia Veterinaria* 2005;49:117-120.
17. Sileshi Z, Owen E, Dhanoa MS, et al. Prediction of in situ rumen dry disappearance of Ethiopian forages from an in vitro gas production technique using a pressure transducer, chemical analyses on in vitro digestibility. *Anim Feed Sci and Tech* 1996; 61:73-87.
18. Cone JW. Degradation of starch in feed concentrates by enzymes, rumen fluid and rumen enzymes. *J Sci Food Agric* 1991; 54: 23-34.
19. Şeker E. Ruminant beslemede kullanılan bazı yemlerin enerji değerlerinin gaz-testi yöntemiyle belirlenmesi. I. Ulusal Hayvan Besleme Kongresi, Elazığ, 2001.