

A COMPARATIVE STUDY OF LOW AND HIGH QUALITY FORAGES FOR CHEMICAL COMPOSITION AND *IN VITRO* DEGRADABILITY

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ABSTRACT

Four low quality forages (LQF) alongside three high quality forages (HQF) were analysed for their chemical components and *in vitro* degradability (IVD) to establish their complementary properties in ruminant diets. The HQF involved dried ryegrass; rapeseed plant and ryegrass silage and LQF involved rice straw, wheat straw, ryegrass hay and sugarcane bagasse. The HQF had significantly more CP, EE, soluble sugar, total phenolic, tannin, saponin and mineral contents than LQF ($P < 0.05$). The variations for oxalate contents were not significant ($P > 0.05$) between two groups. Among minerals Ca, P, S, Mg, K, Cu and Co were significantly higher but Mn was lower in HQF than LQF ($P < 0.05$). The LQF contained large amount of saturated fatty acids, especially palmitic acid (C16:0) and HQF contained large amount of poly-unsaturated fatty acids especially α -linolenic acid (C18:3). About 61% of fatty acids in ryegrass were represented by α -linolenic acid. Due to higher nutrient and lower fibre contents, the IVD and IVOMD were higher in HQF than LQF ($P < 0.001$). Among the minerals, PHOS was found to be more effective to increase the IVD of forages. It appeared that the selected forages can offer complementary properties for their use to formulate forage based balanced diets to optimize the degradability and utilization of LQF in ruminants.

Key words: Chemical composition; Forage quality; Fatty acids; Minerals, In vitro degradability.

INTRODUCTION

In the developed countries ruminants depend mainly on grazing pasture land or conserved grass and cereal grains. The high price and demand of cereals for human consumption and production of bio-fuel from grains make it difficult to supply enough grains for animals feeding (Santos *et al.*, 2010). The current scenario demands alternative feed sources to maintain ruminant production. Conversely, in developing countries ruminant animals mostly depend on low quality forages (LQF) and agricultural by-products such as cereal straws and products like sugar-cane tops, bagasse, tree leaves and stovers etc. However, these forages are either low in nutrient contents or most of these are bound to compounds like lignin, silica, phenolic and oxalate etc., that reduce their digestibility and nutrient availability. Therefore, the ruminants cannot utilize these forages effectively and consequently animal longevity and production are adversely affected when ruminants are reared with poor quality forages (Chaudhry, 1998). To get more production from these ruminants it is necessary to enhance the utilization of these LQF. The quality of LQF can be improved by using supplements that are rich in energy, protein, vitamin and minerals (Chaudhry, 2008). These supplements can increase the utilization of LQF, but most of these conventional supplements are costly and are not available in most developing countries (Devendra and Sevilla, 2002). However, some non-

conventional and novel feed supplements could be used due to their relatively low cost and availability. It is also important to know the availability and shortage of various nutrients in LQF as compared to high quality forages (HQF) so that the researchers in the future can develop most appropriate strategies for their use in ruminant diets. This study, therefore, evaluated four different LQF alongside three HQF for their chemical components and *in vitro* rumen degradability (IVD).

MATERIALS AND METHODS

In the present study HQF comprised dried ryegrass (RG = *Lolium perenne*), rape seed plant (RP = *Brassica napus*) and RG silage (SIL) and LQF involved rice straw (RS = *Oryza sativa*, Asian rice), wheat straw (WS = *Triticum Sp*), RG hay (HAY) and sugarcane bagasse (SB = *Saccharum officinarum*).

Collection of forages and Chemicals analysis: Representative samples of rice straw (Variety, IR50) were collected from the fields of Bheramara, Kushtia, Bangladesh and sugarcane bagasse was collected from Natore, Sugar Mill, Natore Bangladesh, whereas those of wheat straw (Variety, Einstein), ryegrass hay, dried ryegrass, ryegrass silage and rapeseed plant at its green and pre-flower stage were collected from the Newcastle University's Farm in the UK. The samples of dried ryegrass were prepared after cutting random samples of fresh grass with a hand cutter directly from a field in mid

May 2008 before their oven drying, at 60°C followed by grinding through a 1 mm sieve by using a grinder (Christy mill, Christy and Norris Ltd, Suffolk, United Kingdom). The chemicals and reagents used in the present study were obtained either from Fischer Scientific UK or VWR unless otherwise stated for different chemical analysis.

The forage samples were analyzed in triplicate for dry matter (DM), ash and ether extract (EE) by using the methods of AOAC (1990). Nitrogen contents of these samples were determined by using Leco (FP-428, Leco Corporation St. Joseph, MI, USA) analyzer where the samples were combusted by the Dumas method to obtain nitrogen values which were multiplied with 6.25 to determine crude protein (CP). Acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) of different forages were determined by appropriate methods of Van Soest, (1963), Goering and Van Soest (1970) and Van Soest *et al.* (1991) without using dekalin and sodium sulphite for NDF.

Estimation of soluble sugars, total phenolics, tannins and saponins contents: Soluble sugars (SS) were determined by using the method described by Khan and Chaudhry (2010). The Folin-Ciocalteu method (Makkar, 2003) was used to determine total phenolics (TP). Extracts for the determination of total phenolics were prepared by suspending 0.1 g samples in 10 ml of 75% aqueous acetone in test tubes which were vortexed (Whirlimix, Fison Limited) for 30 min. Aliquots (0.1 ml) of each extract were mixed with 0.4 ml of water, 0.25 ml of Folin-Ciocalteu reagent and 1.25 ml of sodium carbonate solutions (20%). After standing for 40 min at around 21°C, the absorbance was read at a wavelength of 760 nm by using a UV/Vis-spectrophotometer (Biochrom Libra, S12, UK). Gallic acid was used as standard and the results obtained were expressed as mg gallic acid equivalent/g of sample DM.

Tannins were determined according to Makkar (2003) by the difference between total phenolics before and after mixing about 1 ml extract with 1 ml water and 0.1g polyvinyl pyrrolidone (PVP). The mixtures were vortex mixed (Whirlimix, Fison Limited), incubated at 4°C for 30 min, centrifuged for 5 min and supernatants were collected. The supernatants were centrifuged again for 3 min to remove any remaining insolubles. About 0.1 ml aliquot of each this supernatant were mixed with 0.4 ml of water, 0.25 ml of Folin-Ciocalteu reagent and 1.25 ml of sodium carbonate solutions (20%) and the absorbance of the resultant colour was read as described earlier. Total tannins were determined by subtracting the value of total phenol after using PVP from the value of total phenol before using the PVP. Gallic acid was used as a standard and the results were expressed as mg gallic acid equivalent g of sample DM.

Saponins (SP) were determined as described by Makkar *et al.* (2007) and Khan and Chaudhry, (2010). Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent/g of sample DM.

Determination of mineral and oxalate contents: Mineral contents were determined as described by Khan and Chaudhry (2010). Each forage sample was extracted in triplicate with 3 N HCl to obtain the relevant precipitates as calcium oxalate which was treated with dilute sulfuric acid to form oxalic acid. The oxalic acid was then quantified by titration with a standard KMnO₄ solution (Makkar *et al.*, 2007).

Determination of fatty acid composition Fatty acid compositions of forages was determined by using the method as described by Sukhija and Palmquist (1988) with some modifications. All samples were extracted by using petroleum ether with boiling temperature of 40-60°C. About 0.5 g of the sample extract was transferred to a Soveril tube (washed with DeCon 90 and dried) to which 1.7 ml of methanol:toluene (4:1) solution were added, and vortex mixed. To this mixture, 250 µl of acetyl chloride were slowly added using a glass pipette. The test tube containing this mixture was vortexed for 30 seconds and placed in a hot block (Techne Dri-Block^R BD-3D, UK) at 100 °C for 1 h. The sample was then removed from the hot block and cooled for 20 min before mixing it with 5 ml of potassium chloride solution (5% KCl). The tubes were then gently shaken before their centrifugation at 1000g for 5 min. The supernatant was then transferred to a brown glass vial with a glass insert (Chromacol Ltd., Hertfordshire, UK) which was then analysed by a gas chromatograph (GC) (Shimadzu GC-2014, Japan by using helium as the carrier gas and BPX 70 column (30 m x 0.25 mm ID x 0.25 µm film thickness) (SGE Europe Ltd, Milton Keynes, UK). Peaks were identified by using a 38 external FAME standard (C4-C24, Supelco; Sigma-Aldrich). The initial temperature of the column was 50°C which was held for 1 min before it was raised at 2°C / min to 188°C which was held for 10 min and then increased at the same rate to 240°C where it was held for 4 min, and then returned to the initial temperature. For each analysis 1 µL of the sample was injected into the GC. The results were processed by using GC solution software and the fatty acids were expressed as the relative percentage of each fatty acid. The amounts of saturated, monounsaturated and polyunsaturated fatty acids were calculated as % of total fatty acids according to the presence or absence of their double bond.

In vitro degradability trial: Rumen fluid was obtained from two fistulated sheep (Lleyen breed) with mean live-weight of 81 kg about 60 min before their morning feeding. These sheep were managed under the Animal and Scientific Procedures Act 1986 of the UK. These

sheep were consuming fixed amounts (1200 g/day) of a diet comprising 65% chopped hay and 35% concentrate to fulfill their maintenance requirements (AFRC, 1993). The rumen fluid was transported in insulated flasks under anaerobic conditions to the laboratory. The rumen fluid was strained through four layers of a cheese cloth into pre-warmed flasks under CO₂ before its mixing with the pre-warmed phosphate-bicarbonate buffer (McDougall, 1948) at 1:4 ratio to prepare the inoculum. The flasks were then screw capped and kept at 39°C in a water bath until this buffered inoculum was used for its incubation with the forage samples.

Samples of about 0.4 g dried ground forage were separately weighed into test tubes to which 40 ml of the inoculum were added under CO₂. The tubes were sealed with rubber stoppers containing pressure release valves and incubated at 39°C for the fixed time periods of 48 and 96 hours. After each time the tubes were submerged in ice to stop fermentation. The liquids and residues were separated by centrifuging the tubes at 3000 rpm for 10 min. The residues were washed with distilled water and first dried at 60°C for 48 h and then ignited at 600°C for 5 h to determine their DM and OM contents, respectively. These DM and OM values were then used to estimate *in vitro* DM (IVD) and OM (IVOMD) degradability.

Statistical Analyses: The data were analysed by using analysis of variance in General Linear Model of Minitab to compare forage groups of HQF and LQF for their compositions. The means of each chemical component for different materials within each forage group were also compared for their significance by using the Tukeys post-hoc t-test at P<0.05. The stepwise regression analysis was also performed to examine potential relationships between selected minerals and IVD of these forages at each of the 2 incubation times.

RESULTS

Proximate compositions, fibre fractions, soluble sugars, total phenolics, tannins and saponins: The differences in the mean DM, CP, EE, NDF, ADF, ADL, SS, TP and SP contents of two forage groups (P<0.04) are shown in Table 1. The HQF contained higher CP, EE, SS, TP and SP contents than that of LQF. However, the LQF contained higher DM, NDF, ADF and ADL when compared with the HQF. The ash contents were highest in rice straw while lowest in sugarcane bagasse. However, the difference between LQF and HQF for ash content was not significant (P>0.05). Among the LQF, CP was highest in hay and lowest in sugarcane bagasse. The NDF and ADF contents were highest in sugarcane bagasse but ADL was highest in wheat straw. Although the difference between LQF and HQF for the tannin contents was not significant, HQF contained more tannins than LQF. The SP and SS contents were higher in hay

whereas TP and tannins were higher in rice straw. Within LQF, the oxalate contents were significantly (P<0.05) higher for hay than other LQF. Amongst HQF, the highest amounts of CP, EE, TP, tannins and SP were in ryegrass but the highest amount of ash was present in rapeseed plant; however the difference of EE was not significant among the HQF. The ADF and ADL contents were highest in silage as compared to the other HQF.

Mineral components: The mineral contents of low and high quality forages are shown in Table 2. In general, Calcium (Ca), Phosphorus (PHOS), Sulfur (S), Magnesium (Mg), Potassium (K) and Cobalt (Co) were significantly higher in HQF than LQF (P<0.05) whereas Manganese (Mn) content was higher in LQF. Most HQF contained more Copper (Cu) than LQF but overall hay contained the highest and rapeseed plant the lowest Cu contents. Within LQF, sugarcane bagasse contained the lowest amount of most minerals than other LQF (P<0.05). Hay was highest in Ca, PHOS, S, Cu, Co and Zinc (Zn) whereas rice straw contained higher Na, K, Mg and Mn contents than other LQF. Within HQF, the S, K, Ca, Selenium (Se) and PHOS contents were highest in rapeseed plant, Co was highest in ryegrass and Cu and Mn were highest in silage.

Fatty acid composition of different forages: Individual fatty acid contents of different forages are shown in Table 3, whereas different types of fatty acids in different forages are shown in Figure 1. Amongst the two forage groups, the LQF contained more Saturated Fatty Acids (SFA) whereas HQF contained more Poly-unsaturated Fatty Acids (PUFA). Within LQF, SFA were highest in rice straw and hay. These two forages contained a large amount of palmitic acid (C16:0), followed by stearic acid (C18:0). Palmitic acid (C16:0) was also higher in wheat straw; however wheat straw also contained large amount of PUFA like α -linolenic (C18:3) and arachidonic acid (C20:4). Among the LQF, sugarcane bagasse contained high amount of linoleic acid (C18:2) followed by palmitic acid. Hay was lower in PUFA content, however, it contained the highest amount of Docosahexaenoic acid (C22:6). Among the PUFA, α -linolenic acid was most abundant in the HQF. About 61% of fatty acids in ryegrass were represented by α -linolenic acid. Beside α -linolenic acid, silage contained large amount of palmitic and linoleic acid and rapeseed plant contained large amount of heneicosanoic (C21:0) and linoleic acids. Rapeseed plant also contained large amount (9.7%) of an unexpected fatty acid that was not used in the standard. The retention time for this fatty acid was 132.42 min which suggests that this fatty acid was either a much longer chain fatty acid or that it had more double bonds. The unidentified fatty acid was assumed as PUFA and so it was included as PUFA of rapeseed plant.

***In vitro* degradability of forages:** There were statistically significant differences between the IVD of the forages as shown in Table 4, where HQF had higher values than those of LQF ($P < 0.001$). Within LQF, the IVD was highest for hay and lowest for sugarcane bagasse ($P < 0.002$) at both incubation times and within HQF the IVD of ryegrass and rapeseed plant were higher than silage. The IVD and IVOMD were greater at longer incubation period but for rapeseed plant the difference of IVD at 48 h and 96 h was very low.

Relationship of IVD with mineral contents of forage: A combination of Ca, PHOS and Cu appeared to have shown the best relationships with IVD at 48 and 96h of incubation (Table 5).

DISCUSSION

It appeared that the presence of higher CP, SS and EE and lower fibre in HQF are the main reasons of better nutritive value of HQF. However, to optimize rumen microbial yield through better utilization of CP in HQF, additional soluble sugars may be needed (Bach *et al.*, 2005). Conversely, to increase the nutritive value and utilization of LQF in ruminants, additional supplements containing CP, EE and SS should have to be added when LQF are offered as a feed to the ruminants.

The higher amount of silica (Jackson, 1977; Van Soest, 2006) and other minerals perhaps caused highest ash content in rice straw (Table 1). Conversely, the lowest mineral contents in sugarcane bagasse might have resulted in its lower ash content. Among the four LQF, ryegrass hay appeared to contain relatively a better mix of CP and SS than the other LQF whereas out of the three HQF, dried ryegrass had a better nutrient mix comprising CP, EE and SS than other two HQF. The ryegrass used in the present study was comparatively higher in quality than the average quality ryegrass due to the presence of large amount of CP (Kaur *et al.*, 2010). However, its supplementation with carbohydrate containing feeds will be required to balance the oversupply of CP before feeding to ruminants in different situations (Bach *et al.*, 2005). The ash, ADF and soluble sugars of rapeseed plant of present study were similar with the value of rapeseed plant obtained from Australia (Kaur *et al.*, 2010), but the CP value was higher than the Australian rapeseed plant.

While Tannins, TP and SP are known as antinutritional factors for their detrimental effects (Patra, 2007), their use in smaller amounts to reduce nitrogen loss during mastication and rumen proteolysis has also been reported (Min, *et al.*, 2003). In the present study tannin contents (>10 g kg/ DM) of HQF were within the acceptable range. The mean tannin contents were higher in HQF than LQF ($P > 0.05$). Tannins produced higher molar proportion of propionate and lower protozoal counts in the *in vitro* fermentation system. Makkar *et al.*

(1995) found more short-chain fatty acid production per unit of microbes in the presence of tannins. In fact, Waghorn and Shelto (1997) observed higher animal performance when the diet contained low levels of tannins. While almost tannin free wheat straw had low digestibility (Canbolat, *et al.*, 2007), the high tannin containing HQF of this study appeared to have higher nutritive value.

In the present study saponin contents (>35 g/ kg DM) of HQF were also within the acceptable range (>35 g/ kg DM). In contrast the low SP contents (<10 g/ kg DM) of LQF might be of less interference for rumen fermentation. Makkar and Becker (1996) found that the efficiency of *in vitro* microbial protein synthesis was linearly increased by the addition of Quillaja saponins (0.4-1.2 mg/ ml) to a hay based substrate.

There were not much published data available on oxalate composition of forages. Garcia- Rivera and Morris (1955) observed 0.2-25.7 mg oxalate/g different tropical grasses in USA which compared well with the oxalate results of this study. Some researcher (Jackson, 1977) assumed that rice straw and some other grasses might contain 20 mg oxalate/g, however, in the present experiment low amount of oxalate in rice straw or any other forages were noted.

The LQF being low in most minerals, supplements containing Ca, PHOS, S, Cu, and Co are required to balance the nutrient supply of LQF in order to increase their utilization. Phosphorus deficiency is most widespread and economically important for livestock (Rasby *et al.*, 1998). Phosphorus has been called the master mineral because it is involved in most metabolic processes (Rasby *et al.*, 1998). Animal fed low PHOS diet cannot use energy properly which results in an energy deficiency (Ammerman and Goodrich, 1983). Underwood and Suttle, (1999) reported that temperate forages generally contained more PHOS than tropical forages. Generally, PHOS supplementation increased bacterial population, total VFA concentration and degradability of ammoniated rice straw (Zain *et al.*, 2010). Ferguson and Sklan (2005) reviewed the influence of PHOS on reproduction in cattle and concluded that dairy cattle can safely be fed dietary PHOS concentrations of 0.33% to 0.40% with no negative effects on reproduction or milk production. Sulphur is a component of the amino acids methionine, cysteine and cystine and also some organic substances like B-vitamins, thiamin and biotin (McDonald *et al.*, 2002). Ruminant microorganisms are capable of synthesizing most of the organic sulphur containing organic substances from inorganic sulphur. Sulphur is also required by ruminant microorganisms for their growth and normal cellular metabolism (Meschy, 2000) but its deficiency can cause depressed appetite and digestibility in sheep and cattle. The Cu addition to a ruminant diet can increase rumen microbial activity and forage digestion (Harris, *et al.*,

2003). Khan and Chaudhry (2010) observed positive correlations between IVD and PHOS and Cu of forages. MacPherson, (2000) reported lowest Cu in fresh grass followed by silage and hay which agreed with the present findings. Adding Co to the diet of ruminants also had increased rumen microbial activity and enhanced forage digestion (Harris, *et al.*, 2003). Rasby *et al.*, (1998) reported Ca deficiency in LQF which can cause Ca

deficiencies if fed to high yielding dairy cattle due to potential Ca depletion in the form of milk resulting in milk fever (Wilde, 2006). So, PHOS, S, Cu, Ca and Co supplementation of LQF would be needed to increase the degradability and utilization of LQF in ruminants. PHOS was also found to be more effective to increase the IVD of forages.

Table 1 Proximate composition (mg/ g DM, unless stated otherwise), fibre contents, total Phenolics (TP), Tannins (T), Saponins (SP), Soluble Sugars (SS) and oxalate (mg/ g DM) of different forages (Updated from Khan and Chaudhry, 2010)

Forage Groups	Forage Types	DM Mg/ g	Ash	CP	EE	NDF	ADF	ADL	TP GE	T GE	SP DE	SS	Oxalate
LQF	Rice straw (RS)	951	161	40.8	12.1	676	481	153	5.3	1.25	3.3	34.7	0.444
	Wheat straw (WS)	939	59	23.2	15.6	790	522	160	3.0	0.56	4.3	18.7	0.512
	Ryegrass hay (HAY)	954	68	66.4	14.0	747	413	156	4.7	1.09	8.3	161.5	0.658
	Sugarcane bagasse (SB)	955	15	18.9	29.0	881	592	71	2.9	0.78	4.5	52.0	0.293
	SEM to compare means within LQF	1.9	19.9	6.9	0.25	30.5	26.5	11.9	0.43	0.12	0.77	2.1	0.05
Within LQF P<		0.03	0.001	0.001	0.002	0.002	0.05	0.05	0.04	0.2	0.08	0.008	0.001
HQF	Silage (SIL)	914	79	183	36.0	524	340	163	7.8	1.00	14.0	134	0.293
	Rapeseed plant (RP)	945	135	283	36.5	184	141	50	8.6	1.08	14.0	164	0.512
	Dried Ryegrass (RG)	943	89	286	37.1	548	220	39	17.5	2.0	33.9	164	0.440
	SEM to compare means within HQF	5.7	4	21	0.034	78.7	26.3	20.5	1.97	0.21	4.5	0.91	0.04
	Within HQF P<	0.003	0.03	0.001	0.6	0.002	0.009	0.004	0.001	0.004	0.05	0.4	0.001
SEM to compare LQF v HQF	3.35	11.2	30.4	2.95	61.7	39.5	13.0	1.31	0.122	2.84	17.2	33.3	
Between LQF & HQF P<	0.02	0.93	0.001	0.001	0.001	0.001	0.04	0.002	0.08	0.003	0.007	0.4	

LQF = Low quality forages; HQF = High quality forages;
 GE = Gallic acid equivalent; DE = Diosgenin equivalent;
 SE=Standard error mean within groups; SEM= Standard error mean between groups

Table 2 Mean mineral components (mg/ kg DM) of different forages

Feed Groups	Feed Types	Ca	K	Mg	Na	S	P	Cu	Co	Mn	Se	Zn
LQF	RS	2588	13308	2071	3057	949	861	1.81	1.023	142.8	4.46	24.5
	WS	1602	2332	368	230	694	225	0.94	0.516	14.3	3.22	11.4
	Hay	3354	9279	993	1215	1535	1313	7.33	1.167	69.9	4.72	29.2
	SB	524	1099	183	133	355	191	0.05	1.104	7.7	3.60	10.4
	SEM	321	1512	222	354	131	141	0.86	0.08	16.4	0.8	2.5
Within LQF P<		0.001	0.94	0.001								
HQF	SIL	2533	17742	926	715	1664	2081	2.65	1.335	26.3	4.61	24.4
	RG	1918	22638	795	590	3049	2067	2.14	1.791	15.7	1.48	15.0
	RP	8385	27639	1308	536	4460	3453	0.18	1.479	14.6	6.08	18.1
	SEM	951	1279	72.2	23.8	404	252	0.34	0.07	0.52	0.495	1.88
	Within HQF P<	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.08
SEM for LQF v HQF	504	2017	123	199	303	233	0.49	0.084	9.80	0.487	1.43	
Between LQF v HQF P<	0.05	0.001	0.8	0.2	0.001	0.001	0.5	0.001	0.04	0.6	0.98	

*Updated from Khan and Chaudhry, 2010

LQF = Low quality forages; HQF = High quality forages

RS = Rice straw; WS = Wheat straw; Hay = Ryegrass hay; SB = Sugarcane bagasse; SIL = Ryegrass silage; RP = Rapeseed plant and RG = Dried ryegrass

SE =Standard error mean within groups; SEM = Standard error of means to compare means within LQF or HQF and between LQF v HQF

Table 3: Fatty acid composition (% of total fatty acids) of different forages

Fatty Acids	Carbon no.	RS	WS	HAY	SB	SIL	RP	RG
Caprylic	C6:0	0.00	0.00	0.00	0.00	0.04	0.00	0.00
Capric	C8:0	0.00	0.00	0.00	0.00	0.03	0.00	0.00
Lauric	C12:0	1.39	0.82	0.76	0.37	0.63	0.47	0.98
Tridecanoate	C13:0	0.00	0.00	0.00	0.00	0.11	0.14	0.00
Myristic	C14:0	1.94	5.69	0.88	0.31	0.52	0.20	0.34
Pentadec	C15:0	2.46	1.22	0.00	0.23	0.15	0.14	0.08
Palmitic	C16:0	35.59	20.08	33.89	18.78	25.43	7.71	13.34
Heptadeca	C17:0	3.94	2.24	3.04	0.98	0.61	0.27	0.39
Stearic	C18:0	10.70	7.39	11.78	9.04	5.04	1.47	3.01
Arachidic	C20:0	8.23	6.51	6.66	2.17	1.36	0.56	0.92
Heneicosanoic	C21:0	0.00	0.00	7.08	1.49	0.65	26.91	1.52
Behenic	C22:0	6.74	7.24	5.90	2.07	1.95	0.43	1.46
Tricosanoic	C23:0	0.00	0.00	0.00	1.03	1.43	0.00	0.00
Lignoceric	C24:0	6.34	4.15	3.72	3.11	1.01	0.02	0.87
Total saturated fatty acids		77.33	55.34	73.71	39.58	38.96	38.32	22.91
Myristoleic	C14:1	0.00	0.00	0.00	0.00	0.06	0.08	0.00
cis Palmitic	C16:1 Δ^9	0.41	0.00	0.00	0.10	0.19	2.24	2.41
cis-Heptadeca	C17:1 Δ^{10}	0.00	1.02	0.00	0.00	0.00	0.00	0.00
Elaidic	C18:1 $\Delta^9(t)$	0.00	0.00	0.00	0.00	0.00	0.80	0.00
Oleic	C18:1 Δ^9	7.97	6.49	6.12	16.03	6.33	7.39	1.35
Eicosenoic c11	C20:1 Δ^{11}	0.00	0.00	0.00	0.28	0.00	0.12	0.00
Erucic	C22:1 Δ^{13}	1.14	0.00	0.00	0.11	0.00	0.09	0.00
Total mono unsaturated fatty acids		9.52	7.51	6.12	16.52	6.58	10.72	3.76
Linolelaidic	C18:2 $\Delta^9\Delta^{12}(t)$	0.00	0.00	0.00	0.00	0.17	0.00	0.00
Linoleic	C18:2 $\Delta^9\Delta^{12}$	3.65	3.42	3.29	37.41	18.50	11.50	10.29
α Linolenic	C18:3 $\Delta^9\Delta^{12}\Delta^{15}$	5.05	17.54	2.72	4.79	34.69	28.42	60.91
γ Linolenic	C18:3 $\Delta^6\Delta^9\Delta^{12}$	0.00	0.00	0.00	0.00	0.00	0.08	0.20
Eicosadienoic c11,14	C20:2 $\Delta^{11}\Delta^{14}$	0.00	0.00	0.00	0.03	0.00	0.09	0.00
cis-11,14,17 Eicosatrienoic	C20:3 $\Delta^{11}\Delta^{14}\Delta^{17}$	0.00	0.00	0.00	0.16	0.00	0.00	0.00
Arachidonic	C20:4 $\Delta^5\Delta^8\Delta^{11}\Delta^{14}$	4.45	14.43	8.48	1.51	0.16	1.15	1.00
Docosahexaenoic	C22:6 $\Delta^4\Delta^7\Delta^{10}\Delta^{13}\Delta^{16}\Delta^{19}$	0.00	1.76	5.69	0.00	0.93	0.00	0.96
RT132.417		0.00	0.00	0.00	0.00	0.00	9.72	0.00
Total poly unsaturated fatty acids		13.15	37.15	20.18	43.9	54.45	50.96	73.36
		100.00	100.00	100.00	100.00	100.00	100.00	100.00

RS = Rice straw; WS = Wheat straw; Hay = Ryegrass hay; SB = Sugarcane bagasse; SIL = Ryegrass silage; RP = Rapeseed plant and RG = Dried ryegrass

Normally it is believed that forages represent a high proportion of fatty acids, particularly PUFA (Boufaied *et al.*, 2003). In grass fed ruminants, the major dietary lipids are chloroplast membrane containing galactolipid, sulpholipid and phospholipids (Harfoot, 1981). The main fatty acids being present in glycolipids and phospholipids were α -linolenic acid (Hawke, 1973), whereas animals receiving dietary lipid supplements were linoleic acid. As linoleic acid has two double bonds it can easily be biohydrogenated to stearic acid but α -linolenic acid has three double bonds, so the process of biohydrogenation is complex (Harfoot and Hazelewood, 1997). Recent studies showed dietary PUFA, specially n-3 had positive effects on reproduction (Coyne *et al.*, 2008; Petit *et al.*, 2002) and immunity (Lessard *et al.*,

2004) in dairy cows. In the present study large amount of PUFA in HQF also suggested that the HQF had a better nutritive value. The α -linolenic acid of ryegrass in the present study were comparable to those that were reported by Palladino *et al.*, (2009) for twelve different cultivators of perennial ryegrass and Hawke, (1973) for different herbage. The greater variation in the fatty acid contents of silage could be due to the ensiling process which results in the loss of nutrients during, the anaerobic fermentation of freshly cut or wilted grass. Rapeseed plant contained large amount of exceptionally long chain fatty acid Heneicosnoic acid.

In contrast LQF contained more SFA that means during maturation and processing many PUFA were oxidized to SFA. Likewise, Dewhurst *et al.* (2006), Aii *et*

al. (1988) and Boufaied *et al.* (2003) also reported losses or changes of fatty acids during forage conservation and storage. Khan *et al.* (2009) reported substantial decrease in the concentrations of C18:3, C18:2 and total FA of grass silages during even 24 h of their exposure to air. The fatty acids of rice straw had similarities with the finding of Shahjahan *et al.* (1992) who also found large amount of C18:0 in rice straw. Among the LQF only sugarcane bagasse contained large amount of PUFA especially linoleic acid that had two double bonds. When sugarcane was processed to obtain sugar containing liquids, its PUFA were perhaps less oxidized and so contained greater amount of PUFA. Higher docosahexaenoic acid in hay could contribute in the reproduction process of dairy cows (Coyne *et al.*, 2008). As PUFA can increase the quality of meat and milk by increasing n-3 PUFA, vaccenic acid and conjugated

linoleic acid (Dewhurst *et al.*, 2006; Scollan *et al.*, 2006) they may also help reduce methane (Giger-Reverdin *et al.*, 2003; Dong *et al.*, 1997) but improve reproduction (Coyne *et al.*, 2008; Petit *et al.*, 2002) and immunity (Lessard *et al.*, 2004). Therefore, PUFA containing supplements could help increase the utilization of LQF.

Due to the higher nutrient and lower fibre contents in HQF, their IVD and IVOMD were higher in this study. Khan and Chaudhry (2010) reported positive correlations of IVD and IVOMD of forages with their CP and SS. It was mentioned earlier that, the nutritive value of ryegrass was more acceptable and sugarcane bagasse was least acceptable which was re-confirmed by their respective IVD and IVOMD in this study. Indeed the forages containing more CP were found to be more degradable than others.

Table 4: IVD and IVOMD (mg/g) of different types of forages and pH of rumen fluid incubated with different types of forages at 48 and 96 h

Time hour		48			96		
Forage Groups	Forage Types	IVD	IVOMD	pH	IVD	IVOMD	pH
LQF	RS	423	402	7.24	568	565	7.03
	WS	300	265	7.22	479	482	7.10
	Hay	557	518	7.04	739	714	6.98
	SB	172	107	7.32	256	231	7.19
	SEM	55.7	58.1	0.039	65.8	66.4	0.032
Within LQF P<		0.002	0.001	0.001	0.001	0.001	0.06
HQF	SIL	793	763	6.98	835	823	6.96
	RP	891	885	7.10	906	903	7.12
	RG	886	880	7.05	941	920	7.01
	SEM	20.7	25.7	0.022	22.3	22.0	0.03
Within HQF P<		0.02	0.007	0.02	0.2	0.2	0.003
SEM for LQF v HQF		75.4	78.9	0.032	64.8	64.9	0.022
LQF v HQF P<		0.001	0.001	0.007	0.001	0.001	0.4

LQF = Low quality forages; HQF = High quality forages

RS = Rice straw; WS = Wheat straw; Hay = Ryegrass hay; SB = Sugarcane bagasse; SIL = Ryegrass silage; RP = Rapeseed plant and RG = Dried ryegrass SEM= Standard error of means to compare means within LQF or HQF and means of LQF v HQF

Table 5: The stepwise regression equations, R² and P values for selected minerals and IVD at 48 and 96 h of forage incubations

Predictor	Equation	R ²	P <
IVD (48 h)	226 + 14.3 Cu - 0.0595 Ca + 0.337 PHOS	95.1	0.001
IVD (96 h)	346 + 26.3 Cu - 0.0281 Ca + 0.242 PHOS	88.8	0.001

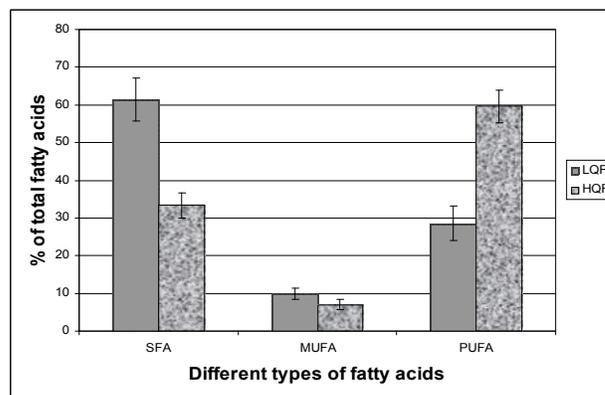


Figure 1: Different categories of fatty acids in different types of forages

LQF = Low quality forages; HQF = High quality forages
SFA = Saturated fatty acids; MUFA = Mono unsaturated fatty acids; PUFA = Poly unsaturated fatty acids

Conclusion: LQF were not only deficient in energy and CP but also other nutrients. It is recognized that the deficiency of these nutrients can cause reduction in the degradability of these LQF. Therefore, to increase the degradability and optimum utilization of these LQF, appropriate amounts of HQF can be tested as potential additives in the future studies.

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REFERENCES

- Aii, T., S. Takahashi, M. Kurihara, and S. Kume (1988), The effects of Italian ryegrass hay, haylage and fresh Italian ryegrass on the fatty acid composition of cows' milk, *Japanese J. Zootechnical Sci.*, 59(8): 718-724.
- Ammerman, C. B., and R. D. Goodrich (1983), Advances in Mineral Nutrition in Ruminants, *J. Anim. Sci.*, 57(Supp.2): 519-533.
- AFRC (1993), Energy and protein requirements of ruminants., 175 pp., CAB International.
- AOAC. (1990), Official methods of Analysis, 15th ed., Association of Official Analytical Chemists, Washington D.C.
- Bach, A., S. Calsamiglia, and M. D. Stern (2005), Nitrogen metabolism in the rumen, *J. Dairy Science*, 88(esuppl-1): E9-21.
- Boufaied, H., P. Y. Chouinard, G. F. Tremblay, H. V. Petit, R. Michaud, and G. Belanger (2003), Fatty acids in forages. I. Factors affecting concentrations, *Canadian J. Anim. Sci.*, 83(3): 501-511.
- Canbolat, O., C. O. Ozkan, and A. Kamalak (2007), Effects of NaOH treatment on condensed tannin contents and gas production kinetics of tree leaves. (Special issue: Nutrition technologies in animal feed science and technology.), *Anim. Feed Sci. and Tech.*, 138(2): 189-194.
- Chaudhry, A. S. (1998), Chemical and biological procedures to upgrade cereal straws for ruminants, *Nutrition Abstracts and Reviews*, 68(5): 319-331.
- Chaudhry, A. S. (2008), Slaughtered cattle as source of rumen fluid to evaluate supplements for in vitro degradation of grass nuts and barley straw, *The Open Vet. Sci. J.*, 2:16-22
- Coyne, G. S., D. A. Kenny, S. Childs, J. M. Sreenan, and S. M. Waters (2008), Dietary n-3 polyunsaturated fatty acids alter the expression of genes involved in prostaglandin biosynthesis in the bovine uterus, *Theriogenology*, 70(5): 772-782.
- Devendra, C., and C. C. Sevilla (2002), Availability and use of feed resources in crop-animal systems in Asia *Agricultural Systems*, 71(1-2): 59-73.
- Dewhurst, R. J., K. J. Shingfield, M. R. F. Lee, and N. D. Scollan (2006), Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems, *Anim. Feed Sci. and Tech.*, 131(3-4): 168-206.
- Dong, Y., H. D. Bae, T. A. McAllister, G. W. Mathison, and K. J. Cheng (1997), Lipid-induced depression of methane production and digestibility in the artificial rumen system (RUSITEC), *Canadian J. Anim. Sci.*, 77(2): 269-278.
- Ferguson, J. D., and D. Sklan (2005), Effects of dietary phosphorus and nitrogen on cattle reproduction, in *Nitrogen and phosphorus nutrition of cattle*, edited by E. Pfeffer and A. Hristov, CABI Publishing, Wallingford, Oxfordshire, UK.
- Garcia-Rivera, J., and M. P. Morris (1955), Oxalate content of tropical forage grasses, *American Association for the Advancement of Science. Science*. 122: 1089-1090.
- Giger-Reverdin, S., P. Morand-Fehr, and G. Tran (2003), Literature survey of the influence of dietary fat composition on methane production in dairy cattle, *Livestock Prod. Sci.*, 82(1): 73-79.
- Goering, H. K., and P. J. Van Soest (1970), Forage fibre analysis, in *Agriculture Hand book*, edited, US Department of Agriculture.
- Harfoot, C. G. (1981), Lipid metabolism in the rumen in Lipid metabolism in ruminant animals, edited by W. W. Christie, pp. 21-55, Pergamon Press, Oxford, UK.
- Harfoot, C. G., and G. P. Hazlewoodm (1997), Lipid metabolism in the rumen, in *The Rumen Microbial Ecosystem*, edited by P. N. Hobson and C. S. Stewart, pp. 382-426, Blackie Academic and Professional, London, UK.
- Harris, B., A. L. Adams, and H. H. Van Horn (2003), *Mineral Needs of Dairy Cattle*, edited, Animal Science Department, Institute of Food and Agricultural Sciences, University of Florida, Florida, USA.
- Hawke, J. C. (1973), Lipids, in *Source Chemistry and biochemistry of herbage*, edited by G. W. Butler and R. W. Bailey, pp. 213-263, Academic Press Inc., London, UK.
- Jackson, M. G. (1977), Rice straw as livestock feed, *World Animal Review*, 23: 25-29.
- Kaur, R., S. C. Garcia, W. J. Fulkerson, and I. Barchia (2010), Utilisation of forage rape (*Brassica napus*) and Persian clover (*Trifolium resupinatum*) diets by sheep: effects on whole tract digestibility and rumen parameters, *Animal Prod. Sci.*, 50(1): 59-67.
- Khan, M. M. H., and A. S. Chaudhry (2010), Chemical composition of selected forages and spices and the effect of these spices on in vitro rumen degradability of some forages Asian-Australasian *J. Anim. Sci.* 23(7): 889-900.

- Khan, N. A., J. W. Cone, and W. H. Hendriks (2009), Stability of fatty acids in grass and maize silages after exposure to air during the feed out period, *Anim. Feed Sci. and Tech.*, 154(3-4): 183-192.
- Lessard, M., N. Gagnon, D. L. Godson, and H. V. Petit (2004), Influence of parturition and diets enriched in n-3 or n-6 polyunsaturated fatty acids on immune response of dairy cows during the transition period *J. Dairy Sci.*, 87(7): 2197-2210.
- MacPherson, A. (2000), Trace-mineral status of forages, in *Forage evaluation in ruminant nutrition*, edited by D. I. Givens, E. Owen, R. F. E. Axford and O. H.M., pp. 345-371, CABI Publishing, Wallingford, Oxfordshire, UK.
- Makkar, H. P. S. (2003), Quantification of tannins in tree and shrub foliage: a laboratory manual Kluwer Academic Publishers, AA Dordrecht, The Netherlands.
- Makkar, H. P. S., P. Siddhuraju, and K. Becker (2007), *Plant Secondary Metabolites*, Humana Press Inc., Totowa.
- Makkar, H. P., M. Blummel, and K. Becker (1995), In vitro effects of and interactions between tannins and saponins and fate of tannins in the rumen, *J. Sci. Food and Agri.*, 69(4): 481-493.
- McDonald, P., R. A. Edwards, J. F. Greenhalgh, and C. A. Morgan (2002), *Animal Nutrition*, Ed. 6, x + 607 pp., Longman Group Limited Harlow.
- McDougall, E. I. (1948), Studies on ruminant saliva. I. The composition and output of sheep's saliva, *Biochemistry Journal*, 43: 99-109.
- Meschy, F. (2000), Recent progress in the assessment of mineral requirements of goats, *Livestock Prod. Sci.*, 64(1): 9-14.
- Min, B. R., T. N. Barry, G. T. Attwood, and W. C. McNabb (2003), The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review, *Anim. Feed Sci. and Tech.*, 106(1-4): 3-19.
- Palladino, R. A., M. O'Donovan, E. Kennedy, J. J. Murphy, T. M. Boland, and D. A. Kenny (2009), Fatty acid composition and nutritive value of twelve cultivars of perennial ryegrass, *Grass and Forage Sci.*, 64(2): 219-226.
- Patra, A. K. (2007), Nutritional management in organic livestock farming for improved ruminant health and production - an overview, *Livestock Res. Rural Develop.*, 19(3): 41.
- Petit, H. V., R. J. Dewhurst, N. D. Scollan, J. G. Proulx, M. Khalid, W. Haresign, H. Twagiramungu, and G. E. Mann (2002), Milk production and composition, ovarian function, and prostaglandin secretion of dairy cows fed omega-3 fats, *J. Dairy Sci.*, 85(4): 889-899.
- Rasby, R., D. Brink, I. Rush, and D. Adams (1998), *Minerals and vitamins for beef cows*, edited, Institute of Agriculture and Natural Resources, University of Nebraska, Nebraska, USA.
- Santos, M. B., G. A. Naderc, P. H. Robinsona, D. Kirand, U. Krishnamoorthy, and G. M.J. (2010), Impact of simulated field drying on in vitro gas production and voluntary dry matter intake of rice straw, *Anim. Feed Sci. and Tech.*, 159: 96-104.
- Scollan, N., J. F. Hocquette, K. Nuernberg, D. Dannenberger, I. Richardson, and A. Moloney (2006), Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality, *Meat Science*, 74(1): 17-33.
- Shahjahan, M., M. Mosihuzzaman, and A. J. Mian (1992), Analysis of fatty acid in some local and high yielding varieties of rice straw (*Oryza sativa*), *Bangladesh J. Scientific and Industrial Res.*, 27(3/4): 148-153.
- Sukhija, P. S., and D. L. Palmquist (1988), Rapid method for determination of total fatty acid content and composition of feedstuffs and feces, *J. Agri. and Food Chem.*, 36(6): 1202-1206.
- Underwood, E. J., and N. F. Suttle (1999), *The Mineral Nutrition of Livestock*, 3 ed., 614 pp., CABI Publishing.
- Van Soest, P. J. (1963), Use of detergents in the analysis of fibrous feeds. 2. A rapid method for the determination of fiber and lignin, *Journal of the Association of Official Agricultural Chemists*, 46: 829-835.
- Van Soest, P. J. (2006), Rice straw, the role of silica and treatments to improve quality, *Anim. Feed Sci. and Tech.*, 130(3/4): 137-171.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis (1991), Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition, *J. Dairy Sci.*, 74(10): 3583-3597.
- Waghorn, G. C., and I. D. Shelton (1997), Effect of condensed tannins in *Lotus corniculatus* on the nutritive value of pasture for sheep, *J. Agri. Sci.*, 128(3): 365-372.
- Wilde, D. (2006), Influence of macro and micro minerals in the peri-parturient period on fertility in dairy cattle, *Anim. Reprod. Sci.*, 96(3-4): 240-249.
- Zain, M., R. Ninggrat, N. Jamarun, and T. A (2010), Effect of phosphorus supplementation of ammoniated rice straw on rumen fermentability, synthesised microbial protein and degradability in vitro, paper presented at Annual Conference, British Society of Animal Science, Belfast, Northern Ireland, UK, 12th to 14th April.