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The Determination of Incubation Time and Amount of Faecal Content of Horse Faeces as an Inoculum Source for Digestibility Determination of Forages with *in vitro* Procedure

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Abstract: This study was carried out to determine incubation time and amount of horse fecal content for measuring *in vitro* dry matter digestibility of roughages (barley straw, wheat straw, lentil straw, wheat silage, corn silage and alfalfa hay) and their comparison with *in vivo* digestibility values. In *in vitro* study, inoculums were prepared using 100, 150, 200, 250, 300, 330, 500, 660 ve 1000 g faeces for per liter of buffer. Roughages were incubated with these inoculums at two incubation times (48 vs 72 h) before 48 h pepsin-HCl digestion. Six fat tailed Awassi rams (42.7±2.5 kg) at the age of two years were used as experimental animals (3 per treatments) in an apparent digestibility experiment. According to result of this study, the best equation for estimating *in vivo* DM digestibilities was obtained with usage of inoculum containing 250 g faeces for per liter buffer solution at length of 48 h incubation ($r = 0.93$, $Y = 24.11 (\pm 2.57) + 0.62 (\pm 0.05) X$, $R^2 = 0.87$ ve RSD = 3.19) and 660 g faeces for per liter buffer solution at length of 72 h incubation ($r = 0.94$, $Y = 16.08 (\pm 2.88) + 0.72 (\pm 0.05) X$, $R^2 = 0.89$ ve RSD = 2.88). Even though, result from this study showed that horse faecal fluid had high potential to be used for predicting *in vivo* DM digestibility, further researches dealing with larger number of feeds with known *in vivo* DM digestibility are required to improve usage horse faecal fluid for *in vitro* inoculums source.

Key words: Horse, faeces, *in vitro* digestibility

INTRODUCTION

In vivo determinations of digestibility in ruminants are expensive, labour intensive and time consuming. This creates a need for a simple, cheap and reliable laboratory technique to evaluate the nutritive value of forages for ruminants (Van Der Baan *et al*., 2004). The techniques include the *in vivo* and *in vitro* methods, which necessitate the use of live intact or surgically modified animals (Chikwanda and Mutisi, 2001). Two-stage procedure for the *in vitro* digestion of feedstuffs described by Tilley and Terry (1963) has been widely accepted as a standard procedure for the estimation of digestibility. Most *in vitro* digestibility techniques rely on fermentation of feeds with buffered rumen fluid. In order to obtain rumen fluid, ruminally cannulated animals are required, which are expensive to maintain and in some circumstances unavailable such as surgical facilities, constant care to avoid infections, long-term maintenance and ethical issues of using these animals (Mauricio *et al*., 1999; El-Meadaway *et al*., 1998).

One method of over coming the need for rumen-fistulated animals is to use freshly voided feaces from the sheep, cow and horse as the source of inocolum (Denek and Can, 2007; El Shaer *et al*., 1987; Chikwanda and Mutisi, 2001; Akhter *et al*., 1999). It was reported that faeces has a high potential as an alternative source of inoculum for the Tilley and Terry (1963) *in vitro* digestibility technique in former studies (El Shaer *et al*., 1987; Omed *et al*., 1998; Manyuchi *et al*., 1991; El-Meadaway *et al*., 1998). Faeces are a cheap, readily available source of microorganisms, do not require the use of surgically prepared (fistulated) animals and can be collected from any individual or several animals, thereby, minimizing the effects of animal to animal variation. Equine faeces should also be a readily available source of viable microorganisms for use *in vitro*, particularly as equines are hindgut fermenters with little or no post fermentative digestion and absorption of microbial cells as is the case with ruminants (El-Shaer *et al*., 1987; Harris *et al*., 1995; Theodorou *et al*., 1994).

The aim of this study was to determine incubation time and amount of horse fecal content for measuring *in vitro* dry matter digestibility of roughages and their comparison with *in vivo* digestibility values via regression parameters.

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MATERIALS AND METHODS

Feed samples: Total 6 roughages commonly used in ruminant nutrition were chosen as feed materials in current study. The roughages were barley straw, wheat straw, lentil straw, wheat silage, maize silage ve alfalfa hay. The feeds were ground in a laboratory mill fitted with a 1-mm sieve and analyzed for Dry Matter (DM), Organic Matter (OM) and ash (AOAC, 1990). Acid (ADF) and Neutral Detergent Fibre (NDF) were analyzed according Van Soest *et al.* (1991) with exclusion of residual ash. Nitrogen was determined with a nitrogen analyzer (Model FP-328, Leco, Mönchengladbach, Germany) and CP expressed as N×6.25. The chemical composition of the roughages is presented in Table 1.

***In vivo* digestibility:** Six fat tailed Awassi rams (42.7±2.5 kg) at the age of two years were used as experimental animals (3 per treatments) in an apparent digestibility experiment. The rams were subjected to a 31-day trial period, consisting of a 10-day adaptation period, 10-day feed intake calculation, followed by 4 days at 85% of ad libitum intake prior to 7-day faeces (Cochran and Galyean, 1994). During the collection period, the experimental animals were fed ad libitum intake in 2 equal portions at 8:00 and 16:00 h. They had free access to water at all times. Faeces were collected immediately after excretion and bulked daily for total weight determination and then a 10% representative sample was taken to make running composite samples for individual animals. All the diet and faecal samples were preserved in sealed polyethylene bags stored in freezers until chemical analyses. Dry Matter (DM) content of and faecal samples were determined (AOAC, 1990).

Faecal inoculum: Feaces required for the production of faecal inoculums were collected within 1 h of defecation from the three Arabic horses which were fed 4 kg/d rolled barley and ad libitum access to grass hay, barley straw and water. Faecal samples were pooled within animals and mixed thoroughly. There after the samples were put in a prewarmed flask until used to prepare the inoculum.

***In vitro* digestibility determination:** Inoculums from wet faeces were prepared the method described by the El-Shaer *et al.* (1987) using 100, 150, 200, 250, 300, 330, 500, 660 and 1000 g wet faeces for per liter of buffer. Then two different incubation times were chosen as 48 or 72 h before 48 h pepsin-HCl digestion. Four replicates of each amount of feaces and incubation times were used for chosen each roughage sources. Blanks consisting of buffer and inoculums without the roughages were included.

Table 1: The chemical composition of the roughages (% of DM)

Feeds	DM	OM	CP	ADF	NDF
Barley straw	94.02	9.32	2.68	46.71	79.19
Wheat straw	93.92	11.23	2.78	60.07	77.06
Lentil straw	93.52	13.76	6.08	44.22	57.07
Wheat silage	46.04	7.81	8.20	38.81	55.13
Maize silage	30.41	9.94	8.58	39.74	59.78
Alfalfa hay	93.99	10.28	10.95	43.80	60.99

In vitro digestibility estimates derived from the use of horse faecal fluid techniques were regressed on the values derived from the *in vivo* experiment using SAS program (SAS, 1989).

RESULTS AND DISCUSSION

The relationships between *in vivo* digestibility (y) and *in vitro* digestibility (x) during 48 h incubation from different inoculums and two incubation times (48 vs 72 h) are presented in Table 2. The best equation for predicting *in vivo* DM digestibility was obtained from 250 g faeces/per liter of buffer containing inoculums with r = 0.93 for 48 h incubations. In contrast, usage of 100 or 1000 g faeces/per liter of buffer resulted with poorest relationship (r = 0.68 and 0.69, respectively). When 72 h incubation time was considered, the best equation for predicting *in vivo* DM digestibility was obtained from 660 g faeces/per liter of buffer containing inoculums with r = 0.94 and poorest with 200 g/per liter of buffer (r = 0.77).

Mauricio *et al.* (1999) reported that usage of rumen liquid instead of faecal suspension resulted with better relationship due to lower microbial activity in feacal source. They also suggested that longer incubation time is required when faecal materials are chosen as source of inocolum for *in vitro* digestibility determination. Additionally, Mauricio *et al.* (2001) explained that microorganisms in bovine faeces are likely to be in a 'state of suspended animation' from which it takes longer lag time to begin growing and dividing. Therefore, microorganisms in faeces have lower metabolic activity compared to those in rumen fluid. It is unknown that how activity of faecal inocula might be increased (Rymer *et al.*, 2005). Sunvold *et al.* (1995) evaluated the influence of gastrointestinal tract microflora from several species (dog, cat, human, swine and cattle) on fiber fermentation characteristics *in vitro*. Disappearances of organic matter following 6, 12 and 24 h were the lowest with horse feacal microflora comparison with other species. Desired fermentation with horse faecal flora was obtained with after 48 h incubation in their study. Chikwanda and Mutisi (2001) observed low gas production values of grass samples incubated in the faecal fluid due to lower microbial population in the fluid compared to the

Table 2: The relationships between in vivo digestibility (y) and *in vitro* digestibility (x) using different inocolums including varying amount wet faeces at two incubation times (48 vs 72 h)

Wet faeces (g/per litre buffer)	Regression equations	R^2	r	RSD	p-value
48 h incubation					
100	Y = 33.45 (±4.93) + 0.46 (±0.11) X	0.46	0.68	6.43	0.0003
150	Y = 24.23 (±4.68) + 0.60 (±0.09) X	0.66	0.81	5.11	0.0001
200	Y = 24.30 (±3.39) + 0.63 (±0.07) X	0.79	0.89	4.03	0.0001
250	Y = 24.11 (±2.57) + 0.62 (±0.05) X	0.87	0.93	3.19	0.0001
300	Y = 14.40 (±5.10) + 0.80 (±0.10) X	0.74	0.86	4.67	0.0001
330	Y = 24.51 (±4.75) + 0.63 (±0.10) X	0.65	0.81	5.19	0.0001
500	Y = 35.01 (±3.32) + 0.44 (±0.07) X	0.62	0.79	5.36	0.0001
660	Y = 30.60 (±4.52) + 0.55 (±0.10) X	0.57	0.75	5.77	0.0001
1000	Y = 26.20 (±6.28) + 0.65 (±0.14) X	0.48	0.69	6.30	0.0002
72 h incubation					
100	Y = 15.52 (±4.02) + 0.70 (±0.07) X	0.81	0.90	3.79	0.0001
150	Y = 19.18 (±4.28) + 0.70 (±0.08) X	0.76	0.87	4.30	0.0001
200	Y = 26.34 (±4.98) + 0.55 (±0.10) X	0.60	0.77	5.56	0.0001
250	Y = 25.88 (±3.99) + 0.57 (±0.08) X	0.71	0.84	4.75	0.0001
300	Y = 15.99 (±3.76) + 0.73 (±0.07) X	0.83	0.91	3.63	0.0001
330	Y = 24.90 (±2.88) + 0.57 (±0.06) X	0.83	0.91	3.58	0.0001
500	Y = 29.10 (±2.85) + 0.55 (±0.06) X	0.79	0.89	4.00	0.0001
660	Y = 16.08 (±2.88) + 0.72 (±0.05) X	0.89	0.94	2.88	0.0001
1000	Y = 19.50 (±4.35) + 0.86 (±0.11) X	0.75	0.87	4.39	0.0001

population in rumen fluid. Chen and Zhao (2004) studied the suitability of using a faecal suspensions of 35, 50, 100 and 150 g wet faeces of sheep of the utilizable crude protein of feeds. The highest regression coefficient (R^2) was calculated between gas productions after inoculation with suspension of 100 g wet faeces per litre McDougall's buffer. El-Meadaway *et al.* (1998) reported that IVDMD values obtained with either rumen fluid or faecal suspension containing 3% faeces were not different. In contrast, Faecal suspension containing 6 or 9% fresh cattle faeces resulted in lower IVDMD than those obtained with rumen fluid. They suggested that faecal suspension can not be used for low quality roughages (such as barley straw) for IVDMD as an inocolum source. This study shows that incubation time is varying according to amount of wet faeces used in inocolum preparation.

El Shaer *et al.* (1987) indicated that fermentation period should be extended from 48-72 h when barley straw samples were subjected to *in vitro* procedure with faecal inocolum source. Akhter *et al.* (1999) invesatigated whether bovine faeces could replace rumen liquor collected from fistulated sheep in the determination of OM digestibility of forages with *in vitro*. Two inocula were prepared by mixing 83 and 333 g of cattle faeces with 1 L of artificial saliva. Inocula made from 333 g of faeces gave higher and closer values of OM digestibilities of rumen liquor than those values from 83 g faeces containing inocula values. Omed *et al.* (1989) found that the method using a buffered faecal suspension as the inoculum for the fermentation *in vitro* was almost as accurate as the method using rumen fluid. In their experiments, they found good estimation equation for roughage samples, but unsatisfactory results for concentrates was reported.

There are only few reports on the use of faecal fluid instead of rumen fluid as an inoculum source. Denek and Can (2007) indicated that horse faecal suspension had more potential for predicting *in vivo* DM digestibility than faecal suspensions from sheep and cattle (r = 0.83). Lowman *et al.* (1999) reported that equine faeces are available source of viable microorganism for use *in vitro* because equids are hindgut fermenters with little or no post fermentative digestion of microbial cells comparison with microbial cells in ruminant feaces. Each segment of the hindgut is host to a large population of bacteria and protozoa that are similar to the population hosted in rumen (Henneke *et al.*, 1983). Thus it might have more potential for replacing rumen fluid as inoculum sources. In contrast, Total viable and sellulolytic counts were determined to be 10-1000 times higher in rumen content than caecal/faecal materials (Latham *et al.*, 1971; Kern *et al.*, 1974). Physical characteristics of horse (large faecal ball-shape; Holter, 1991) which obviously provide more stable environments for survival of faecal microbes compared with cattle faeces. Current study is in agreement with above reports because of having high correlation coefficients (r) and R^2 with *in vivo* and *in vitro* DM digestibility values for roughages.

According to results of this study, correlation coefficient (r), R^2 and Residual Standard Deviation (RSD) of 100, 150, 200, 250, 300, 330, 500, 660 and 1000 g wet faeces per 1 L buffer including *in vitro* digestibility values and *in vivo* values were 0.68, 0.81, 0.89, 0.93, 0.86, 0.81, 0.79, 0.75 and 0.69; 0.46, 0.66, 0.79, 0.87, 0.74, 0.65, 0.62, 0.57 and 0.48; 6.43, 5.11, 4.03, 3.19, 4.67, 5.19, 5.36, 5.77 and 6.30, respectively. Denek and Can (2007) indicated that correlation coefficient (r), R^2 and RSD were 0.83, 0.90 and 4.89 between *in vitro* DM digestibility of horse faecal suspension (16% wet faeces including) during 48 h incubation and *in vivo* DM digestibility.

In this study, usage of 150, 200, 250, 300, 330 and 500 g wet faeces had similar coefficient values (0.81, 0.89, 0.93, 0.86, 0.81 and 0.79) with that reported by Denek and Can (2007).

CONCLUSION

As a conclusion, the best equation for estimating *in vivo* DM digestibilities was obtained with usage of inoculum containing 250 g faeces for per liter buffer solution at length of 48 h incubation (r = 0.93, Y = 24.11 (±2.57) + 0.62 (±0.05)X, R^2 = 0.87 and RSD = 3.19) and 660 g faeces for per liter buffer solution at length of 72 h incubation (r = 0.94, Y = 16.08 (±2.88) + 0.72 (±0.05) X, R^2 = 0.89 ve RSD = 2.88). Even though, result from this study showed that horse faecal fluid had high potential to be used for predicting *in vivo* DM digestibility, further researches dealing with larger number of feeds with known *in vivo* DM digestilities are required to improve usage horse faecal fluid for *in vitro* source.

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