Chemical composition of selected forages and spices and the effect of these spices on *in vitro* rumen degradability of some forages

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Running heading: Spice supplementation and *in vitro* forage degradability

ABSTRACT: Spices can be used as novel supplements to enhance the utilization of low quality forages (LQF) and reduce nutrient wastage by the ruminant animals. However, it would be essential to characterize these spices alongside LQF before testing their potential use as supplements in ruminant diets. This study characterized four spices (cinnamon, cumin, clove and turmeric) alongside three forages (rice straw, wheat straw and hay) for their chemical components before evaluating their effect at four different doses (0, 10, 30 and 90 mg/g forage DM) on the *in vitro* rumen degradability of dry matter (DM) (IVD) and organic matter (OM) (IVOMD) of these forages at various incubation times. It appeared that some spices could provide complementary nutrients which could improve the utilization of LQF where hay had better chemical composition than the other two forages. Cumin contained more crude protein (CP), ether extract and mineral contents whereas turmeric contained more soluble sugars than the other spices. Cinnamon was least acceptable as a ruminant supplement due to its higher condensed tannin and saponin and lower CP and mineral contents. The IVD and IVOMD were highest for hay and lowest for wheat straw with all spices at all incubation times ($P<0.001$). Due to relatively better nutrient profiles, cumin and turmeric had greater effect on IVD and IVOMD of the forages. In contrast**,** cinnamon had negative effect on IVD and IVOMD. IVD and IVOMD were greater at 10 mg/g than other levels of most spices suggesting that using certain amounts of spices can increase forage degradability. However, the choice of a spice will depend upon the forage type being offered to the ruminants. Further studies will examine the effect of these spices on fermentation profile, methane production and nitrogenous loss by ruminants.

(**Key words:** Spices, Low quality forages, Nutritive value, *In vitro* degradability, Ruminant)

INTRODUCTION

Ruminant animals are mainly dependent on forages as these are essential to maintain their health and production at various stages of their development and growth. In developed countries, sufficient grazing land is available so ruminants can get adequate amount of green grasses during grazing seasons and when it is not possible in other season they are supplied with silage and other high quality conserved forages. Conversely, green forages are not abundantly available in some developing countries, so ruminants are mainly supplied with low quality forages (LQF) like cereal straws (Makkar, 2005). The longevity and production are adversely affected when ruminants are reared with poor quality forage. To get more production from these ruminants it is necessary to enhance the utilization of these LQF. It may be possible to increase the nutritive value of these low quality forages through either biological and chemical procedures (Chaudhry, 1998) . During the last five decades many studies were done to improve the quality of these forages by using different biochemical treatments. But improving the quality of forages by using these treatments was not always successful. Supplementation is another tool to improve the quality of LQF by adding nutrients that otherwise are low in these forages (Chaudhry, 2008, Khandaker et al., 1998, Muetzel et al., 2003). Supplements increase the utilization of LQF, but the requirement for these supplements is more than their availability in many developing countries (Devendra and Sevilla, 2002).

Spices which have long been safely used for human consumption could be tested as alternative supplements to enhance forage utilization and reduce nutrient wastage from ruminant livestock. Recently several researchers have used some plant extracts to manipulate rumen fermentation (Patra et al., 2006a; Busquet et al., 2005, Cardozo et al., 2004). But obtaining these extracts from plants will be costly as the extraction process will require expensive instruments and the farmers from developing countries will not be able to afford such technology. Besides, only a small quantity of these plants will be available as extracts and the rest of such plants will be unused and wasted. Furthermore, the whole spices may contain some other useful components that can differ from their small amounts of extracts and these also can have more desirable impacts on degradability and fermentation. Therefore, it is necessary to chemically analyze these spices before testing their potential use as supplements for LQF consuming ruminants to enhance forage degradability. Therefore this study evaluated four different spices alongside three LQF for their chemical components in order to examine the suitability of different levels of these spices on the *in vitro* rumen degradability of LQF at different incubation times.

MATERIALS AND METHODS

Experimental work plan

A series of laboratory experiments were conducted to characterize selected spices and LQF for their chemical composition and then the effect of these spices on *in vitro* rumen degradability of LQF. Different methods were used to determine chemical compositions of three forages; rice straw (*Oryza sativa*), wheat straw (*Triticum Sp*) and rye grass hay (*Lolium perenne*) and four spices; turmeric (*Curcuma longa*), cinnamon (*Cinnamomum cassia*), cumin (*Cuminum cyminum*) and clove (*Syzygium aromaticum*). A 3x4x4 factorial arrangement in duplicate for each incubation time of 20 h, 40 h and 60 h was used to assess the degradability of three forages with four spices at four levels of 0, 10, 30 and 90 mg/g forage DM. Further details are given in the following sections:

Collection of forages and spices

Representative samples of rice straw (Variety, IR50) were collected from Bangladesh, whereas those of wheat straw (Variety, Einstein), and ryegrass hay were collected from the Newcastle University's Farm. Spice samples (cinnamon, cumin, clove, coriander and turmeric) of Indian origin were collected from the local market of Newcastle upon Tyne. The samples of forages and spices were dried at 60^{o}C in an oven and ground through a 1 mm sieve by using a grinder (Christy mill, Christy and Norris Ltd, Suffolk, United Kingdom)

Chemical composition of forages and spices

All chemicals and reagents used in this study were obtained either from Fischer Scientific UK or VWR unless otherwise stated for the determination of different chemical components as described in the following sections.

Proximate and Cell wall composition

All samples of forage and spices were analyzed in triplicate for DM, ash and ether extract (EE) by using methods of AOAC (1990). All these samples were analyzed for EE with the help of soxhlet apparatus. Nitrogen (N) contents of these samples were determined by using Leco (model FP-428, Leco corporation St. Joseph. MI, USA) N determinator (Sweeney and Rexroad, 1987) where the samples were combusted by the Dumas method to obtain N values. The N contents were multiplied with 6.25 to determine the CP. Acid detergent fibre (ADF), acid detergent lignin (ADL) and neutral detergent fibre (NDF) of different forages and spices were determined by the methods of Van Soest (1991) and Goering and Van Soest (1970) .

Total soluble sugars

Total soluble sugars (SS) were determined according to the method of Hall and Haczkaylo (1963) by mixing 1 g ground sample with 40 ml 80% ethanol while sonicating the mixture for 1 h in a conical flask. The contents were then filtered where the residue was used for starch determination and the supernatant was used for SS determination after evaporating the ethanol by using a vacuum evaporator. The evaporated aqueous solution was carefully transferred to a 100 ml volumetric flask and made up to volume with water. Diluted solution of 1 ml was taken in a test tube to which 4 ml anthrone reagent (2% into concentrate H_2SO_4) was added and the contents mixed thoroughly. The intensity of the colour developed due to the presence of sugar was measured at 620 nm in a spectrophotometer (Biochrom Libra, S12). A standard curve was prepared from the known concentration of pure glucose and SS in the sample was calculated with reference to the standard curve.

Starch, oligosaccharides and non cellulose carbohydrates

The starch and oligosaccharides (SO) analysis was performed according to the method of Kent– Jones and Amos (1967). The residue of an alcohol washed sample was refluxed for 2.5 h with 100 ml/L HCl solution. It was cooled and neutralized with 5N NaOH solution. The hydrolyzed sample was then titrated against the standardized freshly made Fehling's solution to determine the reducing sugar (Lewis and Lane, 1931). It comprised of equal parts of the two solutions; Fehling's solution A (6.928 % $CuSO_4.5H_2O$ in distilled water) and Fehling's Solution B (34.6 % Rochelle salt and 12% NaOH distilled water). During refluxing the residue some other carbohydrates were also converted to monosaccharides. To correct it, the values for reducing sugars were converted into starch content by multiplying with the factor 0.9 (Kent-Jones and Amos, 1967). Non cellulose carbohydrates (NCC) were calculated by adding the value of SS and SO.

Total phenolics

The Folin-Ciocalteu method (Singleton and Rossi, 1965) with some modification was used to determine total phenolics (TP). Extracts for the TP determination were prepared by suspending 0.125 g samples in 6.25 ml of 75% aqueous acetone in test tubes. The contents of the test tubes were mixed at 5 minutes (min) intervals for 2 h and 20 min using a vortex mixer (Whirlimix, Fison Limited). Aliquots (1ml) of each extract were mixed with 5 ml Folin-Ciocalteu reagent in 100 ml volumetric flasks that contained 70 ml of deionised water. After waiting for up to 8 minutes 15 ml Na_2CO_3 solution (20%) was added. The volumetric flasks were then made up to volume with deionised water. After standing for 2 hours at room temperature, the absorbance was read at a wavelength of 760 nm in the visible range of the spectrum using a UV/VIS-spectrophotometer (Biochrom Libra, S12). The estimation of total phenolics (TP) in extracts was carried out in duplicate for all samples. Gallic acid was used as a standard and the results obtained were expressed as mg gallic acid equivalent per g of sample DM.

Condensed tannins

Total condensed tannins (CT) were determined according to Osman (2004) with some modification. Extracts for the determination of CT were prepared by suspending 0.125 g samples in 6.25 ml of acidified methanol (1% HCl in methanol) in test tubes for 24 h. The contents of the test tubes were vortex mixed at 5 min and centrifuged for 6 min at 3000 rpm. After centrifugation, extracts were assayed for CT. Vannilin HCl reagent was freshly prepared by mixing equal volumes of 8% HCl in methanol with 2% vanillin in methanol. One ml of supernatant was mixed with 5 ml of vanillin HCl reagent. The absorbance was read at 500 nm after 20 min of incubation at room temperature. Catechin was used as a standard and the results obtained were expressed as mg catechin equivalent per g of sample DM.

Total saponins

Total saponins (SP) were determined by the method of Hiai et al. (1976) described by Makkar et al. (2007) with some modification. Each sample of 0.5 g was vortex mixed in 10 ml of 80% aqueous methanol in separate test tubes for 4 h. The mixtures were kept at room temperature for 15 h after which the contents of the test tubes were centrifuged for 10 min at 3000 rpm. The supernatant were collected in 25 ml volumetric flasks. The residue was washed three times, with 5 ml 800 ml/L aqueous methanol followed by vortex mixing. The supernatants were collected in the above volumetric flasks and made volume up to 25 ml with 80% aqueous methanol. In a test tube an aliquot (0.25 ml) was taken and 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml 72% aqueous H_2SO4 were added. The contents in the tube were heated by placing it in a water bath at 60°C for 10 min. The tube was cooled in ice for 4 min and then it was kept at room temperature. The intensity of the colour developed due to the presence of SP was measured as optical density in a spectrophotometer (Biochrom Libra, S12) at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of sample DM.

Macro and micro mineral contents

About 1 g dried and ground sample was placed into a Kjeldahl tube and 20 ml pure HNO_3 were added. The sample was digested in Kjeldahl digestion chamber at 100°C and the digested sample was diluted to the original volume of 20 ml with water. The samples were filtered through Whatman filter papers no 541 and the concentrations of selected minerals were determined with inductively coupled plasma optical emission spectroscopy (ICP-OES) with Unicam 701 ICP-OES. The machine was calibrated over the relevant concentrations using individually certified standards obtained from Sigma-Aldrich, UK.

***In vitro* degradability trial**

Collection of rumen fluid from fistulated sheep

Rumen fluid (RF) was obtained from two fistulated sheep (Lleyn breed) with mean live-weight of 81 kg just 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 requirement (AFRC, 1993). The concentrate consisted of (% DM) soybean meal (20), maize gluten feed (15), rolled barley (27.5), sugar beet pulp (25), soy pass (2.5), molasses (7.5) and vitamin and mineral supplement (2.5). The RF was transported in insulated flasks under anaerobic conditions to the laboratory. The RF was strained through four layers of a cheese cloth into pre-warmed flasks under CO_2 before its mixing with the pre-warmed phosphate-bicarbonate (McDougall, 1948) buffer at 1:4 ratio to prepare the inoculum. The flasks were then screw capped and kept at 39°C in a water bath until used.

***In vitro* incubations, chemical analysis and calculations**

Samples of about 0.4 g dried ground forage were separately weighed into test tubes, dried and ground spices were added according to the experimental work plan in the tubes to which 40 ml of the inoculum were added under CO_2. The tubes were sealed with rubber stoppers containing pressure release valves and incubated at 39°C for the pre-determined times. 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. Residues were washed with distilled water and first dried at 60°C for 48 h and then ignited at 600ºC 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. Degradability of each treatment combination by using the following equation where A is either DM or OM:

$$IVD \text{ or } IVOMD\ (g/kg) = \{(g\ Sample\ A - g\ Residue\ A) / g\ Sample\ A\} * 1000$$

Statistical Analyses

The data for the chemical composition were analyzed by using the analysis of variance in General Linear Model of Minitab to compare different materials within each group of forages and spices for each chemical component at P<0.05. Individual means of chemical components within each group of feeds were compared by using the Tukey's t-test at P<0.05. Standard errors were also calculated where needed to show variation within and between different feed sample groups for the means of different components at P<0.05. The data of IVD and IVOMD were also analyzed by using General Linear Model of Minitab in a 3x4x4 factorial arrangement. The main effects of three forages, four spices and four levels and their interactions on each of the IVD and IVOMD were considered for each incubation time. The means of each treatment factor and combination were tested for significance at P<0.05 by using the Tukey's test. The data were further analyzed by using the Pearson's Correlation in Minitab to study possible relationships as determined by 'r' between different chemical components and in vitro degradability of various forages of this study. However, only the satisfactory correlation coefficients are presented in this paper.

RESULTS

Chemical compositions of forages and spices

Mean proximate and fibre contents of different forages and spices are given in Table 1. Significant differences (P<0.03) were observed between forages for CP, ash, NDF and ADF where hay contained more CP but less ADF than other two forages (P<0.002). In contrast hay contained less NDF than wheat straw but more than rice straw (P<0.03). Among forages, rice straw had more CP and less NDF, ADF and ADL than wheat straw but more ash than wheat straw and hay. These forages did not differ significantly for EE (P>0.05). The spices also differed significantly (P<0.003) for all chemical components. Maximum CP and EE were found in cumin (P<0.002) which contained more than double the amount of CP than other spices. Clove contained the highest and cinnamon contained the lowest amount of ash (P<0.001). Turmeric contained very high NDF and very low ADL whereas cinnamon contained the highest amount of ADL (P<0.001) but cumin contained the lowest NDF and ADF compared to the other spices (P<0.003).

Table 2 shows significant differences between different forages and spices for TP, CT, SS, SO and NCC (P<0.02). The forages differed significantly (P<0.001) but not spices (P>0.05) for SP.

Among forages hay contained the highest and wheat straw the lowest TP, CT, SP, SS and NCC (P<0.05). In spices, TP was highest in clove and lowest in cumin (P<0.001) whereas CT and SP were highest in cinnamon and SS, SO and NCC were highest in turmeric (P<0.001). Cumin contained the second highest whereas cinnamon the lowest SS but cumin contained the lowest SO and NCC contents than other spices.

Different forages and spices varied significantly (P<0.001) for most minerals (Table 3). Within forages, hay contained the highest Ca, PHOS, Cu, Co and Zn whereas rice straw contained the highest Na, K, Mg and Mn and wheat straw contained the lowest amounts of most minerals. Within spices, cumin contained the highest amounts of PHOS, Mg, Se and Zn whereas cinnamon contained the lowest amounts of most minerals except Ca which compared well with clove (P>0.05) and Mn which was much greater than turmeric and cumin (P<0.001). Clove contained the highest Ca, Mn and Mg and turmeric was highest for K and Cu.

Effects of spice supplementation on *IVD and IVOMD* of forages

The main effects of forage type, spice type and spice levels and most of their 2 and 3 way interactions were significant for IVD and IVOMD at each incubation time (P<0.001) as shown in Table 4 and 5, respectively. IVD and IVOMD were greater for longer incubation times which were not statistically compared in this study. IVD and IVOMD were highest for hay and lowest for wheat straw at all the incubation times (P<0.001) with all spices. IVD and IVOMD were greater at 10 mg/g forage DM than other spice levels. Cinnamon, clove and turmeric were more effective for 10 mg/g level but cumin was more effective at higher spice levels, hence suggesting spice x level interactions at different incubation times (Tables 4 and 5). At 20 and 60 h, IVD was highest for turmeric whereas at 40 h IVD was highest for cumin (P<0.001). IVOMD was highest for turmeric and cumin at 20 h, for cumin and cinnamon at 40 h and for turmeric at 60 h (Table5).

At 20 h incubation, the maximum IVD of hay, rice straw and wheat straw were in the presence of turmeric, cumin and clove respectively. At 40 h the maximum IVD of hay and rice straw were found in cumin and maximum IVD of wheat straw was found in cinnamon with a significant Forage x Spice interaction (P<0.001). The IVD and IVOMD values changed with the spice type and spice level but the extent of changes depended on the forage type specially at 40 and 60 h of *in vitro* rumen incubations with a significant 3 way interaction (P<0.01; Tables 3-4).

Relationships between forage IVD and saponins, condensed tannins, total phenolics, CP and soluble sugars of forage and supplement

Forage IVD were negatively correlated with SP, CT and TP when each forage IVD was separately correlated with these components (Figures 1-8). However, this correlation did not exist when the mean IVD being averaged over all forages were correlated with these components. At 20 h all the forage showed negative correlation (r) between IVD of forage and SP and the r values (RS = -0.297; WS = -0.390; Hay = -0.505) were significant ($P < 0.03$) for wheat straw and hay. At 40 h the r values were not significant but at 60 h negative correlation between IVD of forage and SP was observed where the r values (RS = -0.434; WS = -0.364; Hay = -0.397) were significant ($P<0.05$) for all forages Like SP, CT also had negative correlation with IVD of forages at 20 h. The r values (RS = -0.517; WS = -0.412; Hay = -0.569) were significant ($P<0.02$) for all forages at 20 h. The correlations between CT and IVD of forages were negative at 40 h for rice straw and hay but not statistically significant. At 60 h the r values (RS = -0.523; WS = -0.504; Hay = -0.461) were negative and significant ($P<0.05$) for all forages. The correlations between TP and IVD of forages were also negative but not statistically significant; however the r values were significant for rice straw at 60 h and for hay at 20 h ($P < 0.04$).

The CP and SS had positive correlations with IVD of forages. When all the three forages were considered together in the presence of spices then significantly ($P<0.001$) positive correlation was observed between total CP and SS content and IVD of forages. The correlation, r, values between total CP and IVD of forages were 0.538, 0.648 and 0.521 at 20, 40 and 60 h respectively and correlation values between SS and IVD of forages were 0.678, 0.702 and 0.7 at 20, 40 and 60 h respectively. If the individual forage is considered then the correlation between total CP and IVD of wheat straw and hay were significant ($P < 0.04$) at 40 h and the r values were 0.481 and 0.371, respectively. The correlation between SS and IVD of forages were significantly ($P<0.02$) positive for rice straw at 20 h and for hay at 20 and 60 h and the r values were 0.410, 0.501 and 0.489, respectively.

Relationshis between Forage IVD and mineral contents of forage and supplement

Some minerals like calcium, phosphorus and copper showed positive correlation with IVD of forages. When all the three forages were considered together in the presence of spices then significantly ($P<0.001$) positive correlation was observed between the above mentioned minerals and IVD of forages. The correlation between PHOS and IVD of forages were significantly ($P<0.03$) positive for rice straw and hay at 20 and 60 h. The correlation values between PHOS and IVD of rice straw were

0.439 and 0.4 at 20 and 60 h respectively and the correlation values between PHOS and IVD of hay were 0.528 and 0.397 at 20 and 60 h respectively. Ca showed significantly ($P<0.001$) positive correlation with IVD of wheat straw at 40 h. The correlation between Cu and IVD of forages were not significant for individual forages.

DISCUSSION

Chemical composition of the forages

The aim of the present study was to characterize the forages and spices and then test the effect of different levels of these spices on the *in vitro* degradability of these forages. The ash, CP, EE, NDF, ADF, of rice and wheat straw of this study were similar to the finding of other researchers (Jackson 1977; Khandaker, et al. 1998; Pan and Sano 2005). The higher ash content of rice straw than all other forages or spices might have been partly due to the higher amount of silica (Jackson 1977; Van Soest 2006) and other minerals.

Phenolic compounds, tannins and SP are known as antinutritional factors due to their detrimental effects on ruminant nutrition (Makkar, 2003, Patra, 2007). High level of tannins in diet (6-12% DM) may depress digestive efficiency and animal productivity (Patra, 2007). But recently it was reported that low amount of tannins and SP had some positive effect on ruminant nutrition (Min et al., 2003, Muetzel et al., 2003). Low level of CT (2-4.5% DM) improved efficiency of N use and increased daily weight gain in lambs consuming temperate forages (Min et al., 2003). The low CT and SP contents (<1% DM) of LQF might be of less interference for rumen fermentation. Due to very low amount of tannins wheat straw is used as a tannin free component in ruminant nutrition research (Canbolat et al., 2007). In the present study TP and SP contents of hay were higher than the other two forages and CT content was higher than wheat straw but these values were within the acceptable range for its use as ruminant feed.

Higher amount of certain minerals increased the nutritive value of hay because these minerals are essential for ruminants for their normal functioning but these minerals are normally deficit in low quality forages. Positive correlation between the forage IVD and Ca, PHOS and Cu showed that these minerals were necessary to increase the forage degradability. Rice straw being an exceptional forage containing high levels of most minerals could be under utilized as ruminant feed due to its deficiency in some vital minerals like PHOS and Cu as reported by McDonald et al., (2002) and other researchers

(Jackson, 1977). If animal feed is low in phosphorus, the animal cannot use energy properly which results in an energy deficient animal (Ammerman and Goodrich, 1983). Adding Cu to the diet of ruminants increased rumen microbial activity and enhanced forage digestion (Harris et al., 2003). So, PHOS and Cu supplementation should be able to increase the degradability of rice straw in ruminants. Wheat straw was low in most of the minerals which was also reported by Makled (1974). In the absence of other supplements, grass hay may be able to slightly compensate for the mineral deficiency of cereal straws if it is offered to the ruminants that are consuming cereal straws. From the chemical composition it can be assumed that among these three forages the nutritive value was highest for hay and lowest for wheat straw.

Chemical composition of the spices

It appears that the spices have the potential to be used as supplements due to their relatively higher CP, EE and SS than forages being observed in this study. Several researchers (Ali et al., 1992, Khanum et al., 2001) also found higher amount of EE, CP and SS in cumin. Although cinnamon and turmeric contained lower amounts of CP and EE than other spices these values were comparable to those of other researchers (Braga et al., 2003, Khanum et al., 2001) for similar spices but these values were higher than the experimental forages. Indeed, the large amounts of SS and SO in turmeric of this study compared well with the values of Braga et al. (2003). The higher CP and EE of clove than cinnamon and turmeric were also comparable to the previous report (Khanum et al., 2001). Due to the better nutrient composition compared with these forages, appropriate amounts of spices could be used as supplements to improve the utilization of these forages by ruminants.

The higher amounts of TP, CT and SP in cinnamon, clove and turmeric of this paper were comparable to the values reported by other researchers (Bamdad et al., 2006, Jayaprakasha et al., 2005, Singh et al., 2004, Variyar and Bandyopadhyay, 1995) who also found higher amount of TP in clove, cinnamon and turmeric. The greater amounts of TP in cinnamon and clove may also have contributed to their high ADL contents (Table 1).

Here spices being rich in many of these minerals could be used as another source to compensate for the deficits of relevant minerals in these forages. The mineral composition of these spices were comparable to the result of other researchers (Ozcan and Akbulut, 2008, Ozkutlu et al., 2007). If combined with forages cumin containing highest amount of Mg, Zn, PHOS and Se and higher amount of Ca, Cu and Co would increase the utilization of low quality forages in ruminants. Higher amount of Ca, Mg, Cu and PHOS in cinnamon and higher Cu and PHOS in turmeric than low quality forages might also be helpful to increase the utilization of low quality forages in ruminants. However, extremely high amount of Mn (NRC, 1996) in clove and cinnamon deserves careful attention if these two spices to be used as supplements for low quality forages in order to avoid any potential detrimental effect of these high Mn containing spices on forage utilization.

Effect of spices on forage degradability

The better nutrient composition of hay might have helped increase the IVD and IVOMD of hay especially in the presence of spices than other two forages. Conversely the lower IVD and IVOMD of wheat straw with or without spices might be due to its lower CP, SS and minerals and higher fibre contents (Table 1-3). Wheat straw showed positive response with spices at 40 h only. The response of spices on forages was greater at 40 h than 20 h where IVD and IVOMD of all forages were higher in the presence of spices than the absence of spices. This longer duration of 40 h might have given the anaerobic microbes in the rumen fluid an opportunity to adapt better to provide more favourable *in vitro* fermentation conditions (Cardozo et al., 2004).

The antimicrobial effect of TP, CT and SP of clove and cinnamon might have reduced the IVD and IVOMD of forages in the presence of higher level of these spices as higher CT, SP and TP showed negative correlations with IVD of forages. Patra, (2007) reported that, high level of tannins in diet (60-120 g/kg DM) might depress digestive efficiency. SP can kill or damage protozoa (Hu et al., 2005), which have some important role in fibre digestion (Mould and Ørskov, 1983). As cinnamon was highest in SP and CT contents its higher level might caused decreases in IVD and IVOMD of forages at all incubation times. Busquest et al. (2005) reported that cinnamaldehyde, an active compound of cinnamon, decreased fibre digestibility in a dual flow continuous culture.

Higher level of clove containing high amount TP might have reduced the forage IVD and IVOMD at high level as TP had negative correlation with IVD of some forages. Clove was lower in CP and EE than cumin but higher than cinnamon and SS was lower than turmeric and cumin but higher than cinnamon (Table 1 and 2), which might have affected forage IVD. Patra et al. (2006b) observed that methanol and ethanol extracts of clove depressed IVD of wheat straw based forage. They also observed lower protozoa counts in the presence of clove extract. Clove was extremely high in Mn and Ca contents but lower in PHOS, Cu and Zn contents (Table 3) which are necessary for proper microbial growth and many enzymatic reactions both in ruminants and microbes (McDonald et al., 2002) and low amount of these minerals in cloves might have reduced IVD of forages in presence of clove.

The higher amount of CP, EE and SS and important minerals like Ca, PHOS, Cu, Co, Mn and Zn of cumin (Table 1-3), that were deficit in low quality forages, might have increased the IVD and IVOMD of forages. These higher mineral contents might have compensated the mineral deficiency in low quality forages which in combination with higher CP might have caused increased IVD and IVOMD as Ca, PHOS and Cu showed positive correlations with the IVD of forages.

The high amount of SS of turmeric might have increased the IVD and IVOMD initially at 20 h, but when the sugar was utilized by the microbes IVD was leveled off at 40 h while its SO contents could have been degraded into glucose which helped increase IVD and IVOMD again at 60 h. Higher Cu content of turmeric (Table 3) also might have increased the forage degradability at longer time. Higher amount of CT and SP of turmeric (Table 2) might have reduced the IVD and IVOMD of forages in the presence of high level; on the other hand lower TP, CT containing cumin increased IVD and IVOMD of forages at higher spice level.

For optimizing rumen microbial yield through better utilization of high CP containing forage, additional SS are needed as a supplement (Bach et al., 2005, Hoover and Stokes, 199). This argument was supported by the results of this study where higher CP containing forage hay showed better performance in the presence of turmeric at 20 h as this spice had higher iSS and SO. IVD of hay also showed significantly positive correlation with SS. On the other hand, as rice straw was lower in CP content it performed better in the presence of higher CP containing cumin at both 20 and 40 h.

It appears that SS might be beneficial in improving the IVD and IVOMD during shorter incubations as SS had positive correlation with the forage IVD at 20 h, whereas starch and CP would be more effective in improving the IVD over longer incubation times as CP showed positive correlation with IVD of forages at 40 h. Higher CT, SP and TP had a negative effect on forage degradability as these three components showed negative correlation with IVD of forages. The study suggested that the spices containing reasonable amounts of minerals such as Ca, PHOS, Cu, Co and Zn can play an important role in the forage degradation by the ruminant animals.

CONCLUSIONS

It appears that the spices being moderate to high nutrient containing materials have the potential for their use as supplements for forages. As these spices also contained low to high amounts of phenolics, tannins, SP and essential minerals their use in ruminant diets may help modify the rumen fermentation and so the utilization of low quality forages in ruminants. This study showed that spices can manipulate rumen degradation but the extent of their effect varied with the spice type and level, incubation time and also forage types. Among the spices turmeric and cumin showed more effect on the IVD and IVOMD of the forages. While higher amounts of TP, CT, SP and Mn in clove and cinnamon did not show much effect on the *in vitro* rumen degradability of forages, their careful use in ruminant diets may help modify the rumen fermentation process in order to modify forage utilization and reduce the nutrient wastage by the ruminants. Further studies are looking at the effect of different levels of these spices on forage fermentation profiles and total gas and methane production in ruminants.

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Table 1. Proximate composition (% DM, unless stated otherwise) and fibre content (% DM) of different forages and spices

Feed Groups	Feed Types	DM %	Ash	CP	EE	NDF	ADF	ADL
Forages	Rice straw (RS)	95.1	16.1	4.08	1.21	67.6	48.1	15.3
	Wheat straw (WS)	93.9	5.9	2.32	1.56	79.0	52.2	16.0
	Ryegrass hay (HAY)	95.4	6.8	6.64	1.40	74.7	41.3	15.6
	SEM	0.24	2.03	0.74	0.007	2.21	2.79	0.626
	P<	0.03	0.002	0.002	0.2	0.03	0.02	0.93
Spices	Cinnamon (CIN)	95.1	4.28	7.4	3.4	43.2	53.7	30.7
	Clove (CLO)	92.3	8.50	10.2	7.3	28.0	25.6	18.5
	Turmeric (TUR)	95.2	6.94	8.6	2.6	38.1	19.9	6.9
	Cumin (CUM)	96.4	8.00	22.3	14.6	55.2	24.1	12.1
	SEM	0.58	0.62	2.41	1.79	3.76	5.06	3.41
	P<	0.002	0.001	0.001	0.002	0.001	0.001	0.003

SEM=Standard error mean within groups

Table 2. Mean (mg/g DM) Total Phenolics (TP), Tannins (CT), Saponins (SP), Soluble Sugars (SS), Starch and oligosaccharides (SO) and Non Cellulose Carbohydrates (NCC) of different forages and spices

Feed Groups	Feed Types	TP GE	CT CE	SP DE	SS	SO	NCC
Forages	RS	4.6	9.2	3.3	34.7	455	489.7
	WS	3.3	4.5	4.3	18.7	164	182.7
	HAY	6.3	8.0	8.3	161.5	352	513.5
	SEM	0.561	0.888	0.982	2.87	5.53	6.90
	p<	0.002	0.001	0.001	0.002	0.02	0.02
Spices	CIN	73	53	62	25.5	169	194.5
	CLO	168	27	47	53.0	103	156.0
	TUR	22	39	38	67.9	542	609.9
	CUM	18	10	44	65.4	80	145.4
	SEM	22.6	4.39	18.09	6.33	70.6	73.1
	p<	0.001	0.03	0.3	0.001	0.001	0.001

GE = Gallic acid equivalent; CE = Catechin equivalent; DE= Diosgenin equivalent;

Table 3. Mean mineral components (mg/kg DM) of different forages and spices

Feed Groups	Feed Types	Ca	K	Mg	Na	P	Cu	Co	Mn	Se	Zn
Forages	RS	2588	13308	2071	3057	861	1.81	1.023	142.8	4.46	24.5
	WS	1602	2332	368	230	225	0.94	0.516	14.3	3.22	11.4
	Hay	3354	9279	993	1215	1313	7.33	1.167	69.9	4.72	29.2
	SEM	254	1603	249	414	158	1.00	0.102	18.6	0.96	2.7
	p<	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.84	0.001
Spices	CIN	9630	3758	735	360	442	2.93	2.111	196	3.56	18.2
	CLO	9999	16403	2794	2700	1135	4.08	1.967	612	6.65	17.7
	TUR	1539	24126	2418	788	2309	8.24	0.684	22	1.94	11.1
	CUM	8303	14180	2700	2296	3969	5.50	1.778	50	9.20	50.4
	SEM	688	1463	168	198	269	0.40	0.120	47	0.81	3.1
	p<	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.5	0.001

Table 4. IVD (g/kg) for different forages with different levels (mg/g) of spices at 3 incubation hours (means with SEM and significance for main effects and interactions)

Spices	Forages	20 h				40 h				60 h			
		Level of spices				Level of spices				Level of spices			
		0	10	30	90	0	10	30	90	0	10	30	90
CIN	RS	215	220	193	145	239	330	305	246	462	467	415	364
	WS	229	166	159	138	241	293	256	283	422	317	298	289
	Hay	290	329	235	135	320	424	449	328	562	439	524	396
CLO	RS	215	244	213	245	239	287	280	249	462	404	440	439
	WS	229	203	207	196	241	269	237	271	422	395	413	391
	Hay	290	311	270	287	320	439	413	332	562	523	568	560
CUM	RS	215	254	276	265	239	307	266	270	462	444	462	463
	WS	229	178	192	199	241	253	261	303	422	415	410	415
	Hay	290	291	326	317	320	360	457	460	562	550	546	513
TUR	RS	215	286	257	210	239	291	263	238	462	482	441	480
	WS	229	190	181	219	241	220	223	216	422	446	412	391
	Hay	290	351	293	341	320	322	246	292	562	581	520	585
SEM and significance for main effects and interactions		SEM = 6.28 F; p < 0.001 S; p < 0.001 L; p < 0.003 FxS; p < 0.4 FxL; p < 0.005 SxL; p < 0.001 FxSxL; p < 0.3				SEM = 6.85 F; p < 0.001 S; p < 0.001 L; p < 0.001 FxS; p < 0.001 FxL; p < 0.001 SxL; p < 0.001 FxSxL; p < 0.001				SEM = 7.53 F; p < 0.001 S; p < 0.001 L; p < 0.001 FxS; p < 0.001 FxL; p < 0.06 SxL; p < 0.001 FxSxL; p < 0.001			

F= Forage; S= Spices; L= Level of spices;

Table 5. IVOMD (g/kg) for different forages with different spices at different levels (mg/g) and for 3 incubation hours.

Spices	Forages	20 h				40 h				60 h			
		Level of spices				Level of spices				Level of spices			
		0	10	30	90	0	10	30	90	0	10	30	90
CIN	RS	157	150	119	79	181	303	272	232	404	412	366	284
	WS	161	100	101	113	207	271	231	246	382	275	257	273
	Hay	217	218	182	109	303	378	447	324	522	421	491	353
CLO	RS	157	172	137	181	181	258	236	178	404	333	377	385
	WS	161	136	156	169	207	255	191	248	382	355	371	343
	Hay	217	246	209	222	303	441	410	331	522	475	499	518
CUM	RS	157	206	211	175	181	303	245	245	404	394	414	410
	WS	161	115	184	139	207	213	230	285	382	381	367	393
	Hay	217	272	253	251	303	355	424	412	522	490	482	477
TUR	RS	157	208	190	145	181	262	233	182	404	465	395	428
	WS	161	125	132	176	207	192	205	190	382	403	397	368
	Hay	217	290	274	262	303	278	238	259	522	531	427	514
SEM and Significance for main effects and interactions		SEM = 5.60 F; $p < 0.001$ S; $p < 0.001$ L; $p < 0.3$ FxS; $p < 0.3$ FxL; $p < 0.002$ SxL; $p < 0.006$ FxSxL; $p < 0.8$				SEM = 7.86 F; $p < 0.001$ S; $p < 0.001$ L; $p < 0.001$ FxS; $p < 0.001$ FxL; $p < 0.001$ SxL; $p < 0.001$ FxSxL; $p < 0.004$				SEM = 6.80 F; $p < 0.001$ S; $p < 0.001$ L; $p < 0.001$ FxS; $p < 0.002$ FxL; $p < 0.2$ SxL; $p < 0.001$ FxSxL; $p < 0.001$			

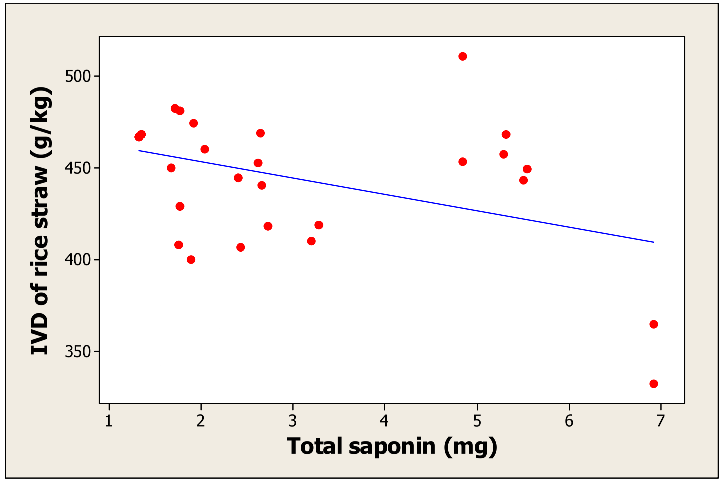

Figure 1. Relationship between SP and IVD of rice straw at 60h (r = -0.434; P<0.02)

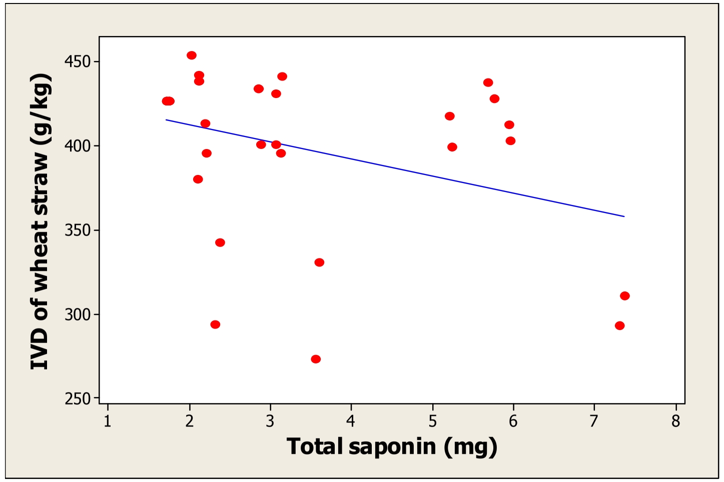

Figure 2. Relationship between SP and IVD of wheat straw at 60h (r = -0.364; P<0.05)

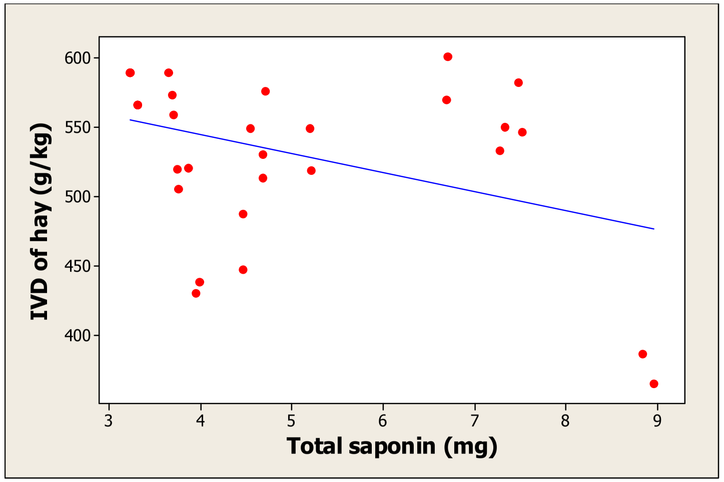

Figure 3. Relationship between SP and IVD of hay at 60h (r = -0.397; P<0.03)

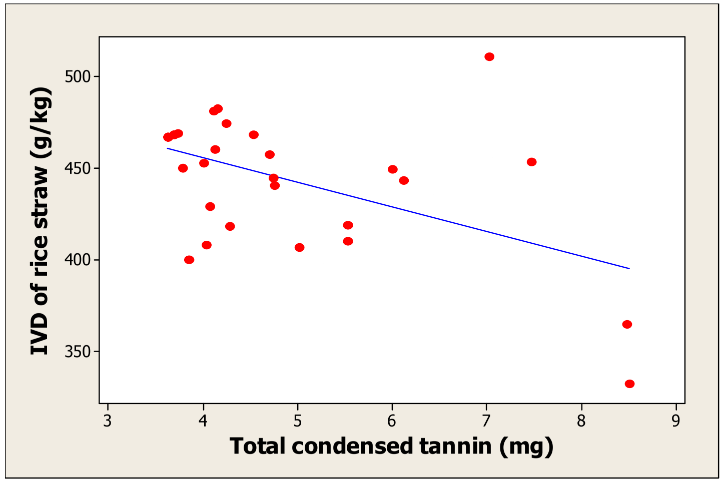

Figure 4. Relationship between CT and IVD of rice straw at 60h (r = -0.523; P<0.003)

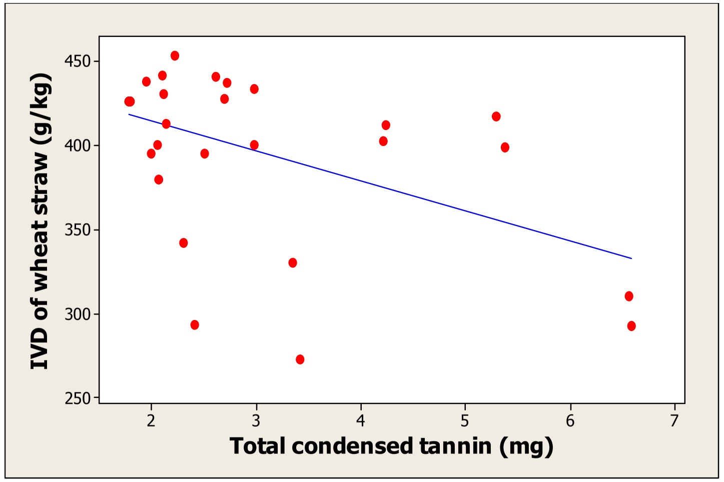

Figure 5. Relationship between CT and IVD of wheat straw at 60h (r = -0.504; P<0.004)

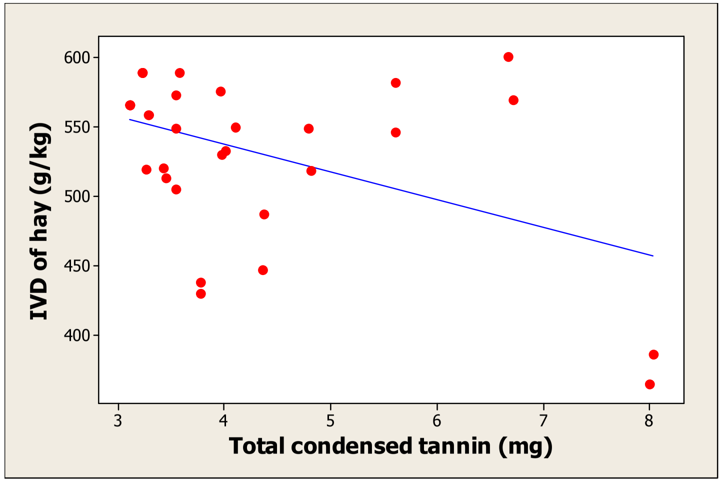

Figure 6. Relationship between CT and IVD of hay at 60h (r = -0.461; P<0.009)

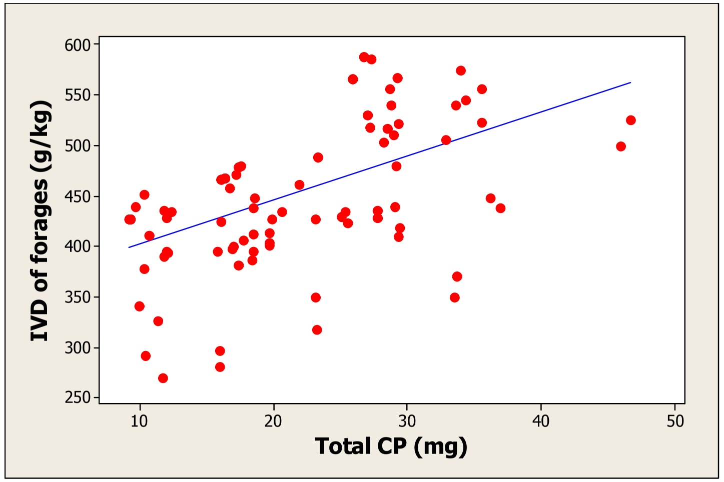

Figure 7. Relationship between CP and mean forage IVD at 60h (r = 0.521; P<0.001)

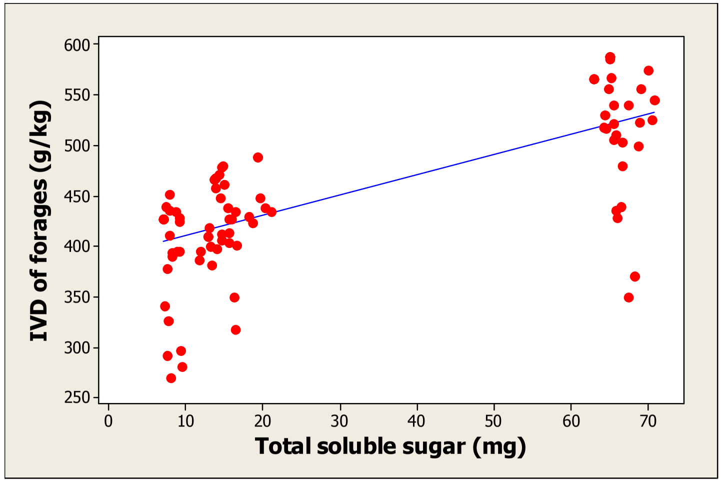

Figure 8. Relationship between SS and mean forage IVD at 60h (r = 0.7; P<0.001)