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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 Nguarea (Diet-MN), diet from Ta Pelarea (Diet-TP) and diet from Amarapuraarea (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

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nutrition of animals. Most of smallholder dairy farmers in Myanmar have been used 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 un degradable 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 Central Myanmar through *in vitro* gas method.

Materials and methods

Experimental diets

The diets used in *experiment 1* and *experiment 2* were 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 (Makkarand 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 CO_2. 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 ($p>005$) 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 CO_2 and H_2 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,

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
Roughage: 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	58.9^{a}	91.0^{a}	16.1^{b}	60.6^{bc}	40.1^{b}	1.94
Diet-MN	54.0^{b}	90.3^{a}	16.5^{b}	59.1^{c}	34.7^{c}	2.06
Diet-TP	41.7^{d}	90.2^{a}	18.0^{a}	64.2^{a}	38.9^{b}	2.02
Diet-AM	46.7^{c}	88.1^{b}	11.5^{c}	61.3^{b}	48.0^{a}	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	0.001	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 fibre (%); 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	6.33^{a}	24.5^{a}	36.4^{a}	47.5^{a}	51.3	52.6	38.0^{a}
Diet-MN	5.31^{a}	21.8^{b}	33.0^{ab}	43.9^{ab}	48.0	49.5	35.1^{ab}
Diet-TP	3.56^{b}	19.2^{b}	30.3^{b}	42.0^{b}	46.8	48.7	33.2^{b}
Diet-AM	2.10^{b}	21.7^{b}	35.0^{a}	47.8^{a}	52.3	54.0	37.2^{a}
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	4.19^{a}	49.1^{b}	0.0446^{a}	53.3^{ab}
Diet-MN	3.41^{a}	47.0^{b}	0.0414^{a}	50.4^{b}
Diet-TP	1.80^{b}	48.3^{b}	0.0371^{b}	50.1^{b}
Diet-AM	1.62^{b}	55.4^{a}	0.0428^{a}	57.1^{a}
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	Estimated parameters					
	ME (MJ Kg^{-1} DM)	**OMD (%)**	**SCFA (mmol 200 mg^{-1} DM)**	**Methane gas concentration (%)**	**IVDMD (%)**	**PF**
Diet-ST	7.90^{ab}	54.0^{ab}	0.78^{b}	31.9^{ab}	63.0^{a}	3.29
Diet-MN	7.61^{b}	52.1^{b}	0.73^{b}	25.3^{b}	60.8^{ab}	3.45
Diet-TP	7.94^{a}	54.4^{a}	0.77^{b}	40.0^{a}	61.8^{a}	3.64
Diet-AM	8.07^{a}	54.9^{a}	0.85^{a}	34.6^{ab}	58.5^{b}	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); CH_4 – 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 SeresinheandIben (2003); Ammar *et al.* (2004); Njiddaand Nasiru (2010). They indicated that forage digestibility is mainly affected by the cell wall contents (NDF and ADF) and its lignifications. Madibela and Modiakgotla (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. Blümmel *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 vit*ro 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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