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Relationships between yield and quality related traits of annual medics species using multivariate statistical techniques under different sowing methods

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Abstract

In order to identify the relationships between yield and quality related traits of three annual medics species (*Medicago rigidula, Medicago polymorpha and Medicago scutellata*), a 2-years field study was conducted in 2010 and 2011 growing season. The experiment was conducted as split plot experiments based on randomized complete block (RCB) design with three replications. Two sowing methods containing broadcast planting (BP) and row planting (RP) were taken as main plot and the medics species as sub plot, with their related traits, Four statistical techniques consisted of canonical correlation (CC), principal component analysis (PC), cluster analysis, and stepwise regression were undertaken for exploring the relationships. Principal component analysis and canonical correlation showed that OM, CP, EE, NFE, and ME have the positive effect in contributing of digestible energy, and SDW and SFW most important traits toward total fresh weight of annual medics species. Dendrogran of cluster analysis showed that OM and ME are the most important factors affecting digestible energy, while SDW and SFW had closest the distance with total shoot weight. On the other hand, it can be concluded that ordinal regression analysis is not a very effective method for detecting relationships among medics traits and multivariate statistical methods are more reliable.

Keywords: method planting, crude protein, digestible energy, annual medics species.
Abbreviations: OM_ Organic matter; ME_ Metabolic energy; EE_ Extract ether DE_ Digestible energy;. CF_ Crude fiber; CP_ Crude protein; NFE_ Nitrogen free extract; TFW_ Total fresh weight; SFW_ Shoot fresh weight; SDW_ Shoot dry weight; RDW_ Root dry weight; S/R_ Shoot to root dry weight ratio; Ca_ Calcium; P_ Phosphorous; NDF_ Natural detergent fiber; WCf_ Water content based on fresh weight.

Introduction

Annual medics (*Medicago* Spp) species are belonging to leguminous family that are native to semiarid areas of the Mediterranean region and have long been used in pastures in the region (Raven et al., 2005). Annual medics' species is now widely distributed throughout the world, largely in areas with mild, rainy winters and alkaline soils (Dorry, 2008). In Michigan, annual Lucerne and berseem clover provide an extra source of emergency forage when Lucerne was winter killed (Shrestha et al., 1998). *Medicago* in Western Montana can provide the benefits of perennial legumes in annual cropping systems as a low input systems as green manures without expending an entire growing season (Westcott et al., 1991). Annual medics' species are found in almost all region of Iran indicating these plants are appropriate for Iran pastures. Annual medics' species have several annual species, such as *M. rigidula, M. polymorpha and M. scutellata. M. scutellata* was able to produce a high number of seeds. It establishes relatively easily but its early growth in autumn is rapid and erect making it susceptible to overgrazing (Smeltekop et al., 2002). This species can enhance water permeation into the soil; consequently improve the soil structure and protecting the soil surface (Dorry, 2008). Although most of Annual medics' species are special to tropical areas, however *M. rigidula* is native to cold and temperate zones (Carter, 1981). *M. rigidula* is a species that found at high latitudes and elevations in Eurasia. *M. polymorpha* has a very low forage production compared with other cultivars, resulting in low protein and seed production (Dorry, 2008). Study of Sukhchain and Sidhu (1992) showed a significant correlation between total crude protein per plant (CP) and digestible dry matter per plant with dry matter yield per plant (DMY). They reported a high relationship between morphological traits with CP and DMY. Based on the Monirifar (2011) CP content was correlated directly with acid detergent fiber (ADF) and natural detergent fiber (NDF) while its correlation with crude fiber (CF) was inversed. Leaf dry weight had negative relationship with CP. ADF, CF, NDF (Monirifar, 2011). There are several systems used to measure the availability of energy from feeds. Digestible energy (DE) is an indicator in a forage crops (NRC, 1984).
Sowing method is a major contributing factor to affect crop vigor and ultimately leads to better yield (Korres and Froud-Williams, 2002). Collins and Fowler (1992) indicated that the broadcasting planting method was considered inferior to other methods (row planting and intercropping). These results confirm that sowing method was responsible for *Medicago* growth and performance. The most common method in Iranian pasture is broadcast planting; however it seems that this method lacks efficiency. So, this study was conducted to evaluate the effect of sowing methods such as broadcast planting and row planting on yield and quality of annual *Medicago* species and determine relationship among quality related and yield related traits, and to investigate their impact

on total shoot yield and quality related traits of annual medics species under different sowing methods.

Results

Principal component (PC) analysis and trait's scores in the first two components

In principal component (PC) analysis, the first two PC explained about 91 percent of variability among the traits (Table 1) and the Biplot of PCs showed that the first two PCs are sufficient for considering relationships among traits, so that the loading plot for these first two components was carried out (Fig. 1). The PC1 showed high positive correlation (higher than 0.6) with quality determining traits such as OM, CP, EE, CF, NDF, ash, NFE, Ca, P, DE and ME while its correlation with yield related traits such as TFW, SFW, SDW, RDW, DM, S/R and WCf was low. The PC1 indicated that quality determining traits especially OM (0.94), CP (0.95), NFE (0.93), P (0.83), ME (0.96) and digestibility (0.96). PC2 showed positive and high correlation with the yield related traits indicating that TFW (0.86), SFW (0.96), SDW (0.97), RDW (0.87) and S/R (0.61). WCf showed no clear relationship with both PCs (PC1=-0.34 and PC2=0.37). Loading plot for first two PCs confirmed abovementioned results and showed that WCf is more connected to yield related (TFW, Total fresh weight; SFW, Shoot fresh weight; SDW, Shoot dry weight; RDW, Root dry weight; S/R, and Shoot to root dry weight ratio; WCf) traits (Fig. 2).

Stepwise regression selection method for indicating most important traits on the total fresh weight and quality traits

Digestible energy and total fresh weight had +1 coefficient in the first and second canonical correlation (CC) respectively. Similar to the results of the PC analysis, quality determining traits; OM (0.98), CP (0.89), NFE (0.92), EE (0.78), and ME (0.99) had high coefficients in first CC while low coefficient in the second one. Yield related traits; SFW (0.87) and SDW (0.86) had a high coefficient in contributing second CC (Table 2). The highest coefficient of first PC was belonged to shoot digestible energy, so that the name of this component can be nominated as digestible energy, where traits with higher coefficient had a higher effect on digestible energy. Based on the results of the canonical correlation, OM, CP, EE, NFE, and ME had a high positive contribution in digestibility characterization, while root dry weight (RDW) had a negative effect. NFE had a value near to 1 first canonical vector, therefore it has great relationship with digestibility, and annual medics species plant with higher NFE probably has higher digestible energy. Considering total fresh weight and digestible energy as dependent variables, separately, stepwise regression was carried out. This method took Ash (F=75.66), Ca (F=3.74), SFW (F=4.26), SDW (F=3.67), RDW (F=3.88), and WCf (F=2.6) as the most important traits affecting total shoot fresh weight of the annual medics species. Stepwise model selection for modeling digestible energy, selected SFW (F=232.7), Ca (F=34.63), Ash (F=501.65), RDW (F=81.98), WCf, (F=961.16) and SDW (F=322.54) as the most effective traits (Table 3 and 4). Fig. 3 showed cluster analysis based on the Ward method for measured traits of the annual medics species. This technique extracted three groups for measured traits. First group consisted of OM, ME, DE, EE, and CF. The second group contained mineral element content of annual medics species including CP, NFE, P, ash, Ca, and NDF. Finally, SWF, SDW, TFW, RDW, S/R and WCF were clustered in the third group, where the closest distance with total shoot fresh weight was related to SDW and SFW.

Discussion

First two components related to all measured traits

PCA showed that the first two PC explained about 91 percent of variability among the traits, thus, these PCs could be used for considering relationship among measured traits. The PC1 revealed a high positive correlation (higher than 0.6) with quality determining traits while its correlation with yield related traits was low. Results of this PC indicated that quality determining traits especially OM, CP, NFE, P, and ME have a great relationship with digestible energy of the annual medics species. On the other side, TFW and other yield related traits of the annual medics species had a low contribution in digestible energy. Thus, it can be concluded that yield related traits are not important variables to be consisted as important variables. Davodi et al (1998) by considering quality and quantity traits of alfalfa get the same results. Their study showed that the results of factor analysis based on the principal component and Varimax rotation were accounted for 81% of total variance for first six factors. Factor 1 which was accounted for 19% of variation was associated with DMD, WSC and ADF. This factor was regarded as quality factors. Factor 2 accounted for 17% of variation was named as the productivity factor since it included DM yield, vegetation score and leaf/stem ratio Considering this PC also showed that mineral elements have a great contribution in digestible energy of annual medics species, and must be reviewed as important variables. Second PC showed positive and high correlation with the yield related traits. Because of high coefficient of shoot measured traits, PC2 can be named as shoot weight.PC2 clearly shows that quality determining traits of annual medics species have no confident effect on total shoot fresh weight. Except of the coefficient of EE, other negative trait's coefficients of PC2 were not significant, probably indicating negative effect of EE on total shoot fresh weight. In the study of Bhattarai et al (2008) on *Astragalus filipes*, the first principal component described 60.5% of the total variation among accessions, whereas the second principal component described an additional 15.4% of the total variation. The PC1 loadings were high for biomass (0.94), seed yield (0.87), combined plant height and vigor score (0.80), and combined number of stems and inflorescences (0.93), and low for crude protein (20.67) and over winter mortality (20.63). The PC2 loadings were high for seed mass (0.71).They stated that the number of stems is a reliable predictor of high biomass and seed yield. For improvement and understanding of relationship among traits regarding principal component, loading plot for the first two PCs was figured out. In loading plots, more closer vector of the traits, more closer relationship and vice versa. With the regards to loading plot, OM and ME are the most important factors affecting digestible energy. Also, it could be comprehended that because of conversed vectors related to ECF and EE, these traits have inverse effect on each other. WCf had a contrary vector with EE, measured mineral elements and quality related traits. This result could determine that WCf negative on digestible energy. WCf vector was more close to yield related traits, and it has a positive effect on annual medics species yield. Similar to PC analysis, in CC analysis, digestible energy and shoot fresh weight had highest (1) coefficient in first and second

Table 1. Principal component (PC) analysis and trait's scores in the first two components.

Traits	PC1	PC2
OM (%)	0.940	-0.160
CP (%)	0.954	0.041
EE (%)	0.672	-0.443
CF (%)	0.610	-0.223
NDF (%)	0.651	0.120
Ash (%)	0.767	0.296
NFE (%)	0.932	0.0145
Ca (%)	0.725	-0.028
P (%)	0.838	0.133
DE (Kcal/kg)	0.961	-0.108
ME(Kcal/kg)	0.955	-0.129
TFW(g)	0.124	0.862
SFW(g)	0.181	0.965
SDW(g)	0.151	0.979
RDW(g)	-0.07	0.870
S/R(g)	0.206	0.613
WC*f* (%)	-0.338	0.371
Variance accounted	67%	24%

OM, Organic matter; ME, Metabolic energy; DE, digestible energy;EE, Extract ether Digestibility;. CF, Crude fiber; CP, Crude protein; NFE, Nitrogen free extract; TFW, Total fresh weight; SFW, Shoot fresh weight; SDW, Shoot dry weight; RDW, Root dry weight; S/R, Shoot to root dry weight ratio; Ca, Calcium; P, Phosphorous; NDF, Natural detergent fiber; WCf, Water content based on fresh weight.

Table 2. Correlation of traits with the two canonical correlations.

Traits	CC1	CC2
OM (%)	0.980	-0.004
CP (%)	0.898	0.131
EE (%)	0.777	-0.227
CF (%)	0.615	-0.041
NDF (%)	0.583	0.164
Ash (%)	0.559	0.279
NFE (%)	0.918	0.013
Ca (%)	0.584	-0.116
P (%)	0.671	0.122
ME(Kcal/kg)	0.999	-0.016
SFW(g)	0.018	0.873
SDW(g)	0.029	0.857
RDW(g)	-0.395	0.166
S/R(g)	0.330	0.283
WC*f* (%)	-0.046	-0.029
DE (Kcal/kg)	1.000	0.000
TFW(g)t	-0.0052	1.000
P-value	0.003	0.009
Variability accounted	69%	22%

OM, Organic matter; ME, Metabolic energy; DE, digestible energy;EE, Extract ether Digestibility;. CF, Crude fiber; CP, Crude protein; NFE, Nitrogen free extract; TFW, Total fresh weight; SFW, Shoot fresh weight; SDW, Shoot dry weight; RDW, Root dry weight; S/R, Shoot to root dry weight ratio; Ca, Calcium; P, Phosphorous; NDF, Natural detergent fiber; WCf, Water content based on fresh weight.

canonical vectors, respectively. Based on the results of the canonical correlation, OM, CP, EE, NFE, and ME had a high positive contribution in digestible energy, while root dry weight (RDW) had a negative effect. NFE had a value near to 1 first canonical vector, therefore it has great relationship with digestible energy, and annual medics species with higher NFE probably has higher digestible energy. Sukhchain and Sidhu (1992) by using correlation statistical method stated that there are significant correlations between total CP and digestible dry matter per plant with dry matter yield per plant. They reported a high relationship between morphological traits with CP and dry matter yield. Monirifar (2011) showed that CP content was positively correlated with NDF while negative correlation with CF. Leaf dry weight had negative relationship with CP. ADF, CF, NDF (Monirifar, 2011). Julier et al (1998) reported that Stem digestible energy was negatively correlated to forage yield.

Cluster analyzing of measured traits

Analysis of clusters based on the Ward method for measured traits in annual medics species extracted three main groups (clusters) for traits. First group was consisted of OM, ME, DE, EE, and CF showing most important factors on the digestible energy characteristic. This dendrogram also showed that OM and ME are the most important factors affecting shoot digestibility. The second group truly was contained mineral elements content of annual medics species indicating direct effect of these elements one another. Yield related traits with WCf were grouped in the third cluster the same results showed by (Bozickovic et al., 2013). Ordinal stepwise regression had a converse result with PC, CC and cluster analysis. SFW, Ca, Ash, RDW, WCf and SDW are selected variables affecting both yield and digestible energy of annual medics species. The conversed result of stepwise regression with other multivariate statistical techniques

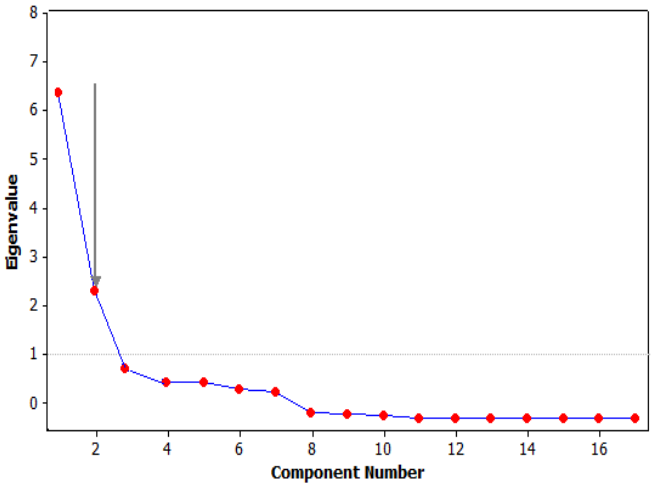

Fig 1. Screeplot for component extracted from principal component analysis of three annual medics species

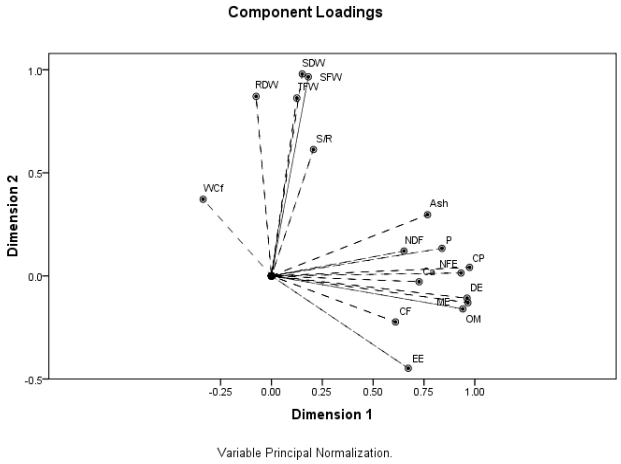

Fig 2. Loading plot for the first two components related to all measured traits of three annual medics species
Organic matters: OM, crude protein: CP, extract ether: EE, crude fiber: CF, natural detergent fiber: NDF, nitrogen free extract: NFE, calcium: Ca, phosphorous:P, digestible energy: DE, metabolic energy: ME , total and shoot fresh weight: TFW and SFW respectively, shoot and root dry weight: SDW and RDW respectively, dry matter: DM, shoot to root weight ratio: S/R and water content: WC*f*.

probably is related to exciting high correlation between traits known as coleanirity, in such situation, results of regression are less valuable than those in multivariate techniques. Hence, it can be concluded that ordinal regression analysis is not a very effective method for reviewing relationships among annual medics species traits and multivariate statistical method are more reliable. Oba and Allen (1999) considered effects of the digestibility of NDF from forage on performance of dairy cows. They reported that Enhanced NDF digestibility of forage significantly increased dry matter intake (DMI) and milk yield. A one-unit increase in NDF digestibility in vitro or in situ was associated with a 0.17-kg increase in DMI and a 0.25-kg increase in 4% fat-corrected milk. On the other hand, Regarding to regression analysis for modeling the dependent traits, other modified regression methods or removing some other traits from the model may be satisfied a good model but for considering relationship among traits, multivariate techniques are suitable and our results could be explained in the same way.

Materials and Methods

Site Description and Trial Design

In order to detect the relationships between total shoot weights and shoot digestibility of three annual Lucerne species with the related traits, a 2-yreas field study was conducted at Shiraz, Fars Province of Iran during the 2010 and 2011 growing seasons. The experimental site was located at the Research Farm of College of Agriculture, Shiraz University (29°43´ N and 52°35´ W). The experiment was conducted as a split plot experiment based on randomized complete block (RCB) design with three replications (blocks). Three annual medics species consisted of *Medicago rigidula*, *Medicago polymorpha and Medicago scutellata* were planted under two sowing methods containing broadcast planting (BP) and row planting (RP). Descriptions of the soil characteristics and weather properties are given in Tables 5 and 6, respectively. In each of the three blocks, the three main plots were randomly assigned to two sowing methods: broadcast planting and row planting. Each the main plot was divided into three sub-plots and each sub-plot (Plots size were 6 × 8 m) were randomly assigned to one of three species. In two years, the viable scarified seeds were sown at 25 May and plants harvested at late of September. In RP, row spacing and plant space on row were 75 and 20 cm, respectively, with 3 cm sowing depth, also in BP, the seeds dispersed on soil surface handy and then covering with soil by a rake. Seed density in RP and BP were about 130 and 120 kg/ha, respectively. Hand weeding was conducted during growing season and plots were irrigated with a garden hose, with 10 days distances.

Crop production measurements

Total and shoot fresh weight (TFW and SFW respectively), shoot, root dry weight (SDW and RDW respectively), dry matter (DM), shoot to root weight ratio (S/R) and water content (WC*f*) were measured as quantitative traits. From each plot ten plants were selected randomly and all above- and below ground parts of the plants were harvested. The samples were dried at 70°C for 24 hours. Relative water content measured using the following formula.

$$WCf = \frac{SFW - SDW}{SFW}$$

Where WC*f* is water content based on fresh weight, SFW and SDW are shoot fresh and shoot dry weight.

Quality traits measurements

Quality traits were included organic matters (OM), crude protein (CP), extract ether (EE), crude fiber (CF), natural detergent fiber (NDF), ash, nitrogen free extract (NFE), calcium (Ca), phosphorous (P), digestible energy (DE) and metabolic energy (ME). The dried samples were analyzed for Lucerne quality by an NIR Systems 5000 scanning monochromatic (NIR Systems Inc., Silver Spring, MD, USA), applying the Near Infrared Reflectance Spectroscopy (NIRS) methodology (Shenk and Westerhaus, 1991; Pecetti et al., 2001).

Table 3. Stepwise regression selection method for indicating most important traits on the total fresh weight of annual medics species.

Pr	F	C(p)	Model R-Square	Partial R-Square	Number Removed	Variable Entered	Variable Step
<.0001	75.66	29.6602	0.690	0.690	-	Ash	1
0.0618	3.740	25.3859	0.7215	0.0315	-	Ca	2
0.0471	4.260	20.8743	0.7542	0.0327	-	SFW	3
0.0646	3.670	17.6971	0.7803	0.026	-	SDW	4
0.0582	3.880	14.6931	0.8054	0.0252	-	RDW	5
0.1179	2.60	13.5134	0.8214	0.016	-	WCf	6

SDW, Shoot dry weight; RDW, Root dry weight; WC*f*, Water content based on fresh weight; Ca, Calcium.

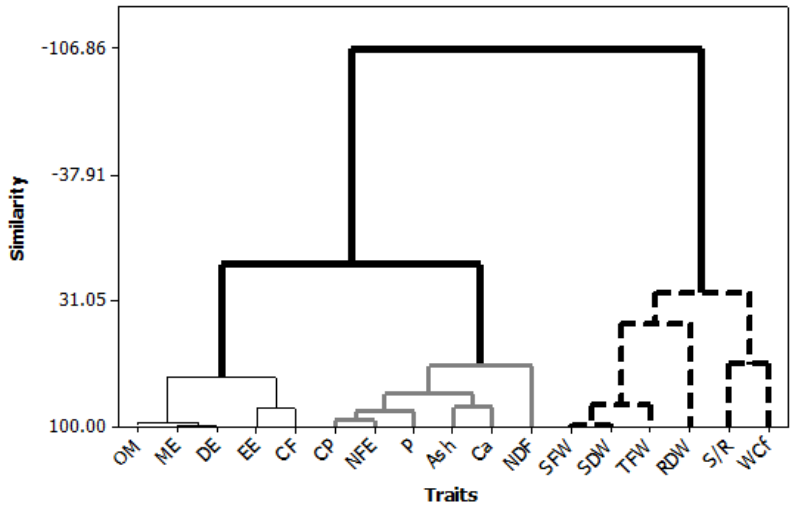

Fig 3. Dendrogram for cluster analyzing of quality and related traits of three annual medics species
Organic matters: OM, crude protein: CP, extract ether: EE, crude fiber: CF, natural detergent fiber: NDF, nitrogen free extract: NFE, calcium: Ca, phosphorous:P, digestible energy: DE, metabolic energy: ME , total and shoot fresh weight: TFW and SFW respectively, shoot and root dry weight: SDW and RDW respectively, dry matter: DM, shoot to root weight ratio: S/R and water content: WC*f*.

Table 4. Stepwise regression selection method for indicating most important traits on the annual medics species.

Pr	F	C(p)	Model R-Square	Partial R-Square	Number Removed	Variable Entered	Variable Step
<.0001	232.7	23479	0.8985	0.8985	-	SFW	1
<.0001	34.63	11415	0.9404	0.0419	-	Ca	2
<.0001	501.65	6832.5	0.9715	0.0327	-	Ash	3
<.0001	81.98	18786	0.9922	0.0217	-	RDW	4
<.0001	961.16	545.391	0.9978	0.0156	-	WCf	5
<.0001	322.54	-16.999	1	0.0022	-	SDW	6

SDW, Shoot dry weight; RDW, Root dry weight; WC*f*, Water content based on fresh weight; Ca, Calcium.

Table 5. Soil properties (0-30 cm) of the experimental site at Badjgah, Shiraz, Iran before plant sowing.

Year	pH†	Soil Texture	Sand	Silt	Clay	OC	Total N	P	K	EC
			Percentage (%)					mg kg^{-1}		dSm^{-1}
2010	7.1	Silty loam	17.5	61.2	21.3	0.83	0.09	15.5	470	0.54
2011	7.8	Silty loam	17.30	61.50	22.20	1.23	0.23	16.7	483	0.61

†- pH, Soil Acidity; OC, Organic Carbon; N, Nitrogen; P, Phosphorous; K, Potassium; EC, Electrical Conductivity.

Table 6. Weather characteristics of the experimental site at Badjgah, Shiraz, Iran during the study period.

Month	Temperature °C		Relative Humidity (%)		Precipitation (mm)	
	2010	2011	2010	2011	2010	2011
May	19.59	18.8	42.37	54.59	0	14
June	21.99	23.48	36.91	40.1	0	0
July	25.5	26.82	35.91	37.58	0	0
August	25.66	23.5	41.38	44.36	0	10
September	19.89	20.76	38.00	45.79	0	0

Statistical analysis

Four multivariate statistical technique such as principal component analysis (PC), clustering analysis, stepwise regression and canonical correlation were used for studying relationship between total yield and digestible energy of shoot in annual medics' species with measured traits. Principal component analysis (PC) is a variable reduction procedure and is useful when obtained data have large number of variables, and can be explained as a method that reduces data dimensionality by performing a covariance or correlation analysis between variables. Performing PC analysis helps researchers to use simple diagrams such as factors loading to explain relationships among each number of variables (Everitt and Dunn, 1992; Saed-Moucheshi et al., 2013). In canonical correlation (CC; multiple correlation), the data can be divided into two sets of variables, dependent and independent both consisting more than two variables, where its goal is to describe the relationships between these two sets of variables using some statistical equations (Bozickovic et al., 2013). Regression analysis is usually referred to one dependent variable and one or more independent variables. Stepwise regression technique is a method for selecting most important variable affecting dependent variable (Dong et al., 2008; Saed-Moucheshi et al., 2013). Cluster analysis or clustering is the task of assigning a set of objects into groups (called clusters) so that the objects in the same cluster are more similar (in some sense) to each other than to those in other clusters (Hakl et al., 2010). Clustering was performed using Minitab 14 while other techniques were fulfilled by SAS 9.2.

Conclusion

Principal component analysis and canonical correlation showed that qualified determining traits of medics especially organic matter (OM), crude protein (CP), extracted ether (EE), natural detergent fiber (NFE), and metabolic energy (ME) have the positive effect in contributing of digestible energy. Stepwise regression selected Ash, calcium (Ca), shoot fresh weight (SFW), shoot dry weight (SDW), root dry weight (RDW), and water content based on fresh weight (WCf) for digestible energy and SFW, Ca, Ash, RDW, WCf, and SDW for total fresh weight as variables having high effect on the related mentioned dependent variables. Dendrogran of cluster analysis showed that OM and ME are the most important factors affecting digestible energy while SDW and SFW had closest distance with total shoot weight. On the other hand, it can be concluded that ordinal regression analysis is not a very effective method for reviewing relationships among medics traits and multivariate statistical method are more reliable.

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