In vitro fermentation of conventional diets commonly fed to dairy cows in Central Myanmar

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Abstract

This study consisted of two experiments conducted to evaluate the effective net gas, fermentation kinetics (experiment 1), methane gas concentration, partitioning factor (PF) for microbial protein synthesis and in vitro dry matter digestibility (IVDMD) (experiment 2) of conventional diets commonly fed to dairy cows in Central Myanmar. The conventional diets from four areas [diet from Sin Tel area (Diet-ST), diet from Myay Ngu area (Diet-MN), diet from Ta Pel area (Diet-TP) and diet from Amarapura area (Diet-AM)] were used as experimental diets in this study. In most of conventional diets, rice straw, sorghum stover and natural grass were used as roughage source and cotton seed cake and broken rice were used as concentrate. However in some diets, sesame residue and butter bean residue were used as roughage source instead of sorghum stover and natural grass. The roughage to concentrate ratio and crude protein (CP) content of conventional diets ranged from 53:47 to 72:28 and 11.46 to 17.96%, respectively. In experiment 1, the effective net gas volume of Diet-TP was lower (p<0.05) than Diet-ST and Diet-AM and generally, the fermentation kinetics (a, b, c and a+b) of Diet-TP were also lower than those of other diets. In the experiment 2, the lower value (p<0.05) of short chain fatty acid (SCFA) was found in Diet-MN and Diet-TP while the higher values (p<0.05) of metabolizable energy (ME) and organic matter digestibility (OMD) were observed Diet-AM and Diet-TP. Although methane gas concentration of Diet-TP was higher (p<0.05) than those of other diets, the greater values of IVDMD and PF were observed in Diet-TP. According to these findings, it was perceived that all conventional diets have different nutritional qualities which are useful for production and health of dairy cows; however the Diet-TP possessed the highest nutritional qualities among the conventional diets.

Key words: Conventional diets, effective net gas, fermentation kinetics, partitioning factor, in vitro dry matter digestibility

Introduction

The smallholder farmers of developing countries have limited resources available for feeding their animals. The available resources are low digestible forages such as tropical pastures (both green and mature), crop residues and agricultural by-products which are generally low in protein (Leng, 1991). Khan et al. (2009) also reported that the traditional feeding system for dairy cattle is based on the use of rice straw, natural grasses supplemented with a little or no concentrates. It is well recognized that cereal crop residues are of low nutritive value (Sundstøl and Owen, 1984) because of fibrous in nature, bulky and more of lignin content. However, the level of incorporation of crop residues in the complete diet is influenced by the quality of crop residue (Anandan et al., 2010).

One of many constraints to livestock production is the scarcity and fluctuation of the quality and quantity of animal feed supply throughout the year. Thus, evaluating the nutritive values of those feed supply are important as these could make an important contribution to the
nutrition of animals. Most of smallholder dairy farmers in Myanmar have been using the conventional diets for their dairy cows, however the efforts concerning the determination of nutritive values of those diets were still limited. Chemical composition, in combination with in vitro gas production, OMD and ME content were widely used to determine the potential nutritive value of feedstuffs which are previously limited or uninvestigated (Ammar et al., 2004; Kaya et al., 2016).

According to the report of Aung et al. (2015a), a survey of regional feed resources and milk production level of dairy cows and in Central Myanmar, the highest milk production was observed in the Tapel area and the lowest production was found in Amarapura area. Therefore, it is needed to clarify the causes of differences in milk production of dairy cows from study areas and the nutritional status of conventional feeds offered to those cows. Aung et al. (2015b) presented that the nutritive values such as fermentation kinetics, metabolizable energy (ME), short chain fatty acid (SCFA) and organic matter digestibility (OMD) of feedstuffs from Tapel area were higher than those of feedstuffs from Amarapura area. Moreover, Aung et al. (2015c) reported that rumen undegradable protein (UDP) and energy protein synchronization values of conventional diet from Tapel area were significantly higher than those of other diets. However, it was still needed to determine the fermentation kinetics, partitioning factor for microbial protein synthesis and methane gas concentration of conventional diets because of their important roles in evaluating the nutritional qualities of diets. Therefore, this experiment was intended to evaluate the effective net gas, fermentation kinetics (experiment 1), methane gas concentration, partitioning factor (PF) for microbial protein synthesis and in vitro dry matter digestibility (IVDMD) (experiment 2) of conventional diets commonly fed to dairy cows in the central Myanmar and those diets are as follows:

1. Diet-ST: Common diet from Sin Tel village, Tatar U Township
2. Diet-MN: Common diet from Myay Ngu village, Tatar U Township
3. Diet-TP: Common diet from Ta Pel village, Tatar U Township
4. Diet-AM: Common diet from Amarapura Township

The ingredient composition and chemical composition of conventional diets were presented in Table 1 and 2, respectively.

In vitro gas production and measurements

Experiment 1

Rumen fluid was collected from fistulated bull (280 Kg Body Weight) before morning feed. The in vitro gas production method was done as the procedures reported by Menke and Steingass (1988). Incubation was carried out at 39 °C and gas production was read at 1, 12, 24, 48, 72 and 96 hours. The extent and rate of gas production was determined by exponential model of Ørskov and McDonald (1979); \( Y = a + b (1 - e^{-ct}) \). Where: \( a \) = The gas production (ml) from rapidly fermentable fraction, \( b \) = The gas production (ml) from slowly fermentation fraction, \( c \) = The gas production rate constant for the slowly fermentation fraction, \( a+b \) = Potential gas production (ml), \( t \) = Incubation time (h) and \( Y \) = Gas production at time \( t \). After 96 hrs of incubation, the total gas volumes, effective net gas volumes and fermentation kinetics of conventional diets were calculated.

Experiment 2

In these incubations, the sample weights were increased from 200 mg to 500 mg so as to increase the mass of residue to minimize the analytical error. The double strength medium (40 ml) was incubated with 500 mg air-dry substrate. After 24 hrs incubation, partitioning factor, methane gas concentration, in vitro dry matter digestibility and other estimated parameter such as short chain fatty acid (SCFA), organic matter digestibility (OMD) and metabolizable energy (ME) values of conventional diets were calculated.
**Partitioning factors (PF) for microbial protein synthesis efficiency**

After 24 hours of incubation, the contents were centrifuged and the discarded supernatant. The pellet washed with distilled water followed by centrifugation (20,000 g, 30 min, 4 °C) at least 3 times. The undigested substrate and microbial mass are dried in the oven at 135 °C for 2 hrs. The residue has been termed as apparent undigested residue. The residue was refluxed with NDS solution (70 ml) for 1 hr for the determination NDF and NDF ash. From them, microbial protein synthesis (MPS) efficiency was calculated step-by-step calculation (Makkar and Becker, 1996).

**Methane gas production**

In order to estimate methane production by the substrate and immediately after evacuation from the incubator, 4 ml of NaOH (10 M) was introduced using 5 ml capacity syringe as reported by Fievez et al. (2005). The content was inserted into the silicon tube, which are fastened to the 120 ml capacity syringe. The clip was then opened while the NaOH was gradually released. The content was agitated while the plunger began to shift position to occupy the vacuum created by the absorption of CO₂. The volume of methane was read on the calibration.

**In vitro dry matter digestibility (IVDMD)**

After 24 hrs digestion, the samples were transferred into test tube and centrifuge for 1 h in order to obtain the residues which was then filtered using Whatman No. 4 filter paper by gravity and the residues placed in for drying at 65 °C for 24 hrs. The dry residues were weighted and digestibility was calculated using the equation as follows: IVDMD (%) = Initial DM input − (DM residue – Blank)/ Initial DM input ×100

**Estimation of other parameters**

The other parameters such as short chain fatty acid (SCFA), organic matter digestibility (OMD) and metabolizable energy (ME) values of conventional diets were estimated using equations as below: SCFA (mmol) = 0.0222Gp − 0.00425 (Makkar, 2005); OMD (%) = 14.88 + 0.889Gp + 0.458CP +0.0651XA (Menke and Steingass,1988); ME (MJ Kg⁻¹ DM) = 2.20 + 0.136Gp + 0.057CP (Menke and Steingass,1988). Where; CP = % crude protein in DM, Gp = gas production from 200mg sample at 24 hours and XA = % ash in DM.

**Chemical analysis**

Ground samples of feeds were analyzed for dry matter (DM), organic matter (OM), ether extract (EE) by the method described by AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analyzed according to the method of Goering and van Soest (1970). All feeds were analyzed for nitrogen (N) by using Kjeldahl method (Fross, 2020 digester and Foss 2100 Kjeltec distillation unit) and crude protein (CP) was calculated as 6.25 x N (AOAC, 1990).

**Statistical analysis**

The data obtained from experiment 1 and 2 were subjected to the analysis of variance (ANOVA) and the significance of differences between means was compared by Duncan’s Multiple Range Test (DMRT) (Steel and Torrie, 1980) using SPSS (version 16.0) software.

**Results**

**Experiment 1**

The cumulative gas production of conventional diets at the different incubation times are shown in Table 3. The result points out that the cumulative gas volumes at all incubation times were different (p<0.05) except 72 and 96 hrs of incubation times. The gas volumes at 24 and 48 hrs ranked from the highest to the lowest; Diet-ST, Diet-AM, Diet-MN and Diet-TP respectively. For the effective net gas volume, Diet-TP was lower (p<0.05) than Diet-ST and Diet-AM, while the latter two diets were not different (p>0.05) from each other.

The kinetic fermentation parameters (a, b, c) of conventional diets are presented in Table 4. The rapidly fermentable fraction “a” was highest in Diet-ST and Diet-MN (4.19 and 3.14%, respectively) and they were higher (p<0.05) than those of other two diets. The slowly fermentable fraction “b” was greater (p<0.05) in Diet-AM in comparison with other three diets which were not different (p>0.05) each other. The potential gas production (a+b) of Diet-AM was higher (p<0.05) than Diet-MN and Diet-TP while it was not
different (p>0.05) from that of Diet-ST (57.1 vs 53.3). The rate of fermentation “c” of Diet-TP was lower (p<0.05) than other three diets while the highest was found in Diet-ST.

Experiment 2

The amount of ME, OMD, SCFA, IVDMD, PF and methane gas concentration of conventional diets are presented in Table 5. The values of ME and OMD of Diet-AM and Diet-TP were 8.07 and 7.94 MJ kg⁻¹ DM and 54.9 and 54.4%, respectively and these values were higher (p<0.05) than other two diets, Diet-ST and Diet-MN. The Diet-AM showed the highest value of SCFA (0.85 mmol200 mg⁻¹ DM) and this value was greater (p<0.05) than other diets which were not different (p>0.05) from one another. The methane concentration of Diet-TP was found to be the highest (40.0%) and it was higher (p<0.05) than those of other diets. The IVDMD of Diet-ST and Diet-TP were 63.0 and 61.8%, respectively and higher (p<0.05) than those of other two diets. The values of PF among the diets were not found to be different (p>0.05) to each other.

Discussions

Experiment 1

Generally, Diet-ST gave the highest values of cumulative gas volume up to 48 hrs of incubation time and effective net gas, higher amount of rapidly fermentable fraction “a” and faster fermentation rate “c”. Conversely, the lowest amounts of those values were observed in Diet-TP which had the greater amount of cell wall contents (NDF and ADF). Moreover, the lowest cumulative gas volume at early incubation time and the highest volume at later incubation time were observed in Diet-AM because of its high cell wall contents, the lowest value of rapidly fermentable fraction “a”, highest value of slowly fermentable fraction “b” and faster fermentation rate “c”.

Therefore, it could be assumed that the gas production and their kinetics were influenced by the level of quickly soluble carbohydrate fraction readily available to the microbial population and level of slowly fermented carbohydrates (NDF) which needs more time to attachment of microorganism. This assumption was supported with the finding of van Soest, (1994), who reported that the low content of fibre can facilitate the utilization of feed by ruminal microbes, which in turn might induce higher fermentation rates, therefore improving digestibility. De Boever et al. (2005) also reported that gas production was negatively related with NDF content and positively with starch. Furthermore, the gas production of feed in buffered rumen fluid was associated with feed fermentation and carbohydrate fraction (Sallam et al., 2008). The gas production kinetics of feeds could be affected by carbohydrates fraction (Deaville and Givens, 2001). Moreover, the highest protein content was found in Diet-TP; however the values of cumulative gas volume and effective net gas of that diet were lower than those of other diets. This is because the protein fermentation does not lead to excessive gas production (Khazaal et al., 1995).

Experiment 2

The lower value of SCFA predicted for Diet-MN and Diet-TP was due to a lower gas production which was evident in the first 24 hrs of incubation. This is supported to the finding of Blümmel et al. (1990), who suggested that gas production from cereal straws and different class of feeds incubated in in vitro buffered rumen fluid was closely related to the production of SCFA which was based on carbohydrate fermentation. A high value of SCFA is an indication of energy availability to the animal. The in vitro gas production method has been widely used to evaluate the energy value of several classes of feed (Getachew et al., 1999, 2002). The ME and OMD values of Diet-AM and Diet-TP were higher (p<0.05) than those of Diet-ST and Diet-MN. It might be due to the higher level of starch, the major carbohydrate of these diets which were fermented by amylolytic bacteria and protozoa (Kotarski et al., 1992).

The methane gas concentration of Diet-TP was found to be the highest (40.0%) and it was greater (p<0.05) than those of other diets. That diet possessed the higher amount of fibre content (NDF and ADF) which convert CO₂ and H₂ to methane instead of acetate (Miller, 1995). Fermentation of cell wall carbohydrates produces more methane than fermentation of soluble sugar, which produces more methane than fermentation of starch (Johnson et al., 1996). Diets rich in starch favor the propionate production and decrease methane production and conversely,
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| Table 1. Ingredient compositions (%) of conventional diets |
|-----------------------------------------------------------|
| **Conventional feedstuffs**                               | **Conventional diets** |
|                                                           | Diet-ST  | Diet-MN  | Diet-TP  | Diet-AM  |
| Rice straw                                              | 23.0     | 21.0     | 23.0     | 33.0     |
| Sorghum stover                                          | 14.0     | _        | 36.0     | 9.00     |
| Sesame residue                                          | 25.0     | _        | _        | _        |
| Natural grass                                           | _        | 17.0     | _        | 11.0     |
| Butter bean residue                                     | _        | 34.0     | _        | _        |
| Cottonseed cake                                         | 29.0     | 20.0     | 35.0     | _        |
| Broken rice                                             | 8.00     | 8.00     | 6.00     | 7.00     |
| Chickpea mill                                           | _        | _        | 40.0     | _        |
| Roughtage: Concentrate                                   | 63:37    | 72:28    | 59:41    | 53:47    |

All ingredient compositions are on DM basis.

| Table 2. Chemical composition (%) of conventional diets |
|--------------------------------------------------------|
| **Conventional diets**                                 | **Chemical compositions (%)** |
|                                                        | DM     | OM     | CP     | NDF   | ADF   | EE    |
| Diet-ST                                                | 58.9a  | 91.0c  | 16.1a  | 60.6c | 40.1b | 1.94  |
| Diet-MN                                                | 54.0b  | 90.3b  | 16.5b  | 59.1c | 34.7c | 2.06  |
| Diet-TP                                                | 41.7c  | 90.2a  | 18.0a  | 64.2a | 38.9b | 2.02  |
| Diet-AM                                                | 46.7c  | 88.1b  | 11.5c  | 61.3b | 48.0a | 1.78  |
| SEM                                                    | 2.05   | 0.41   | 0.74   | 0.62  | 1.47  | 0.05  |
| P Value                                                | 0.001  | 0.038  | 0.001  | 0.001 | 0.112 |

a, b, c, d, – Means within the same column with various superscripts are different (p<0.05); DM – dry matter (%); OM – organic matter (%); CP – crude protein (%); NDF – neutral detergent fibe (%); ADF – acid detergent fibre (%); EE – ether extract (%); SEM – standard error mean; All values except DM are on DM basis.

| Table 3. Cumulative gas production (ml 200 mg⁻¹ DM) of conventional diets at the different incubation times |
|----------------------------------------------------------------------------------------------------------|
| **Conventional diets**                                                                                   | **Incubation times (hrs)**  | **Effective net gas**   |
|                                                                                                          | 1     | 12    | 24    | 48    | 72    | 96    |
| Diet-ST                                                | 6.33a | 24.5a | 36.4a | 47.5a | 51.3  | 52.6  | 38.6a |
| Diet-MN                                                | 5.31a | 21.8b | 33.0b | 43.9b | 48.0  | 49.5  | 35.1ab|
| Diet-TP                                                | 3.56a | 19.2b | 30.3b | 42.0b | 46.8  | 48.7  | 33.2b |
| Diet-AM                                                | 2.10b | 21.7b | 35.0a | 47.8a | 52.3  | 54.0  | 37.2a |
| SEM                                                    | 0.54  | 0.67  | 0.83  | 0.93  | 0.92  | 0.90  | 0.74  |
| P Value                                                | 0.002 | 0.012 | 0.018 | 0.040 | 0.073 | 0.104 | 0.050 |

a, b, c – Means within the same column with various superscripts are different (p<0.05); SEM – standard error mean.

| Table 4. Kinetic fermentation of conventional diets through in vitro gas method |
|--------------------------------------------------------------------------------|
| **Conventional diets**                                                          | **Kinetic fermentation parameters** |
|                                                                              | a (%) | b (%) | c (h⁻¹) | a+b    |
| Diet-ST                                                                        | 4.19c | 49.1b | 0.0446c | 53.3bc |
| Diet-MN                                                                        | 3.41c | 47.0b | 0.0414a | 50.4b  |
| Diet-TP                                                                        | 1.80b | 48.3b | 0.0371b | 50.1b  |
| Diet-AM                                                                        | 1.62b | 55.4a | 0.0428a | 57.1a  |
| SEM                                                                            | 0.350 | 1.160 | 0.001   | 1.050  |
| P Value                                                                        | 0.001 | 0.013 | 0.004   | 0.032  |

a, b – Means within the same column with various superscripts are different (p<0.05); a – gas production (ml) from rapidly fermentable fraction, b gas production (ml) from slowly fermentable fraction, c – constant rate of fermentation, a + b – potential gas production; SEM – Standard error mean.

roughage based diets favor acetate production and increase methane production per unit of fermentable OM in rumen (Johnson and Johnson, 1995). Moreover, the higher amount of OMD was observed in Diet-TP. Johnson and Johnson (1995) pointed out that methane production and OMD were positively correlated by affecting the activity of methanogens and protozoa in the rumen.
Table 5. Estimated parameters of ME, OMD, SCFA, IVDMD, PF and methane gas concentration (%) of conventional diets

| Conventional diets | ME (MJ Kg⁻¹ DM) | OMD (%) | SCFA (mmol 200 mg⁻¹ DM) | Methane gas concentration (%) | IVDMD (%) | PF |
|--------------------|----------------|---------|-------------------------|-----------------------------|-----------|----|
| Diet-ST            | 7.90ab         | 54.0ab  | 0.78b                   | 31.9ab                      | 63.0b     | 3.29 |
| Diet-MN            | 7.61b          | 52.1b   | 0.73b                   | 25.3b                       | 60.8ab    | 3.45 |
| Diet-TP            | 7.94a          | 54.4a   | 0.77b                   | 40.0a                       | 61.8a     | 3.64 |
| Diet-AM            | 8.07a          | 54.9a   | 0.85a                   | 34.6ab                      | 58.5b     | 2.92 |
| SEM                | 0.06           | 0.41    | 0.01                    | 2.01                        | 0.58      | 0.11 |
| P Value            | 0.042          | 0.055   | 0.005                   | 0.039                       | 0.013     | 0.097|

a,b,c,d,e – Means within the same column with various superscripts are different (p<0.05); ME – metabolizable energy, OMD – organic matter digestibility; SCFA – short chain fatty acid (mmol 200 mg⁻¹ DM); CH₄ – methane gas concentration (%); IVDMD – *in vitro* dry matter digestibility, PF: partitioning factor; SEM – standard error mean.

The lowest value of IVDMD was found in Diet-AM because of its high content of fibre. This result was consistent with the finding of Seresinhe and Iben (2003); Ammar et al. (2004); Njidda and Nasiru (2010). They indicated that forage digestibility is mainly affected by the cell wall contents (NDF and ADF) and its lignifications. Madibela and Modikagotla (2004) reported that ADF has a negative effect on energy content of forages and caused negative correlation between ADF and IVDMD.

The PF values of conventional diets (range from 2.92 to 3.64) were agreed with the theoretical range for PF (from 2.74 to 4.41). A feed with higher PF means that the efficiency of microbial protein synthesis is higher. Roughage with higher PF has been shown to have higher dry matter intake. Blümml et al. (1999) pointed out that different *in vitro* PF values are also reflected by *in vivo* microbial protein synthesis and in methane production by ruminants. These results show that the PF calculated *in vitro* provides meaningful information for predicting the dry matter intake, the microbial mass production in the rumen and the methane production of the whole ruminant animal.

Conclusions

The roughage to concentrate ratio of all conventional diets were generally higher than the optimum ration (40:60). Among the conventional diets, the highest CP content and lowest effective net gas values were observed in Diet-TP. Therefore, it could be assumed that the CP content of feeds does not correlate with the effective net gas. Generally, in the experiment 1, the lower values of effective net gas and fermentation kinetics were found in Diet-TP while the other diets were possessed the higher values in those parameters. However, in experiment 2, the higher values of partitioning factor (PF) for microbial protein synthesis, *in vitro* dry matter digestibility (IVDMD), methane gas concentration and other estimated parameters were observed in Diet-TP, while lower values were found in other diets.

According to these findings, it was perceived that all conventional diets have different nutritional qualities which are useful for production and health of dairy cows; however the Diet-TP possessed the highest nutritional qualities among the conventional diets.

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References

Ammar, H., Lopez, S., Gonzalez, J.S. & Ranilla, M.J.(2004). Chemical composition and *in vitro* digestibility of some Spanish browse plant species. *Journal of the Science of Food Agriculture*, 84, 197-204.

Anandan, S., Khan, A.A., Ravi, D.,Reddy, J. & Blümml,M.(2010). A comparison of sorghum stover based complete feed blocks with a conventional feeding practice in a peri urban dairy. *Animal Nutrition and Feed Technology*, 10, 23-28.
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AOAC (1990). Official methods of analysis, 15th ed. (pp. 69-88). Washington D.C. Association of official analytical chemists.

Aung, M., Khaing, M., Ngwe, T., Mu, K.S., Htun, M.T., Oo, L.N. & Aung, A. (2015a). Preliminary survey on the dairy cattle production system and conventional feed resources in the central dry zone of Myanmar. Global Journal of Animal Scientific Research, 3(2), 383-387.

Aung, M., Khaing, M., Mu, K.S., Htun, M.T., Oo, L.N. & Aung, A. (2015b). Nutritional evaluation of conventional feedstuffs for ruminants using in vitro gas production technique. Global Journal of Animal Scientific Research, 3(2), 518-523.

Aung, M., Kyawt, Y.Y., Htun, M.T., Mu, K.S. & Aung, A. (2015c). In situ nutrient degradation of conventional diets commonly fed to dairy cows in central Myanmar. Journal of Aridland Agriculture, 1, 36-42.

Blümmel, M., Ørskov, E.R., Becker, K. & Soller, H. (1990). Application of the Hohenheim gas test for evaluating the kinetics of rumen fermentation. Journal of Animal Physiology and Animal Nutrition, 64, 56-57.

Blümmel, M., Mgomezulu, R., Chen, X.B., Makkar, H.P.S., Becker, K. & Ørskov, E.R. (1999). The modification of in vitro gas production test to detect roughage related differences in in vivo microbial protein synthesis as estimated by the excretion of purine derivate. Journal of Agricultural Science, 133, 335-340.

De Boever, J.L., Aerts, J.M. & De Brabader, D.L. (2005). Evaluation of the nutritive value of maize silages using gas production techniques. Animal Feed Science and Technology, 123-124, 255-265.

Deaville, E.R. & Givens, D.I. (2001). Use of the automated gas production technique to determine the fermentation kinetics of carbohydrate fractions in maize silage. Animal Feed Science and Technology, 93, 205-215.

Fievez, V., Babayemi, O.J. & Demeyer, D. (2005). Estimation of direct and indirect gas production in syringes: a tool to estimate short chain fatty acid production requiring minimal laboratory facilities. Animal Feed Science and Technology, 123-124, 197-210.

Getachew, G., Grovetto, G.M., Fondivilla, M., Krishnamoorth, B., Singh Sphaghero, H., Steingass, P.H., Robinson, P.H. & Kailas, M.M. (2002). Laboratory variation of 24h in vitro gas production and estimated metabolizable energy values of ruminant feeds. Animal Feed Science and Technology, 102, 169-180.

Getachew, G., Makkar, H.P.S. & Becker, K. (1999). Stoichiometric relationship between short chain fatty acids and in vitro gas production in presence and polyethylene glycol or tannin containing browses. EAAP satellite symposium.

Goering, K.H. & van Soest, P.J. (1970). Forage fibre analysis, Agricultural Handbook (pp. 8-12). Washington D.C. USDA.

Johnson, D.E., Ward, G.W & Ramsey, J.J. (1996). Livestock methane: current emission and mitigation potential. In E.T. Kornegay (Ed.), Nutrient management of food animals to enhance and protect the environment (pp. 219-234). New York, NY, Lewis publication.

Johnson, K.A. & Johnson, D.E. (1995). Methane emission from cattle. Journal of Animal Science, 3, 2483-2492.

Kaya, E., Canbolat, O., Atalay, A.I., Kurt, O. & Kamalak, A. (2016). Potential nutritive value and methane production of pods, seed and senescent leaves of Gleditsiatriacanthos trees. Livestock Research for Rural Development, 28, Article 123.

Khan, M.J., Peters, K.J. & Uddin, M.M. (2009). Feeding strategies for improving dairy cattle productivity in small holder farm in Bangladesh. Bangladesh Journal of Animal Science, 38(1), 67-85.

Khazaal, K., Dentinho, M.T., Riberio, J.M. & Ørskov, E.R. (1995). Prediction of apparent digestibility and voluntary feed intake of hays fed to sheep: Comparison between using fibre component. In vitro digestibility or characteristics of gas production or nylon bag degradation. Animal Science, 61, 521-538.
Kotarski, S.F., Waniska, R.D.& Thurn, K.K.(1992). Starch hydrolysis by the ruminant micro flora. Journal of Nutrition, 122, 178-190.

Leng, R.A.(1991). Feeding strategies for improving milk production of dairy animals managed by small farmers in the tropics. Feeding dairy cows in the tropics. FAO Animal Production and Health paper, 86, 82-104.

Madibela, O.R.&Modiakgotla, E.(2004).Chemical composition and in vitro dry matter digestibility of indigenous finger millet (Eleusinecoracana) in Botswana. Livestock Research and Rural Development, 16, article 4.

Makkar,H.P.S. (2005).In vitro gas methods for evaluation of feeds containing phytochemicals. Animal Feed Science and Technology, 123-124, 291-302.

Makkar, H.P.S. & Becker, K.(1996). Nutritional value and antinutritional components of whole and ethanol extracted Moringaoleiferalaves. Animal Feed Science and Technology, 63, 211-228.

Menke, K.H.&Steingass, H.(1988). Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Animal Research Development, 28, 7-55.

Miller, T.L.(1995). Ecology of methane production and hydrogen sinks in the rumen. In E. Von Engelhardt, S. Leonhard-Marek, G. Breves & D. Giessecke(Eds.), Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction(pp. 317-331). Stuttgart, Ferdinand EnkeVerlag.

Njidda, A.A.&Nasiru, A.(2010). In vitro gas production and dry matter digestibility of tannin-containing forages of semi-arid region of North-Eastern Nigeria. Pakistan Journal of Nutrition, 9(1), 60-66.

Ørskov, E.R.&McDonald, L.M.(1979). Estimation of protein degradability in the rumen from incubation measurement weighted according to rate of passage. Journal of Agricultural Science, 92, 499-503.

Sallam, S.M.A., Bueno, I.C.S., Godoy, P.B., Nozella, E.F., Vitti, D.M.& Al-Abdalla, S.S.(2008). Nutritive value in the value assessment of the artichoke (Cynarascolyms) by products as an alternative feed resource for ruminant. Tropical and Subtropical Agroecology, 8, 181-189.

Seresinhe, T.&Iben, C.(2003). In vitro quality assessment of tropical shrub legume in relation to their extractable tannin contents. Journal of Animal Physiology and Animal Nutrition, 87, 109-115.

SPSS (2007). Statistical Package for the Social Sciences. Version 16.0. SPSS Inc. United State of America. www.spss.com

Steel, R.G.&Torrie, J.H.(1980). Principles and procedures of statistics. A biometrical approach. 2nd ed. New York, McGraw-Hill Book Company.

Sundstøl, F.&Owen, E.(1984). Straw and other fibrous by-products as feed. Elsevier, Amsterdam.

van Soest, P.J.(1994). Nutritional ecology of the ruminant. Corvallis, 2nd ed. Ithaca, United State of America, Cornell University Press.