

## Effects of Feeding Frequency on Nutrient Digestibility and Feeding Behavior in the Turkmen Horse

E. Direkvandi<sup>1</sup>, Y. Rouzbehan<sup>1\*</sup>, and H. Fazaeli<sup>2</sup>

### ABSTRACT

In this experiment, sixteen Turkmen horses with average weights of  $430 \pm 46$  kg and age  $7 \pm 3$  years were used. Four feeding frequencies of 2, 4, 6, and 8 meals per day were used for a period of 28 days. Data were statistically analyzed based on a completely randomized design, and the results showed that increased feeding frequency led to more stable serum glucose levels throughout the day and an increase in the digestibility of nutrients ( $P < 0.05$ ). However, ether extract digestibility was not influenced by feeding frequency. Means of chewing and swallowing (per kg DM) of alfalfa were not influenced by feeding frequency. Also, the chewing and swallowing rates of concentrate for the treatment of 2 meals per day increased ( $P < 0.05$ ). By increasing the feeding frequency, alfalfa intake decreased, but duration of forage intake increased in the treatment of 8 meals per day ( $P < 0.05$ ). In contrast, by increasing the feeding frequency, concentrate intake was increased, but duration of concentrate intake was decreased in the treatment of 8 meals per day ( $P < 0.05$ ). In conclusion, feeding 2 times per day more often resulted in lower digestibility of different nutrients and less steady-state level of serum glucose than other feeding frequencies.

**Keywords:** Chewing and swallowing rates, Forage intake, Horse management, Serum glucose.

### INTRODUCTION

Modern horse management practices differ from those in the past and often include keeping horses in stalls and feeding them with limited amounts of forage and feeding large amounts of concentrate at limited number of times in the day. Such intensive management feeding practices present many factors which may negatively impact on the welfare of horses. Some of these factors are represented by the provision of meals twice a day which means that feed is consumed rapidly, and eaten while kept within a stall (Søndergaard *et al.*, 2004). Additionally, development of gastric ulcers is perhaps the worst consequence of infrequent feeding. Horses without access to feed for 12 hours

were found to have gastric pH as low as 2 and the development of gastric lesions was imminent (Pearson *et al.*, 2001). Moreover, the limited number of feeding times, e.g. when a large amount of feed is given once a day, can lead to increased rates of gastric bypass (Pearson *et al.*, 2001), which results in reduced feed digestibility (Warner, 1981). Dividing the feed into more meals during the day, which is closer to natural behavior of horses, leads to a decrease in the passage rate of digesta chymous (Pearson *et al.*, 2001), improved digestibility and a reduction in the risk of diseases such as colic (Potter *et al.*, 1992). One of the factors that can be improved by feeding management is chewing activity. Increasing chewing activity can improve the effects of

<sup>1</sup> Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

\* Corresponding author; e-mail: rozbeh\_y@modares.ac.ir

<sup>2</sup> Animal Science Research Institute, Karaj, Islamic Republic of Iran.



enzymatic and microbial digestion in herbivores (Mueller *et al.*, 1998). The chewing activity breaks the forage into smaller pieces (Grenet *et al.*, 1984), and also makes them wet by stimulating salivation. The horse, compared to the ruminant, has only one opportunity to chew its food (McDonald *et al.*, 2011), but the chewing rate in the horse is higher than that of ruminants (Rosenfeld *et al.*, 2006). Hence, finding the right feeding frequency to control physiological, digestive, and behavioral issues is crucial for the health of horses (Clauss *et al.*, 2009). Therefore, the aim of this trial was to assess the effect of increasing the feeding frequency, up to 8 times per day, on the feeding behavior (i.e., chewing and swallowing), nutrient digestibility, and daily serum glucose changes of Turkmen horses.

## MATERIALS AND METHODS

The experiment was performed at Tarbiat Modares University (Tehran, Iran) with cooperation from the Zoljanah Equestrian Club.

### Animals and Diets

Sixteen Turkmen horses (6 stallions and 10 mares) with an average age of  $7 \pm 3$  years were used. Before starting the experiment, all horses were treated against both external and internal parasites using ivermectin (Pandex injectable solution–Biovet, Pestera, Bulgaria), containing 10 mg ivermectin per mL preparation. The preparation was applied subcutaneously in the region of the neck at a dose of  $0.2 \text{ mg kg}^{-1}$  body weight.

The horses (average estimated weights of  $430 \pm 46 \text{ kg}$ ) were placed in individual stalls with dimensions of  $4 \times 5 \text{ m}$  with a straw and sawdust bedding. During the experiment, water and salt/mineral licks were freely available at all times. The weight of horses (BW) was calculated according to Hall (1971), as follows:

$$\text{BW (kg)} = \left( \frac{(\text{Chest width})^2 \times (\text{Body length})}{11880} \right) \quad (1)$$

In this experiment, the horses were randomly assigned to each of four treatments (2, 4, 6, and 8 meals per day) and fed individually for 21 days adaptation period, followed by seven days for the experiment. During the adaptation period, the horses were exercised by longeing twice a day (morning and evening for 20 minutes at each occasion). The four feeding frequencies were used for a total period of 28 days (Table 1). The diets were formulated according to NRC (2007) and are shown in Table 2. The diets were based on 70% alfalfa and 30% concentrate, and on average, the quantity (per kg DM) of alfalfa and concentrate was equivalent to 1.5-2.0 and 0.75-0.85% of body weight, respectively).

### Behavioral Observations of the Horses

Chewing and swallowing rates in horses were observed and recorded by two observers who also recorded the duration of alfalfa hay and concentrate intake per meal at all meals for each horse. The chewing rate (number of chews  $\text{min}^{-1}$ ) was measured for alfalfa and concentrate. Alfalfa chewing rates recorded at 4 times

**Table 1.** Treatments and number of meals per day (for both alfalfa hay and concentrate).

Treatment	Explanation
2 times per day	Feeding every 12 hours (07:00 and 19:00)
4 times per day	Feeding every 6 hours (07:00, 13:00, 19:00 and 01:00)
6 times per day	Feeding every 4 hours (07:00, 11:00, 15:00, 19:00, 23:00 and 03:00)
8 times per day	Feeding every 3 hours (07:00, 10:00, 13:00, 16:00, 19:00, 22:00, 01:00 and 04:00)

**Table 2.** Ingredients, chemical composition (g kg<sup>-1</sup> DM) and digestible energy (DE MJ kg<sup>-1</sup> DM) used in the experimental diets.

Ingredients concentrate feed (g kg <sup>-1</sup> DM)		
Alfalfa	462	
Wheat straw	238	
Micronized wheat	24	
Micronized barley and Steam flake	108	
Extruded corn	30	
Roasted soybeans	15	
Rice bran	15	
Beet pulp	15	
Pelleted concentrate <sup>a</sup>	54	
Oats	24	
Vegetable oil mixture <sup>b</sup>	9	
Vitamin and mineral supplements <sup>c</sup>	6	
Chemical composition (g kg <sup>-1</sup> DM)		
Dry matter	Concentrate	Alfalfa hay
Organic matter	985	939
Ash	949	833
Crude protein	36	106
Ether extract	127	110
NDFom	88	17.7
ADFom	250	622
Lignin	77	449
Digestible energy (MJ kg <sup>-1</sup> DM)	19.2	104
	14.5	7.76

<sup>a</sup> A mixture of soybean; canola, and sunflower meal; <sup>b</sup> A mixture of soybean; sunflower, and canola oil, <sup>c</sup> 14,000 IU vitamin A; 455 IU vitamin E; 1,500 IU vitamin D; 15 mg vitamin B<sub>1</sub>; 12 mg vitamin B<sub>2</sub>; 0.3 mg vitamin Biotin; 2.5 gr Ca, 1.1 gr P; 5 gr Mg; 28 gr K; 8.5 gr Cl, and 4.4 gr Na per kg feed.

per one minute each time (total 4 minutes), and concentrate chewing rates were recorded at 2 separate times for one minute each time (total 2 minutes). An example of how chewing rate in horses was measured is shown schematically in Figure 1, which represents the measurement of chewing rates of alfalfa hay when horses were fed 2 times per day (*i.e.*, measuring one horse four times within one meal in 4 consecutive minutes). In other words, the horses were fed and chewing rate was measured for 1 minute, then the horses were let to eat a little bit longer and measured again (measure no. 2), then the horses were let to eat longer and the measurement no. 3 was taken, and so on. The swallowing rate also was observed per minute. In addition, the

chewing and swallowing rates were calculated per kg DM and NDF.

### Digestibility of Nutrients

Fresh fecal grab samples were collected from the rectums of all horses three times per day post-feeding on days 1–7 of the experimental period. Fecal samples were transferred to aluminum pans and dried at 50°C in a forced-air oven for 48 hours. Fecal samples were then ground to pass a 1-mm Wiley mill screen, and a single composite sample was prepared for each horse by mixing equal amounts (on DM basis) from the three samples. Fecal samples were analyzed for content of DM, OM, EE, ash-free Neutral Detergent Fiber (NDFom), ash-



free Acid Detergent Fiber (ADFom), and total N. Lignin content of feed and feces was used as an internal marker to determine apparent total tract nutrient digestibility coefficients. Digestibility Coefficient of Nutrient (DCN) was calculated using the equation (2) (Church, 1993):

$$\text{DCN (\%)} = 100 - \left[ 100 \times \left( \frac{\% \text{ M D}}{\% \text{ M f}} \right) \times \left( \frac{\% \text{ NF}}{\% \text{ ND}} \right) \right] \quad (2)$$

Where MD is Marker in Diet, MF is Marker in feces, NF is Nutrient in feces and ND is Nutrient in Diet.

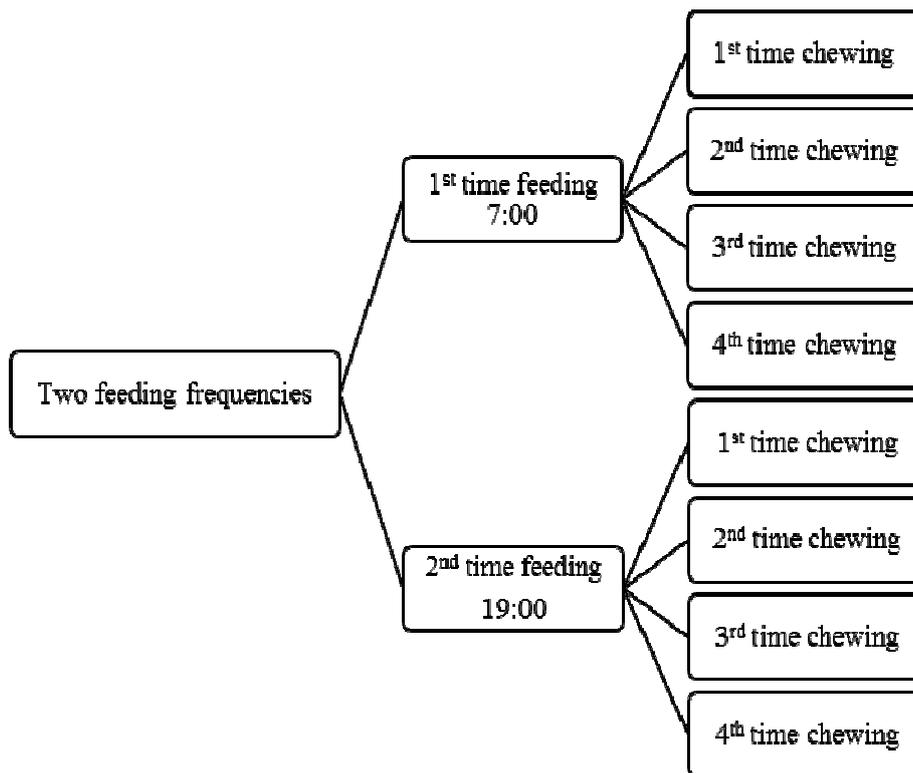
Digestible energy of the diet was calculated as follows (NRC, 2007):

$$\text{DE (Mcal kg}^{-1} \text{ DM)} = 2.118 + (0.01218 \times \text{CP}) - (0.00937 \times \text{ADF}) - [0.00383 \times (\text{NDF} - \text{ADF})] + (0.04718 \times \text{EE}) +$$

$$(0.02035 \times \text{NSC}) - (0.0263 \times \text{ASH}) \quad (3)$$

### Laboratory Analysis

Before the chemical analysis, feed and feces samples were oven-dried at 55°C for 48 hours and then were passed through a 1 mm sieve (Wiley mill, Swedesboro, USA). Content of Dry Matter (DM), ash, Organic Matter (OM), Crude Protein (CP) and Ether Extract (EE) were analyzed according to the methods of AOAC (1990). Ash-free Neutral Detergent Fiber (NDFom) and ash-free Acid Detergent Fiber (ADFom) were analyzed using the method proposed by Van Soest *et al.* (1991). Lignin was determined by solubilization of cellulose with sulfuric acid (Robertson and Van Soest, 1981).



**Figure 1.** Schematic representation of the measurement of chewing rate of alfalfa when horses were fed 2 times per day. The chewing rate (number of chews  $\text{min}^{-1}$ ) was measured for alfalfa hay concentrate. Alfalfa hay chewing rates were recorded 4 separate times for one minute each time and the concentrate chewing rates were recorded at 2 separate times for one minute each time.

### Serum Glucose

On day 27 of the trial, blood samples were taken from the jugular vein into vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) containing no anticoagulant. Blood samples were drawn 13 times throughout the day (06:30, 07:30, 09:30, 11:30, 13:30, 15:30, 17:30, 19:30, 21:30, 23:30, 01:30, 03:30 and 05:30). The blood sample was left to coagulate for a minimum of 30 minutes and was then centrifuged (Hitachi, CT15RE, Koki, Japan) (10 minutes at 3,000 rpm) within one hour of sampling. Serum glucose concentration was measured immediately after centrifuging using a rapid test kit (Bayer, Zurich, Switzerland).

### Statistical Analysis

The data obtained from assessing nutrient digestibility and feeding behavior of horses were analyzed as a randomized complete design using General Linear Models (GLM) procedure in SAS software (SAS Institute, 2008), which is based on the following statistical model:

$$Y_{ij} = \mu + T_i + e_{ij} \quad (4)$$

Where,  $Y_{ij}$  is observation (nutrient digestibility, feeding behavior and serum glucose level),  $\mu$  is the general mean,  $T_i$  is the effect of feeding frequency and  $e_{ij}$  is the standard error term. Furthermore, a polynomial contrast was used to test the linear or quadratic effects of feeding

frequency on the measured traits.

## RESULTS

### Digestibility of Nutrients

Nutrient digestibilities are shown in Table 3. Feeding 2 times per day more often resulted in lower digestibility for dry matter, organic matter, crude protein, NDFom, ADFom and estimated DE than the other feeding frequencies. However, digestibility coefficients of ether extract was not influenced by feeding frequency.

### Feeding Behavior

#### Alfalfa Hay Intake Behavior

Table 4 shows the alfalfa consumption behavior. Number of chews per kg DM and NDF increased when 6 meals per day was fed compared to 2 or 4 times day<sup>-1</sup> ( $P < 0.05$ ). There was no difference between the different feeding frequencies in the number of chews at the fourth time of measurement or in the average number of chews per minute. When feeding 8 meals per day, the number of swallows per kg DM and kg NDF increased by increasing feeding frequency ( $P < 0.05$ ). However, the mean of the swallowing rate of alfalfa was not affected by feeding frequency. Also, by increasing the feeding frequency, alfalfa hay intake rate

**Table 3.** Effect of feeding frequency on nutrient digestibility (g kg<sup>-1</sup> DM).

	Feeding frequency per day				SEM <sup>a</sup>	P value	Contrast	
	×2	×4	×6	×8			L <sup>b</sup>	Q <sup>c</sup>
Dry matter	426 <sup>b</sup>	476 <sup>a</sup>	480 <sup>a</sup>	501 <sup>a</sup>	7.4	0.001	0.001	0.06
Organic matter	479 <sup>b</sup>	485 <sup>a</sup>	491 <sup>a</sup>	497 <sup>a</sup>	2.6	0.01	0.004	0.93
Crude protein	476 <sup>b</sup>	501 <sup>a</sup>	535 <sup>a</sup>	518 <sup>a</sup>	11.4	0.018	0.09	0.09
Ether extract	421	415	427	422	7.4	0.03	0.31	0.19
NDFom	228 <sup>b</sup>	276 <sup>a</sup>	270 <sup>a</sup>	278 <sup>a</sup>	4.5	0.001	0.01	0.05
ADFom	204 <sup>b</sup>	247 <sup>a</sup>	266 <sup>a</sup>	271 <sup>a</sup>	10.9	0.004	0.001	0.10
DE (MJ kg <sup>-1</sup> DM)	6.71 <sup>c</sup>	7.38 <sup>b</sup>	7.38 <sup>b</sup>	7.89 <sup>a</sup>	0.27	0.03	0.01	0.80

<sup>a</sup> Standard Error of Means; <sup>b</sup> Linear effect of feeding frequency, <sup>c</sup> Quadratic effect of feeding frequency.

**Table 4.** Effect of feeding frequency on chewing and swallowing behavior (alfalfa hay).

	Feeding frequency per day				SEM <sup>a</sup>	P value	Contrast	
	×2	×4	×6	×8			L <sup>b</sup>	Q <sup>c</sup>
Chewing rate (chews min <sup>-1</sup> )								
First time	86.3 <sup>ab</sup>	84.1 <sup>b</sup>	85.6 <sup>ab</sup>	88.4 <sup>a</sup>	1.41	0.03	0.22	0.04
Second time	84.6 <sup>ab</sup>	83.8 <sup>b</sup>	86.7 <sup>ab</sup>	89.2 <sup>a</sup>	1.55	0.01	0.04	0.10
Third time	79.8 <sup>c</sup>	83.2 <sup>b</sup>	86.1 <sup>ab</sup>	88.8 <sup>a</sup>	1.41	0.01	0.01	0.40
Fourth time	84.5	85.1	85.9	88.7	1.53	0.07	0.07	0.31
Chewing mean	83.8	84.1	86.1	87.8	2.03	0.37	0.09	0.87
No of chews kg <sup>-1</sup> DM	1972 <sup>b</sup>	1989 <sup>b</sup>	2449 <sup>a</sup>	2548 <sup>a</sup>	51.0	0.01	0.01	0.43
No of chews kg <sup>-1</sup> NDF	3180 <sup>b</sup>	3203 <sup>b</sup>	3943 <sup>a</sup>	4103 <sup>a</sup>	43.5	0.01	0.01	0.13
Swallowing rate (swallow min <sup>-1</sup> )								
Mean swallowing rate	1.62	1.63	1.67	1.72	0.04	0.33	0.08	0.65
No of swallows kg <sup>-1</sup> DM	38.3 <sup>c</sup>	38.2 <sup>c</sup>	42.9 <sup>b</sup>	49.4 <sup>a</sup>	0.98	0.01	0.01	0.02
No of swallows kg <sup>-1</sup> NDF	61.8 <sup>b</sup>	61.5 <sup>b</sup>	76.6 <sup>a</sup>	79.7 <sup>a</sup>	1.07	0.01	0.01	0.14
Alfalfa hay intake rate (g min <sup>-1</sup> )	45.1 <sup>a</sup>	41.9 <sup>ab</sup>	36.3 <sup>bc</sup>	35.4 <sup>c</sup>	1.99	0.04	0.01	0.58
Total intake time for all meals (min)	164 <sup>b</sup>	163 <sup>b</sup>	198 <sup>a</sup>	201 <sup>a</sup>	0.76	0.03	0.08	0.73

<sup>a</sup> Standard Error of Means; <sup>b</sup> Linear effect of feeding frequency, <sup>c</sup> Quadratic effect of feeding frequency.

Means with different superscripts in the same column are different (P<0.05).

decreased in g/min, but in contrast, total intake time (minutes day<sup>-1</sup>) for forage was higher for 6 and 8 feeding times compared to 2 and 4 times (P< 0.05).

### Concentrate Intake Behavior

For concentrate intake, chewing and swallowing means and total intake time did not show any clear pattern with increased

feeding times (Table 5).

### Serum Glucose

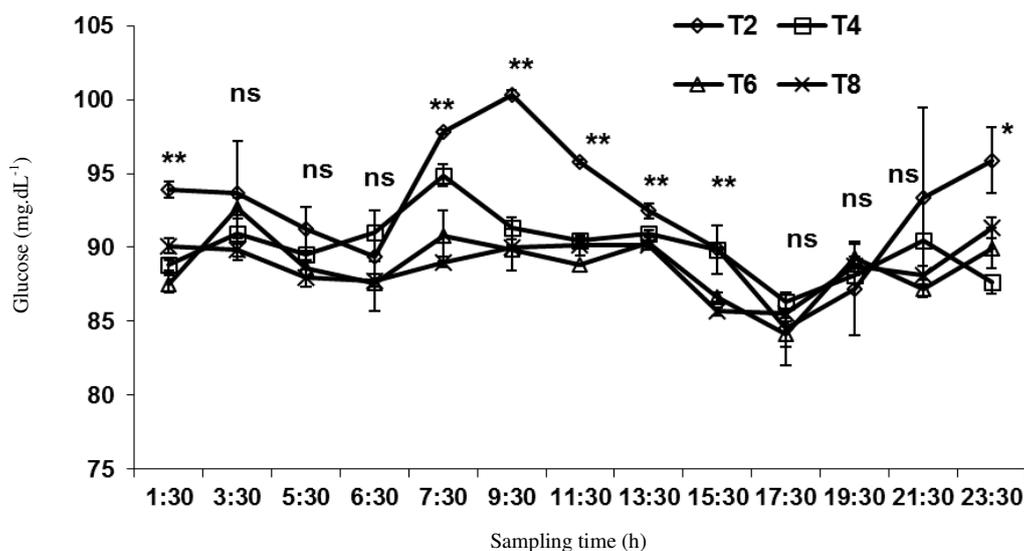
Figure 2 shows the effect of increasing feeding frequency on the serum glucose changes over 24 hours. As the feeding frequency increased, the fluctuation of serum glucose level decreased.

**Table 5.** Effect of feeding frequency on chewing and swallowing behavior (concentrate).<sup>a</sup>

	Feeding frequency per day				SEM <sup>a</sup>	P-value	Contrast	
	×2	×4	×6	×8			L <sup>b</sup>	Q <sup>c</sup>
Chewing rate (chew min <sup>-1</sup> )								
First time	82.3 <sup>a</sup>	79.8 <sup>b</sup>	77.7 <sup>b</sup>	76.9 <sup>b</sup>	1.57	0.01	0.01	0.01
Second time	83.2 <sup>a</sup>	79.1 <sup>b</sup>	76.4 <sup>b</sup>	77.5 <sup>b</sup>	1.55	0.03	0.08	0.01
Mean chewing rate	82.7 <sup>a</sup>	79.5 <sup>a</sup>	77.1 <sup>b</sup>	77.2 <sup>b</sup>	0.88	0.01	0.01	0.31
No of Chewing kg <sup>-1</sup> DM	629 <sup>a</sup>	609 <sup>a</sup>	564 <sup>b</sup>	588 <sup>ab</sup>	14.2	0.02	0.01	0.13
Swallowing rate (swallow min <sup>-1</sup> )								
Mean swallowing rate	1.60 <sup>a</sup>	1.55 <sup>a</sup>	1.49 <sup>b</sup>	1.51 <sup>b</sup>	0.02	0.03	0.01	0.11
No of Swallowing kg <sup>-1</sup> DM	12.2	11.8	10.9	11.6	0.56	0.46	0.32	0.33
Concentrates intake (g min <sup>-1</sup> )	133	137	140	146	5.68	0.47	0.78	0.20
Total intake time for all meals (min)	20.2 <sup>a</sup>	20.0 <sup>a</sup>	19.8 <sup>a</sup>	18.7 <sup>b</sup>	0.17	0.01	0.01	0.11

<sup>a</sup> Standard Error of Means; <sup>b</sup> Linear effect of feeding frequency, <sup>c</sup> Quadratic effect of feeding frequency.

Means with different superscripts in the same column are different (P<0.05).



**Figure 2.** Serum glucose changes during the 24 hours. Diamond represents 2 meals per day, square is 4 meals per day, triangle is 6 meals per day, and crosshairs is 8 meals per day. *T* is effect of feeding frequency; *Ns* means no statistical difference ( $P > 0.05$ ), \* and \*\* means statistically significant difference at  $P > 0.05$  and at  $P > 0.01$ , respectively.

## DISCUSSION

### Digestibility of Nutrients

Apart from ether extract digestibility, the results of this experiment showed that by increasing feeding frequency above 2 times  $\text{day}^{-1}$ , digestibility of nutrients increased ( $P < 0.05$ ). In contrast to our results, previous literature such as Houtp *et al.* (1988), Clarke *et al.* (1990), Jansson *et al.* (2006) and van Weyenberg *et al.* (2007) reported that nutrient digestibility in ponies and horses was not influenced by the feeding frequency. In our experiment, the horses were fed throughout the day and night so there was no feed deprivation. In other experiments involving increased feeding frequency, the horses were fed in 12 hour day periods and there were feed deprivation during the night (Van Weyenberg *et al.*, 2007; Houtp *et al.*, 1988; Gill and Lawrence, 1998). Horses fed a limited number of meals (1-2) over a 12

hour day period spent more time feeding at each meal (Van Weyenberg *et al.*, 2007). Also, in most studies, maintenance diets have been used. In such circumstances, nutrient digestibility is not influenced by feeding frequency (Jansson *et al.*, 2006). The greatest impact of increasing feeding frequency was observed on digestibility of NDFom and ADFom (Table 3). In order to avoid overload of the small intestine, it would from a theoretical point of view be advantageous to feed small meals frequently. That is due to the limited capacity of the small intestine to digest starch and fat. Therefore, frequent small meals, such as when more than 2 meals  $\text{day}^{-1}$  were offered, may improve the efficiency of digestive capability of the small intestine, the result of which is less amount of feed in the hindgut to be fermented by the microbial population (Jansson *et al.*, 2006). In contrast, the quicker the digesta moves through the small intestine, the less time the enzymes in the small intestine have to act and, therefore, leading to lower the small intestinal digestibility. This may also mean that too much undigested feed, particularly



starch, may enter the hindgut and be fermented by the microbial population (Frape, 2004). The risk of fermentation of starch in the large intestine is acidosis by the formation of lactic acid (Frape, 2004).

The use of lignin as an internal marker to predict digestibility in horses has received criticism from the scientific community regarding its efficacy for that task, given the low feces recovery rate it provides, which is around 80% (Araujo *et al.*, 2000; Bergero *et al.*, 2004). In the current experiment, the recovery rate of lignin in feces was 91%. On the other hand, fecal recovery is reported to be more reliable when Acid Insoluble Ash (AIA) was used as an internal marker (Sales, 2012; De Marco *et al.*, 2012). However, digestibility coefficients determined in competitively trained Arab horses, employing naturally occurring markers such as lignin and AIA, turned out to be overestimated in comparison with the total fecal collection when diets consisted of forage and concentrates (Goachet *et al.*, 2009). For these reasons, some researchers recommend that in experiments which are used to provide reliable data serving as models in widely available tables of nutritional values and feeding standards, the classical balance method should be applied (Bergero *et al.*, 2004).

## Feeding Behavior

### Alfalfa Hay Intake Behavior

The increase in the alfalfa hay intake rate when feeding 2 meals per day treatment (Table 4) was due to the tendency to increase hay intake at the beginning of the provision of hay and the higher quantity of hay provided at each meal. When feeding 8 meals per day in which feeding was done every 3 hours, at the time of providing alfalfa, the horses were observed to be calmer than those in other treatments. As anticipated, the horses fed 2 meals per day on average spent 82.4 min meal<sup>-1</sup> for hay

intake, while the average duration of hay intake per meals for treatments of 4, 6 and 8 meals per day were 39.1, 33 and 25.2 min meal<sup>-1</sup>, respectively. In contrast to the results of Willard *et al.* (1977), in our experiment, horses spent less time when fed 2, 4, 6 and 8 meals per day (*i.e.*, respectively 11.4, 11.3, 13.7 and 13.9 percent of their time) in the stalls with hay intake. Also, the intake rate (kg DM per minute) was 24.9, 24.8, 30.1 and 30.6 minutes for treatments of 2, 4, 6, and 8 meals per day, respectively. That was lower than the results of Brussow *et al.* (2005) which were on average 45 minutes per kg DM. Increasing feeding frequency led to an increase in chewing activity. The number of chews in the horse has been reported to be between 72-93 chews per minute for long forage (Frape, 2004). Also, it was reported that the chewing rate was 91 chews per minute for fresh forage (Gross *et al.*, 1993). The results of feeding either 2, 4, 6, or 8 meals per day in our experiment was close to this result. In another experiment, the reported number of chews per kg DM for chopped forage was from 2,368 to 2,441 (Müller, 2009) which was agreement with the results of our experiment. Other research (Ellis, 2003) noted that the number of chewing per kg dry matter obtained was in the range of 3,000 to 3,500, which was higher than our results and the results reported by Müller (2009). However, chewing activity was influenced by body size, animal species, feeding levels, physiological state, amount of fiber in the diet, physical form of feed, and feed particle size (Mueller *et al.*, 1998).

### Concentrate Intake Behavior

There was an increase in the number of chews per min when feeding 2 meals per day compared to the other treatments, probably due to the larger quantity of concentrate (1.350 kg) per meal. In the other treatments, as expected, the horses tended to consume less concentrate per meal in less time. When feeding 8 meals per day, the

duration of concentrate intake were reduced, but the amount of concentrate intake did not differ among treatments (Table 5). However, means of chewing and swallowing rate were reduced when feeding 6 or 8 meals per day compared to those offered 2 or 4 meals per day. Although variation in the mean chewing rate between 2 and 4 meals versus 6 and 8 meals per day existed, the differences were small. In another experiment, the chewing activity of concentrate was between 800 and 1,200 chews per kg DM (Ellis, 2003), which was higher than the results of our experiment. Brøkner *et al.* (2008) reported chewing rate for whole oats, ground oats, muesli feed, and feed pellets to be 95, 95, 90 and 96 chews per minute, respectively, which is higher than the results from our experiment. In another work, Brøkner *et al.* (2006) reported the chewing rates for oats, barley, and wheat to be 92.4, 91.8 and 92.4 chews per minute, respectively, which is also higher than the results of our experiment. The discrepancy between our results and others may be due to the type and processing method of the concentrates used in ration of horses (Brøkner *et al.*, 2006). Moreover, other workers concluded that the method used to estimate the chewing rate affect the estimation of chewing rate in horses (Shingu *et al.*, 2001; Ellis *et al.*, 2005; Brøkner *et al.*, 2008).

### Serum Glucose

Blood concentrations of glucose in all the horses were within the typical ranges (75-115 mg dL<sup>-1</sup>) previously reported for horses (Radostits *et al.*, 2007). With increasing feeding frequency, a more steady-state level of serum glucose (i.e., homeostasis) was observed ensuring a stable blood glucose supply for elongated periods (Youket *et al.*, 1985). This state is likely to improve the wellbeing and activity performance of a horse kept in a stall, and is comparable to the more natural condition where horses evolved to eat small and frequent meals

throughout the day and spend 16-18 hours per day grazing (Youket *et al.*, 1985).

Development of gastric ulcers is perhaps the worst consequence of infrequent feedings during the day (Pearson *et al.*, 2001). Horses without access to feed for 12 hours were found to have gastric pH as low as 2 which may lead to the development of stomach ulcer (Pearson *et al.*, 2001). Wickens and Heleski (2010) reported that stereotypic behaviors like wood-chewing and cribbing may be responses to abnormal acidity in the stomach, although other reasons are also present *i.e.*, equine stereotypies are connected to poor welfare of horse.

In conclusion, feeding 2 times per day more often resulted in lower digestibility for different nutrients and less steady-state level of serum glucose than other feeding frequencies. In regard to the welfare of the animal, horses that are fed less often experience more stress partly from being fasted for an extended period.

### ACKNOWLEDGEMENTS

The authors would like to thank Mr. Gary Easton for his English language correction of the manuscript. Also, I would like to thank Dr. S. Moharrampour (Tarbiat Modares University) for his statistical assistance.

### REFERENCES

1. AOAC. 1990. *Official Method of Analysis*. 15<sup>th</sup> Edition, Association of Official Analytical Chemists, Washington, DC.
2. Araujo, K. V., Lima, J. A. F. and Fialho, E. T. 2000. Comparação Entre os Indicadores Internos e o Método de Coleta Total de Fezes na Determinação da Digestibilidade dos Nutrientes de Alimentos Volumosos, em Equinos. *Revista da Sociedade Brasileira de Zootecnia*, **29**: 745-751.
3. Bergero, D., Meineri, G., Miraglia, N. and Peiretti, G. 2004. Apparent Digestibility of Hays in Horses Determined by Total



- Collection of Feces and Using Internal Marker Methods. *J. Food Agric. Environ.* **3**: 198–201.
4. Brøkner, C., Norgaard, P. and Hansen, H. H. 2008. Effect of Feed Type and Essential Oil Product on Equine Chewing Activity. *J. Anim. Physiol. Anim. Nutr.*, **92**: 621-630.
  5. Brøkner, C., Norgaard, P. and Soland, T. M. 2006. The Effect of Grain Type and Processing on Equine Chewing Time. *Pferdeheilkunde.*, **22**: 453-460.
  6. Brussow, N., Voigt, K., Vervuert, I., Hollands, T., Cuddeford, D. and Coenen, M. 2005. The Effect of the Order of Feeding Oats and Chopped Alfalfa to Horses on the Rate of Intake and Chewing Activity. *Pferdeheilkunde.*, **21**: 37-8.
  7. Church, D. C. 1993. *The Ruminant Animal: Digestive Physiology and Nutrition*. Waveland Press, Illinois.
  8. Clarke, L. L., Argenzio, R. A. and Roberts, M. C. 1990. Effect of Meal Feeding on Plasma Volume and Urinary Electrolyte Clearance in Ponies. *Anim. J. Vet. Res.*, **51**: 571-576.
  9. Clauss, M., Nunn, C., Fritz, J. and Hummel, J. 2009. Evidence for a Tradeoff between Retention Time and Chewing Efficiency in Large Mammalian Herbivore. *Comp. Biochem. Physiol. A. Mol. Integr. Physiol.*, **154**: 376-382.
  10. Crandell, K. 2012. *Why Is Feeding Frequency Important in Horse Management?*. Equineews, Kentucky Equine Research.
  11. De Marco, M., Miraglia, N., Peiretti, P. G. and Bergero, D. 2012. Apparent Digestibility of Wheat Bran and Extruded Flax in Horses Determined from the Total Collection of Feces and Acid-insoluble Ash as an Internal Marker. *Anim.*, **6**: 227-231.
  12. Ellis, A. N. D. 2003. Ingestive and Digestive Processes in the Horse. PhD. Thesis, Writtle College, Essex University, UK.
  13. Ellis, A. D., Thomas, S., Arkell, K. and Harris, P. 2005. Adding Chopped Straw to Concentrate feed: The Effect of Inclusion Rate and Particle Length on Intake Behavior of Horses. *Pferdeheilkunde* **21**: 35–37.
  14. Frappe, D. 2004. *Equine Nutrition and Feeding*. Oxford, United Kingdom.
  15. Gill, A. M. and Lawrence, L. M. 1998. Effect of Feeding Frequency on Feed Digestibility in Horses. In: “*Advances in Equine Nutrition*”, (Ed.): Pagan, J. D. University Press, Nottingham, PP. 89-92.
  16. Goachet, A. G., Philippeau, C., Varloud, M., Julliand, V., 2009. Adaptations to Standard Approaches for Measuring Total Tract Apparent Digestibility and Gastro-intestinal Retention Time in Horses in Training. *Anim. Feed Sci. Technol.* **152**: 141–151.
  17. Grenet, E., Martin-Rosset, W. and Chenost, M. 1984. Compared Size and Structure of Plant Particles in the Horse and Sheep Feces. *Can. J. Anim. Sci.*, **64**: 345-346.
  18. Gross, J. E., Shipley, L. A., Hobbs, N. T., Spalinger, D. E and Wunder, B. A. 1993. Functional Response of Herbivores in Food-concentrated Patches: Tests of a Mechanistic Model. *Ecol.*, **74**: 778-791.
  19. Hall, L. W. 1971. *Wright's Veterinary Anaesthesia and Analgesia*. Baillière Tindall, London, 176 PP.
  20. Houpt, K. A., Perry, P. J., Hintz, H. F. and Houpt, T. R. 1988. Effect of Meal Frequency on Fluid Balance and Behavior of Ponies. *Physiol. Behav.*, **42**: 401-407.
  21. Jansson, A., Sandin, A. and Lindberg, J. E. 2006. Digestive and Metabolic Effects of Altering Feeding Frequency in Athletic Horses. *Equine. Comp. Exerc. Physiol.*, **3(2)**: 83-91.
  22. McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A. and Wilkinson, R. G. 2011. *Animal Nutrition*. 6<sup>th</sup> Edition, Prentice Hall, Harlow.
  23. Mueller, P. J., Protos, P., Houpt, K. A. and Van Soest, P. J. 1998. Chewing Behaviour in the Domestic Donkey (*Equus asinus*) Fed Fibrous Hay. *Appl. Anim. Behav. Sci.*, **60**: 241-251.
  24. Müller, C.E., Von Rosen, D. and Udén, P. 2008. Effect of Forage Conservation Method on Microbial Flora and Fermentation Pattern in Forage and in Equine cColon and Faeces. *Livest. Sci.*, **119**: 116-128.
  25. Müller, C. E. 2009. Long-stemmed vs. Cut Haylage in Bales: Effects on Fermentation, Aerobic Storage Stability, Equine Eating Behaviour and Characteristics of Equine Faeces. *Anim. Feed. Sci. Technol.*, **152**: 307-321.
  26. NRC. 2007. *Nutrient Requirements of Horses*. 6<sup>th</sup> Revised Edition, National Research Council of the National Academies, Washington, DC, USA, PP. 224-226.

27. Pearson, R. A., Archibald, R. F. and Muirhead, R. H. 2001. The Effect of Hay Quality and Level of Feeding on Digestibility and Gastrointestinal Transit Time of Oat Straw and Alfalfa Given to Ponies and Donkeys. *Br. J. Nutr.*, **85**: 599-606.
28. Penning, P. D. and Johnson, R. H. 1983. The Use of Internal Markers to Estimative Herbage Digestibility and Intake. *J. Agric. Sci.*, **100**: 133-138.
29. Potter, G. D., Arnold, F. F., Householder, D. D., Hansen, D. H. and Brown, K. M. 1992. Digestion of Starch in the Small or Large Intestine of the Equine. *Pferdeheilkunde, Sonderheft*, PP. 107-111.
30. Power, M. L. and J. Schulkin. 2008. Anticipatory Physiological Regulation in Feeding Biology: Cephalic Phase Responses. *Appetite.*, **50**:194-206.
31. Radostits, O. M., Gay, C. C., Blood, D. C. and Hinchliff, K. W. 2007. *Veterinary Medicine. A Text Book of the Diseases of Cattle, Sheep, Goats and Horses*. 10<sup>th</sup> Edition, WB Saunders Ltd., London, UK.
32. Robertson, J. B. and Van Soest, P. J. 1981. The Detergent System of Analysis. Chapter 9. In: "The Analysis of Dietary Fibre in Food", (Eds.): James, W. P. T., Theander, O. Marcel Dekker, New York, NY, USA and Basel, Switzerland, PP. 123-158.
33. Rosenfeld, I., Austbo, D. and Volden, H. 2006. Models for Estimating Digesta Passage Kinetics in the Gastrointestinal Tract of the Horse. *J. Anim. Sci.*, **84**: 3321-3328.
34. Sales, J. 2012. A review of Indigestible Dietary Markers to Determine Total Tract Apparent Digestibility of Nutrients in Horses. *Anim. Feed Sci. Technol.*, **174**: 119-130.
35. Shingu, Y., Kondo, S., Hata, H. and Okubo, M. 2001. Digestibility and Number of Bites and Chews on Hay at Fixed Level in Hokkaido Native Horses and Light Half-bred Horses. *J. Equine Vet. Sci.*, **12**: 145-147.
36. Søndergaard, E., Clausen, E., Christensen, J. W. and Schougaard, H. 2004. *Housing of Horses*. Danish Institute of Agricultural Sciences Edition, Tjele, Denmark.
37. Statistical Analysis System (SAS). 2008. *User's Guide: Statistics. Version 9.2*. SAS Institute, Cary, NC, USA.
38. Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber and Non-starch Polysaccharides in Relation to Animal Nutrition. *J. Dairy. Sci.*, **74**: 3583-3597.
39. Van Weyenberg, S., Buyse, J. and Janssens, G. P. 2007. Digestibility of a Complete Ration in Horses Fed Once or Three Times a Day and Correlation with Key Blood Parameters. *Vet. J.*, **173**: 311-316.
40. Vervuert, I and Coenen, M. 2006. Factors Affecting Glycaemic Index of Feds for Horses. *Proceedings of the 3<sup>rd</sup> European Equine Nutrition and Health Congress*, March 17-18, Ghent University, Merelbeke, Belgium.
41. Warner, A. C. I. 1981. Rate of Passage of Digesta through the Gut of Mammals and Birds. *Nutr. Abstract Rev. Serie. B.*, **51**: 799-807.
42. Wickens, C. L. and Heleski, C. R. 2010. Crib-biting Behavior in Horses: A Review. *Appl. Anim. Behav. Sci.*, **128**: 1-9.
43. Willard, J. G., Willard, J. C., Wolfram, S. A. and Baker, J. P. 1977. Effect of Diet on Cecal pH and Feeding Behavior of Horses. *J. Anim. Sci.*, **45**: 87- 93.
44. Youket R. J., J. M. Carnevale, K. A. Houpt and Houpt, T. R. 1985. Humoral, Hormonal, and Behavioral Correlates of Feeding in Ponies: The Effects of Meal Frequency. *J. Anim. Sci.*, **61**: 1003-1110.



## تأثیر دفعات خوراک‌دهی بر قابلیت هضم مواد مغذی و رفتار مصرف خوراک در اسب ترکمن

۱. دیرکوندی، ی. روزبهان، و ح. فضائی

### چکیده

در این آزمایش از ۱۶ رأس اسب نژاد ترکمن با میانگین وزن  $430 \pm 46$  کیلوگرم و سن  $3 \pm 7$  سال استفاده گردید. دفعات خوراک‌دهی ۲، ۴، ۶ و ۸ وعده تغذیه در روز برای مدت ۲۸ روز مورد استفاده قرار گرفتند. داده‌های حاصل از این آزمایش بر پایه طرح کاملاً تصادفی تجزیه و تحلیل شدند. نتایج حاصل از این آزمایش نشان داد، با افزایش دفعات خوراک‌دهی قابلیت هضم مواد مغذی افزایش می‌یابد ( $P < 0.05$ ). با این حال قابلیت هضم عصاره اتری تحت تاثیر دفعات خوراک‌دهی قرار نگرفت. نرخ جویدن در زمان‌های مختلف برای تیمار ۸ وعده تغذیه در روز به طور معنی‌داری افزایش یافت ( $P < 0.05$ ). اما میانگین جویدن و بلعیدن علوفه تحت تاثیر دفعات خوراک‌دهی قرار نگرفت. همچنین نرخ جویدن و بلعیدن کنسانتره برای تیمار ۲ وعده تغذیه در روز به طور معنی‌داری افزایش پیدا کرد ( $P < 0.05$ ). با افزایش دفعات خوراک‌دهی میزان علوفه مصرفی کاهش یافت و مدت زمان مصرف علوفه در تیمار ۸ وعده تغذیه در روز افزایش یافت ( $P < 0.05$ ). در مقابل با افزایش دفعات خوراک‌دهی میزان کنسانتره مصرفی افزایش یافت و مدت زمان مصرف کنسانتره در تیمار ۸ وعده تغذیه در روز کاهش یافت ( $P < 0.05$ ). در پایان می‌توان نتیجه گرفت که افزایش دفعات خوراک‌دهی سبب بهبود قابلیت هضم مواد مغذی و رفتار مصرف خوراک در اسب می‌شود.