

Estimating *In Situ* Effective Crude Protein Degradability with Cornell Net Carbohydrate and Protein System Parameters in Energy-Rich Feedstuffs for Ruminants ^[1]

Muazzez POLAT ¹  Yılmaz ŞAYAN ¹ Hülya ÖZELÇAM ¹

^[1] This study was supported by the TÜBİTAK (VHAG-No:1913), Scientist Development Group (NATO/A2) and Ege University Science and Technology Research and Application Center (EBİLTEM - No: 2004/025)

¹ Department of Animal Science, Faculty of Agriculture, Ege University, TR-35100 Bornova, İzmir - TURKEY

Makale Kodu (Article Code): KVFD-2013-9968

Summary

The objective of this experiment was to estimate *in situ* effective crude protein degradability (EPD) with Cornell Net Carbohydrate and Protein System (CNCPS) parameters [crude protein fractions (A, B₁, B₂, B₃ and C) and degradable protein intake value (DIP) values] of six energy-rich feedstuffs. Four cereals: maize, wheat, barley, rye and two wheat middling (WM-1 and WM-2) were tested. The *in situ* effective protein degradability (EPD) was calculated using the nylon bag method where the test feedstuffs incubated in the rumen of three Tahirova wethers. The EPD's were estimated as EPD₂, EPD₅ and EPD₈ assuming rumen outflow rates of 0.02, 0.05 and 0.08 h⁻¹. The crude protein fractions *i.e.* A=NPN, B₁=fast, B₂=intermediate, B₃=slow and C=not fermented and unavailable to the animal were calculated using the soluble protein (SolP), the non-protein nitrogen (NPN, % of SolP), the neutral detergent insoluble protein (NDIP) and the acid detergent insoluble protein (ADIP=C) values of feedstuffs based on CNCPS. Then, DIP was calculated by using CNCPS crude protein fractions, degradation rate of B fractions (Kd) and coefficients of outflow rate on the different levels of dry matter intake (Kp): (DIP_{1x}=at 1x maintenance level of intake, DIP_{2x}=at 2x maintenance level of intake, and DIP_{3x}=at 3x maintenance level of intake). It was found that there was a significant multiple regression relation between the EPD₈ (g/kg DM) and crude protein fractions (g/kg DM) (R²=0.96, n=18, P<0.001), and simple regression relation between the EPD₈ (g/kg DM) and DIP_{3x} (g/kg DM) (R²=0.98, n=18, P<0.001). These regression relations did not improve when the different rumen outflow rates were used to estimate EPD. In conclusion, we claimed that *in situ* effective protein degradation (EPD) can be reliably and accurately predicted from CP fractions and DIP values in cereals and wheat middling.

Keywords: Nylon bag method, CNCPS parameters, Protein degradation, Energy-rich feedstuffs

Ruminantlarda Enerjice Zengin Yem Hammaddelerin *In Situ* Etkin Ham Protein Yıkımlanabilirliklerinin Cornell Net Karbonhidrat ve Protein Sistemi Kullanılarak Tahmin Edilmesi

Özet

Bu çalışma, enerjice zengin altı adet yem hammaddesinin ruminantlarda *in situ* etkin ham protein yıkımlanabilirliklerinin (EPD), Cornell Net Karbonhidrat and Protein Sistemi (CNCPS) parametreleri [ham protein fraksiyonları (A, B₁, B₂, B₃ and C) ve tüketilen parçalanabilir protein (DIP)] kullanılarak belirlenmesi amacıyla yapılmıştır. Çalışmanın yem materyali mısır, buğday, arpa, çavdar ile iki farklı buğday kepeğinden (WM-1 ve WM-2) oluşturulmuştur. Yem hammaddelerinin *in situ* etkin ham protein yıkımlanabilirlikleri (EPD), üç adet Tahirova koçu kullanılarak nylon kese tekniği ile belirlenmiştir. EPD değerleri (EPD₂, EPD₅ and EPD₈) 0.02, 0.05 ve 0.08 s⁻¹ rumenden geçiş hızı katsayılarında hesaplanmıştır. Ham protein fraksiyonları A=NPN, B₁=Hızlı, B₂=Orta, B₃=Yavaş ve C=yararlanılamayan protein, CNCPS ile tahıllar ve buğday kepeklerinin çözünebilir protein (SolP), protein tabiatında olmayan nitrojen (NPN, SolP'de %), nötral deterjanda çözünmeyen protein (NDIP) ve asit deterjanda çözünmeyen protein (ADIP=C) değerleri kullanılarak hesaplanmıştır. Daha sonra, DIP değerleri ham protein fraksiyonları, B fraksiyonlarının rumende parçalanma hızı katsayıları (Kd) ve farklı kurumadde tüketim düzeylerindeki rumenden geçiş hızı katsayıları kullanılarak hesaplanmıştır (DIP_{1x}=yaşama payı düzeyinde besleme, DIP_{2x}=yaşama payı düzeyinin iki katında besleme ve DIP_{3x}=yaşama payı düzeyinin üç katında besleme). Bulgular, EPD₈ (g/kg KM) ve ham protein fraksiyonları (g/kg KM) (R²=0.96, n=18, P<0.001) ile EPD₈ (g/kg KM) ve DIP_{3x} değerleri (g/kg KM) (R²=0.98, n=18, P<0.001) arasında önemli derecede regresyon ilişkileri olduğunu göstermiştir. Bu regresyon ilişkileri, EPD değerlerini tahminlemek için farklı rumen geçiş hızı katsayıları kullanıldığında geliştirilememiştir. Sonuç olarak, tahıllar ve buğday kepeklerinin *in situ* EPD değerlerinin ham protein fraksiyonları (A, B₁, B₂, B₃ and C) ve tüketilen parçalanabilir protein (DIP) değerleri ile tahmin edilebileceği ileri sürülebilir.

Anahtar sözcükler: Naylon kese tekniği, CNCPS parametreleri, Protein yıkımlanabilirliği, Enerjice-zengin yem hammaddeleri



İletişim (Correspondence)



+90 232 3881867



muazzez.polat@ege.edu.tr

INTRODUCTION

The mathematical models include *in vivo*, *in situ* and *in vitro* methods have been used to determine the ruminal protein digestibility of the feedstuff [1-4]. Although, *in vivo* method is the most proper method for these mathematical models, surgical preparation for animals with duodenal and rumen cannula and suitable markers for calculating flow rate of digesta make it risky, labour-intensive and expensive [5]. *In situ* Nylon Bag Method (NBM) is the most widely used research approach for measuring ruminal CP degradation [6]. This method also requires rumen cannulated animals, but it is relatively simple compared to *in vivo* method [1,7]. The CNCPS estimates the degradable proteins of the feedstuff using five CP fractions based on solubility in protein precipitant agents, buffer and detergent solutions: A represents the soluble non-protein nitrogen (NPN), B₁ (soluble true protein) is the albumins and globulins, B₂ is the most albumins and glutelins, B₃ is the prolamins, extension proteins and heat denatured proteins, and C is the unavailable N (N bound to lignin) [8-10]. These protein fractions present in each feedstuff were important factors influencing N solubility [11]. So far, no single methods has been accepted as being reliably accurate for predicting the rumen CP degradation. In recent years, the CNCPS as an alternative for estimation of degradable protein has become widely accepted in the studies [12-14], because CNCPS can be applied at the farm level and CP fractions could be measured easily in most feed analyses laboratories [15]. Moreover, some researchers stated that *in situ* rumen degradability may be reliably and accurately predicted from CNCPS parameters [1,16,17]. Energy-rich feedstuffs are sources of rumen degradable protein and glutelin levels (B₂ fraction) are generally high in cereals. In addition, there are not enough studies to compare the CP degradabilities of cereals and wheat middling in Turkey.

The objective of this experiment was to estimate *in situ* effective protein degradability (EPD) with Cornell Net Carbohydrate and Protein System (CNCPS) parameters [crude protein fractions (A, B₁, B₂, B₃ and C) and degradable

protein intake value (DIP)] in four cereals and two wheat middlings offered to ruminant animals in Turkey.

MATERIAL and METHODS

Four cereals: maize, wheat, barley, rye and two wheat middlings (WM-1 and WM-2) with three replicates were collected from feed factories in Western Anatolia Region. The chemical compositions: dry matter (DM), crude ash (CA), crude protein (CP) and ether extract (EE) were determined by Weende analyses method [18]. Ankom Fiber Analyzer (Ankom 200, Ankom Technology, Fairport NY) was used to determine neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses [19]. NDF analyses were carried out as alpha amylase pre-treated on test feedstuffs. All chemical analyses were carried out at least in duplicate. The chemical compositions are shown in Table 1. This study was approved by the internal ethical committee of Ege University (Approval no: 2002/06).

In Situ Nylon Bag Method

Three mature Tahirova wethers (average 50 kg body weighed) fitted with a rumen cannula (40 mm diameter) were used. The vaccination and parasite applications were performed based on veterinary recommendations. The wethers fed twice daily at 9:00 AM and 16:00 PM with 60% alfalfa hay and 40% concentrate with the 1.25 x of maintenance requirements. The alfalfa hay contained 145.0 g kg⁻¹ of CP and 8.00 MJ kg⁻¹ of metabolisable energy (ME), the concentrate contained 150.0 g kg⁻¹ of CP and 11.50 MJ kg⁻¹ of ME. Vitamin-mineral composition of concentrate consists of following: Vitamin A 7000 U/kg, Vitamin D₃ 700 U/kg, Vitamin E 25 mg/kg, Ca 1.1%, P 0.4% and Na 0.25%. The animals were kept individually and had free access to fresh water. The CP degradability was determined according to the method of Bhargava and Orskov [20] using the nylon bag 9x14 cm in size with pore diameter of 40 µm. The feedstuffs were grinded using 2.5 mm sieve, weighed 5-6 g, and then incubated in the rumen for periods 4, 8, 16, 24, 48 h. The 72 h incubation

Table 1. Chemical compositions of cereals and wheat middling (based on g/kg DM)

Tablo 1. Tahıllar ve buğday kepeklerinin kimyasal kompozisyonları (g/kg KM)

Chemical Composition	Maize	Wheat	Barley	Rye	WM-1	WM-2	SE (±)
DM, g/kg	890.0	895.0	901.1	896.5	887.2	889.2	2.4
CA	14.9	17.7	27.5	21.5	61.5	49.8	1.4
CP	100.5 ^c	116.9 ^c	114.5 ^c	141.4 ^b	158.8 ^b	183.8 ^a	5.7
EE	38.7	15.3	22.8	19.6	32.2	44.4	2.5
NDF	134.9	245.0	329.6	329.6	457.5	401.6	27.7
NFC	711.0	605.1	505.6	487.9	290.0	320.4	28.1
ADF	32.5	39.0	60.4	49.8	153.4	126.3	4.6

Wheat middling (WM-1 and WM-2), DM: Dry matter, CA: Crude ash, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber (alpha amylase pre-treated), NFC: Soluble carbohydrates in neutral detergent solution (1000 - CA - CP - EE - NDF), ADF: Acid detergent fiber, Different letters (a, b, c) in the same row are statistically different for CP (P<0.05), SE, Standard error of mean

period was only used with wheat middling. After removal from the rumen, the bags were rinsed in cold tap water. The washing losses were determined by measuring one hour incubation in 39°C water. Then, all bags were washed for 10 min in a washing machine, dried at 55-60°C for 48 h and weighed. Finally, the residues in the bags were used to determine CP degradability. Each feedstuff was tested using three animals with the three replicates (three bags per wethers). The CP degradability was determined by “ $p=a+b(1-e^{-ct})$ ” model using Neway package program with the washing loss [21]. The (p) is the CP degradability at time t, a is the fraction of CP immediately soluble protein, b is the fraction of CP insoluble but degradable in the rumen, c is the rate constant of degradability of fraction b and t is the time of incubation on the model. The effective protein degradabilities (EPD2, EPD5 and EPD8) were calculated by “ $EPD=a+(bxc/c+k)$ ” model. The (k) is the rumen outflow rates of 0.02, 0.05 and 0.08 h⁻¹ on the model, which is representative for low, medium and high feeding levels, respectively [21].

The Cornell Net Carbohydrate and Protein System Parameters

The SolP, NPN (% of SolP), neutral detergent insoluble protein (NDIP) and acid detergent insoluble protein (ADIP) were determined standardized method of Licitra et al. [22]. The feedstuffs were ground using 1 mm sieve. NDIP and ADIP were determined by filtering NDF and ADF residue on filter paper followed by Kjeldhal Method [18]. The CP fractions fractionated as a non-protein nitrogen (A fraction) and as true proteins (B and C fractions) [8,11]. Fraction A is rapidly degraded in the rumen. Fraction B can be divided into three subfractions (B₁, B₂ and B₃) based on the rate of ruminal degradation. Fraction B₁ is soluble true protein. The A + B₁ fractions generate the total soluble proteins (SolP). Total SolP was determined as the proportion of CP that is soluble in borate-phosphate buffer (pH = 6.7-6.8). Sodium azide solution was used to control microbial growth. The sample was filtered through Whatman#54 filter paper using several washes of buffer and the residue plus paper transferred into Kjeldhal tube for the estimation of N in residue. Tungstic acid was used as precipitating agent to determine Fraction A. B₂ is degraded in the rumen intermediate level. B₃ is the fraction with the slowest degradation rate. Fraction C (acid detergent insoluble protein = ADIP) is not fermented and unavailable to the animal. The following equations were used to calculate the CP fractions of feedstuff: A (% of CP) = SolP (% of CP) x (NPN (% of SolP)/100); B₁ (% of CP) = (SolP (% of CP) - A (% of CP)); C (% of CP) = ADIP (% of CP); B₃ (% of CP) = (NDIP (% of CP) - ADIP (% of CP)); B₂ (% of CP) = (100 - Fractions (A+B₁+B₃+C)) (% of CP).

Degradable intake protein (DIP) was calculated by using the following equations: RDPA (% of CP) : rumen soluble protein, A fraction (NPN); RDPB₁ (% of CP): (B₁ x (Kd_{1x}/Kd_{1x} + Kp_{B1})) B₁ fraction (fast soluble protein); RDPB₂

(% of CP): (B₂ x (Kd_{1x}/Kd_{1x} + Kp_{B2})) B₂ fraction (intermediate degradable protein); RDPB₃ (% of CP): (B₃ x (Kd_{1x}/Kd_{1x} + Kp_{B3})) B₃ fraction (slow degradable protein); RDP_{TOTAL} (% of CP) = RDPA + RDPB₁ + RDPB₂ + RDPB₃. RDP_{TOTAL} = DIP_{1x} (Degradable intake protein) according to dry matter intake fed at 1x maintenance level). In these calculations (DIP_{1x} = at 1x maintenance level of intake, DIP_{2x} = at 2x maintenance level of intake, and DIP_{3x} = at 3x maintenance level of intake), the values stated in Fox et al. [8] and Sniffen et al. [11] were used for the degradation rate of B fractions (Kd) and the coefficients of outflow rate on the different levels of dry matter intake (Kp), respectively.

Statistical Analyses

The general linear model procedure of statistical package SPSS® was used one-way ANOVA on results [23]. The Duncan test was used to compare the means, when significant differences observed. Stepwise simple and multiple linear regressions were used to predict *in situ* EPD from CP fractions (A, B₁, B₂, B₃ and C) and DIP value based on CNCPS.

RESULTS

***In Situ* Effective Protein Degradability (EPD) Values**

In situ CP degradability with the incubation time were ranged between 22.77-95.99% in cereals for 0-48 h, 50.15-92.81% in wheat middling for 0-72 h. The degradation parameters (a, b, c) and all EPD values were significantly affected by the feedstuffs (Table 2). The WM-2 and rye had the highest EPD2 (P<0.05), while EPD2 values of WM-1, wheat and barley were similar. Maize had significantly the lowest EPD2 values. EPD5 and EPD8 had the same pattern that WM-2 had the highest in compare the others. Rye and WM-1 values of EPD5 and EPD8 were similar and higher than wheat and barley values. While wheat and barley were similar, maize had the lowest EPD5 and EPD8 values (P<0.05).

The Crude Protein Fractions and Degradable Intake Protein Values

CNCPS parameters of cereals and wheat middling were significantly different (Table 3). DIP values decreased in accordance with the increased feeding level of dry matter intake (1x, 2x and 3x). The A fraction results were following trend from the highest to lowest WM-2, WM-1 and wheat which were differ significantly. The rye, barley and maize had the similar A fractions, being the lowest one. Rye had the highest B₁ fraction compare to the others, in consequence, WM-2 and barley had the similar values and significantly higher than wheat. There was no significant differences between the wheat, WM-1, however, only wheat was significantly higher than maize. B₂ fraction results showed no significant differences among maize, barley and wheat. At the same time, only wheat and barley were not significantly higher than WM-1. B₂ fraction of rye

Table 2. In situ crude protein degradation characteristics of cereals and wheat middling (% of CP)**Tablo 2.** Tahıllar ve buğday kepeklerinin in situ ham protein yıkılabilirlik özellikleri (HP'de, %)

Degradation Characteristics	Maize	Wheat	Barley	Rye	WM-1	WM-2	SE(±)
Degradation parameters							
a	15.31 ^d	37.16 ^{cd}	29.84 ^d	48.45 ^{ab}	44.17 ^{bc}	53.79 ^a	2.63
b	54.93 ^b	58.93 ^b	67.17 ^a	46.03 ^c	46.46 ^c	38.74 ^c	2.65
c, h ⁻¹	0.0588 ^c	0.0943 ^b	0.0860 ^{bc}	0.0999 ^b	0.1365 ^a	0.1486 ^a	0.012
RSD	1.47	1.27	1.50	1.56	1.64	1.04	0.14
Effective protein degradability							
EPD2	56.04 ^c	84.16 ^b	84.05 ^b	86.77 ^a	84.32 ^b	87.72 ^a	0.75
EPD5	44.71 ^d	72.48 ^c	71.99 ^c	79.11 ^b	77.67 ^b	82.51 ^a	1.07
EPD8	38.34 ^d	65.16 ^c	64.36 ^c	74.01 ^b	72.99 ^b	78.74 ^a	1.27

Wheat middling (WM-1 and WM-2), **Degradation parameters:** a an intercept representing the proportion of CP solubilized at initiation of incubation time (soluble fraction), b the fraction of CP insoluble but degradable in the rumen, c the rate constant of degradability of fraction b, **RSD:** Residual standard deviation of equation, **effective protein degradability (EPD)** at rumen outflow rate $k = 0.02, 0.05, \text{ and } 0.08 \text{ h}^{-1}$. Different letters (a,b,c) in the same row are statistically different ($P < 0.05$), SE, Standard error of mean

Table 3. CNCPS parameters of cereals and wheat middling (% of CP)**Tablo 3.** Tahıllar ve buğday kepeklerinin CNCPS parametreleri, (HP'de %)

CNCPS Parameters	Maize	Wheat	Barley	Rye	WM-1	WM-2	SE (±)
SolP	13.46 ^d	31.48 ^{bc}	25.74 ^c	55.53 ^a	35.43 ^b	54.12 ^a	2.91
NPN (% of SolP)	67.98 ^a	64.80 ^a	36.48 ^b	18.35 ^c	74.85 ^a	66.09 ^a	2.56
NDIP	15.74 ^a	5.70 ^c	9.97 ^b	9.08 ^{bc}	8.74 ^{bc}	7.05 ^{bc}	1.15
Crude protein fractions							
A = NPN	9.15 ^d	20.40 ^c	9.39 ^d	10.19 ^d	26.52 ^b	35.77 ^a	1.81
B ₁	4.31 ^d	11.08 ^c	16.35 ^b	45.34 ^a	8.91 ^{cd}	18.35 ^b	1.58
B ₂	70.80 ^a	62.82 ^{ab}	64.29 ^{ab}	35.39 ^c	55.83 ^b	38.83 ^c	3.19
B ₃	13.76 ^a	3.77 ^{bc}	6.87 ^b	4.20 ^{bc}	4.89 ^{bc}	2.65 ^c	1.16
C (ADIP)	1.98 ^c	1.93 ^c	3.10 ^{bc}	4.88 ^a	3.85 ^{ab}	4.40 ^{ab}	0.51
Degradable intake protein							
DIP 1x	63.89 ^d	83.84 ^b	79.65 ^c	84.95 ^{ab}	83.94 ^b	87.65 ^a	0.97
DIP 2x	61.01 ^d	82.02 ^b	77.73 ^c	83.83 ^{ab}	82.15 ^b	86.40 ^a	0.98
DIP 3x	58.45 ^d	80.33 ^b	75.94 ^c	82.78 ^{ab}	80.50 ^b	85.24 ^a	1.00

Wheat middling (WM-1 and WM-2), **SolP:** Soluble protein, **NPN:** non-protein nitrogen (% of SolP), **NDIP:** Neutral detergent insoluble protein, **A fraction (NPN):** non-protein nitrogen, **B₁:** fast soluble true protein, **B₂:** intermediate degradable protein, **B₃:** slow degradable protein, **ADIP (C):** acid detergent insoluble protein, **DIP:** Degradable intake protein fed at 1x maintenance level, at 2x maintenance level of intake, and at 3x maintenance level of intake, Different letters (a,b,c) in the same row are statistically different ($P < 0.05$), SE: Standard error of mean

Table 4. The regression equations to predict in situ EPD values by using CNCPS parameters of cereals and wheat middling (n=18) according to level of feeding (g/kg DM)**Tablo 4.** Tahıllar ve buğday kepeklerinin (n=18) yemleme düzeylerine göre in situ EPD değerlerini tahmin etmede kullanılan CNCPS parametreleri (g/kg KM)

Regression Equations	R ²	SE (±)
EPD2 = - 25.495 + 1.035 A + 1.244 B ₁ + 1.137 B ₂ - 0.414 B ₃ - 0.004 C	0.96	8.00
EPD5 = - 37.228 + 1.085 A + 1.259 B ₁ + 1.112 B ₂ - 0.329 B ₃ - 0.049 C	0.96	8.08
EPD8 = - 42.987 + 1.098 A + 1.250 B ₁ + 1.080 B ₂ - 0.266 B ₃ - 0.053 C	0.96	8.09
EPD2 = - 4.774 + 1.048 DIP1x	0.98	5.24
EPD5 = - 16.011 + 1.069 DIP2x	0.98	5.19
EPD8 = - 20.876 + 1.067 DIP3x	0.98	5.36

R²: Determination coefficient, SE: Standard error of the estimate, $P < 0.001$ for each equation

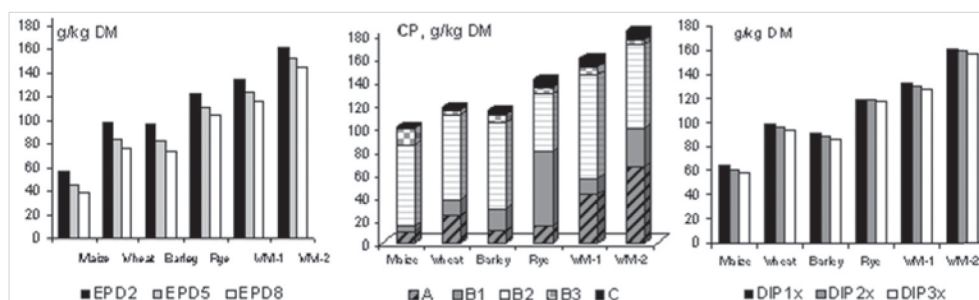


Fig 1. Effective protein degradability (EPD) and CNCPS parameters of cereals and wheat middling (g/kg DM)
Şekil 1. Tahıllar ve buğday kepeklerinin etkin protein yıkılabilirlik (EPD) ve CNCPS parametreleri (g/kg KM)

was similar to WM-2 and the lowest one compared to the others ($P < 0.05$), while maize had the highest value. No significant differences were observed for barley, WM-1, wheat and rye in B_3 fractions, while barley was significantly higher than WM-2. B_3 fraction of maize had the highest value compared to the others ($P < 0.05$). In C fraction, there was no significant differences between rye, WM-2 and WM-1, however, rye was significantly higher than barley, but not WM-1 and WM-2. The C fractions of maize and wheat had no significant differences with barley, but they were lower than the others ($P < 0.05$). The results of DIP1x, DIP2x and DIP3x showed that there were no significant differences found between WM-2 and rye, while WM-2 was significantly higher than WM-1 and wheat. The barley and maize were different each other, being maize had the lowest value ($P < 0.05$).

The Prediction of Effective Protein Degradability (EPD) Values

The EPD2, EPD5 and EPD8 were predicted by using A, B_1 , B_2 , B_3 , C and DIP1x, DIP2x, DIP3x values and these values were shown in Fig. 1. The regression equations to predict EPD's were shown in Table 4. The regression analysis indicated that there was significant multiple regression relationship between EPD values and CP fractions (A , B_1 , B_2 , B_3 and C) ($R^2 = 0.96$, $n = 18$, $P < 0.001$). And also, simple regression relationship is found to predict *in situ* EPD values from DIP values according to level of feeding ($R^2 = 0.98$, $n = 18$, $P < 0.001$).

DISCUSSION

The chemical compositions of energy-rich feedstuffs were varied widely (Table 1), because the chemical compositions of feedstuffs are affected by soil type, fertilizing, climate and processing to by-product. The WM-2 had the highest CP in compared the cereals and WM-1. There were no significant differences between rye and WM-1, even they were higher than barley, wheat and maize ($P < 0.05$). Van Soest [2] and Mc Donald et al. [24] stated similar values for cereals and wheat middling, except rye had higher content of CP (124-138 g/kg DM) compare to available literature.

Effective Protein Degradability (EPD)

The (a) (29.61%) and (b) (63.22%) parameters of barley were close to the our result in Woods et al. [25]. In consistent with Batajoo and Shaver [26], maize (0.041 h^{-1}) had the lowest, while WM (0.1710 h^{-1}) had the highest (c) parameter in our study. In comparison to our result for wheat and barley, Herrera-Saldana et al. [27] showed that the (c) parameter was lower in wheat (0.2536 h^{-1}) and barley (0.1778 h^{-1}). This difference could be attributed to microbial contamination of the feed residues as it stated in Varvikko and Lindberg [28], when estimating *in situ* degradability of CP in starchy feedstuffs. Also, microbial population inside the bag is restricted compared to normal digestion, thus *in situ* protein degradation rates could be lower than actual *in vivo* rates [3]. As the outflow rates (k) increased from rumen to abomasum (i.e from $k = 0.02 - 0.08 \text{ h}^{-1}$), the EPD values decreased (Table 2). EPD values were similar in Cömert and Şayan [29] that maize's being lower than other feeds, higher in wheat middling than for other feedstuffs. It appears that the (c) parameter is important to determine the EPD values of any feedstuffs, because (c) parameter and EPD value were sorted to be the same for maize and wheat middling.

The Crude Protein Fractions and Degradable Intake Protein

The CP fractions and DIP values varied widely among feedstuffs (Table 3), because CP fractions were affected by different protein structure in feedstuffs and processing to by-product. CNCPS parameters of study were compared with the values of Fox et al. [8] (CNCPS ver. 5 feedbank) and those determined by Fortina et al. [12]. The results of our analysis were generally agreed with Fox et al. [8]. However, some differences were observed for SolP of barley, NDIP of wheat middling and ADIP (C fraction) for maize. Fox et al. [8] (17%) reported that SolP of barley was lower than reported values of our study. However, the SolP value of barley reported in Fortina et al. [12] (21.2%) was close to our result. Fox et al. [8] reported that NDIP of wheat middling was lower compare the those of our study (at WM-1 8.74% and at WM-2 7.05% instead of 4%). Fox et al. [8] (5%) and Fortina et al. [12] (6.6%) reported that C fractions of maize was higher than reported values of ours. Regarding NDIP

and C (ADIP) fractions determinations, we suggested that the variability may be imputed to the use of different apparatus (Fibertec vs Ankom) in the laboratory. The results of A, B₁, B₂ and B₃ fractions were also in agreement with Fox et al.^[8] except A fraction of barley. Fox et al.^[8] reported that A fraction for barley (4.9%) was lower than our findings. The differences between the A fraction could be due to use of different reagents (tungstic acid vs trichloroacetic acid) and filtration methods^[12]. Nikokyris and Kandyliis^[30] stated that wheat middling had a higher protein solubility than the unprocessed wheat protein, because soluble proteins such as albumin, globulin and NPN fractions have been increased by processing to by-products. This situation could be explained that A fraction had the highest in wheat middling. Because of the high prolamin and glutelin levels of the energy-rich feedstuffs^[30], B₂ and B₃ fractions were high in cereals. And, B₂ fraction was the highest compare to A, B₁, B₃ and C fractions. The CP fractions of maize were better agreed with Fortina et al.^[12], but barley and wheat middling were not close. B₂ fractions of our study were higher for barley (19%), B₃ fraction of our study were lower for barley (56.1%) and wheat middling (27.3%) than reported in Fortina et al.^[12]. The variability of NDIP and ADIP values were caused the difference in B₂ and B₃ fractions of feedstuffs. Our study agreed with Sniffen et al.^[11] that maize contained high B₃ fractions, because of high zein protein content (prolamins). DIP_{1x} values were highest in WM-2 and lowest in maize. The result of DIP_{1x} values were similar to Fox et al.^[8] in maize (65%), wheat (85%), barley (80%), rye (86%), and wheat middling (86%).

The Estimation of Effective Protein Degradability (EPD)

The all EPD and DIP values are lined up starting from the highest to the lowest as WM-2, rye, WM-1, wheat, barley, maize, similarly. This result disagree with Bach et al.^[31] that some mathematical models may not be appropriate for all types of feedstuffs and the feedstuffs could be ranged in a different order. However, we tested same type of feedstuffs. Results indicated that all determination coefficients were significantly high for the all equations ($R^2 \geq 0.96$) to predict EPD values. These regression relations did not improve when the different rumen outflow rates were used to estimate EPD. In Shannak et al.^[1], Zhoa and Cao^[16] ($n = 30$, $R^2 = 0.90$, $P < 0.0001$) and Westreicher-Kristen et al.^[17] similar to our findings, they reported that *in situ* rumen undegradable protein (1-EPD) obtained from nylon bag method may be the reliable and accurately predicted from CP fractions based on CNCPS. Zhoa and Cao^[14] indicated that the regression equations could be used as a possible alternative, when rumen cannulated sheep or cattle are not available in some laboratories.

The present study showed that *in situ* effective protein degradability (EPD) can be reliably and accurately predicted from CP fractions and DIP values in cereals and wheat middling based on CNCPS. In Turkey, more studies about

feedstuffs based on type are needed to increase the reliability of the regression equation, which is used to estimate the crude protein degradability.

REFERENCES

1. **Shannak S, Südekum KH, Susanbeth A:** Estimating ruminal crude protein degradation with *in situ* and chemical fractionation procedures. *Anim Feed Sci Tech*, 85, 195-214, 2000.
2. **Van Soest PJ:** Nutritional Ecology of The Ruminants. 2nd ed., Cornell Univ. Press, Sage House, 512 East State Street, Ithaca, New York, 14840, p.476, 1994.
3. **Broderick GA, Wallace RJ, Ørskov ER, Hansen L:** Comparison of estimates of ruminal protein degradation by *in vitro* and *in situ* methods. *J Anim Sci*, 66 (7): 1739-1745, 1988.
4. **Schwab CG, Tylutki TP, Ordway RS, Sheaffer C, Stern MD:** Characterization of proteins in feeds. *J Dairy Sci*, 86 (E. Suppl): E88-E103, 2003.
5. **Madsen J, Hvelplund T:** Protein degradation in the rumen, a comparison between *in vivo*, nylon bag, *in vitro* and buffer measurements, *Acto Agric Scand*, 25 (Suppl.): 103-124, 1985.
6. **Stern MD, Bach A, Calsamiglia S:** New concepts, in protein nutrition of ruminants. 21st Annual Southwest Nutrition and Management Conference, February, 23-24, Tempe, Arizona, p.22, 2006.
7. **Gonzalez J, Marmol JF, Matesanz B, Rodriguez CA, Alvir MR:** *In situ* intestinal digestibility of dry matter and crude protein of cereal grains and rapeseed in sheep. *Reprod Nutr Dev*, 43 (1): 29-40, 2003.
8. **Fox DG, Tylutki TP, Tedeschi LO, Van Amburgh ME, Chase LE, Pell AN, Overton TR, Russell JB:** The Net Carbohydrate and Protein System for evaluating herd nutrition and nutrient excretion model documentation CNCPS version 5.0, July 29, Animal Science Mimeo 213 Dep. of Anim Sci, Cornell University, 130 Morrison Hall, Ithaca, New York, 14853-4801, p.381, 2003.
9. **Tylutki TP, Fox DG, Durbal VM, Tedeschi, LO, Russell JB, Van Amburgh ME, Overton TR, Chase LE, Pell AN:** Cornell Net Carbohydrate and Protein System: A model for precision feeding of dairy cattle. *Anim Feed Sci Tech*, 143, 174-202, 2008.
10. **Lanzas C, Broderick GA, Fox DG:** Improved feed protein fractionation schemes for formulating rations with the Cornell Net Carbohydrate and Protein System. *J Dairy Sci*, 91 (12): 4881-4891, 2008, DOI: 10.3168/jds.2008-1440
11. **Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG, Russell JB:** A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *J Anim Sci*, 70 (11): 3562-3577, 1992.
12. **Fortina R, Malfatto V, Mimosi A, Guo K, Tartari E:** The establishment of a database of Italian feeds for the Cornell Net Carbohydrate and Protein System. *Ital J Anim Sci*, 2, 171-179, 2003.
13. **Ghoorchi T, Arbabi S:** Study of protein characteristics of five feeds by CNCPS model. *Asian J Anim and Vet Advan*, 5 (8): 584-591, 2010.
14. **Branco AF, Viana KB, Castañeda RD, Prohmann PE, Coneglian SM, Mouro GF:** Chemical composition and crude protein fractions of coastcross grass under grazing on winter, spring and summer in Southern Brazil. *Acta Sci Anim Sci, Maringá*, 34 (2): 183-187, 2012.
15. **Chalupa W, Sniffen CJ:** Protein and amino acid nutrition of lactating dairy cattle-today and tomorrow. *Anim Feed Sci Tech*, 58, 65-75, 1996.
16. **Zhao GY, Cao JE:** Relationship between the *in vitro* estimated utilizable crude protein and the Cornell Net Carbohydrate and Protein System crude protein fractions in feeds for ruminants. *J Anim Physiol and Anim Nutr*, 88 (7/8): 301-310, 2004.
17. **Westreicher-Kristen E, Steingass H, Rodehutsord M:** Variations in chemical composition and *in vitro* and *in situ* ruminal degradation characteristics of dried distillers' grains with soluble from European ethanol plants. *Arch of Anim Nutr*, 66 (6): 458-472, 2012.
18. **Official Methods of Analysis of AOAC International:** Standard

Compendium of Laboratory Methods for Analyzing Foods and Related Substances. 16th ed., AOAC Publ., Washington, DC, USA. 1995.

- 19. Anonymous:** Acid Detergent and Neutral Detergent Fiber Using Ankom's Fiber Analyzer F200. Ankom Technology Corporation, Fairport, NY, 1995.
- 20. Bhargava PK, Ørskov ER:** Manual for the use of nylon bag technique in the evaluation of feedstuffs. The Rowett Research Institute, Bucksburn, Aberdeen, AB2, 9SB Scotland, p.21, 1987.
- 21. Ørskov ER, McDonald I:** The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage, *J Agric Sci*, 92, 499-503, 1979.
- 22. Licitra G, Hernandez TM, Van Soest PJ:** Standardization of procedures for nitrogen fractionation of ruminant feeds. *Anim Feed Sci Tech*, 57, 347-358, 1996.
- 23. SPSS, for Windows:** Released 15.0 Versions, 233 South Wacker Drive, 11th Floor, IL 60606-6412 Chicago, USA, 2005.
- 24. McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA:** Animal Nutrition. Sixth ed., Pearson Education Limited, Edinburgh Gate, Harlow, Essex CM20 2 JE p.672, 2002.
- 25. Woods VB, Moloney AP, O'Mara FP:** The nutritive value of concentrate feedstuffs for ruminant animals. Part II: *In situ* ruminal degradability of crude protein. *Anim Feed Sci Tech*, 110, 131-143, 2003.
- 26. Batajoo KK, Shaver RD:** *In situ* dry matter crude protein, and starch degradability's of selected grains and by-product feeds. *Anim Feed Sci Tech*, 71, 165-176, 1998.
- 27. Herrera-Saldana RE, Huber JT, Poore MH:** Dry matter, crude protein and starch degradability of five cereal grains. *J Dairy Sci*, 73 (9): 2386-2393, 1990.
- 28. Varvikko T, Lindberg JE:** Estimation of microbial nitrogen in nylon-bag residues by feed 15N dilution. *Br J Nutr*, 54, 473-481, 1985.
- 29. Cömert M, Şayan Y:** A research on degradation of protein in the rumen in some feeds used ruminant nutrition. *Ege Univ J Agric Fac*, 37 (1): 145-152, 2000.
- 30. Nikokyris PN, Kandyliis K:** Feed protein fractions in various solvents of ruminant feedstuffs. *J Sci Food Agric*, 75, 198-204, 1997.
- 31. Bach A, Stern MD, Merchen NR, Drackley JK:** Evaluation of mathematical approaches to the kinetics of protein degradation *in situ*. *J Anim Sci*, 66 (11): 2885-2893, 1998.