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A Comparison of Apparent Digestibility of Nutrient in Caspian Horse Feeds as Determined by Total Collection of Faeces, Acid Insoluble Ash and Lignin Methods

Research Article

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ABSTRACT

The aim of this research is to compare indigestible internal markers acid insoluble ash (AIA) and acid detergent lignin (ADL) and total collection of faeces (TCF) for apparent digestibility estimation in Caspian horse. Analyses were carried out on wheat straw, alfalfa, barley and maize, for dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF) and acid detergent fiber (ADF). Using change over design including 4 ration diet, 4 period and each period was 12 days with 4 Caspian horse with 3-5 years age and reared in individual cages. The results digestibility of DM, OM, NDF, ADF did not show any significant differences among the ADL, AIA and total collection of faeces methods, but digestibility of CP and EE were significantly higher than the ADL and AIA methods. A weak correlation was found between total collection techniques and AIA, for crude fat (EE) and ADF, whereas there was a high correlation (R^2=0.89) between AIA and total collection of faeces for the digestibility of crude protein. The results obtained in this study show that there were no significant difference between internal markers (acid-insoluble ash and acid detergent lignin) and total collection techniques of faeces for estimation apparent digestibility of nutrients in Caspian horses.

KEY WORDS ADL, AIA, Caspian horse, digestibility, internal markers.

INTRODUCTION

Caspian horse is ancient breed of small horse which has been rediscovered in the north of Iran, the Caspian Sea bank. A few of this horses are still at the farms and forests in the Northern provinces of Iran. This horse -also named shortsnout- is mainly used by the villagers as a means of transportation. Caspian horse has the features of a normal horse and like Arabian horse, comes in different sizes. Between the years 1965 and 1998, approximately 700 purebred Caspian horses were registered in Iran, Britain, Australia, New Zealand and the United States and only a few are in Japan, France, Brussels, Norway and Sweden (Firouz, 1972; Dalton, 1999). Digestibility in horses is affected by quality of forage. Horses act like ruminants in response to a decrease in forage quality (increase or decrease the amount of fiber, crude protein) and reduce feed intake (Dulphy *et al.* 1997). Research has shown that the ability of horses in forage consumption varies in different species (Martin-Rosset *et al.* 1996). Different method have been used to evaluate the digestibility coefficients. Total collection of faeces (TCF) is one of the most accurate direct procedures to determine apparent digestibility in horses (Schurg, 1981; Bergero *et al.* 2009). Although considered the most accurate, this procedure is labor-intensive and time-consuming for evaluating a wide range of feed sam-

ples and requires a large number of animals. In addition, animals should be confined in stalls, considering that being surrounded will be accepted by horses for a short period and training is necessary for horses fed with ration containing high amounts of energy (Frape *et al*. 1982). Other disadvantage of keeping the animal trapped includes the potential impact on animal metabolism (Bowers *et al*. 1993). These problems led to separation from the animal's normal behavior. Accurate measurement of food intake and fecal collection is a tough work (Sales and Janssens, 2003). Martin-Rosset *et al*. (1996) recommended a 14-day adaptation period after 6-days of collection of faeces, whereas the Kentucky Equine Research Center (USA) has collected the faeces over a period of 5 days after adaptation for 21 days (Pagan, 1998). Digestibility coefficients can be measured indirectly by markers. Various indigestible markers include polyethylene (Hertel *et al*. 1970), chromium oxide (Cr_2O_3) (Hintz *et al*. 1971), acid detergent lignin (ADL) (Varloud *et al*. 2004) and acid-insoluble ash (AIA) (Varloud *et al*. 2004) have been used to determine the digestibility of nutrients in different parts of gastrointestinal tract of horses. By the end of 19th century, lignin was considered as a potential internal marker for the estimation of digestibility (Kotb and Luckey, 1972). Because no specific enzymes exist for degradation of lignin in mammals or in anaerobic bacteria (Van Soest, 1956), different results have been reported by researchers when using ADL for the estimation of digestibility (Bergero *et al*. 2009; Miraglia *et al*. 1992; Varloud *et al*. 2004).

AIA is the most frequent internal marker to determine apparent digestibility coefficients in horses (Araujo *et al*. 2000a; Araujo *et al*. 2000b; Miraglia *et al*. 1992; Schurg, 1981) and sheep (Van Keulen and Young, 1977). Frape *et al*. (1982) reported that DM digestibility in horses and ponies obtained by AIA was overestimated. Block *et al*. (1981) reported that digestibility was underestimated when using AIA as a natural marker. However, using this method is difficult for grazing animals. When diet contains high forage, estimation of digestibility using total collection of faeces showed similar results. Huhtanen *et al*. (1994) found that AIA was the best method to predict the digestibility of DM. One of the advantages AIA compared to other markers is simplicity of analysis and it does not need special equipment. Bergero *et al*. (2009) found no difference between AIA in feed and faeces and digestibility of dry matter organic matter (OM) and DM in two methods. Santos *et al*. (2005) reported that when using lignin or 2 N-HCl AIA as internal marker, apparent digestibility coefficients of nutrients were similar.

The aim of the present study is to determine apparent digestibility of nutrients in Caspian horse by TCF, AIA and ALA methods.

MATERIALS AND METHODS

This study was conducted at Tehran Agricultural and Natural Resources Research Center-Khojeyr Station, Iran, Using change over design including 4 ration diets, 4 period and each periods was 12 days with 4 Caspian horse with 3-5 years age and reared in individual stalls. The 4 rations varied in nutritional quality were designated (including: diet 1: alfalfa, diet 2: alfalfa+wheat straw, diet 3: alfalfa+wheat straw+maize, diet 4: alfalfa+wheat straw+maize+barley, see Table 1 for chemical composition of ingredients of diets). The ration was formulated to meet the energy and protein requirement. Feed was offered in equal amounts at 08:00 and 16:00. The experiment (12 days) comprised a 6-day period for adaptation of the animals to the diets and experimental conditions followed by 6 days of collection of samples of faeces used for subsequent feed intake and apparent digestibility estimations. During the study, daily total faecal output and feed intake were recorded individually. The AIA content of the feed and the excreta were determined using the procedure of Van Keulen and Young (1977). The ADL was determined according to the method of Van Soest (1965).

Statistical analysis

The data were analyzed withthe PROC GLM of SAS (SAS, 2002). Data were analyzed using the model:

$$Y_{ijkl} = \mu + \tau_i + \beta_k + SUB\ (\beta)_{jk} + t_l + \varepsilon_{ijkl}$$

Where:

Y_{ijkl}: observation for animal i receiving diet j in period l.

μ: overall mean.

τ_i: effect of treatment.

β_k: effect of treatment order.

$SUB\ (\beta)_{jk}$: random effect.

t_l: effect of period.

ε_{ijkl}: residual effect.

Differences among means with ($P<0.05$) were accepted as representing statistically significant differences.

RESULTS AND DISCUSSION

Significant differences were observed in evaluation of apparent digestibility of DM, OM and crude fat (EE) among the three methods of TCF, ADL and AIA procedures in alfalfa diet ($P<0.05$; Table 2). However, the value of TCF was lower than the other two methods. crude protein (CP), ADF and NDF digestibility showed a significant decrease using TCF and ADL methods, compared to AIA techniques ($P<0.05$).

Table 1 Chemical composition ingredient of diets

Nutrient (%)	Alfalfa	Wheat straw	Barley	Maize
Dry matter (DM)	94.00	92.2	94.0	93.0
Organic matter (OM)	89.4	87.5	91.6	90.9
Crude protein (CP)	17.3	1.83	15.6	15.0
Ether extract (EE)	2.5	1.4	2.6	2.7
Acid detergent fiber (ADF)	35.0	50.5	28.0	32.2
Neutral detergent fiber (NDF)	51.0	73.0	40.5	46.2
Acid detergent lignin (ADL)	7.8	9.8	2.1	5.1
Acid-insoluble ash (AIA)	3.8	2.98	0.83	1.8

Table 2 Percentage of apparent digestibility of diets with different methods

Nutrient	TCF	AIA	ADL	SE
Diet 1: (alfalfa)				
Dry matter (DM)	53.83^{c}	82.27^{a}	63.99^{b}	2.9
Organic matter (OM)	53.76^{c}	82.23^{a}	63.97^{b}	2.8
Crude protein (CP)	67.89^{b}	84.94^{a}	64.69^{b}	3.42
Ether extract (EE)	63.17^{c}	68.75^{b}	75.51^{a}	3.70
Neutral detergent fiber (ADF)	37.31^{b}	76.71^{a}	41.18^{b}	4.04
Acid detergent fiber (NDF)	43.57^{b}	78.57^{a}	58.34^{b}	2.7
Diet 2: (alfalfa+ wheat straw)				
Dry matter (DM)	61.62^{a}	52.77^{b}	59.45^{a}	2.29
Organic matter (OM)	62.16^{a}	52.32^{b}	59.95^{a}	2.26
Crude protein (CP)	34.43^{c}	57.54^{b}	70.80^{a}	3.02
Ether extract (EE)	47.00^{a}	30.15^{b}	58.29^{a}	4.2
Neutral detergent fiber (ADF)	64.46^{a}	61.76^{b}	67.23^{a}	2.16
Acid detergent fiber (NDF)	67.15^{a}	62.98^{b}	67.99^{a}	1.61
Diet 3: (alfalfa+wheat straw+maize)				
Dry matter (DM)	76.15^{b}	78.68^{b}	85.57^{a}	2.56
Organic matter (OM)	80.71^{a}	79.08^{a}	74.32^{b}	2.14
Crude protein (CP)	86.50^{b}	88.25^{b}	94.14^{a}	1.19
Ether extract (EE)	70.04^{a}	72.57^{a}	49.12^{b}	4.88
Neutral detergent fiber (ADF)	71.54^{b}	73.87^{b}	79.27^{a}	2.56
Acid detergent fiber (NDF)	69.90^{a}	67.68^{a}	57.45^{b}	3.16
Diet 4: (alfalfa+wheat straw+maize+barley)				
Dry matter (DM)	77.67^{a}	58.84^{b}	87.33^{a}	3.39
Organic matter (OM)	73.48^{b}	55.65^{c}	89.31^{a}	2.17
Crude protein (CP)	84.100^{b}	90.37^{a}	87.06a^{b}	1.00
Ether extract (EE)	45.90^{b}	75.77^{a}	81.66^{a}	2.18
Neutral detergent fiber (NDF)	77.00a^{b}	63.17^{b}	78.59^{a}	3.31
Acid detergent fiber (ADF)	83.60^{b}	64.54^{c}	92.92^{a}	2.33

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
TCF: total collection of faeces; AIA: acid insoluble ash and ADL: acid detergent lignin.
SD: standard deviation.

Apparent digestibility coefficients of all parameters calculated using AIA were higher than TCF and ADL, except for crude protein. Nutrient digestibility with internal markers was lower in diet 2 (alfalfa+straw) than the alfalfa alone (diet 1).

But the digestibility value by TCF was more than the diet 2. The apparent digestibility coefficients of the nutrients in diet 3 and diet 4 with TCF were higher than diet 1 (Table 2).

The correlation between *in vitro* and *in vivo* methods for digestibility of CP, EE and ADF are shown in Figures 1 to 3. Weak correlations were derived between the use of AIA as marker and total collection of faeces for EE and ADF (Figure 2 and 3).

There was a high correlation (R^2=0.89) between AIA and total collection of faeces for the digestibility of crude protein (Figure 2).

No significant differences were observed for the digestibility of DM, OM, ADF and NDF among the ADL, AIA and total collection of faeces methods (P>0.05). However, the digestibility of CP and EE in the collection of faeces were significantly higher than the ADL and AIA methods (P<0.05; Table 3).

In this study, 4 rations offered to the animals because concentrate feed may cause digestive disturbances if given alone to animal, and their digestibility is often determined by giving them in combination with forage of known digestibility (McDonald *et al*. 2010).

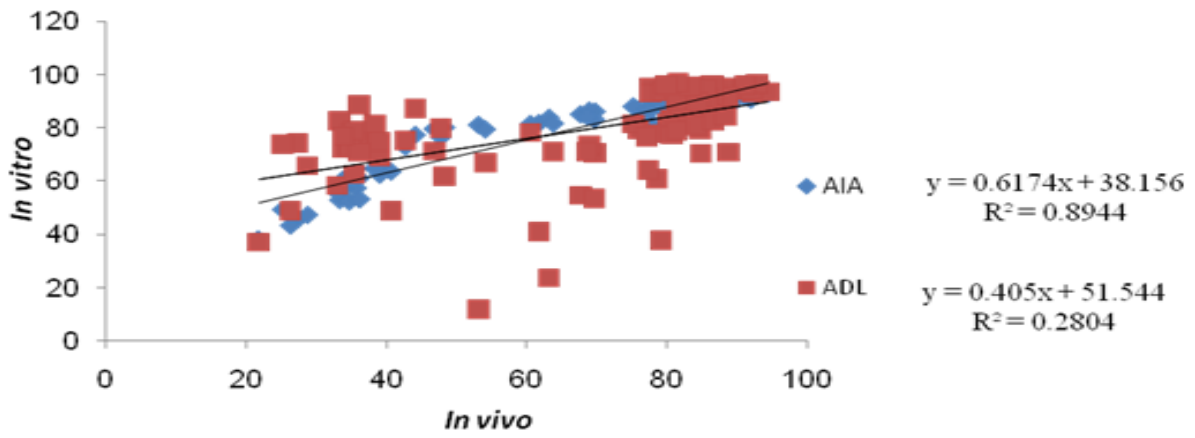

Figure 1 The correlation between *in vitro* and *in vivo* methods for digestibility of crude protein

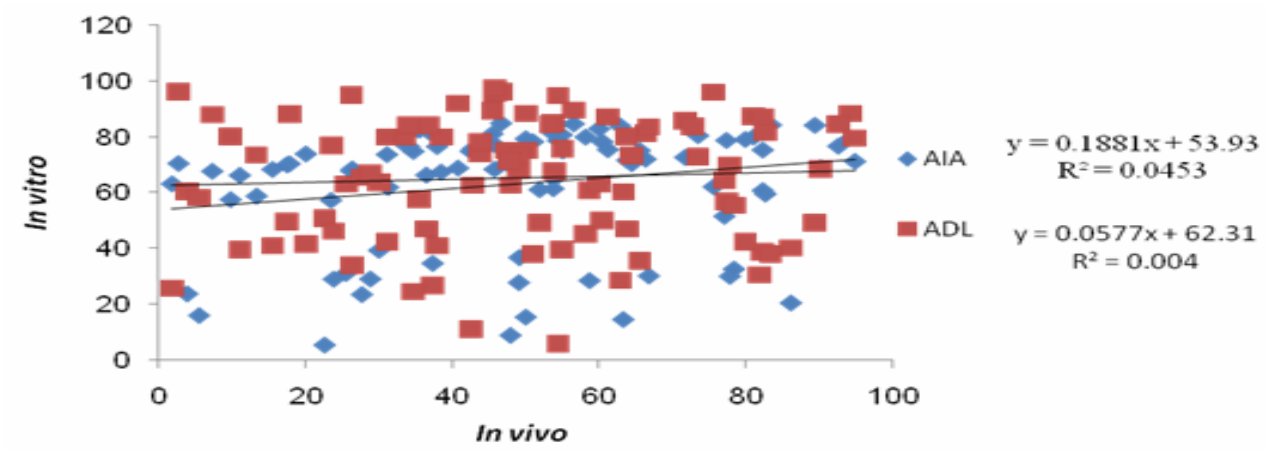

Figure 2 The correlation between *in vitro* and *in vivo* methods for digestibility of crude fat

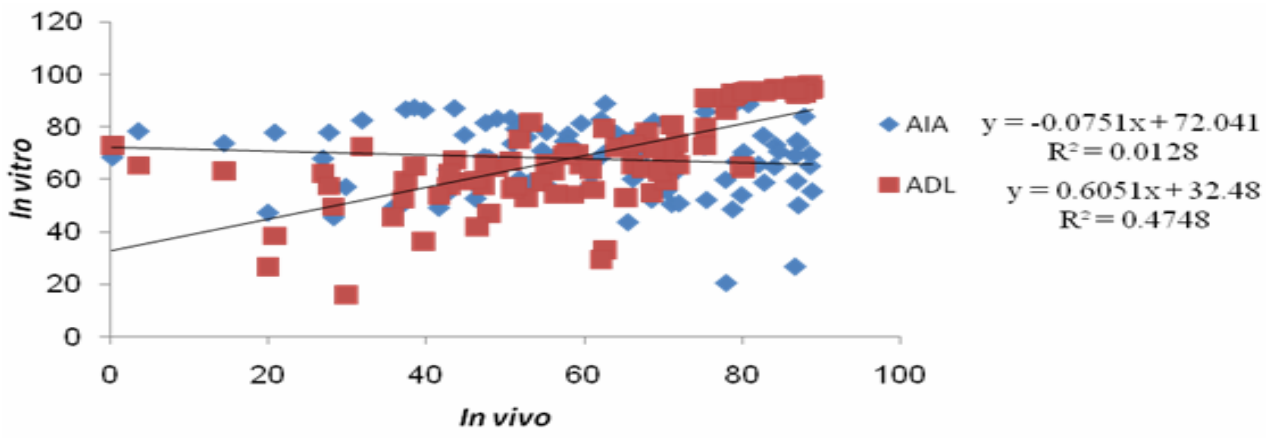

Figure 3 The correlation between *in vitro* and *in vivo* methods for digestibility of crude ADF

Correlation between the ADL and total collection of faeces confirmed the results reported by Miraglia *et al.* (1992), indicating low correlation between the ADL and total collection of faeces for estimation digestibility of nutrients.

They related the cause of this correlation to incomplete recovering of lignin from faeces.

Table 3 A comparison of apparent digestibility of nutrient as determined by total collection of faces, acid insoluble ash, lignin methods

Nutrient	TCF[a]	AIA[b]	ADL[c]
Dry matter (DM)	66.32[a]	67.77[a]	74.08[a]
Organic matter (OM)	64.03[a]	67.53[a]	71.89[a]
Crude protein (CP)	68.23[a]	80.28[b]	79.17[b]
Ether extract (EE)	51.94[a]	63.68[b]	64.40[b]
Neutral detergent fiber (NDF)	60.21[a]	67.30[a]	69.17[a]
Acid detergent fiber (ADF)	62.70[a]	70.10[a]	65.18[a]

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
TCF: total collection of faeces; AIA: acid insoluble ash and ADL: acid detergent lignin.

The ADL due improper recovering of manure cannot be a good marker for determinations of digestibility. This difference is due to partial digestion of lignin caused by bacteria, and herbal source of lignin cause incomplete recovery of lignin. But this effect has not been studied in horses (Goachet *et al.* 2009). AIA as internal marker is used in numerous studies. The important benefit of AIA is simple measurement and lack of need for expensive equipment (Bergero *et al.* 2009). The present study revealed that the highest correlations were obtained by the use of AIA as marker for determination of digestibility of crude protein (CP) relative to crude fat and ADF. In a number of experiments the positive correlations have been attributed to increased recovery of AIA in faeces. In the study of Bergero *et al.* (2009) on horses, no significant differences were observed between two methods of analysis for digestibility of DM and OM (P<0.05). Santos *et al.* (2005) reported no significant differences between two methods lignin and 2 N-HCl AIA as markers for estimation apparent digestibility coefficients of nutrients. Miraglia *et al.* (1992) reported high correlation between AIA and total collection of faeces for CP, ADF and EE, which were 0.84, 0.85 and 0.98, respectively. They concluded that AIA is suitable internal marker for estimation of digestibility in horses. However, the other researchers reported different DM digestibility (Cuddeford and Hughes, 1990; Miraglia *et al.* 1992; Parkins *et al.* 1982). This indicates a high correlation between AIA and TCF. The high correlation between two methods show that coefficients calculated for digestibility are accurate and reliable.

The period of collection of faeces affect the digestibility coefficients of nutrients. In this study a period of 6 days was considered for collection of faeces. Hintz and Loy (1966) did not found differences in DM or crude protein (CP) digestibility coefficients between the period of 3 and 7 days for faeces collection. Goachet *et al.* (2009) recommended 3 days of collection of faeces to determine apparent digestibility of DM, OM and fibre fractions. However, the period of 4 days of collection will be enough to study digestibility of crude fat (Hintz and Loy, 1966). In the horses fed with concentrate rations, gross energy (GE) and nitrogen digestibility coefficients when the faeces were collected 4 days (sample selection), were similar compared to Partial collection of faeces over 7 days when 4 N-HCl AIA was used as marker (Sutton *et al.* 1977). The estimation of digestibility coefficients of DM, OM and fibre with Rectal sampling of faeces for 3 days were 0.90 at 3 days and 0.86 at both 4 and 5 days by lignin as marker (Goachet *et al.* 2009). AIA is not a discrete chemical entity; however it has high the recovery in faeces (Ordakowski *et al.* 2001). High recovery of exogenous sources such as kaolin and saliva contamination by AIA, increase rate of recovery AIA in faeces.

CONCLUSION

The results obtained in this study show that no significant difference was observed between internal markers (AIA and ADL) and techniques of total collection of faeces for estimation of apparent digestibility of nutrients in Caspian horses. However, AIA has proven to be a suitable marker for determining the digestibility of Caspian horses diets compared to ADL. A positive correlation was observed between the TCF and AIA.

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