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Araştırma Makalesi/*Research Article (Original Paper)*

Determination of the Relationships between Crude Protein Content and Other Forage Quality Parameters in Grazed and Nongrazed Pastures by Correlation and Path Analysis

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Abstract: This research was conducted to determine characters effecting crude protein content in different artificial pastures from the years 2010 to 2012 by using simple correlation coefficient and path analysis. Forage samples were collected from grazing and non-grazing areas once every 15 days during the grazing seasons. The crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) contents, *in vitro* dry matter digestibility (IVDMD), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and crude ash ratios were determined on the forage samples. The results showed that there were significant interrelationships between quality parameters and the crude protein content in grazing and non-grazing areas of pastures. Several traits affected the crude protein directly or indirectly. In nongrazed areas, P, K and Mg showed positive direct effect on crude protein whereas there were negative direct effects of NDF, ADF, IVDMD, Ca and ash on crude protein content. In grazed areas, ADF, IVDMD, P and K showed positive direct effect on crude protein whereas there were negative direct effects of NDF, Ca, Mg and ash on crude protein content.

Keywords: ADF, Crude protein, Grazing, Correlation, Path analysis.

Otlanan ve Korunan Meralarda Ham Protein İçeriği İle Diğer Kalite Faktörleri Arasındaki İlişkilerin Korelasyon ve Path Analizleri İle Belirlenmesi

Özet: Bu araştırma, korelasyon ve path analizleri kullanılarak, otlanan ve korunan meralarda ham protein içeriğini etkileyen karakterleri belirlemek amacıyla 2010-2012 yılları arasında yürütülmüştür. Otlatma sezonu boyunca otlanan ve korunan alanlardan her 15 günde bir bitki örnekleri toplanmıştır. Meradan alınan örneklerin ham protein, ADF, NDF, in vitro kuru madde sindirilebilirliği (IVDMD), fosfor, potasyum, kalsiyum, magnezyum ve ham kül içerikleri belirlenmiştir. Araştırma sonuçlarına göre, hem otlatılan hem de korunan alanlarda ham protein içeriği ile diğer kalite kriterleri arasında önemli ilişkiler tespit edilmiştir. Birçok özellik ham protein verimini doğrudan veya dolaylı olarak etkilemiştir. Korunan alanlarda P, K ve Mg ham protein oranı üzerine pozitif doğrudan ilişki gösterirken, NDF, ADF, IVDMD, Ca ve ham kül negatif doğrudan etki göstermiştir. Otlanan alanlarda ise, ADF, IVDMD, P ve K ham protein oranı üzerine pozitif doğrudan etki gösterirken, NDF, Ca, Mg ve kül negatif doğrudan etki göstermiştir.

Anahtar Kelimeler: ADF, Ham protein, Otlatma, Korelasyon, Path analizi.

Introduction

Forage quality can be defined as the relative performance of animals (Buxton et al. 1996). In general, higher levels of cell-soluble, crude protein and minerals are considered as criteria for higher nutritive quality. These components of forage decline substantially with the advanced plant growth and reach the lowest level when plants become quality (Koc and Gokkus 1994) as in all steppe vegetation. The changing trend of nutritive component of forage shows great differences among range types because the timing and length of growing season differ among themdue to climate (Holechek et al. 2004). Most plants show a similarity in declining nutrient composition with advancing development towards maturation (Rama et al. 1973; Stubbendieck and Foster 1978; Rebole et al. 2004).

It has been reported that Path-Coefficient Analyses (PCA) are more informative and useful than simple correlation coefficients and widely used in crop breeding to determine the nature of relationships between seed yield and some yield components (Kang et al. 1983; Samonte et al. 1998). Path-coefficient is a standardized partial regression coefficient that measures the direct influence of one trait upon another and permits the separation of a correlation coefficient into components of direct and indirect effects (Board et al. 1997).

The objectives for this study were to estimate correlation coefficient between crude protein and quality parameters and to evaluate the relative contribution of each component to crude protein using path coefficient analyses.

Material and Methods

This research was conducted at Suleyman Demirel University Research Farm in Isparta Province (37°45′N, 30°33′E, elevation 1035 m) located in the Mediterranean region of Turkey on three consecutive years of 2010 and 2012. The major soil characteristics of the research area, determined based on the method described by Rowell (1994), were as follows: The soil texture was clay loam, the organic matter was 1.3% as determined using the Walkley–Black method, the lime was 7.1% as determined using a Scheibler calcimeter, the total salt was 0.29%, the exchangeable K was 122 mg kg^{-1} by 1 N NH_4OAc, the extractable P was 3.3 mg kg^{-1} by 0.4 N $NaHCO_3$ extraction, and the pH of a soil-saturated extract was 7.7. The soil type was calcareous fluvisol.

In March 2010, two artificial grazed lands, covering 1.5 ha pasture each land were established at university farm. Pasture 1 (P1) was composed of *Medicago sativa* L. (20%) + *Bromus inermis* L. (40%) + *Agropyron cristatum* L. (30%) + *Poterium sanguisorba* (10%); and Pasture 2 (P2) had mixtures of *Medicago sativa* L. (15%) + *Onobrychis sativa* Lam. (15%) + *Agropyron cristatum* L. (35%) + *Bromus inermis* L. (35%), respectively. Animal grazing applications were performed in the second and the third year of the study since the first year covered only the establishment of the artificial pastures. The animals were turned out to pasture for grazing on the 1^{st} of May and the grazing was terminated on the 1^{st} of August each year. 10 Holstein male calves with an average 6 months old were included and allocated evenly to artificially established pasture in the experiment which lasted for 90 days in 2011 and 2012. The animals had a free access to the water during all experimental periods.

In order to monitor chemical composition changes in pastures, 4 non-grazed areas within each pasture were established and fenced with wires by 4×3m size and grass samples were collected by using $0.5m^2$ (0.5x1 m) quadrats fortnightly from May to August each year. The crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) contents, *in vitro* dry matter digestibility (IVDMD), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg), ratios were determined as well.

The collected samples after the harvest were weighed and dried at 70 °C for 48 h. The dried samples were reassembled and ground to pass through a 1-mm screen. The crude protein (CP) content was calculated by multiplying the Kjeldahl nitrogen concentration by 6.25 (Kacar and Inal 2008); K, Ca and Mg contents of samples was determined using an atomic spectrophotometer after digesting the samples with $HClO_4$:HNO_3 (1:4); P content was determined by vanadomolybdophosphoric yellow colour method (Kacar and Kovancı 1982). The acid detergent fiber (ADF) and neutral detergent fiber (NDF) concentrations were measured according to methods from Ankom Technology. Tilley and Terry's (1963) methods were used to determine *in vitro* dry matter digestibility (IVDMD) of samples.

The two years data were combined and subjected to variance analyses (SAS, 1998). The simple phenotypic correlation coefficient among all the observed components were first calculated by the Tarist statistical program and then they were separated into direct and indirect effects via path coefficient analyses as suggested by Dofing and Knight (1992) and Rodriguez et al. (2001).

Results and Discussion

Simple correlation coefficient calculated between the crude protein and other quality parameters are given in Table 1. In nongrazed areas of artificial pastures, while negative and significant relationships were found between the crude protein and NDF (-0.944**), ADF (-0.921**), Ca (-0.918**), positive and significant relationships were found between the crude protein and IVDMD (0.926**), P (0.938**), K (0.946**), Mg (0.794**) and ash (0.844**). In grazed areas of artificial pastures, while negative and significant relationships were found between the crude protein and NDF (-0.951**), ADF (-0.926**), Ca (-0.930**), positive and significant relationships were found between the crude protein and IVDMD (0.947**), P (0.955**), K (0.975**), Mg (0.870**) and ash (0.867**). This indicated that there were significant interrelationships between these quality parameters and the crude protein content. Our results confirm the finding of Tucak et al. (2013), Araujo and Coulman (2004).

Table 1. Correlation coefficients among characters in forage quality in grazing and non-grazing areas of pastures.

	CP	NDF	ADF	IVDMD	P	K	Ca	Mg	Ash
				NONGRAZED					
CP	1.000	-0.944**	-0.921**	0.926**	0.938**	0.946**	-0.918**	0.794**	0.844**
NDF		1.000	0.980**	-0.902**	-0.908**	-0.875**	0.954**	-0.747**	-0.919**
ADF			1.000	-0.870**	-0.890**	-0.843**	0.933**	-0.764**	-0.926**
IVDMD				1.000	0.928**	0.950**	-0.925**	0.849**	0.890**
P					1.000	0.913**	-0.906**	0.830**	0.878**
K						1.000	-0.877**	0.831**	0.814**
Ca							1.000	-0.759**	-0.947**
Mg								1.000	0.796**
Ash									1.000
				GRAZED					
CP	1.000	-0.951**	-0.926**	0.947**	0.955**	0.975**	-0.930**	0.870**	0.867**
NDF		1.000	0.982**	-0.928**	-0.937**	-0.909**	0.965**	-0.886**	-0.947**
ADF			1.000	-0.878**	-0.920**	-0.886**	0.931**	-0.848**	-0.924**
IVDMD				1.000	0.925**	0.953**	-0.952**	0.944**	0.906**
P					1.000	0.930**	-0.916**	0.907**	0.880**
K						1.000	-0.907**	0.889**	0.835**
Ca							1.000	-0.931**	-0.969**
Mg								1.000	0.914**
Ash									1.000

The NDF content was positively and significantly correlated with ADF and Ca contents, while it was negative and significantly correlated with IVDMD, P, K, Mg and ash in grazed and nongrazed areas of pastures. The ADF was positively and significantly correlated with Ca, while it was negative and significantly correlated with IVDMD, P, K, Mg and ash. The IVDMD was positively and significantly correlated with P, K, Mg and ash, while it was negative and significantly correlated with Ca (-0.925**). Phosphorus was positively and significantly correlated with K, Mg and ash, while it was negative and significantly correlated with Ca. Potassium was positively and significantly correlated with Mg and ash, while it was negative and significantly correlated with Ca. Calcium was negative and significantly correlated with Mg and ash. Magnesium was positively and significantly correlated with ash.

Correlations between acid detergent fiber and neutral detergent fiber were significant and positive and were similar to those findings reported in the literature (Stratton et al. 1979). This is expected because the components of acid detergent fiber (mainly lignin and cellulose) are also components of neutral detergent fiber. On the other hand, correlations of crude protein with acid detergent fiber and neutral detergent fiber were significant and negative, similar results have been found by Stratton et al. (1979) and Araujo and Coulman (2002). Thus, selection for increased crude protein would result in lower fiber concentration, which would be desirable from the standpoint of forage quality. Based on the results of the present study the development of higher yielding cultivars with higher crude protein, and lower acid and neutral detergent fibers concentration should be possible.

Correlation coefficient analyses usually show relationships among independent traits and the degree of linear relation between these characters (Turk et al. 2008). Consequently, these interrelationships with regards to sign and magnitude were found to be different when path analyses were performed. The relationships determined by path analysis among the examined characteristics in the nongrazed and grazed areas are shown in Table 2 and 3.

Table 2. Phenotypic path coefficient showing direct and indirect effects of different components on crude protein nongrazed areas of artificial pastures.

	Path Coef.	%		Path Coef.	%
NDF vs crude protein	r=-0.944**		*K vs crude protein*	r=0.946**	
Direct effect	-0.2737	15.94	Direct effect	0.3654	22.37
Indirect effect via ADF	-0.2785	16.22	Indirect effect via NDF	0.2396	14.67
Indirect effect via IVDMD	0.0072	0.42	Indirect effect via ADF	0.2395	14.67
Indirect effect via P	-0.2124	12.37	Indirect effect via IVDMD	-0.0075	0.05
Indirect effect via K	-0.3198	18.62	Indirect effect via P	0.2136	13.08
Indirect effect via Ca	-0.2207	12.85	Indirect effect via Ca	0.2029	12.42
Indirect effect via Mg	-0.0257	1.50	Indirect effect via Mg	0.0286	1.75
Indirect effect via Ash	0.3793	22.09	Indirect effect via Ash	-0.3359	20.57
ADF vs crude protein	r=-0.921**		*Ca vs crude protein*	r=-0.918**	
Direct effect	-0.2842	16.73	Direct effect	-0.2312	13.49
Indirect effect via NDF	-0.2682	15.78	Indirect effect via NDF	-0.2612	15.24
Indirect effect via IVDMD	0.0069	0.41	Indirect effect via ADF	-0.2653	15.47
Indirect effect via P	-0.2080	12.24	Indirect effect via IVDMD	0.0074	0.43
Indirect effect via K	-0.3078	18.12	Indirect effect via P	-0.2118	12.35
Indirect effect via Ca	-0.2158	12.70	Indirect effect via K	-0.3205	18.70
Indirect effect via Mg	-0.0263	1.55	Indirect effect via Mg	-0.0261	1.53
Indirect effect via Ash	0.3820	22.48	Indirect effect via Ash	0.3907	22.79
IVDMD vs crude protein	r=0.926**		*Mg vs crude protein*	r=0.794**	
Direct effect	-0.0079	0.47	Direct effect	0.0344	2.35
Indirect effect via NDF	0.2468	14.72	Indirect effect via NDF	0.2045	13.96
Indirect effect via ADF	0.2473	14.75	Indirect effect via ADF	0.2173	14.83
Indirect effect via P	0.2169	12.94	Indirect effect via IVDMD	-0.0067	0.46
Indirect effect via K	0.3471	20.70	Indirect effect via P	0.1940	13.24
Indirect effect via Ca	0.2140	12.76	Indirect effect via K	0.3037	20.73
Indirect effect via Mg	0.0292	1.74	Indirect effect via Ca	0.1756	11.99
Indirect effect via Ash	-0.3673	21.91	Indirect effect via Ash	-0.3285	22.43
P vs crude protein	r=0.938**		*Ash vs crude protein*	r=0.844**	
Direct effect	0.2338	13.95	Direct effect	-0.4127	24.51
Indirect effect via NDF	0.2487	14.83	Indirect effect via NDF	0.2516	14.95
Indirect effect via ADF	0.2529	15.08	Indirect effect via ADF	0.2631	15.63
Indirect effect via IVDMD	-0.0074	0.44	Indirect effect via IVDMD	-0.0071	0.42
Indirect effect via K	0.3337	19.91	Indirect effect via P	0.2052	12.19
Indirect effect via Ca	0.2094	12.49	Indirect effect via K	0.2974	17.67
Indirect effect via Mg	0.0285	1.70	Indirect effect via Ca	0.2189	13.01
Indirect effect via Ash	-0.3621	21.61	Indirect effect via Mg	0.0274	1.63

In nongrazed areas of pastures, several traits affected the crude protein directly or indirectly. Results indicated that P, K and Mg showed positive direct effect on crude protein whereas there were negative direct effects of NDF, ADF, IVDMD, Ca and ash on crude protein content. Path coefficient analyses revealed that K (0.3654 and 22.37%) had higher positive direct effect on crude protein. The path coefficient value of K was found to be the result of strong negative indirect effect via ash (-0.3359 and 20.57%). The direct effects of NDF (-0.2737 and 15.94%) and ADF (-0.2842 and 16.73%) on crude protein were found to be negative and high. The trends in ADF and NDF contents with increasing maturity are normally the reverse of protein (Rebole et al. 2004). Young plant cells has the primary cell wall, but also the secondary cell wall occurs with maturing. This causes being the more fibrous of mature plants (Arzani et al. 2004).

Table 3. Phenotypic path coefficient showing direct and indirect effects of different components on crude protein grazed areas of artificial pastures.

	Path Coef.	%		Path Coef.	%
NDF vs crude protein	r=-0.951**		*K vs crude protein*	r=0.975**	
Direct effect	-0.3768	20.58	Direct effect	0.5389	29.69
Indirect effect via ADF	0.1101	6.01	Indirect effect via NDF	0.3427	18.88
Indirect effect via IVDMD	-0.0783	4.27	Indirect effect via ADF	-0.0994	5.47
Indirect effect via P	-0.2694	14.71	Indirect effect via IVDMD	0.0804	4.43
Indirect effect via K	-0.4900	26.77	Indirect effect via P	0.2675	14.74
Indirect effect via Ca	-0.1763	9.62	Indirect effect via Ca	0.1657	9.13
Indirect effect via Mg	0.2427	13.26	Indirect effect via Mg	-0.2433	13.41
Indirect effect via Ash	0.0875	4.78	Indirect effect via Ash	-0.0771	4.25
ADF vs crude protein	r=-0.926**		*Ca vs crude protein*	r=-0.930**	
Direct effect	0.1122	6.28	Direct effect	-0.1827	10.00
Indirect effect via NDF	-0.3699	20.71	Indirect effect via NDF	-0.3636	19.89
Indirect effect via IVDMD	-0.0741	4.15	Indirect effect via ADF	0.1045	5.72
Indirect effect via P	-0.2644	14.81	Indirect effect via IVDMD	-0.0803	4.39
Indirect effect via K	-0.4774	26.73	Indirect effect via P	-0.2634	14.41
Indirect effect via Ca	-0.1702	9.53	Indirect effect via K	-0.4888	26.74
Indirect effect via Mg	0.2323	13.01	Indirect effect via Mg	0.2549	13.95
Indirect effect via Ash	0.0854	4.78	Indirect effect via Ash	0.0895	4.90
IVDMD vs crude protein	r=0.947**		*Mg vs crude protein*	r=0.870**	
Direct effect	0.0844	4.62	Direct effect	-0.2738	15.41
Indirect effect via NDF	0.3496	19.12	Indirect effect via NDF	0.3340	18.80
Indirect effect via ADF	-0.0985	5.39	Indirect effect via ADF	-0.0952	5.36
Indirect effect via P	0.2659	14.55	Indirect effect via IVDMD	0.0796	4.48
Indirect effect via K	0.5136	28.10	Indirect effect via P	0.2607	14.67
Indirect effect via Ca	0.1739	9.51	Indirect effect via K	0.4789	26.95
Indirect effect via Mg	-0.2584	14.13	Indirect effect via Ca	0.1701	9.58
Indirect effect via Ash	-0.0837	4.58	Indirect effect via Ash	-0.0844	4.75
P vs crude protein	r=0.955**		*Ash vs crude protein*	r=0.867**	
Direct effect	0.2875	15.80	Direct effect	-0.0924	5.25
Indirect effect via NDF	0.3531	19.40	Indirect effect via NDF	0.3569	20.28
Indirect effect via ADF	-0.1032	5.67	Indirect effect via ADF	-0.1037	5.89
Indirect effect via IVDMD	0.0780	4.29	Indirect effect via IVDMD	0.0765	4.34
Indirect effect via K	0.5013	27.55	Indirect effect via P	0.2530	14.38
Indirect effect via Ca	0.1674	9.20	Indirect effect via K	0.4499	25.57
Indirect effect via Mg	-0.2482	13.64	Indirect effect via Ca	0.1770	10.06
Indirect effect via Ash	-0.0813	4.47	Indirect effect via Mg	-0.2503	14.23

Although very strong positively correlations (0.844**) between crude protein and ash ratios were found, ash ratio showed the highest negative direct effect (-0.4127 and 24.51%) on crude protein ratio. As in ash, if the correlation coefficient is positive, but the direct effect is negative or negligible, the indirect effects seem to be reason of correlation. In such situations, the indirect causal factors must be considered simultaneously (Singh and Chaudhary, 1977).

The direct effects of IVDMD on crude protein were found to be negative and low (-0.0079 and 0.47%). The path coefficient value of IVDMD was found to be the result of strong positive indirect effect via K (0.3471 and 20.70%). The direct effects of P on crude protein were found to be positive and high (0.2338 and 13.95%). The path coefficient value of P was found to be the result of strong negative indirect effect via ash (-0.3621 and 21.61%). The direct effects of Ca (-0.2312 and 13.49%) on crude protein were found to be negative and high. The path coefficient value of Ca was found to be the result of strong positive indirect effect via ash (0.3907 and 22.79%).

In grazed areas of pastures, several traits affected the crude protein directly or indirectly. Results indicated that ADF, IVDMD, P and K showed positive direct effect on crude protein whereas there were negative direct effects of NDF, Ca, Mg and ash on crude protein content. Path coefficient analyses revealed that K (0.5389 and 29.69%) had the highest positive direct effect, while the NDF had the highest negative direct effect (-0.3768 and 20.58%) on crude protein. The path coefficient value of K was found to be the result of strong positive indirect effect via NDF (0.3427 and 18.88%). Although crude protein was negative and significantly correlated with NDF (0.951^{**}), NDF ratio showed high negative direct effect (-0.3768 and 20.58%) on crude protein ratio. The reason for this, the path coefficient value of NDF was found to be the result of strong negative indirect effect via K (-0.4900 and 26.77%). Results indicated that the crude protein was positively and significantly correlated with ash (0.867^{**}), ash ratio showed the negative direct effect (-0.0924 and 5.25%) on crude protein ratio. The path coefficient value of ash was found to be the result of strong positive indirect effect of ash via K (0.4499 and 25.57%), followed by NDF (0.3569 and 20.28%). The direct effects of IVDMD on crude protein were found to be positive and low (0.0844 and 4.62%). The path coefficient value of IVDMD was found to be the result of strong positive indirect effect via K (0.5136 and 28.10%). The direct effects of P on crude protein were found to be positive and high (0.2875 and 15.80%). The path coefficient value of P was found to be the result of strong positive indirect effect via K (0.5013 and 27.55%). The direct effects of Mg (-0.2738 and 15.41%) on crude protein were found to be negative and high. The path coefficient value of Mg was found to be the result of strong positive indirect effect via K (0.4789 and 26.95%).

Path coefficient and correlation analyses are being widely used in many crop species by plant breeders to understand the nature of complex interrelationships among traits and to identify the sources of variation in yield. The knowledge can be utilized to develop selection criteria to improve seed yield with agricultural practices (Finne et al. 2000; Sinebo 2002).

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