RESEARCH PAPER
Research Journal of Animal Husbandry and Dairy Science
Volume 9 | Issue 1 | June, 2018 | 1-7
e ISSN-2231-6442
DOI: 10.15740/HAS/RJAHDS/9.1/1-7
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Comparison of *in situ* nylon bag technique with *in vitro* procedure to estimate dry matter and other nutrients digestibility in buffalo calves

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ABSTRACT : Three male murrah buffalo calves were taken for entire investigation. The average digestibility co-efficient for dry matter, crude protein, ether extract, crude fibre, nitrogen-free extract, total carbohydrates, total ash, acid detergent fibre and neutral detergent fibre were 53.05 ± 11.05, 54.50 ± 0.90, 52.59 ± 11.14, 53.54 ± 1.09, 53.38 ± 1.04, 53.38 ± 1.08, 32.08 ± 2.04, 52.82 ± 1.60 and 60.06 ± 1.19 at 72 hours, respectively by *in situ* nylon bag technique. The average digestibility of above mentioned feed nutrients were recorded to be 52.19 ± 1.03%, 53.09 ± 1.04%, 51.39 ± 1.09%, 52.41 ± 1.07%, 52.56 ± 0.99%, 52.43 ± 1.06, 31.61 ±1.90%, 51.88 ± 1.07% and 59.09 ± 1.17% at 72 hours, respectively by *in vitro* procedure. The dry matter, crude protein and ether extract digestibility estimated by *in situ* nylon bag technique were significantly higher ($P < 0.01$) than the value estimated by *in vitro* procedure. The crude fibre, nitrogen-free extract, total carbohydrates, total ash and neutral detergent fibre digestibility values obtained by *in situ* nylon bag technique vs. *in vitro* procedure did not differ significantly. The acid detergent fibre digestibility was statistically significant ($P < 0.05$) in *in situ* nylon bag technique than *in vitro* procedure.

KEY WORDS : Comparison, Nylon bag, *In vitro,* Digestibility, Dry matter

HOW TO CITE THIS PAPER : Prakash, Om (2018). Comparison of *in situ* nylon bag technique with *in vitro* procedure to estimate dry matter and other nutrients digestibility in buffalo calves. *Res. J. Animal Hus. & Dairy Sci.,* **9**(1) : 1-7 : **DOI: 10.15740/HAS/RJAHDS/9.1/1-7.**

INTRODUCTION

By the early nineteenth century scientists in several European countries were publishing tables of the nutritive value of feedstuffs and were developing methods upon which many of our current techniques are based (For excellent reviews see Tyler 1975 and Blaxter 1980). Quin *et al.* (1938) used the fibre bag technique to investigate the digestion of feeds in the rumen of cannulated sheep. They used cylindrical bags composed of a very fine natural silk. Subsequent workers have used artificial fibres for the bags (Erwin and Ellison, 1959; Johnson, 1966 and Rodriguez, 1968). The artificial fibre bag (dacron bag, nylon bag, rumen bag) technique, provides a powerful tool for the initial evaluation of feedstuffs and for improving our understanding of the processes of degradation which occur within the rumen. The rate and extent of dry matter fermentation in the rumen are very important determinants for the nutrients absorbed by ruminants. The nylon bag technique has been used for many years to estimate both the rate and the extent of dry matter degradation in forages *in situ* (Mehrez and Orskov, 1977).

Therefore, several other techniques have been developed. Menke *et al*. (1979); Menke and Steingass (1988) developed the *in vitro* gas production technique to evaluate the nutritive value of forages and to estimate the rate and extent of DM degradation indirectly using

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the gas production (CO_2) during fermentation. *In vitro* fermentation or the artificial rumen technique has proved to be one of the more promising laboratory methods for estimating forage nutritive value. The two-stage *in vitro* technique for determination of dry matter digestibility (IVDMD), developed by Tilley and Terry (1963), has become recognized as one of the most reliable methods for the estimation of *in vivo* digestibility. Modifications of the two-stage method have been reported by several laboratories, including those of Alexander and McGowan (1966) for *in vitro* organic matter digestibility determination (IVOMD). The objective of this experiment was to determine the dry matter and other nutrients digestibility of feed (concentrates mixture, green Lucerne and wheat straw) samples by *in situ* nylon bag technique and *in vitro* procedure for dry matter and other nutrients digestibility and make comparison of digestibility values determined by *in situ* nylon bag technique with *in vitro* procedure.

MATERIAL AND METHODS

Three male murrah buffalo calves were taken for entire investigation. Commercially available and widely used feedstuffs consisting of wheat straw, green lucerne and concentrates mixture were used in this experiment. After drying forage samples were milled through a 1-mm sieve for chemical analysis. Dry matter was determined by drying the samples at 105°C overnight and ashing the samples in a muffle furnace at 525°C for 8 hours. Nitrogen (N) content was measured by the Kjeldahl method (AOAC, 1990). Crude protein was calculated as N × 6.25. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined by the method of AOAC (1990). Starch content was determined by the method according to MacRea and Armstrong (1968).

In situ nylon bag technique:

The *in situ* DM degradation analysis was carried out according to the procedure described by Mehrez and Orskov (1977). 5 g samples dried and milled through a 3-mm sieve were weighed into nylon bags and incubated in three rumen fistulated buffalo calves for 72 hours. The experimental calves were provided adequate feed containing nutrients computed on the basis of individual body weight (NRC, 2001). In this experiment, animals were offered concentrate mixture, green lucerne along with wheat straw. The wheat straw was fed ad-libitum, after feeding the concentrate mixture daily morning and evening. On removal the nylon bags were thoroughly washed with cold running water until no further coloured liquid could be extruded, and dried at 60°C for 48 hours. The dry matter and other nutrients losses for each incubation time were determined.

In vitro procedure:

Forage samples milled through a 1 mm sieve were incubated *in vitro* in rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass (1988). Rumen fluid was obtained from three fistulated buffalo calves fed twice daily with a diet containing concentrate mixture, green lucerne along with wheat straw. The wheat straw was fed ad-libitum, after feeding the concentrate mixture. The experimental buffalo calves were provided adequate feed containing nutrients computed on the basis of individual body weight (NRC, 2001). Digestion medium was prepared mixing 500 ml of distilled water, 0.1 ml micro-mineral solution, 200 ml buffer solution, 200 ml macro-mineral solution.

The observations recorded during the course of these investigations were summarized in the form of table for the analysis of mean and standard error. Paired 't' test was applied to judge the differences in digestibility values found by *in situ* nylon bag technique and *in vitro* procedure. The significant differences between the average values were tested against critical difference (CD) at 5 and 1 per cent level of probability.

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

Estimation of digestibility by *in situ* nylon bag technique (NBT):

Dry matter:

The digestibility co-efficient of dry matter was recorded in the three fistulated male buffalo calves and the average value is presented in Table 1. The average dry matter digestibility was found to be 53.20, 51.93 and 54.02 per cent in the samples of animal-A, B, C, respectively. The range of variation in the dry matter digestibility among the animals were 51.93 and 54.02 per cent with an overall average 53.05 per cent 72 hours the

dry matter digestibility found in the present study was within the range reported by Shariff and Gupta (1989) who noted that dry matter digestibility of different feeds ranged from 47.11 to 83.70 per cent at 72 hours.

Singh (1993) also reported higher dry matter disappearance of the concentrate mixture (81.93%) at (72 hours intervals) as compared to the digestibility values recorded in the present study. No significant variation in the dry matter digestibility could be recorded among the animal samples at 72 hours. Ozkan and Sahin (2006) reported higher (64.5-69.3%) dry matter disappearance of different oak species in sheep at 72 hours than the present study. It could be due to the feed material and animal were different.

Other nutrients digestibility:

It is apparent from the data presented in Table 1 that the average crude protein digestibility was recorded to be 54.88, 53.47 and 55.16 per cent at 72 hours in samples of animal-A, B and C, respectively. The overall average digestibility co-efficient of crude protein at 72 hours was recorded to be 54.50 per cent. Singh (1993) reported comparatively higher crude protein digestibility per cent at 72 hours with concentrate mixtures incubated in the rumen of cross-bred calves than the crude protein digestibility values found in the present investigation. The lower crude protein digestibility observed in the present study might due to feed sample containing lower percentage of crude protein in the sample. The average ether extract digestibility was found to be 52.76, 51.37 and 53.65 per cent at 72 hours in the animal-A, B and C samples, respectively. The ether extract digestibility among the animals ranged from 51.3 – 53.65 per cent at 72 hours with respective mean value as 52.59 per cent. No significant difference in the ether extract digestibility could be noted between the samples of animal-A and B, B and C and A and C at 72 hours.

A critical examination of data presented in Table 1 reveals that the average crude fibre disappearance was recorded as 53.37, 52.40 and 54.58 per cent at 73 hours in the animal-A, B and C samples, respectively. The average digestibility co-efficient of crude fibre varied from 52.40 to 54.58 per cent, overall average value being 53.45 per cent at 72 hours, respectively. The average digestibility of the nitrogen-free extract was recorded as 53.02, 52.57 per cent at 72 hours in the animal-A, B and C samples, respectively.The average nitrogen-free extract digestibility among the animals *viz.*, A, B and C samples ranged from 52.57 to 54.57 per cent with concomitant overall average figures of 53.38 per cent at 72 hours intervals.

The data presented in Table 1 indicate that the average digestibility total carbohydrates were recorded as 53.15, 52.44 and 54.57 per cent at 72 hours in the animal- A, B and C samples, respectively. The average total carbohydrates digestibility at 72 hr ranged between 52.44 and 54.57 per cent with the mean value of 53.38 per cent. The average total ash digestibility recorded was 32.09, 30.04 and 34.12 per cent at 72 hours in the animal-A, B and C samples, respectively. The total ash digestibility among the animal-A, B and C samples ranged between 30.04 and 34.12 per cent with concomitant overall average figures of 32.08 per cent at 72 hr intervals. The difference in the total ash digestibility values between the animal-A and B samples at 72 hours were statistically

Table 1: Digestibility co-efficient of feed components estimated by *in situ* nylon bag technique (at 72 hour)

Animal No.	Dry matter	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total carbohydrates	Total ash	Acid detergent fibre	Neutral detergent fibre
A	53.20	54.88	52.76	53.37	53.02	53.15	32.09^{B*}	52.68	60.23
	± 1.35	± 1.08	± 0.76	± 1.03	± 1.50	± 1.50	± 1.44	± 0.79	± 1.18
B	51.93	53.47	51.37	52.40	52.57	52.57	30.04	51.29	58.79
	± 1.11	± 1.09	± 0.93	± 0.74	± 0.75	± 0.75	± 1.17	± 1.22	± 1.77
C	54.02	55.16	53.65	54.58	54.57	54.57	34.12^{A**B**}	54.5^{B*}	61.16
	± 1.18	± 1.15	± 1.03	± 0.82	± 0.96	± 0.96	± 1.08	± 1.02	± 2.48
Average	53.05	54.50	52.59	53.45	53.38	53.38	32.08	52.82	60.06
S.E. ±	± 1.05	± 0.90	± 1.14	± 1.09	± 1.04	± 1.08	± 2.04	± 1.60	± 1.19
C.D. (P=0.05)	-	-	-	-	-	-	1.530	1.789	-
C.D. (P=0.01)	-	-	-	-	-	-	2.318	-	-

* and ** indicate significance of values at P < 0.05 and P < 0.01, respectively

(P < 0.05) significant. The differences in the values between the samples of animal-A and C and B and C were found to be statistically (P < 0.01) significant.

It is apparently clear from the data presented in Table 1 that the average acid detergent fibre digestibility were recorded as 52.68, 51.29 and 54.40 per cent at 72 hours in animal-A, B and C samples, respectively. The overall average digestibility of acid detergent fibre was 52.82 per cent at 72 hours. The neutral detergent fibre digestibility was recorded as 60.23, 58.79 and 61.16 per cent at 72 hours in the animal-A, B and C, samples, respectively with overall average digestibility of neutral detergent fibre was 60.06 per cent at the time intervals of 72 hour. Singh and Gupta (1996) reported comparatively higher neutral detergent fibre digestibility (62.5 % at 72 hours) as compared to the finding of present investigation. No significant differences could be seen in the neutral detergent fibre digestibility values obtained among the animal-A, B and C samples at 72 hours of opening.

Estimation of digestibility by *in vitro* procedure (IVP) :

Dry matter:

It is obviously clear from the data presented in Table 2 that the mean dry matter digestibility values in the experiment were 52.08, 51.22 and 53.28 per cent in the animal-A, B and C samples. The differences in these values were statistically not significant. The overall average of dry matter digestibility was 52.19 ± 1.03 per cent. The dry matter digestibility values found in the present study were within the range reported by Pachauri and Patil (1985), who recorded the dry matter digestibility value from 40.2 to 78.71 per cent (one month); 39.40 to 71.31 per cent (two months) and 37.9 to 58.0 per cent (three months), regrowth legume samples.

In other earlier experiment, the dry matter digestibility of untreated rice straw was found to be 40.63 ± 1.57 per cent (Tripathi *et al.*,1987) whereas, in the untreated wheat straw (Tripathi *et al.*, 1988) it was observed as 41.96 per cent by *in-vitro* procedure which were lower than the values recorded in the present study. In the experiments conducted on the dry matter disapprearance in the treated wheat straw, Tripathi *et al.* (1988) found result (51.98%) as dry matter digestibility recorded in the present investigation. However, higher (from 57.54 to 76.05%) dry matter digestibility reported by Gupta and Pradhan (1975), Mudgal and Singh (1978) and Singh and Katiyar (1999) in the legume and non-legume samples to the findings of the present study.

Other nutrients digestibility:

It is obviously clear from the data presented in Table 2 that the crude protein digestibility was recorded to be the highest as 54.16 per cent and the lowest 52.07 per cent. The average value of all the animal liquor samples was 53.07 ± 1.04 per cent. The differences in the crude protein digestibility values between the animal (A, B and C samples) were not significant. When the ether extract digestibility was recorded in the samples of individual animals, the average ether extract digestibility was found to be 51.28, 50.36 and 52.53 per cent in the liquor samples of animals-A, B and C, respectively. The overall average ether extract digestibility was 51.39 ± 1.09 per cent. The

Table 2 : Digestibility co-efficient of feed components estimated by *in vitro* procedure (at 72 hour)

Animal No.	Dry matter	Crude protein	Ether extract	Crude fibre	Nitrogen-free extract	Total carbohydrates	Total ash	Acid detergent fibre	Neutral detergent fibre
A	52.08	53.05	51.28	52.17	52.53	52.23	31.15^{B*}	51.79	58.96
	± 1.42	± 1.18	± 0.98	± 1.07	± 1.09	± 1.13	± 1.05	± 1.42	± 1.43
B	51.22	52.07	50.36	51.48	51.59	51.48	29.89	50.86	57.99
	± 0.93	± 1.35	± 0.73	± 1.10	± 0.69	± 0.93	± 0.88	± 1.05	± 1.14
C	53.28	54.16	52.53	53.58	53.58	53.58	33.79^{A**B**}	53.00	60.32
	± 1.12	± 1.31	± 0.84	± 0.54	± 1.07	± 1.23	± 0.63	± 1.65	± 0.87
Average	52.19	53.09	51.39	52.41	52.56	52.43	31.61	51.88	59.09
S.E. ±	± 1.03	± 1.04	± 1.09	± 1.07	± 0.99	± 1.06	± 1.90	± 1.07	± 1.17
C.D. (P=0.05)	-	-	-	-	-	-	1.234	-	-
C.D. (P=0.01)	-	-	-	-	-	-	1.870	-	-

* and ** indicate significance of values at P < 0.05 and P < 0.01, respectively

differences in the ether extract digestibility values between the animal (A, B and C samples) were not significant.

It is apparently clear from the data presented in Table 2 that the digestibility of crude fibre values varied from 51.48 per cent to 53.58 per cent with an overall average of 52.41 ± 1.07 per cent. The average crude fibre digestibility of animal-A sample was found to be 53.37 per cent. The difference in the average in the value of crude fibre digestibility among the animal-A, B and C samples was found to be statistically not significant. As regards nitrogen-free extract digestibility, it was noted observed the values ranged between 51.59 and 53.58 per cent when individual buffalo liquor was used. The overall average nitrogen-free extract digestibility was recorded to be 53.56 ± 0.99 per cent. The average value of third animal liquor sample (animal-A) was 53.02 per cent. The differences observed in the digestibility values of nitrogen-free extract were statistically not significance among the animals *viz.*, A, B and C sample.

The data presented in Table 2 indicate that the total carbohydrates digestibility of the feed samples incubated with buffalo calves liquor varied from 51.48 to 53.58 per cent, the overall average value being 52.43 ± 1.06 per cent.The differences in the total carbohydrates digestibility values obtained from the sample of animal-A, B and C were statistically not significant. Observation recorded in above mentioned table reveals that the average digestibility of total ash ranged from 29.89 to 33.79 per cent with the mean value of 31.61 ± 1.90 per cent in the animals-A, B and C sample. The total ash digestibility in the sample of animal-C was very high (P < 0.01) than the value obtained in the sample of animal-B. The significant (P<0.05) difference was also found between the animals- B and A and A and C samples.

A critical examination of data presented in Table 2 reveals that the average acid detergent fibre digestibility of the individual rumen liquor inoculated samples ranged between 50.86 and 53.00 per cent with an average of 51.88 ± 1.07 per cent. No significant differences were observed in the acid detergent fibre digestibility among the animals (A, B and C) samples. The average neutral detergent fibre digestibility average ranged between 57.99 and 60.32 per cent showing insignificant variation in the values obtained in the animals-A, B and C samples. The overall average of the neutral detergent fibre digestibility was recorded to be 59.09 ± 1.17 per cent. Ramachandra and Krishnamoorthy (2000) have reported comparatively higher neutral detergent fibre digestibility (from 64.34 to 81.19%) than the values obtained in the present study. Geisert *et al.* (2007) have also observed lower range of

Table 3 : Comparison of average digestibility co-efficient of feed constituent estimated by *in situ* nylon bag technique (NBT) and *in vitro* procedure (IVP) at 72 hours

Animal No.	Method	Dry matter	Crude protein	Ether extract	Crude fibre	Nitrogen-free extract	Total carbohydrates	Total ash	Acid detergent fibre	Neutral detergent fibre
A	NBT	53.20	54.88	52.76	53.37	53.02	53.15	32.09	52.68	60.23
		± 1.35	± 1.08	± 0.76	± 1.03	± 1.50	± 1.41	± 1.44	± 0.79	± 1.18
	I VT	52.08	53.05	51.28	52.17	52.53	52.23	31.15	51.79	58.96
		± 1.42	± 1.18	± 0.98	± 1.07	± 1.09	± 1.13	± 1.05	± 1.42	± 1.43
B	NBT	51.93	53.47	51.37	52.40	52.57	52.44	30.04	51.29	58.79
		± 1.11	± 1.0	± 0.93	± 0.74	± 0.75	± 2.23	± 1.17	± 1.22	± 1.77
	I VT	51.22	52.07	50.36	51.48	51.59	51.48	29.89	50.86	57.99
		± 0.93	± 1.35	± 0.73	± 1.10	± 0.69	± 0.93	± 0.88	± 1.05	± 1.14
C	NBT	54.02	55.16	53.65	54.58	54.57	54.57	34.12	54.50	61.16
		± 1.18	± 1.15	± 1.03	± 0.82	± 0.96	± 0.96	± 1.08	± 1.02	± 2.48
	I VT	53.28	54.16	52.53	53.58	53.58	53.58	33.79	53.00	60.32
		± 1.12		± 0.84	± 0.54	± 1.07	± 1.23	± 0.63	± 1.65	± 0.87
Average	NBT	53.05**	54.50**	52.59**	53.45	53.38	53.38	32.08	52.82*	60.06
S.E. ±		± 1.05	± 0.90	± 1.14	± 1.09	± 1.04	± 1.08	± 2.04	± 1.60	± 1.19
Average	I VT	52.19	53.09	51.39	52.41	52.56	52.43	31.61	51.88	59.09
S.E. ±		± 1.03	± 1.04	± 1.09	± 1.07	± 0.99	± 1.06	± 1.90	± 1.07	± 1.17

* and ** indicate significance of values at P < 0.05 and P < 0.01

neutral detergent fibre digestibility (43.8-54.0%) than the values founded in this experiment.

Comparison between *in situ* nylon bag technique (NBT) and *in vitro* procedure (IVP) to estimate dry matter and other nutrients digestibility:

The digestibility co-efficients of feed components examined by *in situ* nylon bag technique and *in vitro* procedure have been compared and the results have been as under.

Dry matter:

The average digestibility co-efficient of dry matter (Table 3) was the high (53.05%) in *in situ* nylon bag technique than the value found in *in vitro* procedure (52.19%). The average digestibility co-efficient of dry matter was significantly higher ($P < 0.01$) in *in situ* nylon bag technique than the *in vitro* procedure.

Other nutrients digestibility:

A perusal of the data presented in Table 3 depicts that the digestibility of crude protein and ether extract were recorded higher (54.50%) and (52.59%) in *in situ* nylon bag technique than the values recorded in *in vitro* procedure (53.09%) and (51.39%), respectively. The average crude protein and ether extract digestibility by *in vitro* procedure were significantly lower ($P < 0.01$) than the value obtained by *in situ* nylon bag technique.

A perusal of the data presented in Table 3 clearly depicts that the higher average crude fibre, nitrogen-free extract, total carbohydrates, total ash and neutral detergent fibre digestibility values (53.45%), (53.38%), (53.38%), (32.08%) and (60.06%) were obtained in *in situ* nylon bag technique whereas, the lower values (52.41%), (52.56%), (52.43%), (31.61%) and (59.09%) were recorded in *in vitro* procedure, respectively. It is also observed that the crude fibre, nitrogen-free extract, total carbohydrates, total ash and neutral detergent fibre digestibility values obtained by *in situ* nylon bag technique vs. *in vitro* procedure did not differ significantly.

The higher digestibility of acid detergent fibre (52.82%) was obtained by *in situ* nylon bag technique and lower value was obtained by *in vitro* procedure (51.88%). The acid detergent fibre digestibility was statistically significant ($P < 0.05$) in *in situ* nylon bag technique than the value found in *in vitro* procedure (Table 3).

Implications:

Including concentrates mixture, green lucerne and wheat straw samples with *in-vitro* nutrients digestibility analyze as standards will allow prediction of *in situ* nylon bag nutrients digestibility for feedstuffs. This is important in research settings where a large number of samples are collected and cannot be included within the same *in vitro* run. Samples can be analyzed at different times and the adjustment allows us to compare different runs.

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Received : *28.12.2017;* ***Revised:*** *15.05.2018;* ***Accepted :*** *22.05.2018*