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Determination of *In vitro* Nutrient Digestibility of Silage based Diets with Varying Levels of Crude Fibre using Pig Faecal Inoculum

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ABSTRACT

Background: The *in-vitro* nutrient digestibility and fermentation mimicking gastrointestinal process of pigs is a convenient method for rapid and cheaper measurement of degradation of feed ingredients. In the present study silage based diets containing varying levels of crude fibre were subjected to *in vitro* digestibility evaluation using pig faecal inoculum to determine the best crude fibre level for feeding pigs.

Methods: Eight diets were prepared namely, B1, B2, V1, V2, V3, M1, M2 and M3 having 6.28, 8.43, 8.03, 10.0, 11.96, 8.17, 10.31 and 12.06 per cent crude fibre (on dry matter basis) respectively, where diets B1 and B2 were basal diets, V1, V2 and V3 contained vegetable waste silage; M1, M2 and M3 contained maize silage -as additional fibre source. An *in-vitro* study was carried out to investigate the nutrient digestibility of diets involving enzymatic hydrolysis and fermentation using pig faecal inoculum.

Result: The *in-vitro* total tract digestibility (IVTTD) of dry matter (DM), crude protein (CP) and organic matter (OM) was significantly higher (P<0.001) in B1 diet than the other diets. The digestibility of DM, CP and OM of B2 diet was also significantly higher (p<0.001) than the M2, M3, V2 and V3 diets but comparable to M1 and V1 diets. The IVTTD of crude fibre (CF) was higher in V3 diet (25.85%) and lowest (P<0.001) in M1 diet (11.65%) and both showed a highly significant (P<0.001) difference (to other diets. The DM, CP and OM digestibility were significantly higher (P<0.001) in the diets containing normal fibre sources than silage containing diets. Cumulative gas production (ml/0.5g feed) at 24 h of *in-vitro* fermentation were significantly higher (P<0.001) in B2 (34.95) and B1 (31.00) diets than other diets. As the CF level in diets increased from 6 to 12%, the digestibility of nutrients linearly decreased except CF digestibility. It was revealed from the study that the level and sources of crude fibre in diets influenced the digestibility of nutrients.

Key words: Dietary CF, faecal inoculum, *in-vitro* Digestibility, Pig, Silage.

INTRODUCTION

The use of fibre rich feed ingredients in pig diets especially in small scale farming is rising in recent times due to their wide availability and relatively low cost as compared to commercial feeds. Similarly, offering silages of forages and vegetables in the diets of pigs is becoming an efficient practice in organic pig production system (Edwards, 2002; Bikker *et al*., 2014; Wüstholz *et al*., 2017 and Friman *et al.,* 2021). High fibre diets increase cellulolytic bacteria in the large intestines of pigs (Anugwa *et al*., 1989) and have prebiotic effects and improve gut-associated immune system (Lindberg, 2014). The fermentation products of crude fibre at large intestine such as volatile fatty acids contribute 7 to 25% of maintenance energy needs of pigs (Yen *et al*., 1991) and butyric acid acts as instant energy source for the colonocytes and regulates cell growth and differentiation (Roediger, 1982). As a part of plant cell wall, crude fibre is generally less digestible in pigs and it adversely effects nutrient utilization (Zhang *et al*., 2013; Wilfart *et al*., 2007). The impact of fibre level on nutrients digestibility may vary with fibre source, properties and composition of fibre (Hogberg and Lindberg, 2004; Van Soest, 1978).

Hence, it is imperative to understand the effect of fibre level on nutrient digestibility and voluntary feed intake for optimum swine production. Numerous studies by different scientists regarding digestibility of fibre and fibrous feeds in pigs have been executed. However, information about nutrient digestibility and fermentation characteristics of fibre

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from vegetable waste silages and maize silages are meagre.

Determination of digestibility of nutrients is an important criterion for quality evaluation and feeding value. Digestibility evaluation using animal is a time-consuming and tedious process and requires large quantities of feeds and animals. In pigs, digestibility of nutrients starts in the stomach (pepsin and hydrochloric acid), continues in small intestine (enzymatic hydrolysis) and finally in caecum and colon fermentation occurs and help in the breakdown of fibre and other nutrients that had escaped enzymatic hydrolysis. The *in vitro* nutrient digestibility and fermentation that mimics gastrointestinal process of pigs are convenient methods for rapid and inexpensive measurement of the degradation of feed ingredients (Boisen and Fernandez, 1997; Guo *et al*., 2004). There may be numerous modifications, where some may use commercially available multi-enzymes (Boisen and Fernandez, 1997) or microbial inoculum either rumen or faecal inoculum (Dung *et al*., 2002; Bindelle *et al*., 2007; Youssef and Kamphues, 2020) for simulation of large intestinal fermentation processes. Therefore, vegetable waste silage and maize silage were incorporated as a source of fibre to increase the CF level in experimental diets and were subjected to *in-vitro* digestion comprising two steps enzymatic hydrolysis and fermentation to investigate the higher level of incorporation of CF in pig feeds.

MATERIALS AND METHODS

The study was conducted at Indian Council of Agricultural Research-National Research Centre on Pig, Rani, Guwahati, Assam, India (91°17′ 49″ E longitude, 25°48′ 30″ N latitude and 467m altitude) during the month of April-September, 2021. The experiment was carried out taking prior approval of the Institutional Animal Ethics Committee (IAEC) of Indian Council of Agricultural Research-National Research Centre on Pig, Rani, Guwahati and ensured that no potential harm toward animal welfare would be done.

Silages were prepared from maize fodder (*Zea mays*) and vegetable waste (cabbage and cauliflower at the ratio 60:40 in raw basis) individually in silage bags following standard procedure (3 kg jaggery and 250 g salt per 100 kg raw materials) and opened for sampling after 4 weeks. Feed ingredients were analysed for proximate principles (AOAC, 2012) and neutral detergent soluble and fibre fractions as per the procedure of Van Soest *et al.* (1991). Energy content of feeds was calculated from the proximate composition using the formula as mentioned by NRC (2012). The pH of silages was estimated following the method described by Bernardes *et al*. (2019) using digital pH meter (pHTestr 10, Eutech Instruments). The silage quality was determined by using Flieg's score formula (Kilic, 2010). All feed ingredients were dried in oven following standard procedure and ground through a 1mm mesh screen with Wiley No. 4 Laboratory Mill. Low temperature (60°C) oven drying method was followed for estimation of dry matter (DM) in silages.

Eight types of diets were formulated as per the nutrient requirement of swine (ICAR, 2013) for *in-vitro* studies with commonly available feed ingredients and silages (vegetable waste and maize silage) as fibre source as mentioned in Table 1.

Table 1: Ingredient and nutrient composition of diets used for *in-vitro* digestibility experiments.

Particulars	Basal diets		Vegetable waste silage diets			Maize silage diets		
	B1	B2	V1	V2	V3	M1	M2	M3
Maize (crushed)	58.00	45.50	51.50	42.00	30.50	53.50	44.50	37.50
Soybean meal (deoiled)	12.00	8.50	4.50	3.50	4.50	6.50	7.00	7.00
Wheat bran	12.50	36.00	7.00	7.00	12.00	3.50	8.00	7.00
Groundnut cake (solvent extracted)	15.50	8.00	16.00	11.00	6.00	20.00	15.00	14.00
Vegetable waste silage	0.00	0.00	18.50	34.00	44.50	0.00	0.00	0.00
Maize silage	0.00	0.00	0.00	0.00	0.00	14.00	23.00	32.00
Oil	0.00	0.00	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mixture*	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Common salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Nutrient composition								
Crude protein (%)	19.05	17.04	18.53	17.73	17.37	18.72	17.23	16.73
GE (kcal/kg)**	4284.5	4209.6	4258.9	4203.4	4152.0	4287.9	4241.2	4213.9
ME (kcal/kg)**	3221.4	2890.8	3163.3	3020.9	2944.3	3169.1	2970.2	2846.1
Calorie : Protein	169.1	169.6	170.7	170.4	169.5	169.3	172.4	170.1
Crude fibre (%)	6.28	8.43	8.03	10.0	11.96	8.17	10.31	12.06
OM (%)	93.06	92.35	92.20	91.2	90.21	92.90	92.42	92.03
NDF (%)	20.99	27.41	20.69	21.85	24.08	22.26	26.00	28.25
ADF (%)	6.23	7.68	8.81	11.53	13.87	8.37	10.57	12.46

OM-Organic matter; NDF-Neutral detergent fibre; ADF-Acid detergent fibre; ***Composition of mineral mixture Each 1 kg contains: vitamin A 750,000 IU; vitamin D3 150,000 IU; vitamin C 0.75 g; vitamin E 0.25 g; Niacin 0.5 g; Biotin 0.005 g; calcium 300 g; phosphorus 60 g; magnesium 7.2 g; manganese 1.5 g; iodine 0.15 g; sodium 4 g; sulphur 7.2 g iron 3 g; zinc 3.7 g; copper 1.2 g; cobalt 0.10 g; selenium 0.02 g; L-lysine mono HCl 2 g; DL- methionine 1 g; protein hydrolysate 1.2 g; BHT 0.25 g; Betaine 0.75 g.;**Calculated value.

Representative samples from formulated diets were subjected to a 2-step *in-vitro* enzymatic hydrolysis to mimic the gastro-intestinal digestion using pepsin and pancreatin solutions (Boisen and Fernández, 1997) followed by *in-vitro* fermentation as per the procedure described by Youssef and Kamphues (2020) using faecal materials from pigs slaughtered in the institute slaughter house of ICAR-NRC on Pig, Rani, Guwahati.

In-vitro total tract digestibility of DM, CP, OM and CF were obtained after enzymatic hydrolysis and *in-vitro* fermentation. Cumulative gas production (ml/0.5 g feed) during *in-vitro* fermentation of experimental diets was also estimated. The data generated were subjected to one way ANOVA as per the methods described by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Silage parameters

The prepared silages were evaluated for moisture, pH, fleig point, colour, odour and mould growth (Table 2). The average moisture content and pH level of vegetable waste silage (VS) was more (75.35%; 4.9) compared to maize silage (MS, 69.35%; 4.0). The moisture content in silage greatly influenced the pH level. Yahaya *et al*. (2002) opined that at 65-75% moisture level in silage the pH remained below 5; whereas moisture content below or above this value caused increase the pH of orchard grass silages. The average fleig point for maize silage (MS) and vegetable waste silage (VS) was 106 and 59.3, respectively and as per fleig score maize silage was excellent and vegetable silage was fair in quality (Kilic, 2010). VS contained comparatively more CP and energy (18.25%; ME 2536 kcal/kg) than MS (CP 8.3%, ME 2000.8 kcal/kg). Inversely, MS was richer in CF (24.35 vs18.5) and NDF (45.5 vs 28.5) content than VS. Literature pertaining to the chemical composition of vegetable waste silage is scanty to relate the observed findings and it differs as per the content and type of vegetable waste used for silage preparation. The chemical composition of MS was comparable to the report of NDDB (2012) and Htet *et al*. (2016).

Table 2: Chemical and physical qualities of silages.

Particulars	Moisture (%)	pH(range)	Fleig point	Colour	Odour	Mouldgrowth
Maize silage	69.0±0.42	4.0±0.09 (3.7-4.2)	106±2.84	Brownish green	Sweet	No
Vegetable waste silage	75.35±0.63	4.9±0.07 (4.7-5.1)	59.3±3.91	Dark green	Sweet	No

Table 3: *In-vitro* dry matter digestibility ($IVDMD_h$) and total tract digestibility of nutrients (IVTTD) of experimental diets.

Diet	$IVDMD_h$ (%)	IVTTD (%)			
		DM	CP	OM	CF
A. Diet containing various sources and levels of CF					
B1	62.57^{a}	76.54^{a}	85.65^{a}	76.47^{a}	18.31^{bc}
B2	55.03^{b}	70.21^{b}	80.95^{b}	67.44^{b}	22.49^{a}
M1	53.81^{bc}	66.49^{bcd}	79.60^{bc}	64.50^{bc}	11.65^{d}
M2	50.82^{cd}	63.68^{de}	76.45^{cd}	60.73^{cd}	16.32^{bd}
M3	45.50^{f}	60.80^{e}	73.80^{d}	57.95^{d}	16.70^{b}
V1	54.61^{b}	69.34^{bc}	80.25^{bc}	67.83^{b}	15.71^{bd}
V2	50.13^{de}	64.49^{cde}	77.13^{bcd}	62.53^{bcd}	18.18^{bc}
V3	47.44^{ef}	61.60^{de}	74.40^{d}	59.94^{cd}	25.85^{a}
SEm	0.572	0.94	0.72	1.07	0.79
P value	<0.001	<0.001	<0.001	<0.001	<0.001
B. CF source					
Normal fibre source	58.80^{a}	73.38^{a}	83.30^{a}	71.95^{a}	20.40^{a}
Maize silage	50.04^{b}	63.66^{b}	76.67^{b}	61.06^{b}	14.89^{b}
Vegetable waste silage	50.73^{b}	65.14^{b}	77.26^{b}	63.43^{b}	19.91^{a}
SEm	0.572	0.94	0.72	1.07	0.79
P value	<0.001	<0.001	<0.001	<0.001	<0.01
C. CF level					
Diet with 6% CF	62.57^{a}	76.54^{a}	85.65^{a}	76.47^{a}	18.31
Diet with 8% CF	54.48^{b}	68.68^{b}	80.27^{b}	66.59^{b}	16.61
Diet with 10% CF	50.47^{c}	64.09^{c}	76.79^{c}	61.63^{c}	17.25
Diet with 12% CF	46.47^{d}	61.20^{c}	74.10^{d}	58.94^{c}	21.28
SEm	0.572	0.94	0.72	1.07	0.79
P value	<0.001	<0.001	<0.001	<0.001	0.128

B1 and B2- Basal diet; M1, M2 and M3- diets with maize silage; V1, V2 and V3- diets with vegetable waste silage; [abcdef]Means bearing different superscripts in a column within a subhead (A, B, C) differs significantly (P<0.001; P<0.01).

In-vitro dry matter digestibility in enzymatic hydrolysis ($IVDMD_h$)

Diet B1 (62.57%) had significantly higher (P<0.001) $IVDMD_h$; whereas M3 and V3 diets had significantly lower (P<0.001) $IVDMD_h$ as compared to other diets (Table 3). The $IVDMD_h$ of diets with normal fibre source was found to be significantly higher (50.80%; P<0.001) as compared to MS or VS containing diets. The CF levels irrespective of fibre sources had significant influence on $IVDMD_h$ as shown in Table 4 and digestibility was significantly (P<0.001) reduced as CF level increased in the diet. It may be due to negligible action of pepsin and pancreatin enzymes on fibre and fibre bound nutrients in upper digestive tract.

In-vitro total tract digestibility (IVTTD) of different nutrients

The IVTTD of DM of different diets ranged from 60.80 to 76.54%, where B1 diet showed a significantly higher value (P<0.001) than the other diets. The digestibility of B2 diet was significantly higher (P<0.001) than the M2, M3, V2 and V3 but it was non-significant to M1 and V1 diets. The IVTTD of CP and OM were also significantly higher (P<0.001) for B1 diet compared to other diets, which showed similar trend as the digestibility of DM. The IVTTD of CF was higher in V3 diet (25.85%) and lowest (P<0.001) in M1 diet (11.65%) and showed a highly significant difference to B1, M1, M2, M3, V1 and V2 diets. The higher CF digestibility of high fibre diets may be due to higher concentration of CF as substrate for microbial fermentation (Anguita *et al*., 2006). The DM, CP and OM digestibility were significantly higher (P<0.001) in the diets that contained normal fibre sources. The CF digestibility of diet containing MS was significantly lower (P<0.01) than the diet with normal feed or vegetable silage as fibre sources. This could be due the quality of fibre and degree of lignification. The diet containing 6% CF level showed a significantly higher (P<0.001) IVTTD of DM and OM followed by 8% CF level; while comparable in diets with 10 and 12% CF level (Table 3). A highly significant difference was recorded for CP digestibility (P<0.001) among the diets with different CF level and diet with 6% CF level showed higher CP digestibility. As the level of CF increased in the diets the IVTTD of CP significantly decreased. On the other hand, the IVTTD of CF was comparable among the diets with different levels of CF (P>0.05). The result obtained is comparable with the outcomes from previous experiment (Gürsoy *et al*., 2021). Youssef and Kamphues (2020) also reported similar findings when fibre rich ingredients were exposed to *in vitro* fermentation with faecal inoculum of swine

Table 4: Cumulative gas production (ml/0.5g feed) during *in-vitro* fermentation of experimental diets.

Diet	Cumulative gas production (ml/0.5 g feed)			
	6 h	12 h	18 h	24 h
A. Diet containing various sources and levels of CF				
B1	8.20^{a}	15.10^{a}	22.80^{a}	31.00^{b}
B2	8.10^{a}	15.03^{a}	24.68^{a}	34.95^{a}
M1	3.25^{cd}	7.00^{bc}	13.50^{b}	20.15^{c}
M2	2.08^{f}	4.00^{d}	11.00^{cd}	18.50^{cd}
M3	2.50^{ef}	4.50^{d}	10.05^{d}	16.00^{d}
V1	4.50^{b}	8.00^{b}	14.00^{b}	20.50^{c}
V2	3.75^{c}	7.00^{bc}	12.50^{bc}	18.50^{cd}
V3	3.00^{de}	6.03^{c}	11.05^{cd}	16.10^{d}
SEm	0.41	0.74	0.95	1.20
P Value	<0.001	<0.001	<0.001	<0.001
B. CF sources				
Normal fibre source	8.15^{a}	15.06^{a}	23.74^{a}	32.98^{a}
Maize silage	2.61^{b}	5.17^{b}	11.52^{b}	18.22^{b}
Vegetable waste silage	3.75^{c}	7.01^{c}	12.52^{b}	18.36^{b}
SEm	0.41	0.74	0.95	1.20
P value	<0.001	<0.001	<0.001	<0.001
C. CF levels				
Diet with 6% CF	8.20^{a}	15.10^{a}	22.80^{a}	31.00^{a}
Diet with 8% CF	5.28^{b}	10.01^{b}	17.39^{b}	25.20^{ab}
Diet with 10% CF	2.91^{c}	5.50^{c}	11.75^{c}	18.50^{bc}
Diet with 12% CF	2.75^{c}	5.26^{c}	10.55^{c}	16.05^{c}
SEm	0.41	0.74	0.95	1.20
P value	<0.001	<0.001	<0.001	<0.001

*B1 and B2- Basal diet; M1, M2 and M3- diets containing maize fodder silage; V1, V2 and V3- diets containing vegetable waste silage; [abcdef] Means bearing different superscripts in a column within a subhead (A, B, C) differs significantly (P<0.001).

and turkey. The IVTTD of CF was significantly lower (P<0.01) in diets containing MS than normal diets or VS containing diets. The overall IVTTD of nutrients in all the formulated diets were found satisfactory because the diets were properly balanced as per the requirement of growing-finishing pigs.

Cumulative gas production during fermentation

Cumulative gas production at 24 h of *in-vitro* fermentation ranged from 16.00 to 34.95 (ml/0.5 g feed) and gas production in B2 diet was significantly higher (P<0.001) followed by B1 diet (Table 4). Cumulative gas production of diets with normal fibre source was significantly higher (P<0.001) than MS or VS added diets. It was found that increase fibre level in diet reduces the gas production (Table 4). The higher production of gas in B1 and B2 diets may be due to higher content of fermentable nutrients and easily digestible fibre. However, cumulative gas production (ml/0.5g feed) in *in-vitro* fermentation was less compared to experiments of Youssef and Kamphues, (2020) who used substrate without enzymatic hydrolysis; but results were comparable to that reported by Bachmann *et al.* (2021) for fibre rich ingredients like sugar beet pulp, soybean shell, lucerne and wheat bran after pre-digestion with enzymes. This may be due to the fact that pre-digested substrate mostly produces less gas, NH_3 and short chain fatty acids as compared to substrate without pre-digestion (Bachmann *et al.,* 2021).

CONCLUSION

The present study revealed that the level and sources of crude fibre in diets influence the digestibility of nutrients. The results obtained from this experiment confirmed the possibilities to use maize silage and vegetable waste silage as CF source in diets of growing-finishing pigs. It is suggested that for better understanding of the role of vegetable waste silage and maize silage as fibre source and their level of incorporation in diets for better health and production needs extensive *in-vivo* studies in pigs.

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