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Comparative evaluation of *in vitro* techniques for predicting metabolizable energy content of total mixed ration for Murrah buffaloes

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ABSTRACT

The present study was conducted to ascertain the sensitivity of *in vitro* techniques namely, *in vitro* gas production (IVGPT) and Cornell Net Carbohydrate and Protein System (CNCPS) fraction for predicting the metabolizable energy (ME) content of total mixed ration for Murrah buffaloes. The total mixed ration forming dietary treatment were TMR1, TMR2 and TMR3 having mixture of maize silage and concentrate in ratio of 30:70, 40:60 and 50:50, respectively. The diets formulated were isocaloric and isonitrogenous. The *in vitro* gas production was studied by incubation of diet with rumen liquor as inoculum to predict the metabolizable energy content of ruminant feeds. The CNCPS fraction analysing ME content of feed samples was carried out in laboratory. Lactating Murrah buffaloes (n = 18) having similar lactation yield (MY 9 kg±2.5) were allotted to three groups in a completely randomised design and fed TMR as per the dietary treatments. The feeding trial was carried out for 3 months and nutrient utilization was analysed. The efficiency of ME utilization *in vivo* were compared to *in vitro* gas production and CNCPS fraction. Correlation coefficient (r) between *in vitro* techniques and in vivo trial in evaluating ME content demonstrated a significantly (P<0.01) high correlation of 0.734 with IVGPT and 0.752 with CNCPS fraction indicating that both *in vitro* techniques were reliable in predicting metabolizable energy content of feedstuffs for ruminant feeding.

Keywords: CNCPS, *In vitro* gas production, Metabolizable energy, Total mixed ration

Formulation of ration for livestock involves knowledge on protein and energy of the forage being consumed. Determining the energy content of diet the animal is consuming, is difficult to comprehend, as forage quality keeps changing with its growth (NASEM 2016), affecting the TDN and ME content. Initially, formulation of diet by nutritionists was aimed to optimize production responses and predicted by empirical equations (Mertens 1997). The real-world variations in type of animal, feed, environment and management conditions were accounted by empirical equations producing nutritional recommendations that included significant safety factors (Geisert 2007). But these extra nutrients included as safety factors often increased nutrient excretion by animal. Recently, several different methods to estimate the chemical composition of diet have been established. But, debate over which method meticulously determines the energy value of ration for feeding ruminants still continues.

A number of *in vitro* techniques were available to predict the protein and energy content of ration. Techniques like *in vitro* gas production (Menke and Steingass 1988), CNCPS fraction (Sniffen *et al.* 1992) and NIRS (Norris 1996) are receiving considerable research interest and practical utility in ruminant feeding. The *in vitro* gas method estimates digestion of feed based on relationships between *in vivo* digestibility of feeds and *in vitro* gas production, along with feed's chemical composition (Menke and Steingass 1988). The Cornell Net Carbohydrate and Protein System (CNCPS) accounts for effects of variation in feed CHO fractions, predicting feed ME supply and amino acid balances when developing diets to meet animals' nutrient requirement (Fox *et al.* 2004). Keeping the techniques in mind, objective of the present study was to predict *in vitro* techniques, viz. *in vitro* gas production (IVGPT) and Cornell Net Carbohydrate and Protein System (CNCPS) fraction, in evaluating the metabolizable energy content of total mixed ration (TMR) for feeding Murrah buffaloes.

MATERIALS AND METHODS

The present experimental study for *in vitro* gas production and CNCPS fraction was performed in the Animal nutrition department, NDRI, Karnal. The feed samples forming dietary total mixed ration included TMR1, TMR2 and TMR3 having maize silage plus concentrate in ratio of 30:70, 40:60 and 50:50 respectively (Table 1 and Table 2). The feed samples were collected from cattle yard and brought to the laboratory. The samples were dried (hot air oven at 100°C for 24 h) for dry matter estimation (AOAC

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Table 1. Chemical composition of total mixed ration

Nutrient (%)	TMR1	TMR2	TMR3
DM	42.30	41.21	40.59
OM	88.04	89.15	89.45
CP	13.57	13.61	13.40
EE	02.52	02.29	02.20
NDF	30.21	34.50	37.11
ADF	17.62	20.31	21.36
NDICP (% CP)	11.21	09.72	08.58
ADICP (% CP)	03.43	03.24	02.57

*TMR, Total Mixed Ration having maize silage and concentrate; *TMR1, maize silage + concentrate (30:70); TMR2, maize silage + concentrate (40:60); TMR3, maize silage + concentrate (50:50).

Table 2. Ingredient composition (%) of concentrate feed

Ingredient	Concentrate 1	Concentrate 2	Concentrate 3
Maize	38.0	40.5	40.0
Wheat bran	20.0	14.5	9.5
Rice polish	14.0	8.5	8.0
Mustard oil cake	13.0	21.0	23.0
Soyabean meal	8.0	8.0	11.5
Groundnut cake	4.5	4.5	5.0
Mineral mixture	2.0	2.0	2.0
Salt	1.0	1.0	1.0
Oil	–	–	1.0

2005). After drying, the samples were ground in laboratory mill to pass through 1-mm sieve and stored in zip lock bags, properly labeled for further analysis. The *in vivo* trial on 18 lactating Murrah buffaloes was conducted in Livestock Research Centre, NDRI, Karnal.

Ethical approval: The present study strictly followed the norms of Institutional Animal Ethics Committee (IAEC) of National Dairy Research Institute (NDRI), Karnal, Haryana. The study primarily involved laboratory analysis of ruminant feeds, for which requisite permission for rumen liquor collection was granted by academic council and IAEC of NDRI, Karnal, Haryana.

Chemical analysis of Total Mixed Ration (TMR): The proximate analysis (crude protein [CP], ether extract [EE] and ash) of TMR was as per the standard procedures of AOAC (2005). The CP content was calculated indirectly by multiplying a factor of 6.25 to estimated Nitrogen (N) content (N × 6.25). The cell wall constituents' neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) of feeds were estimated by the methods of Van Soest *et al*. (1988). Insoluble crude protein concentrations in ADF (ADICP) and NDF (NDICP) were analyzed according to procedures described by Licitra *et al*. (1996). The chemical composition of TMR is given in Table 1.

In vitro *gas production method: In vitro* gas production of dietary TMR was determined according to Menke and Steingass (1988). *In vitro* trials were conducted to estimate the total gas production, *in vitro* (DM/OM) digestibility and metabolizable energy (ME) content. Rumen fistulated buffaloes were used as rumen fluid donors. About 800 ml rumen liquor was obtained from the animal before morning feeding. The rumen liquor was filtered through two layers of muslin cloth into a pre-warmed thermos flask punching out free air with carbon dioxide. The thermos flask was put in a pre-heated plastic box and immediately transported to the laboratory. Preparation of incubation media, filling of syringes and all other procedures were done according to Menke and Steingass (1988) protocol.

Three replicates each of TMR1 (30:70), TMR2 (40:60) and TMR3 (50:50) respectively were weighed (200 mg) and placed in 100 ml calibrated glass syringes (Fortuna Optima, Germany). The glass syringes were kept for incubation in a water bath at 39±0.5°C (Blummel 1997a). The measurement of gas production values was carried out after 24 h. The values of chemical constituents (CP, EE and ash) were used to arrive at the feed ME values using the equation given by Menke *et al*. (1979). *In vitro* DM and OM digestibility were estimated using methods of Menke *et al*. (1979).

ME (MJ/kg DM) = 2.20 + 0.1357 × Gas Produced (ml/200 mg DM) + 0.0057 × CP (g/kg DM) + 0.0002859 × EE² (g/kg DM)

OMD (%), 14.88+0.889 × Gas Produced (ml/200 mg DM) + 0.45 CP+0.0651 XA

where, XA = Ash content (%)

CNCPS fractionation for determining total digestible nutrients (TDN) and metabolizable energy (ME): Cornell Net Carbohydrate and Protein (CNCP) system (Sniffen *et al*. 1992) fractionates the carbohydrate of feed according to their degradation rate. Total digestible nutrient (True digestibility) was calculated according to NRC (2001): TDN = tdCP + tdNFC + tdNDF + (tdEE×2.25), where tdCP, tdNFC, tdNDF and tdEE corresponds to: truly digestible crude protein, truly digestible non-fibrous carbohydrates, truly digestible neutral detergent fibre and truly digestible ether extract respectively. 1 kg TDN contains 4.409 Mcal of digestible energy (NRC 1996) therefore for estimating the metabolizable energy of diets; value of 82% of digestible energy was considered (NRC 1996).

8.08±1.5) 9 *kg*± 2.5

In vivo *experiment*

Experiment design, animals and feeding: Eighteen mid lactating Murrah buffaloes (Body wt. 591±12.1; Milk yield 9 kg ±2.5) were divided into three groups of six animals each in a completely randomized design. Dietary treatments were TMR1 (30:70), TMR2 (40:60) and TMR3 (50:50) having maize silage and concentrate (Table 2). The diets had varying NDF levels but were isocaloric and isonitrogenous to avoid variation in feed intake through diet. The diets were formulated to have 13% CP (DM basis) as per ICAR, 2013. Murrah buffaloes were kept in hygienic, well ventilated experimental sheds for study duration of 90 days. Animals were shifted to metabolic cages during mid of the trial allowing for separate collection of faeces and urine. Weighed quantities of respective ration and clean drinking water was offered. Two days preliminary period and 6 days collection period was followed during the metabolic trial. The feed and faecal samples were collected and analyzed for proximate constituents (AOAC 2005) and fiber fractions (Van Soest 1991). TDN is a measure of apparent digestibility (NRC 2001); therefore comparison of ME value through *in vitro* techniques require conversion of apparent digestible TDN (TDNa) to true digestible TDN (TDNt) value. A factor for endogenous fecal energy equaled 0.30 Mcal/kg of DM (Weiss and Tebbe 2019) was added to metabolizable energy of feed converting TDNa to TDNt. Metabolizable energy (ME, MJ/kg DM) value was calculated from TDNt using the factors given by NRC (1988).

Statistical analysis: All the data generated was subjected to ANOVA (Snedecor and Cochran 1994) using software package SPSS version 20.0 and differences in mean were assessed by using Tukey's multiple range test. Correlation coefficient (r) between *in vitro* techniques and *in vivo* trial were determined using CORR procedure of SAS version 9.1 of SAS system for window, copyright© (2011), SAS Institute Inc., CARY, NC, USA.

RESULTS AND DISCUSSION

In vitro *experiment:* Result of *in vitro* feed fermentation as affected by dietary treatments is given in Fig. 1. Total gas production in 24 h ranged from 31.10 to 32.16 (ml per 200 mg DM). No significant changes (P>0.05) in the level of gas production was observed in TMR1, TMR2 and TMR3 respectively. This could be attributed to the availability of uniform energy to microbial population through the diet (Dung 2014). The organic matter digestibility and *in vitro* true digestibility was significantly (P<0.05) depressed in TMR2 and TMR3, ranging from 67.28 to 66.3% and 59.74 to 58.76% respectively as the concentrate level in diet decreased. Similar results were obtained by Zicarelli *et al*. (2011) where organic matter digestibility (OMD) and total gas production increased as the amount of concentrate in the diet increased. Kumar *et al*. (2013) reported *in vitro* dry matter digestibility increased with decreased roughage ratios [47.67, 61.67, 67.33% for diets 1(80:20), 2 (50:50) and 3 (20:80), respectively]. A possible explanation for this might be that a higher level of concentrate in diet contributes to increased level of soluble substrate in feed improving DM and OM digestibilities (Kumar *et al*. 2013).

The ME content of diet showed no significant (P>0.05) change with changing concentrate to roughage ratio, however the ME content was numerically higher in TMR1 as available energy from concentrate rich diet is more than roughage.

CNCPS fractionation for ME estimation: CNCPS fraction of dietary treatment is presented in Table 3. The NDICP and ADICP content ranged from 11.21, 9.72, 8.58 and 3.43, 3.24, 2.57 in TMR1, TMR2 and TMR3 respectively. A strong negative relationship exists between ADICP and N digestibility in ruminant diets, as ADICP is assumed to be completely unavailable to the animal (Yu and Thomas 1976, Licitra *et al*. 1996). Therefore, lower level of ADICP in feed indicates higher protein digestibility. The digestible tdNFC, tdFA and tdNDF were 51.92, 47.59, 44.33; 1.50, 1.20, 1.30 and 11.27, 15.98 and 18.51 in TMR1, TMR2 and TMR3 respectively. TDN (%) content of TMR1, TMR2 and TMR3 was 76.57, 76.48 and 76.41 respectively and their corresponding ME (MJ/kg DM) values were 12.34, 12.33 and 12.31 respectively. The results were in agreement with Tariq *et al*. (2018) who analysed TMR for its ME content using the CNCPS fractions. Cornell Net Carbohydrate and Protein System (Sniffen *et al*. 1992) predicts carbohydrate fermentation, microbial growth and digestible energy derived from feeds by their carbohydrate fractions. It follows integration of feed digestion and passage rates on the basis of level of feed intake (Russell *et al.* 1992).

In vivo *trial:* The chemical composition of dietary treatments (TMR) was presented in Table 1. Nutrient intake during 90 days experimental trial was presented in Table 4. The body weight of animals in three treatment groups was uniform throughout study period. A significant difference in the DM (kg/d) and TDN (g/d) intake was observed between the groups. DMI was significantly (P<0.01) higher in groups fed TMR2 and TMR3 (14.16 kg/d and 14.35 kg/d) when compared to TMR1 (13.57 kg/d). Similar findings were reported by Zhang *et al.* (2017) where decrease in

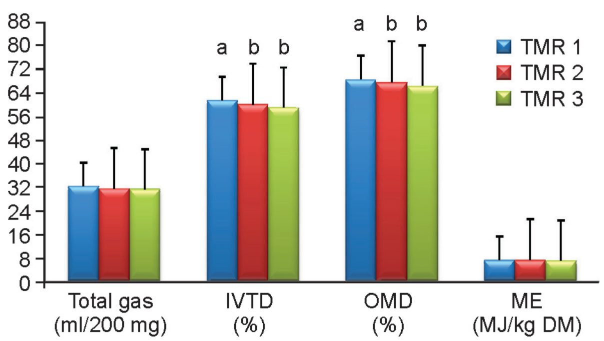

Fig. 1. *In vitro* feed fermentation and energy content of Total Mixed Ration (TMR) in IVGPT.

Table 3. CNCPS fraction for estimating ME content of TMR diet

Parameter	TMR1	TMR2	TMR3
tdNFC (%)	51.92	47.59	44.33
tdFA (%)	01.50	01.20	01.30
tdNDF (%)	11.27	15.98	18.51
TDN (%)	76.57	76.48	76.41
DE (Mcal/kg DM)	03.37	03.37	03.36
ME (Mcal/kg DM)	02.95	02.95	02.95
ME (MJ/kg DM)	12.34	12.33	12.31

*TMR1, maize silage + concentrate (30:70); TMR2, maize silage + concentrate (40:60); TMR3, maize silage + concentrate (50:50).

DMI was observed with increasing concentrate content in diet of HF cows owing to availability of a larger amount of fermentable carbohydrate in rumen, altering ruminal microbiota. Forage content being rich in hemicelluloses and celluloses serve as practical nutritive source for dairy cows, products of their fermentation in rumen are mostly short chain VFA's that can be utilized by host animal for production purposes (Sanh *et al.* 2002).

TDNI (g/d) also significantly (P<0.01) improved in TMR2 and TMR3 (8.95 and 8.97) when compared to TMR1 (8.68 g/d). The utilization of feed nutrients by lactating Murrah buffaloes i.e TDN (%) and ME (MJ/kg DM) is presented in Table 4. TDN (%) ranged from 65.68, 64.59 and 63.52 in TMR1, TMR2 and TMR3 respectively. ME (MJ/kg DM) requirement of lactating Murrah buffaloes was 11.35, 11.18 and 11.02 in TMR1, TMR2 and TMR3 respectively. The results were in agreement with previous studies of Santra and Karim (2009), Malisetty *et al*. (2013). Gafaar *et al*. (2009) also observed significant increase (P<0.05) in the intake of DM, TDN and ME when fed a diet with 60% concentrate and 40% roughage to lactating buffaloes. Dietary variation in concentrate to roughage is one of the means which influence microbial population for rumen fermentation and the constituents of concentrate in treatment diet affect its digestibility (Sinha *et al*. 2017).

Table 4. Nutrient intake and utilization by Murrah buffaloes during experimental trial (90 days)

Attribute	TMR1	TMR2	TMR3	P value
BW (kg)	589± 17.3	590± 14.2	589± 19.8	0.99
DMI (kg/d)	13.57^{a}± 0.02	14.16^{b}± 0.17	14.35^{b}± 0.14	0.00
TDNI (kg/d)	08.68^{a}± 0.04	08.95^{b}± 0.06	08.97^{b}± 0.07	0.00
Nutrient utilization				
TDN (%)	65.68	64.59	63.52	0.33
DE (%)	2.88	2.84	2.79	0.42
ME (Mcal/kg DM)	2.71	2.67	2.63	0.86
ME (MJ/kg DM)	11.35	11.18	11.02	0.86

^{ab}different superscripts in a row differ significantly; *TMR1, maize silage + concentrate (30:70); TMR2, maize silage + concentrate (40:60); TMR3, maize silage + concentrate (50:50).

Predicting the accuracy of in vitro *technique in determining ME requirement of animal:* Mean values for ME observed in the present study were used to generate a correlation between the *in vitro* techniques and *in vivo* utilization of metabolizable energy by Murrah buffaloes (Table 5). A high correlation (P<0.01) of *in vivo* trial was observed with *in vitro* gas production (0.734) and CNCPS fraction (0.752) in evaluating the ME content of total mixed ration for Murrah buffaloes. IVGP technique estimates ME content based on gas production which is a reflection of degradable carbohydrate present in feedstuffs (Menke *et al*. 1979). However, difference among the metabolizable energy estimation of feed samples studied by *in vitro* gas production and CNCPS fractionation may be attributed to difference in processing procedures (Reddy *et al*. 2017).

Table 5. Correlation coefficients between *in vitro* techniques and *in vivo* trial in predicting metabolizable energy content of TMR for lactating Murrah buffaloes

	IVGPT	CNCPS	*In vivo*
IVGPT	1	0.854**	0.734**
CNCPS		1	0.752**
In vivo			1

IVGPT (*in vitro* gas production technique); CNCPS (Cornell net carbohydrate and protein system); **Correlation is significant at 0.01 level.

The results of present experimented concluded that both *in vitro* techniques can be utilized for estimating metabolizable energy concentrations in total mixed rations for lactating Murrah buffaloes. The correlation of CNCPS fraction with *in vivo* trial was numerically higher; however, the result was statistically insignificant. Further, the comparative evaluation of *in vitro* techniques namely *in vitro* gas production and Cornell net carbohydrate system revealed that both methods were reliable in evaluating ME content of dietary feedstuffs for feeding Murrah buffaloes.

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