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NUTRITIVE VALUE, FIBER DIGESTIBILITY AND METHANE PRODUCTION POTENTIAL OF TROPICAL FORAGES IN RABBITS: EFFECT OF SPECIES AND HARVEST MATURITY

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ABSTRACT

The aim of this study was to quantify the nutrient composition, neutral detergent fibre (NDF) digestibility (*in vitro*) and methane (CH_4) emission potential of commonly used tropical forages in rabbits. Seven fodder species, namely, *Trifolium alexandrinum, Trifolium resupinatum, Avena sativa, Triticum aestivum, Hordeum vulgare, Brassica campestris, Cichorium intybus;* and seven grass species, namely, *Pennisetum purpureum, Panicum antidotale, Cenchrus ciliaris, Pennisetum orientale, Setaria anceps and Atriplex lentiformis* were evaluated at early, mid and late stages of maturity. At each maturity, samples were collected from four replicate plots of each species, and subsequently analyzed for the contents of dry matter (DM) and nutrient composition. The DM and NDF degradability, and CH_4 emission was measured using an *in vitro* gas production system. The CH_4 concentration in the gas was measured using Gas Chromatography. Large variation ($P < 0.001$) was observed for contents of all measured chemical components among the forage species. With advancing maturity, the contents of crude protein (CP), ether extract, and hemicelluloses decreased ($P < 0.001$) with concomitant increase ($P < 0.001$) in NDF content. Moreover, *in vitro* DM and NDF degradability decreased ($P < 0.001$) and CH_4 emission increased ($P < 0.001$) with increasing maturity. Overall, the fodder species had higher fiber digestibility and produce less CH_4 as compared to grass species, and among the fodder species *T. alexandrinum* had higher fiber degradability (48%) and produced less CH_4 (7.2 ml/ 100 g organic matter) at early maturity, and could be an ideally fed to rabbits as forage sources. This study highlights that forage species and harvest maturity has a profound influence on nutrients supply to rabbit and on CH_4 emission to the environment.

Keywords: Nutrient composition, *In vitro* digestion, Methane production, Harvest maturity, Tropical forages.

INTRODUCTION

Domestic rabbit *(Oryctolagus cuniculus)* is an unexploited, micro-livestock species that possesses many characteristics for low-cost meat production such as early sexual maturity, high reproductive efficiency and short (30.2-30.4 days) gestation length (Ghosh *et al*., 2008; Rommers *et al*., 2010). Notably, rabbits can obtain energy and nutrients from fibrous feedstuffs (Finzi, 2008), and as such they can fulfill their nutritional requirements from forages and other organic wastes with a small amount of concentrate supplementation (Irlbeck, 2001; Khan *et al*., 2016). In many developing countries such as Pakistan, rabbits are predominantly fed on forages, because of their low cost availability and their ability to effectively utilize and digest leaf protein (Musa, 2003); however, they are often criticized for their lower digestibility and higher CH_4 emission.

In livestock species, there is a growing demand in sustainable land use to optimize animal production while reducing the emission of greenhouse gases (Waghorn *et al*., 2002; De-Fries and Rosenzweig, 2010).The most important greenhouse gases are carbon dioxide, nitrous oxide and methane (CH_4), all of which are increasing in the last few years (Merbold *et al*., 2015). Among these, CH_4 is most potent gas due to its global warming potential (Hook *et al*., 2010). Extensive research has shown that the forage yield and quality is mostly affected by the botanical composition, stage of harvest maturity and soil nutrient availability (Aumont *et al*., 1995; Perez Corona *et al*., 1998). This creates a challenge in term of ensuring high quality forage-based diets with optimum stage of harvest maturity to support a sustainable and economical rabbit's production (Linga *et al.*, 2003), particularly in the resource scarce developing countries.

Northern Pakistan hosts the largest rabbit population, because of suitable agro-climatic condition and feed resources (Khan *et al.*, 2017). However, to the author's knowledge, neither *in vivo* nor *in vitro* experiments have been conducted to compare the digestibility of locally grown fodder and grass/pasture species at different stages of maturity for rabbit's production. Consequently, a detailed screening of forage species is important to identify the better forage and optimum stage of maturity to support a sustainable and economical rabbit's production. We hypothesized that the forage species grown in Northern Pakistan at different harvest maturity show large variation in their nutritive value and CH_4 emission potential, and these differences can be potentially exploited to improve rabbit productivity at lower cost and with a minimum burden on environment. The present study was therefore, designed to (i) quantify the nutrient composition; (ii) dry mater and neutral detergent fiber (NDF) digestibility (*in vitro*); and (iii) CH_4 emission potential of commonly used forage species in rabbits, in an effort to identify suitable forages for domestic and commercial rabbit's production.

MATERIALS AND METHODS

Research site and forage management: A database was built using fourteen forage species, comprising of seven fodders and seven grass species (Table 1). Each fodder species was sown on 17th October, 2014 in four replicate plots (20 m × 25 m) at the experimental station (34°00′ N latitude, 71°30′ E longitude) of the University of Agriculture, Peshawar, while, each grass species was sown on 15th March, 2014 in four replicate plots (12 m × 20 m) at the range research station of the Pakistan Forest Institute Peshawar. The growing area is located at the altitude of 350 m above the sea level.

The plots were fertilized with 130 kg P_2O_5 /ha, 36 kg K_2O/ha and 30 kg N/ha before sowing. The fodder plants (50%) emerged on November 7, 2014 and the range grasses emerged (50%) on April 9, 2014. Two weeks after emergence, the plots of range grasses were fertilised with 120 kg N/ha, while the fodder plots were fertilized with 90 kg N/ha. For the fodder plots a similar dose of fertilization was repeated after each cut.

Sampling: All forages were manually cut at early, mid and late stages of maturity at a height of 5 cm above the ground. At each harvest maturity, four samples were randomly collected from a 1 m^2 area of each replicate plot of each species. Samples were kept in a pre-labelled separate polythene zip-bags, vacuumed closed, and immediately transported to the laboratory in oxygen-free (flushed with CO_2) cooling boxes. At each harvest, the samples were pooled by the replicate plot of each forage type, mixed, and representative subsamples of approximately 2 kg were collected for further processing and chemical analysis.

Chemical analysis: The samples were oven dried at 60 ºC for 72 h, ground in Thomas Willy mill at ca. 1 mm. The samples were then analysed according to the standard methods of AOAC (1990) for the contents of DM (method 930.15), ash (method 942.05), ether extract (EE, method 920.39), crude protein (CP; method 984.13; using a Kjeltec™ 2400 autoanalyzer; Foss Analytical A/S, Hillerød, Denmark) and acid detergent fibre (ADF; method 973.18). The NDF content was analysed according to Van Soest *et al*. (1991), with some modification as described by Habib *et al*. (2016).

***In vitro* digestibility and methane production:** The 3-step *in vitro* procedure of Tilley and Terry (1963) was adopted for determining the DM and NDF digestibility, with modification in the use of inoculums. In this study we used feces of rabbit for microbial inoculation. To determine CH_4 concentration, small aliquots of gas (10 µl) was sampled at (24 h) from headspace using a gas tight syringe (Hamilton 1701N, point style 5 needles) and was subjected to gas chromatography for immediate analysis.

Rabbit fecal inoculum was prepared using feces of three multiparous rabbits. The rabbits received no antibiotics. Berseem hay was fed *ad-libitum* as a basal diet, supplemented with a calculated amount of pelleted concentrate supplying ca. 60% of the total metabolizable energy and protein requirements of the rabbits as previously reported by (Khan *et al*., 2017). Immediately after defecation, feces were collected in a plastic container prefilled with CO_2. Extra CO_2 was decanted into the container with feces to ensure anaerobic storage conditions. The container with feces were closed and immediately placed in a thermostat bottle. After pooling, the feces (5%) was mixed with buffer solution, and subsequently, homogenized and filtered using a filter bag and a stomacher. All liquids were kept at 40 °C under anaerobic conditions. The care, live rabbits handling, welfare, and standard laboratory protocols were in accordance with ethical committee of the Department of Animal Science, the University of Agriculture Peshawar.

The buffer solution was prepared by dissolving 5.794 g sodium bi phosphate (Na_2HPO_4), 9.810 g Sodium bicarbonate (Na_2HCO_3), 0.479 g Sodium chloride (NaCl), 0.577 g potassium chloride (KCl), 0.060 g calcium chloride ($CaCl_2$) and 0.132 g magnesium chloride ($MgCl_2$) in distilled water to a total fluid volume of 1 litre. The final pH of the solution was adjusted to 6.9 and saturated with CO_2 gas.

Statistical analysis: The effect of forage type and maturity stages on the nutritive value, and *in vitro* DM digestibility and methane emission was determined using PROC MIXED Procedure (Littell *et al*., 2006) of Statistical Analysis System (SAS, 2003). Forage type, stage of maturity, and their interaction were measured as fixed effect while replications were measured as random effect. The model used was as follow,

$$Y_{ijk} = \mu + F_i + M_j + FM_{ij} + \epsilon_{ijk}$$

Where, Y_{ijk} is response of the treatment; µ is the overall mean; F_i is the effect of forage species; M_j is effect of harvest maturity; FM_{ij} is effect of interaction of specie and harvest maturity; ϵ_{ijk} is random error

RESULTS

Nutritive value and *in vitro* methane emission of fodder species: Large variations ($P < 0.001$) in chemical composition, *in vitro* DM and NDF digestibility was observed due to fodder species and harvest maturity (Table 2). The contents of CP, EE and hemicellulose (HC) decreased ($P < 0.001$), while those of NDF, ADF and CHO increased ($P < 0.001$) with maturity of the offer species (Table 2). The decrease in CP contents with advancing maturity ranged from 0.25 g/g DM (*C. intybus*) to 0.62 g/g DM (*A. sativa*). There was a large variation in the increase in NDF content with advancing maturity among the fodder species, and the minimum (0.20 g/g DM) increase was recorded for *H. vulgar* and maximum (0.45 g/g DM) for *B. campestris.*The DM and NDF digestibility (*in vitro*) decreased ($P < 0.001$) with increasing forage maturity. Among the forage species, *C. intybus* had a minimum (0.07 g/g DM) and *T. aestivum* had a maximum (0.40 g/g DM) decrease in *vitro* DM digestibility. On the other hand, the lowest decrease in NDF digestibility (0.12 g/g NDF) was observed for *C. Intybus* and maximum (0.65 g/g NDF) for *B. campestris*. Large variation in the extent of *in vitro* CH_4 emission was observed in all fodder species. The CH_4 emission increased ($P < 0.001$) with maturity in all fodders, ranging from 0.42 (ml/100 g OM) in *H. vulgar* to 1.44 (ml/100 g OM) in *A. sativa*. Overall, among the fodder species, the highest DM and NDF digestibility was observed in *T. alexandrinum* and produced less methane at the three stages of maturity. Whereas, the lowest fiber degradability and highest CH_4 emission was recorded for *H. vulgar* (Table 2).

Nutritive value and *in vitro* methane emission of grass species: Data on the chemical composition, in vitro DM and NDF digestibility and CH_4 emission of grass species as affected by stage of maturity is summarized in Table 3. The content of CP decreased ($P < 0.001$) with maturity in all grasses, ranging from 0.17 g/g DM in *P. entidotale* to 0.56 g/g DM in *P. orientale.*The NDF content increased ($P < 0.001$) with maturity, and a minimum increase of (0.01 g/g DM) was recorded for *A. lentiform* and a maximum (0.21 g/g DM) for *C. ciliaris*. The content of EE decreased ($P < 0.001$) with maturity, ranging from 0.06 g/g DM in *P. purpureum* to 0.72 g/g DM in *C. ciliaris*. Because of the decrease in CP content and increase in NDF content, the *in vitro* DM digestibility decreased ($P < 0.001$) with maturity, ranging (g/g DM) from 0.12 (*P. maximum*) to 0.52 (*P. orientale*) g/g DM. Moreover, the *in vitro* NDF digestibility decreased with grass maturity, ranging (g/g DM) from 0.20 in *P. maximum* to 0.70 in *A. lentiformis.* In contrast, the CH_4 emission increased with maturity in all grasses, ranging from 0.14 ml/100g OM in *A. lentiformis* to 0.50 ml/100g OM in *P. purpureum.* Among the grass species, the lowest NDF degradability and highest CH_4 emission was recorded for *S. anceps* at all stages of maturity, whereas the highest NDF digestibility and lower CH4 emission was observed for *P. purpureum* (Table 3).

Table 1. Nomenclature of the tropical forage species used in this study.

Fodder species	Common name	Forage type
Trifolium alexandrinum	Barseem	Legume
Trifolium resupinatum	Shaftal	Legume
Avena sativa	Oat	Cereal
Triticum aestivum	Wheat	Cereal
Hordeum vulgar	Barley	Cereal
Brassica campestris	Mustard	Non legume
Cichorium intybus	Kasni	Forb
Grass species		
Pennisetum purpureum	Mott grass	Grass
Panicum antidotale	Bansi/Perenial Sodan grass	Grass
Cenchrus ciliaris	Buffel grass	Grass
Panicum maximum	Guinea grass	Grass
Pennisetum orientale	Oriental fountain grass	Grass
Setaria anceps	Golden bristle grass	Grass
Atriplex lentiformis	Quail bush	Grass

Table 2.Dry matter (DM; % of fresh matter), chemical composition (% of DM), *in vitro* DM (DMD), NDF (NDFD) digestibility (%), and methane (CH_4; ml/ 100 g organic matter) emission potential of fodder species as affected by stage of maturity

Fodder Species	Maturity	DM	Ash	EE	CP	NDF	ADF	HC	CHO	*In vitro* DMD	*In vitro* NDFD	CH_4
	Early	10.6	11.0	9.11	18.0	41.9	22.3	26.8	59.1	54	29.0	19.3
Hordeum vulgar	Medium	13.1	10.8	7.22	13.4	50.3	26.8	20.0	67.7	51.8	26.8	25.4
	Late	17.3	8.73	6.01	7.01	52.6	34.1	17.2	77.5	39.6	12.6	27.5
	Early	13.0	12.0	7.71	12.4	32.1	17.6	15.2	67	69.9	48.4	7.21
Trifolium alexandrinum	Medium	13.7	11.1	7.42	9.89	32.3	23.5	15.3	70.7	65.4	31.8	11.2
	Late	17.5	10.9	5.91	6.09	44.5	30.2	9.78	76.1	59.8	29.0	14.2
	Early	8.81	12.6	9.24	15.3	24.3	13.9	11.3	60	56.8	41.4	12.5
Brassica campestris	Medium	10.0	12.5	9.41	14.4	24.5	16.4	8.88	62.8	50.4	24.5	15.5
	Late	13.3	11.7	7.72	11.3	44.1	37.1	7.88	68.1	44.2	14.6	19.6
	Early	6.01	15.0	8.61	15.5	37.5	16.9	21.4	60	52.3	27.3	9.21
Cichorium intybus	Medium	8.71	12.4	8.71	15.1	44.9	29.6	16.2	62.9	50.3	25.3	14.2
	Late	10.3	11.6	6.30	11.7	50.8	39.2	12.5	69.5	48.9	23.9	16.2
	Early	12.1	12.0	8.52	16.6	35.8	25.4	11.3	62	57.6	32.6	13.2
Avena sativa	Medium	12.5	11.5	7.32	12.0	45.8	37.7	8.98	68.3	50.3	25.3	27.2
	Late	14.2	11.3	7.24	6.23	56.6	49.3	8.18	74.4	39.1	14.1	32.2
	Early	10.9	9.13	5.71	12.3	22.6	14.6	8.88	71.9	68.8	31.8	11.2
Trifolium resupinatum	Medium	11.4	8.43	5.20	11.2	25.5	18.8	7.58	74.2	54.4	25.4	14.2
	Late	13.9	8.33	4.41	7.79	31.4	25.3	6.98	78.6	47.9	19.2	19.2
	Early	8.72	9.03	6.51	14.4	45.4	22.8	23.5	69.1	66.4	43.8	11.6
Triticum aestivum	Medium	13.8	8.93	6.12	10.6	54.5	34.3	21.1	73.4	49.5	29.4	16.4
	Late	15.8	7.93	4.72	6.39	66.8	48.1	19.6	80	39.6	22.9	20.3
SEM		0.37	0.55	0.18	1.48	1.01	0.82	1.26	1.58	2.29	1.89	1.54
Species		***	***	***	***	***	***	***	***	***	***	***
Maturity		***	**	***	***	***	***	***	***	***	***	***
Species × Maturity		***	ns	***	*	***	***	***	**	***	***	***

CP, crude protein; EE, Ether extract; NDF, nutrient detergent fibre; ADF, acid detergent fibre; HC, hemi cellulose; CHO, total carbohydrate; ns, non significant ; *, P<0.05; **, P<0.01; ***, P<0.001; SEM; standard error of mean

Table 3. Dry matter (DM; % of fresh matter), chemical composition (% of DM), *in vitro* DM, neutral detergent fibre (NDF) digestibility (% of DM) and methane (CH_4; ml/ 100 g organic matter) emission of grass species as affected by stage of maturity.

Grasses	Maturity	DM	Ash	EE	CP	NDF	ADF	HC	CHO	*In vitro* DMD	*In vitro* NDFD	CH_4
	Early	15.6	15.6	7.37	9.32	53.1	30.3	21.6	66.1	40.2	23.7	37.1
Cenchrus ciliaris	Medium	20.8	15.3	4.07	8.32	63.4	42.5	19.7	71.2	32.8	16.3	49.2
	Late	21.1	14.1	2.08	5.52	67.4	50.1	16.0	77.5	25.9	9.4	52.0
	Early	12.4	13.4	9.07	10.3	62.6	27.1	34.3	66.2	35.8	19.3	50.0
Setaria anceps	Medium	14.2	11.4	5.97	10.3	70.9	35.9	33.8	70.5	33.1	16.6	55.3
	Late	15.3	10.3	4.27	7.22	76	45.2	29.6	76.5	21.4	8.5	58.2
	Early	17.8	13.8	5.67	14.2	67.4	22.9	44.3	64.8	38.4	21.4	48.3
Penicum entidotale	Medium	19.7	13.4	4.27	13.8	69.3	30.9	38.4	67.1	33.1	16.1	59.1
	Late	21.2	12.6	4.07	11.8	69.4	36.6	32.8	69.7	25.6	8.6	59.4
	Early	13.8	14.7	7.37	13.8	60.6	31.0	29.7	63	41.5	24.5	47.5
Penicum maximum	Medium	16.2	14.2	4.07	11.4	63.2	37.9	25.3	68.8	37.8	20.8	53.3
	Late	18.3	13.9	3.87	9.62	74.5	52.6	21.9	70.7	36.5	19.5	60.1
	Early	12.4	15.6	5.17	16.6	48	24.7	23.4	61.1	48	31.3	33.3
Pennisetum purpureum	Medium	13.5	15.0	5.07	13.8	55.1	32.4	22.8	64.4	45.1	28.4	44.4
	Late	15.9	13.4	4.87	11.8	60	39.6	20.5	68.5	40.5	23.8	50.1
	Early	16.8	15.3	6.77	11.4	57.4	30.0	27.5	65.5	45.8	29.1	44.1
Pennisetum orientale	Medium	18.6	14.8	4.27	9.92	65	39.3	25.8	69.8	32.3	15.6	50.2
	Late	23.8	14.6	3.37	5	71.5	48.8	22.8	76	21.9	8.9	59.5
	Early	20.7	15.6	5.87	9.72	59.9	32.5	27.6	67.5	40.2	19.7	45.1
Atriplex lentiformis	Medium	23.7	14.6	5.27	7.92	60.6	38.4	22.3	70.7	24	7.3	48.2
	Late	26.9	14.9	3.97	5.52	60.6	40.4	20.3	74.3	20	5.9	51.5
SEM		0.33	0.20	0.18	1.04	1.03	1.09	1.36	1.10	2.79	1.78	1.34
Species		***	***	***	***	***	***	***	***	***	***	***
Maturity		***	***	***	***	***	***	*	***	***	***	***
Species x Maturity		***	***	***	ns	***	*	***	**	***	***	***

CP, crude protein; EE, Ether extract; ADF, acid detergent fibre; HC, hemi cellulose; CHO, total carbohydrate; ns, non significant ; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; SEM; standard error of mean

DISCUSSION

The present study developed the first database on nutrient composition, *in vitro* DM and NDF digestibility, and CH_4 emission of tropical forages in rabbits. Forage yield and nutritive value are the two main criteria in the selection of forage crops. However, the quality of forage is mostly determined by the nutrients they supply. Among these nutrients, the content of CP is of great importance and generally it is observed that forage with higher content of CP had superior feeding value. The nutritive values of forages are mostly influenced by forage types, maturity stage, environment and fertilizer (Hatfield *et al*., 2007). However, forge species, maturity and fiber lignification are the foremost sources of variation in the chemical profile of forages (Khan *et al*., 2015a). In our database, large variation in nutrient profile of forages was observed due to forage maturity at harvest. The content of CP gets lower, and those of NDF increased due to forage maturity at harvest. Our results are supported by earlier findings of (Minson, 2012). Notably, the leaf fraction of forages is higher in cell contents (soluble protein, fat and sugars) and lowers in cell wall content (NDF and ADF) compared to stem. During vegetative development, a decline in forage quality generally occurs, due to modification of plant morphology, primarily a decrease of the leaf to stem ratio, and to lignification's which becomes particularly pronounced during the reproductive and senescent stage (Bovolenta *et al.*, 2008).

Indirect comparison shows that a delayed in forage harvesting, significantly increase the concentration of cell wall and lignin fraction and reduce the concentration of easily digestible cell content, and consequently decreased the digestibility in dairy cows (Khan *et al*., 2015b). In the present study, the DM and NDF digestibility reduced due to forage maturity at harvest that could be related, in part, to the synergistic effect of a decrease in CP content, and increase in NDF and lignin contents. Our results are consistent with the finding of Arthington and Brown (2005). Moreover, the higher CH_4 emission due to late forage maturity at harvest could be related to higher NDF content and forage digestibility, a factor favouring higher acetate to propionate ratio, thereby making hydrogen available for methanogenesis (Mceniry and O'kiely, 2013). Earlier findings have shown that CH_4 production was lower in forages harvested at the vegetative stage than the reproductive stage of maturity (Al-Masri, 2009), which are consistent with the findings of our study.

In addition to maturity at harvest, types of forage had a significant effect on the nutrient composition, fiber digestibility and CH_4 production potential. Compared to grasses, leguminous fodders are more palatable, rich in CP and rapidly digestible. In our database, fodders had significantly higher CP content and lower fibre/cell wall contents (NDF and ADF), a factor that could account for the lower CH_4 production as compared to range grasses. In agreement with our findings, earlier studies have shown that tropical grasses has lower CP and high NDF content than legumes and produces more methane than legumes (McCaughey *et al.*, 1999; Singh *et al.*, 2012). These results highlight the large scope for the selection forages with higher nutritive value and fiber degradability for rabbits, which will not only improve animal productivity but will also reduce CH_4 emission to the environment.

Conclusions: The results showed a large variation in chemical composition due to forage species and harvest maturity that resulted in large difference in fiber digestibility and methane emission. The content of CP decreased, and that of NDF increased, and as a consequence the DM and NDF digestibility (*in vitro*) decreased, and the methane emission increased. Overall, the fodder species had higher fiber digestibility and produce less CH_4 as compared to grass species, and among the fodder species *T. alexandrinum* had higher fiber degradability (48%) and produced less CH_4 (7.2 ml/ 100 g organic matter) at early maturity, and could be a potential forage source for rabbits. The current study presents an opportunity to select best forage species and to identify optimum harvest stage of maturity that has high nutritional quality and lower CH_4 emission potential. This study highlights that forage species and harvest maturity has a profound influence on nutrients supply to rabbit and on CH_4 emission to the environment.

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