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Determination of The Direct and Indirect Effects of Feed Characteristics on the Metabolizable Energy of Natural Pasture Hay

Mera Samanı İçin Yem Özelliklerinin Metabolize Edilebilir Enerjisi Üzerine Path Analizi

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DETERMINATION OF THE DIRECT AND INDIRECT EFFECTS OF FEED CHARACTERISTICS ON THE METABOLIZABLE ENERGY OF NATURAL PASTURE HAY

ABSTRACT:

The aim of this study is to examine the direct, indirect and total effects of nutrient content properties on metabolizable energy contents of natural pasture feeds using path analysis. For this aim, the relationship between metabolizable energy and eight feed nutrient characteristics such as acid detergent lignin (ADL), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber, crude protein, ether extract, ash, and the nitrogen-free extract (NFE) was used for 48 grassland plants samples obtained from natural pastures of Ankara. According to the results of path analysis, the most significant effects on metabolizable energy were reported in crude fiber, acid detergent lignin, crude protein, ash, and nitrogen-free extract. In addition, both acid detergent lignin and neutral detergent fiber had a negative and significant impact on metabolizable energy. As a result, acid detergent lignin and neutral detergent fiber can be presented as prediction criteria to predict metabolizable energy.

Keywords: *Correlation, Path Analysis, Path Coefficient, Direct Effects, İndirect Effect.*

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MERA SAMANI İÇİN YEM ÖZELLİKLERİNİN METABOLİZE EDİLEBİLİR ENERJİSİ ÜZERİNE PATH ANALİZİ

ÖZ:

Bu çalışmanın amacı, doğal mera samanının metabolize edilebilir enerji içerikleri üzerine besin maddesi içeriği özelliklerinin doğrudan, dolaylı ve toplam etkileri Path analizi kullanarak incelemektir. Bu amaçla, Ankara'nın doğal meralarından alınan 48 bitki örneği için metabolize edilebilir enerji ile ADL, ADF, NDF, ham selüloz, ham protein, eter ekstrakt, kül ve NFE gibi yem besin maddesi özelliği arasındaki ilişkiler kullanılmıştır. Path analizi sonuçlarına göre, ham selüloz, ADL, ham protein, kül ve NFE'nin metabolize edilebilir enerji üzerinde en önemli etkileri olduğu bulunmuştur. Ayrıca ADL ve NDF, metabolize edilebilir enerji üzerinde negatif ve anlamlı bir etkiye sahip olduğu belirlenmiştir. Sonuç olarak, metabolize edilebilir enerjiyi tahmin etmek için ADL ve NDF tahmin kriteri olarak önerilebilir.

Anahtar Kelimeler: *Korelasyon, Path Analizi, Path Katsayısı, Doğrudan Etki, Dolaylı Etki.*

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1. INTRODUCTION

The general aim of animal nutrition is to provide nutrients that will increase the productivity of the livestocks. Feed intake is one of the most important factors that will increase the productivity of animals. Variation in feed quality due to a variety of factors such as genetic structure of feed material, season, differences in storage conditions, and sampling errors of feed material used can have a significant impact.. As a result of the evaluation of all these factors, it is difficult to find the standard feeding values of feeds. There are many factors affecting the quality of feed that this will increase the quality of livestock. These factors, such as acid detergent lignin (ADL), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude fiber (CF), crude protein (CP), ether extract (EE), ash (A) and nitrogen-free extract (NFE), metabolizable energy (ME) can affect the productivity and the quality of food from animal origins.

There is a close relationship between the nutrient content of feeds and energy. ADF and NDF affect the digestibility of feeds. Thus, they also affects energy values. ADF and NDF can be determined in a short amount of time. In addition, relative feed value (RFV) can be calculated by ADF and NDF which help to determine the feed quality of roughages as given below (Rohweder et al. 1978).

$$\text{Dry matter intake (DMI)}=(\text{LiveWeight}=\text{LW\%})=120/(\text{NDF\%}) \quad (1)$$

$$\text{Dry matter digestibility (DMD)(\%)}=88.9-(0.779\times\text{ADF\%}) \quad (2)$$

$$\text{Relative Feed Value (RFV)}=(\text{DMD}\times\text{DMI})/1.29 \quad (3)$$

In most cases, those with higher potential for these factors are chosen as feed materials. Therefore, it is necessary to determine the factors affecting these features, which play a crucial role in the selection, and the effect levels of these factors.

In statistical models with two or more variables, cause-effect relationships are generally emphasized, and if there is a functional relationship between variables, the degree and functional form of the relationship are tried to be determined. To determine these effects of factors, many statistical methods such as regression and correlation analysis can be used (Onder and Abaci, 2015). The common method to determine these relationships is correlation analysis (Gul et al., 2019). It is not always possible to explain the relationships between the factors affecting the feature under consideration with a simple correlation coefficient. Because the relationship between two variables can be affected by a third variable or more variables (Gul et al., 2019; Tahtali et al., 2011). These effects, which are defined as direct and indirect effects, need to be determined. Path analysis was used to determine the direct and indirect effects between variables more accurately (Alpar, 2013). Path analysis differs from other multivariate methods in that it considers not only direct but also indirect effects in order to determine the effects and artificial effects (Isci Guneri et al., 2016).

When the literature is examined, no other study has been found that examines the direct and indirect effects on the relationships between feed nutrient contents and metabolizable energy. For this reason, the aim of this study was to determine direct and indirect effects of the feed nutrient content characteristics such as acid detergent lignin, acid detergent fiber, neutral detergent fiber, crude fiber, crude protein, ether extract, ash, and nitrogen-free extract, and metabolizable energy.

2. MATERIAL AND METHODS

The feed material used in the study consists of natural pasture feeds taken from various parts of Ankara province in Turkey (Saricicek, 2020). Plant samples from natural meadows were taken every 15 days in May, June, July and August and analyzed. Plant nutrient analysis (CP, ash, EE, CF) were performed according to AOAC (1990).

Metabolic energy value was determined for ruminants according to Menke et al. (1979) using the following formula.

$$ME, MJ / kgDM = 2.20 + 0.136GP + 0.057CP + 0.002859EE^2 \quad (4)$$

where; GP: 24 h net gas production (ml/200mg DM), CP: Crude protein (%), EE: Ether extract (%).

NEL value was determined using the formula MJ / kgDM = 0.101GP + 0.051CP + 0.11EE for ruminants, according to Menke and Steingass (1988). In addition, ADF, NDF and ADL analysis were made according to Van Soest et al. (1991) by using Ankom device.

Determining the relationship between metabolic energy and other nutrient contents is important for the preparation of the correct ration formulation. Multivariate statistical methods can be used to determine this relationship. There are many multivariate statistical tecniques such as regression analysis, correlation analysis. Investigating the relationship and the degree of this relationship is within the scope of correlation analysis. The correlation coefficient obtained, one of the variables being the result and the other being the explanatory variable, appears as a measure that also expresses the level of influence of these variables on each other. However, even in this case, it is not possible to determine the relationship between the two variables completely. However, there are also nonlinear relationships apart from linear relationships between variables. The process of analysis and interpretation of nonlinear relationships is difficult. Therefore, a path analysis technique, in which all relationships are considered linear, is proposed according to this assumption (Kayali, 2013).

The path coefficient, whose formula is given below where Y and X used as a dependent variable and independent variable respectively, was proposed by (Wright, 1921). Path analysis is a multivariate technique that enables to estimate direct and indirect effects between explanatory and response variables in the model (Mitchell, 1992). There are two main components of path analysis, which are namely the path coefficients representing the mathematical part and the path diagram representing the visual part (Isci Guneri et al., 2016). The standardized regression coefficient found by Wright, which is also a path coefficient, was used to calculate the direct and indirect effects of the correlation coefficient.

$$P_{YX} = \frac{\sigma_{YX}}{\sigma_Y} \quad (5)$$

The path coefficient including direct and indirect effects can be shown with the path diagram. The important and hardest part of path analysis is to logically construct a path diagram. Even if there is no need for a path diagram in practical studies, it would be useful to find direct and indirect effects, from the type of correlation, between explanatory and response variables (Pedhazur, 1997; Isci Guneri et al., 2016). If there is a path coefficient greater than 1 in the path diagram, this situation indicates that there is a balancing mechanism (negative effect) in the system. Path coefficients greater than 1 are not unilaterally significant due to this negative effect (Li, 1975; Isci Guneri et al., 2016).

Figure 1 shows that a path diagram to reveal direct and indirect effects between some feed characteristics and metabolizable energy content. In Figure 1, direct and indirect effects are shown as a one-way arrow and two-way arrow, respectively.

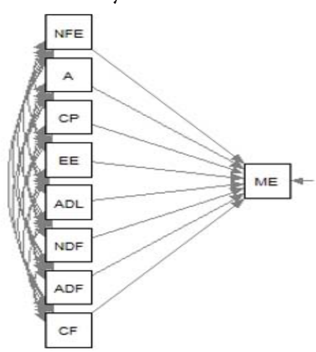

Figure 1. Path diagram of metabolizable energy and other feed nutrient content characteristics

$$r_{NFE-ME} = p_{ME-NFE} + r_{A-NFE} * p_{ME-A} + r_{CP-NFE} * p_{ME-CP} + r_{EE-NFE} * p_{ME-EE} + r_{ADL-NFE} * p_{ME-ADL} + r_{NDF-NFE} * p_{ME-NDF} + r_{ADF-NFE} * p_{ME-ADF} + r_{CF-NFE} * p_{ME-CF} \quad (6)$$

$$r_{A-ME} = p_{ME-A} + r_{NFE-A} * p_{ME-NFE} + r_{CP-A} * p_{ME-CP} + r_{EE-A} * p_{ME-EE} + r_{ADL-A} * p_{ME-ADL} + r_{NDF-A} * p_{ME-NDF} + r_{ADF-A} * p_{ME-ADF} + r_{CF-A} * p_{ME-CF} \quad (7)$$

$$r_{CP-ME} = p_{ME-CP} + r_{NFE-CP} * p_{ME-NFE} + r_{A-CP} * p_{ME-A} + r_{EE-CP} * p_{ME-EE} + r_{ADL-CP} * p_{ME-ADL} + r_{NDF-CP} * p_{ME-NDF} + r_{ADF-CP} * p_{ME-ADF} + r_{CF-CP} * p_{ME-CF} \quad (8)$$

$$r_{EE-ME} = p_{ME-EE} + r_{NFE-EE} * p_{ME-NFE} + r_{A-EE} * p_{ME-A} + r_{CP-EE} * p_{ME-CP} + r_{ADL-EE} * p_{ME-ADL} + r_{NDF-EE} * p_{ME-NDF} + r_{ADF-EE} * p_{ME-ADF} + r_{CF-EE} * p_{ME-CF} \quad (9)$$

$$r_{ADL-ME} = p_{ME-ADL} + r_{NFE-ADL} * p_{ME-NFE} + r_{A-ADL} * p_{ME-A} + r_{CP-ADL} * p_{ME-CP} + r_{EE-ADL} * p_{ME-EE} + r_{NDF-ADL} * p_{ME-NDF} + r_{ADF-ADL} * p_{ME-ADF} + r_{CF-ADL} * p_{ME-CF} \quad (10)$$

$$r_{NDF-ME} = p_{ME-NDF} + r_{NFE-NDF} * p_{ME-NFE} + r_{A-NDF} * p_{ME-A} + r_{CP-NDF} * p_{ME-CP} + r_{EE-NDF} * p_{ME-EE} + r_{ADL-NDF} * p_{ME-ADL} + r_{ADF-NDF} * p_{ME-ADF} + r_{CF-NDF} * p_{ME-CF} \quad (11)$$

$$r_{ADF-ME} = p_{ME-ADF} + r_{NFE-ADF} * p_{ME-NFE} + r_{A-ADF} * p_{ME-A} + r_{CP-ADF} * p_{ME-CP} + r_{EE-ADF} * p_{ME-EE} + r_{ADL-ADF} * p_{ME-ADL} + r_{NDF-ADF} * p_{ME-NDF} + r_{CF-ADF} * p_{ME-CF} \quad (12)$$

$$r_{CF-ME} = p_{ME-CF} + r_{NFE-CF} * p_{ME-NFE} + r_{A-CF} * p_{ME-A} + r_{CP-CF} * p_{ME-CP} + r_{EE-CF} * p_{ME-EE} + r_{ADL-CF} * p_{ME-ADL} + r_{NDF-CF} * p_{ME-NDF} + r_{ADF-CF} * p_{ME-ADF} \quad (13)$$

The matrix given below is used to calculate the path coefficients [16].

$$\begin{bmatrix} p_{ME-NFE} \\ p_{ME-A} \\ \vdots \\ p_{ME-CF} \end{bmatrix} = \begin{bmatrix} 1 & R_{NFE-A} & \cdots & r_{NFE-CF} \\ r_{A-NFE} & 1 & \cdots & r_{A-CF} \\ \vdots & \vdots & \ddots & \vdots \\ r_{CF-NFE} & r_{CF-A} & \cdots & 1 \end{bmatrix}^{-1} * \begin{bmatrix} r_{ME-NFE} \\ r_{ME-A} \\ \vdots \\ r_{ME-CF} \end{bmatrix} \quad (14)$$

The path equations for the path diagram in Figure 1 showing the relationship between ME and feed properties are as follows.

All statistical analysis were performed by using R software (R Core Team, 2020). For descriptive statistics “psych” package were used (Revelle 2020). The package for path analysis was used “lavaan” and the related packages for drawing path diagrams, “semPlot” and “lavaanPlot” were used (Rosseel, 2012; Epskamp, 2019; Lishinski, 2018).

3. RESULTS AND DISCUSSION

Shapiro-Wilk normality test was used to test of normality of the variables. All the variables were determined to be normally distributed (p>0.05). Descriptive statistics for data set were given in Table 1.

Table 1. Descriptive statistics of the data

	CF (%)	ADF (%)	NDF (%)	ADL (%)	CO (%)	CP (%)	CA (%)	NFE (%)	ME (kcal/kg DM)
N	48	48	48	48	48	48	48	48	48
Mean	39.92	43.52	53.27	9.38	1.57	13.15	7.56	37.80	2190.90
Std. Deviation	7.06	7.82	10.13	3.68	0.22	2.06	4.48	8.49	330.36
Minimum	29.55	29.79	36.31	4.40	1.30	10.44	1.46	22.29	1580.45
Maximum	54.57	59.67	75.08	16.28	2.13	18.41	18.14	52.12	2993.71

Pearson correlation coefficients and significance level of correlation coefficients between whole variables are given in Table 2.

Table 2. Pearson correlation coefficient between feed characteristics and metabolizable energy of feed nutrient contents

	CF (%)	ADF (%)	NDF (%)	ADL (%)	CO (%)	CP (%)	CA (%)	NFE (%)	ME (kcal/kg DM)
CF (%)	1								
ADF (%)	.939**	1							
NDF (%)	.928**	.975**	1						
ADL (%)	.933**	.913**	.910**	1					
CO (%)	0.106	-0.008	0.052	0.152	1				
CP (%)	-.657**	-.572**	-.595**	-.686**	-0.265	1			
CA (%)	.427**	0.272	.291*	.423**	.302*	-.607**	1		
NFE (%)	-.906**	-.792**	-.789**	-.843**	-0.211	.637**	-.743**	1	
ME (kcal/kg DM)	-.894**	-.966**	-.955**	-.880**	0.088	.584**	-.322*	.776**	1

**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).

Table 2 shows that the correlation coefficients between all variables, with the exception of ether extract, and between crude protein and ether extract, NFE and ether extract, metabolizable energy and ether extract, ash and ADF, were statistically significant (p<0.05). Among the significance correlations, the coefficients between crude fiber and ADF (r= 0.939**) were determined to be the highest, whereas the coefficients between ash and NDF (r= 0.291*)were found to be the lowest.

Direct, indirect and total effects calculated between each explanatory variable and ME are shown in Table 3.

Table 3. Direct and indirect effect of the feed nutrient contents characteristics on metabolizable energy

Path ways	Effect value	p-value
The relations of crude fiber and metabolizable energy		
Direct effect	-0.999	0.454
Indirect effect over ADF	-0.756	0.000
Indirect effect over NDF	-0.339	0.000
Indirect effect over ADL	-0.019	0.000
Indirect effect over crude oil	0.008	0.469
Indirect effect over crude protein	0.216	0.000
Indirect effect over crude ash	-0.394	0.007
Indirect effect over NFE	1.389	0.000
Total indirect effect	0.105	
Total correlation	-0.894	0.000

The relations of ADF and metabolizable energy		
Direct effect	-0.806	0.000
Indirect effect over crude fiber	-0.938	0.000
Indirect effect over NDF	-0.356	0.000
Indirect effect over ADL	-0.018	0.000
Indirect effect over crude oil	-0.001	0.954
Indirect effect over crude protein	0.187	0.000
Indirect effect over crude ash	-0.251	0.072
Indirect effect over NFE	1.215	0.000
Total indirect effect	-0.161	
Total correlation	-0.967	0.000
The relations of NDF and metabolizable energy		
Direct effect	-0.365	0.008
Indirect effect over crude fiber	-0.927	0.000
Indirect effect over ADF	-0.786	0.000
Indirect effect over ADL	-0.018	0.000
Indirect effect over crude oil	0.004	0.724
Indirect effect over crude protein	0.195	0.000
Indirect effect over crude ash	-0.269	0.055
Indirect effect over NFE	1.210	0.000
Total indirect effect	-0.590	
Total correlation	-0.955	0.000
The relations of ADL and metabolizable energy		
Direct effect	-0.020	0.834
Indirect effect over crude fiber	-0.932	0.000
Indirect effect over ADF	-0.736	0.000
Indirect effect over NDF	-0.332	0.000
Indirect effect over crude oil	0.012	0.303
Indirect effect over crude protein	0.225	0.000
Indirect effect over crude ash	-0.390	0.008
Indirect effect over NFE	1.293	0.000
Total indirect effect	-0.860	
Total correlation	-0.880	0.000
The relations of crude oil and metabolizable energy		
Direct effect	0.077	0.181
Indirect effect over crude fiber	-0.106	0.469
Indirect effect over ADF	0.007	0.954
Indirect effect over NDF	-0.019	0.724
Indirect effect over ADL	-0.003	0.303
Indirect effect over crude protein	0.087	0.079
Indirect effect over crude ash	-0.279	0.048
Indirect effect over NFE	0.324	0.156
Total indirect effect	0.011	
Total correlation	0.088	0.552
The relations of crude protein and metabolizable energy		
Direct effect	-0.328	0.401
Indirect effect over crude fiber	0.657	0.000
Indirect effect over ADF	0.461	0.000
Indirect effect over NDF	0.217	0.000
Indirect effect over ADL	0.014	0.000
Indirect effect over crude oil	-0.020	0.079
Indirect effect over crude ash	0.561	0.000
Indirect effect over NFE	-0.977	0.000
Total indirect effect	0.912	
Total correlation	0.584	0.000
The relations of crude ash and metabolizable energy		
Direct effect	-0.923	0.280
Indirect effect over crude fiber	-0.426	0.007
Indirect effect over ADF	-0.219	0.072
Indirect effect over NDF	-0.106	0.055
Indirect effect over ADL	-0.008	0.008
Indirect effect over crude oil	0.023	0.048
Indirect effect over crude protein	0.199	0.000
Indirect effect over NFE	1.139	0.000
Total indirect effect	0.601	
Total correlation	-0.322	0.026
The relations of NFE and metabolizable energy		
Direct effect	-1.533	0.347
Indirect effect over crude fiber	0.905	0.000
Indirect effect over ADF	0.639	0.000
Indirect effect over NDF	0.288	0.000
Indirect effect over ADL	0.017	0.000
Indirect effect over crude oil	-0.016	0.156
Indirect effect over crude protein	-0.209	0.000
Indirect effect over crude ash	0.686	0.000
Total indirect effect	2.309	
Total correlation	0.776	0.000

According to Table 3, when the direct effects are examined, it is seen that all variables except for ether extract have negative effects. Ether extract has a positive direct effect on metabolizable energy with a value of 0.077 and the p-value of this effect is 0.181. NFE has a negative direct effect on metabolizable energy with a value of -1.533.

In the present study, path analysis was used to determine the direct and indirect effects for the feed nutrient contents characteristics such as ADL, ADF, NDF, CF, CP, EE, A and NFE and ME. According to Li (1975) and Isci Guneri et al. (2016), path coefficients greater than 1 are not unilaterally significant due to the negative effects of the mechanism. Other negative direct effects between feed characteristics and metabolizable energy are ADF, NDF, ADL, crude protein and ash with the values of -0.806, -0.365, -0.020, -0.328, -0.923, respectively. Of these negative direct effects, only ADF and NDF were found to be statistically significant ($p<0.05$). There is no study about the naturel pasture hay within the scope of path analysis. Sayan et al. (2004) reported the correlations between ME and in vitro characteristics such as A, CP, EE, CF, NFE, ADF and NDF. The highest and the most significant correlations are between EE(0.783), CF(-0.851), ADF (-0.892) and NDF (-0.760), respectively. However, Sayan et al. (2004) emphasized that according to Menke and Steingass (1988), it would be more appropriate to consider the amount of NDF or ADF compared to EE, CF, A in meeting the roughage fiber needs of ruminants, therefore, ADF and NDF are more important parameters. Furthermore, ADF and NDF affect the digestibility of feeds. Thus, it also affects energy values. ADF and NDF can be determined in a short time. In addition, in the present study, there is a significant indirect effect on metabolizable energy for ether extract, crude fiber, ADL, crude protein, ash and NFE ($p<0.05$). Canbolat and Kahraman (2009) compared the in vitro gas production, metabolizable energy (ME), organic matter digestibility (OMD), relative feed values (RFV) for the legume forages and they reported the relationship between ME and CP (0.756), EE (0.353), ADF (-0.832), NDF (-0.795) within the scope of correlation analysis, respectively. Of these relationships, only EE was found to be statistically insignificant ($p<0.01$).

4. CONCLUSION

In vivo digestion trials are expensive and require a long time to determine the metabolizable energy (ME) value in feed. However, it is very important that the parameters to be used in determining the ME values of feed materials such as roughages and industrial residues are reliable and that they are obtained economically and practically. For this reason, researchers have conducted many studies on which parameters are more important in predicting ME.

According to the results, ADF and NDF have the highest effect on metabolizable energy in the feed evaluation process. In addition to the direct effects of ADF and

NDF on metabolizable energy, other feed properties have indirect effects through ether extract, crude fiber, ADL, crude protein, ash and NFE. Especially in the literature, NDF or ADF amounts instead of crude protein, ether extract, crude fiber are shown as reliable parameters to estimate metabolizable energy. Therefore, researchers interested in animal nutrition should take into account that ADF and NDF are important selection criteria in their work to predict metabolizable energy. The RFV method developed for quality control in roughage is used. The ADF and NDF contents of feeds are used to calculate the RFV value. RFV is currently an important guide in marketing and determining the quality of roughage. In this way, ADF and NDF, which are among the nutrient contents of the feed, support the results as important criteria as feed evaluation criteria.

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Author Contribution Rates

Design of Study (Çalışmanın Tasarlanması): CT (%25), ZS (%50), HÖ (%25)

Data Acquisition (Veri Toplanması): ZS (%100)

Data Analysis (Veri Analizi): CT (%50), HÖ (%50)

Writing up (Makalenin Yazımı): CT (%33), ZS (%34), HÖ (%33)

Submission and Revision (Makalenin Gönderimi ve Revizyonu): CT (%40), ZS (%20), HÖ (%40)

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