Research Article

A Simplified Medium for *In Vitro* Digestion of Ruminants: Cattle-Fed Rice Straw with Supplementation of Legume Foliage

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Abstract | An experiment was carried out to evaluate the effect of a supplement of *Sesbania grandiflora* L. foliage (SF) on the nutrient digestibility of crossbred (local × Red Sindhi) cattle fed basal diets of rice straw (RS) in Southwestern Vietnam. The experiment was designed to change each of the 4 cattle fed 6 diets over 6 periods, the dietary treatments were RS with the supplementation of SF from 0, 7, 14, 21, and 28 to 35% of total dry matter (DM) intake. Urea was also used to adjust the crude protein content in diets to approximately 11% of DM. The dietary organic matter digestibility (OMD) and metabolizable energy (ME) were determined by the *in vivo*, formal *in vitro* (F_iv) and *in vitro* procedure of simplified medium (S_iv) by eliminating trypticase, minerals and reducing solutions and replacing them with rumen fluid from slaughterhouses of cattle with unknown dietary histories. The results showed that the dietary OMD, total digestible nutrients, and ME values significantly ($P<0.05$) increased according to the rise of the SF supplement. Still, the digestible fiber had a significant ($P<0.05$) decreasing trend. The OMD and ME values (n=24) estimated by the S_iv procedure had a close linear relationship to the *in vivo* (R^2 0.78 - 0.80) and F_iv (R^2 0.86 - 0.87). It is, therefore, concluded that the *in vitro* test of digestion could utilize rumen fluid from slaughterhouses and eliminate some chemicals in the medium to satisfy better concerns associated with animal ethics and welfare, environment and cost.

Keywords | Digestibility, *In vivo*, *In vitro*, Medium, Rumen fluid, Sesbania

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INTRODUCTION

In Southwestern Vietnam, cropland is prioritized above grassland. Roughage supplies for cattle are of low quality, primarily rice straw (RS). In this situation, a high-quality feed supplement would boost performance (Leddin *et al.*, 2011). *Sesbania grandiflora* L., a common legume tree in Southwestern Vietnam, produces edible blooms, and foliage is a high-quality ruminant feed (Thu and Dong, 2017). Digestibility tests are crucial for evaluating the quality of roughages for ruminants since they show what the animal can digest and use. *In vivo* experiments are the most reliable technique to acquire data on animal diets' digestible nutrients (McDonald *et al.*, 2010). However, the *in vivo* procedure has been regarded as costly because it requires caring for live animals in cages for a long time.

Many attempts have been made to create easier methods for determining digestibility. Tilley and Terry's (1963) two-stage *in vitro* technique, refined by Goering and Van Soest (1970), is one such technique that evaluates digestion by replicating the processes in the animal's alimentary

canal in the laboratory. This technique has the advantage of incubating multiple samples at once, which is less expensive, less arduous, and produces findings faster than the *in vivo* procedure (Stern *et al*., 1997). Its disadvantage is requiring maintenance of animals to be fitted with a rumen fistula to allow direct access into the rumen to obtain rumen fluid as a source of inoculum and some chemicals to create a medium such as trypticase, buffer (NH_4HCO_3 and $NaHCO_3$), macro- (Na_2HPO_4, KH_2PO_4, and $MgSO_4$) and micro- ($CaCl_2$, $MnCl_2$, $CoCl_2$, and $FeCl_3$) minerals, reducing (sodium sulfide, cysteine), resazurin, and carbon dioxide (Goering and Van Soest, 1970). It would, therefore, raise ethical and animal welfare problems and practical considerations for the environment and cost. At the same time, developing countries are likely to have challenges in sourcing chemicals and supporting research. Some attempts have been made to search for new sources of inoculum, such as slaughtered animal rumen liquor and feces (Borba and Ribeiro, 1996; Mohamed and Chaudhry, 2012; Mo and Thu, 2008; Mpemba *et al*., 2018; Lifa *et al*., 2018), as well as simplifying reagents in medium (Mould *et al*., 2005; Mo and Thu, 2008). However, there is a shortage of information on evaluating the association between *in vivo* and these *in vitro* technique alterations in Southwestern Vietnam. This study aims to assess the relationship between the *in vitro* digestibility in a simplified medium and the conventional *in vivo* technique in the case of cattle-fed RS with a supplement of *Sesbania grandiflora* foliage (SF) for addressing both practical and ethical considerations.

MATERIALS AND METHODS

The experiment was located at 10°01'56.4"N and 105°45'57.5"E in Southwest Vietnam and took place from autumn to winter, the coolest period of the year. The climate of Southwest Vietnam is of the tropical delta with temperatures from 25.2 to 28.2 ^{0}C, moisture from 78.2 to 84.0%, sunshine from 1893 to 2340 hours, and rainfall from 1932 to 2679 mm (GSO, 2023). Article 72 of the Vietnamese Law on Animal Husbandry (No. 32/2018/QH14), which regulates the humane treatment of livestock used in scientific research and other activities, guided the ethics and welfare of experimental animals in this study.

Animals and Feeds

Four experimental animals were male crossbred (local × Red Sindhi) cattle approximately 1 year of age with a live weight (W) of 136 to 185 kg. Cattle knowing birth dates were purchased from farmers in the region. Cattle were quarantined for 15 days to observe the incidence of any disease. During this quarantine period, they were treated with Ivermectin (0.5 ml/50 kg weight) to control internal and external parasites. Each animal grew up in a cage of 3 × 1.5 m in size. The cages were appended feeder and drinking trough separately and disinfected monthly by Virkon'S. The ingredients of diets feeding experimental cattle consisted of RS, SF, and urea. Rice straw was once collected during the experiment from fields near the experimental site in a winter-spring season with a variety of OM7347. The SF was daily picked in gardens surrounding the experimental site and used as a supplement to animals. The chemical composition of RS and SF are shown in Table 1. The urea had a nitrogen (N) content of 46.08 % equivalent to 288 % of crude protein (CP).

Table 1: Nutrient composition (% of DM, except DM as %) of feeds and diets.

Chemical composition	RS	SF	SF0	SF7	SF14	SF21	SF28	SF35
DM	85.5	24.2	84.9	73.4	63.5	56.2	50.5	44.3
OM	84.0	90.2	85.5	83.8	84.6	85.0	85.0	87.3
CP	5.35	22.1	11.2	11.2	11.2	11.2	11.1	11.3
EE	3.13	9.42	3.10	3.43	4.04	4.47	4.82	5.31
NFC	3.42	30.8	0.60	1.27	4.36	7.13	9.28	14.3
NDF	72.1	27.9	70.6	67.9	65.0	62.2	59.8	56.4
ADF	41.7	21.7	40.8	38.8	36.7	34.7	32.9	30.4
ADL	7.57	10.1	7.41	7.61	7.83	8.03	8.22	8.47
Hemicellulose	30.4	6.20	29.8	29.1	28.3	27.5	26.9	26.0
Cellulose	34.1	11.6	33.4	31.2	28.9	26.7	24.7	21.9

DM: dry matter; **OM:** organic matter; **CP:** crude protein; **EE:** ether extract; **NFC:** non-fiber carbohydrate; **NDF:** neutral detergent fiber; **ADF:** acid detergent fiber; **ADL:** acid detergent lignin; **RS:** rice straw; **SF:** Sesbania foliage; **SF0 – SF35:** diets supplemented with Sesbania foliage from 0 to 35% of DM intake.

Design and Feeding

The experiment was a change-over design consisting of four cattle; one each changed throughout six periods to receive six different diets. The diets consisted of basal RS with six levels of SF supplementations ranging from 0, 7, 14, 21, 28, and 35% of total dry matter (DM) intake (DMI), corresponding to the treatments of SF0, SF7, SF14, SF21, SF28, and SF35. The diet's chemical composition is shown in Table 1. Four cattle (numbered 1-4), respectively, were fed the SF0, SF28, SF7, and SF35 diets in the 1st period from Sep 1 to 28; the SF35, SF0, SF14, and SF21 diets in the 2nd period from Sep 29 to Oct 26; the SF21, SF35, SF0, and SF14 diets in the 3rd period from Oct 27 to Nov 23; the SF28, SF7, SF35, and SF0 diets in the 4th period from Nov 24 to Dec 21; the SF7, SF14, SF21, and SF28 diets in the 5th period from Dec 22 to Jan 4 next year; and the SF14, SF21, SF28, and SF7 diets in the 6th period from Jan 5 to Feb 1. In each experimental period, cattle had 14 days for dietary adaptation, 7 days for sampling, and 7 days for far-seeing.

Cattle were fed fresh SF at 8:00 a.m. and 5:00 p.m. For the SF0 treatment, the RS was offered *ad-libitum*, while for other treatments, the RS was offered approximately 93, 86, 79, 72, and 65% DMI of the SF0. Throughout the experiment, water was supplied for animals' free access. Urea was employed to adjust the same CP content (~11% of DM) in dietary treatments. These dietary CP contents could meet Zebu crossbred cattle's maintenance requirements (Filho *et al.*, 2016).

In Vivo Digestibility

The percent ratio of the difference between nutrients of intake and fecal was the digestibility of nutrients (McDonald *et al.*, 2010) calculated as a general equation (Eq.) 1:

$$Digestibility\ of\ nutrients = 100 \times \frac{1 - weight\ of\ nutrient\ in\ feces}{nutrient\ intake} \quad (1)$$

The digestible nutrients including crude protein (DCP), ether extract (DEE), non-fiber carbohydrate (DNFC), neutral detergent fiber (DNDF), and acid detergent fiber (DADF) as % of DM were calculated as a general Eq. 2:

$$Digestible\ nutrients = \frac{nutrient\ content \times digestibility\ of\ nutrient}{100} \quad (2)$$

The feeds, refusals, and feces were daily weighed and sampled each morning for 7 consecutive days (from 15th to 21st day) of each period. The subsamples each day were dried at 55°C for 24 hours to grind fine through a sieve with a size of 1 mm (AOAC, 1990, method 950.02), and stored at -20°C. At the end of each period, the subsamples were pooled and mixed before chemical analysis and determination of the *in vitro* digestibility.

In Vitro Digestibility

There were two *in vitro* procedures with each test of *in vitro* digestibility for each experimental unit reproduced in double and averaged. The formal *in vitro* (F_iv) procedure was done according to Goering and Van Soest (1970) with fermentation of substrate for 48 hours. The second *in vitro* procedure was done at the same time, a modification from the F_iv procedure, utilizing simpler reagents (S_iv) by removing trypticase, macro-, and micro-minerals, and reducing in the medium, and substrate was incubated for 72 hours (Mo and Thu, 2008).

The rumen liquor was freshly removed from three crossbred (local x Red Sindhi) cattle from a slaughterhouse with unknown dietary histories. The animals were performed according to animal ethics and welfare following the Vietnamese standards (TCVN 12448: 2018). About 15 minutes post-slaughter, the rumen was cut open with a knife to collect the contents, which were immediately strained into pre-warmed thermal flasks through three layers of muslin cloth and transported back to the laboratory quickly (Mohamed and Chaudhry, 2012).

In the S_iv process, substrates (500 mg/tube, W_1) were weighed into glass tubes (175 mL) and sealed with rubber stoppers. The glass tubes were pre-warmed in a water bath at 40 °C before adding 8 ml of buffer solution and 1 ml of resazurin (0.1 percent w/v). The tubes were shaken and warmed at 40 °C before being filled with 42 mL of rumen fluid and continuously pumped with CO_2 until the resazurin indicator turned red to colorless. The buffer solution was made in the manner described by Goering and Van Soest (1970) by combining 4 g of ammonium bicarbonate (NH_4HCO_3) and 35 g of sodium bicarbonate ($NaHCO_3$) in 1 liter of distilled water. Following treatment with 50 ml of a neutral detergent solution at 85 °C overnight, the mixture in each tube was filtered in a crucible, rinsed twice with hot water and twice with acetone, and then sucked dry. The crucibles were dried for 3 hours at 105 °C and weighed (W_2). Ultimately, they were furnaced for 2 hours at 500 °C and weighed (W_3). The blanks ($W_0 = W_2 - W_3$ for the blank case) without substrate were included. The *in vitro* organic matter (OM) digestibility (iv OMD) was determined as Eq 3:

$$iv\ OMD = \frac{100 - (W2 - W3 - W0) \times 100}{W1 \times \frac{DM}{100} \times \frac{OM}{100}} \quad (3)$$

Where;
W_0, W_1, W_2 and W_3 are as gram, DM and OM are as %.

Chemical Analysis

The DM was determined by drying at 105 °C in an oven for 3 hours (Shreve *et al.*, 2006). Chemical analyses were done according to AOAC (1990). The Ash was done by the furnace at 600 °C for 4 hours (method 942.05) then the OM was calculated as 100 minus Ash. The N was measured by the Kjeldahl method, and CP was calculated as % N × 6.25 (method 984.13). The ether extract (EE) was recorded by keeping the sample in ethyl ether to extract in the Soxhlet system (method 920.39). Analysis of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) was according to Goering and Van Soest (1970). The non-fiber carbohydrate (NFC), Hemicellulose, and Cellulose were calculated as Eq. 4, Eq. 5, and Eq. 6, respectively:

$$NFC = OM - CP - EE - NDF \quad (4)$$
$$Hemicellulose = NDF - ADF \quad (5)$$
$$Cellulose = ADF - ADL \quad (6)$$

Total digestible nutrients (TDN, Eq. 7) and metabolizable energy (ME, Eq. 8) were calculated (NRC, 2001) as follows:

$$TDN = DCP + DNFC + 2.25 \times DEE + DNDF - 7 \quad (7)$$

$$ME = 1.01 \times (0.04409 \times TDN) - 0.45 \quad (8)$$

where ME is as Mcal/kg of DM, and TDN, DCP, DNFC, DEE, and DNDF are as % of DM.

The *in vitro* ME (iv ME) values were also predicted from the iv OMD according to Robinson *et al.* (2004) as Eq. 9:

$$ivME = 0.82 \times [0.24 \times CP + 0.39 \times EE + 0.18 \times (OM - CP - EE)] \times ivOMD \quad (9)$$

Where;
ivME is as MJ/kg of DM; OM, CP, and EE are as % of DM; and iv OMD is as %.

Data Analysis

The obtained data were subjected to analysis of variance in the General Linear Model (Eq. 10) of Minitab 21 (2022) software. Tukey's multiple comparison tests contrasted the significantly different treatments. The Paired t-test and Regression were also used to find differences and linear relationships between *in vivo* and *in vitro* techniques.

$$y_{ijk} = \mu + A_{i..} + P_{.j.} + T_{..k} + e_{ijk} \quad (10)$$

Where;
y_{ijk} is observations, μ is a general mean, $A_{i..}$ is the animal's effect, $P_{.j.}$ is the period's effect, $T_{..k}$ is the treatment's effect, and e_{ijk} is random error.

RESULTS AND DISCUSSIONS

Feed Intake

Table 2 presents the consumption of feeds, nutrients, digestible nutrients, ME, and weight changes of experimental cattle. The consumption of SF, RS, and urea of cattle was significantly different among treatments (P<0.05) due to the dietary formula. At the same time, the DM, CP, DCP, and ADF intake were not significantly different (P>0.05) probably due to feeding. The NDF intake decreased significantly (P<0.05), but the EE, NFC, and ME intake increased significantly (P<0.05) from the SF0 to SF35 treatment. As a result, the change-weight of cattle gradually improved (P<0.05) as the supplement level of SF increased. Animals lost weight when they were not supplemented with the SF probably due to the unsatisfactoriness of the energy requirement for maintenance. Cammell *et al.* (1993) reported that the ME requirement for daily maintenance of cattle was 0.46 MJ/$W^{0.75}$, while these experimental cattle only consumed 0.44 MJ/$W^{0.75}$ for the SF0 treatment. Thus, the supplementation of SF was necessary for cattle fed RS basally to improve the ME intake and weight gain.

Table 2: Intake of feeds, nutrients, digestible nutrients, energy, and change-weight of cattle.

Daily intake of	SF0	SF7	SF14	SF21	SF28	SF35	P
SF, kgDM	NA	0.20^{e}	0.38^{d}	0.58^{c}	0.78^{b}	0.96^{a}	0.001
RS, kgDM	2.54^{a}	2.52^{a}	2.34^{ab}	2.17^{abc}	2.04^{bc}	1.79^{c}	0.001
Urea, g	53.2^{a}	46.5^{b}	34.9^{c}	23.5^{d}	12.2^{e}	NA	0.001
Total DM, kg	2.59	2.77	2.76	2.76	2.83	2.75	0.493
OM, kg	2.19	2.33	2.35	2.37	2.42	2.37	0.281
CP, kg	0.296	0.296	0.306	0.310	0.307	0.314	0.482
EE, kg	0.079^{e}	0.096^{de}	0.112^{cd}	0.125^{bc}	0.137^{ab}	0.144^{a}	0.001
NFC, kg	0.079^{f}	0.129^{e}	0.221^{d}	0.272^{c}	0.290^{b}	0.361^{a}	0.001
NDF, kg	1.83^{a}	1.89^{a}	1.77^{ab}	1.71^{ab}	1.70^{ab}	1.55^{b}	0.023
ADF, kg	1.05	1.09	1.06	1.03	1.02	0.95	0.133
ADL, kg	0.198^{b}	0.209^{ab}	0.215^{ab}	0.221^{ab}	0.233^{a}	0.232^{a}	0.016
DCP, kg	0.186	0.209	0.206	0.210	0.218	0.211	0.174
TDN, kg	1.32^{b}	1.45^{ab}	1.47^{ab}	1.51^{ab}	1.57^{a}	1.56^{a}	0.019
ME, MJ	19.8^{b}	21.9^{ab}	22.3^{ab}	23.0^{ab}	23.9^{a}	23.8^{a}	0.008
ME, MJ/$W^{0.75}$	0.44^{b}	0.48^{ab}	0.50^{ab}	0.52^{a}	0.53^{a}	0.54^{a}	0.004
Change-W, kg/day	-0.18^{c}	0.01^{b}	0.07^{ab}	0.12^{ab}	0.18^{a}	0.20^{a}	0.001

SF: Sesbania foliage; **RS:** rice straw; **DM:** dry matter; **OM:** organic matter; **CP:** crude protein; **EE:** ether extract; **NFC:** non-fiber carbohydrate; **NDF:** neutral detergent fiber; **ADF:** acid detergent fiber; **ADL:** acid detergent lignin; **DCP:** digestible crude protein; **ME:** metabolizable energy; **W:** live weight; **SF0 – SF35:** diets supplemented with Sesbania foliage from 0 to 35% of DM intake; NA: not applicable; **^{a,b,c,d,e} :** means within a row with different superscripts differ significantly (P<0.05); **P:** significant level.

Digestibility

The dietary OMD, digestible nutrients, and ME values recorded in the experiment are shown in Table 3, which also includes the iv OMDs measured from both *in vitro* procedures. The dietary OMD, DEE, DNFC, and ME values were increased linearly (P < 0.05) according to the rise of SF supplementation from 0 to 35% of DM intake. While the DCP was not changed (P>0.05), the DNDF and DADF values decreased significantly (P < 0.05). Similar trends (P< 0.05) were observed in the iv OMD and iv ME data for both F_iv and S_iv procedures. When comparing the *in vivo* to the F_iv and S_iv techniques (Table 4), there was no significant difference (P>0.05). For this reason, dietary formulas feeding cattle, mostly consisting of RS, should be supplemented with SF to increase digestible nutrients and energy values.

The results of this study are consistent with Dahlanuddin *et al.* (2014) that the supplement of SF to grass for feeding

Bali cattle enhanced the OMD (623 g/kg, respectively) as compared to those without supplement (549 g/kg), while the NDF digestibility was lowered. Shahjalal and Topps (2000) observed a higher OMD for goats fed *Sesbania aculeata* or *Sesbania rostrata* than the grass, but the crude fiber digestibility was lower. The finding of Tekliye *et al.* (2018) on Farta sheep fed urea-treated RS, with a W of 18.9±1.7 kg showed an increase in OMD with the enhancement of the supplemental level of *Sesbania sesban* from 100 to 400 g/day. Also, the SF supplement to swamp buffalo fed RS or grass had a significant improvement in OMD (Thu and Dong, 2017). The better OMD and ME for supplement groups in this study agree with the report of McDonald *et al.* (2010) who noted that the addition of non-fiber organics in the supplements has supplied energy availability to rumen microorganisms to speed up the digestion process.

Table 3: Digestibility, digestible nutrients, and metabolizable energy of diets.

Items	SF0	SF7	SF14	SF21	SF28	SF35	P
OMD, %	54.7^{e}	56.7^{de}	58.4^{cd}	59.2^{bc}	60.1^{ab}	61.3^{a}	0.001
F_iv OMD, %	51.1^{d}	53.1^{cd}	55.6^{cd}	58.3^{bc}	59.8^{ab}	62.8^{a}	0.001
S_iv OMD, %	50.6^{c}	53.7^{cd}	56.1^{abc}	59.6^{ab}	59.7^{a}	62.7^{a}	0.001
DCP, % of DM	7.21	7.48	7.52	7.63	7.74	7.70	0.551
DEE, % of DM	2.10^{f}	2.49^{e}	2.97^{d}	3.40^{c}	3.88^{b}	4.31^{a}	0.001
DNFC,%of DM	3.12^{f}	5.24^{e}	7.05^{d}	8.85^{c}	10.7^{b}	13.0^{a}	0.001
DNDF,%of DM	42.8^{a}	40.9^{ab}	39.5^{bc}	37.5^{cd}	35.3^{de}	33.3^{e}	0.001
DADF,%of DM	24.9^{a}	23.8^{ab}	22.8^{bc}	22.1^{cd}	21.3^{de}	20.6^{e}	0.001
TDN,%of DM	50.9^{d}	52.4^{cd}	53.7^{bc}	54.5^{abc}	55.4^{ab}	56.7^{a}	0.001
ME,MJ/kg DM	7.61^{d}	7.88^{cd}	8.12^{bc}	8.28^{abc}	8.45^{ab}	8.69^{a}	0.001
F_iv ME, MJ/kg DM	7.01^{e}	7.41^{de}	7.76^{cd}	8.14^{bc}	8.67^{ab}	9.05^{a}	0.001
S_iv ME, MJ/kg DM	7.32^{d}	7.54^{cd}	7.80^{bcd}	8.19^{bc}	8.49^{ab}	9.16^{a}	0.001

OMD: organic matter digestibility; **F_iv:** formal in vitro technique of Goering and van Soest; **S_iv:** *in vitro* simplified reagents; **DCP:** digestible crude protein; **DEE:** digestible ether extract; **DNFC:** digestible non-fiber carbohydrate; DNDF: digestible neutral detergent fiber; **DADF:** digestible acid detergent fiber; ME – metabolizable energy; **SF0 – SF35:** diets supplemented with Sesbania foliage from 0 to 35% of DM intake; **^{a,b,c,d,e,f}:** means within a row with different superscripts differ significantly (P<0.05); **P:** significant level.

Moreover, the diet of low SF was grouped under low OMD and ME which could be due to a higher NDF and ADF content in RS than SF, and high DNDF which could be due to a lower ADL content in RS than SF. Contrary to the present study, Thu and Dong (2017), and Tekliye *et al.* (2018) reported higher NDF digestibility for the supplemental treatments than the control, which could be an attribute of the enhancing dietary N in the supplement. The N content in this study was not different among treatments owing to the setup of different urea levels in dietary treatments.

Table 4: Comparison between the *in vivo* and *in vitro* results.

Items	In vivo (1) (Mean ± SE)	F_iv (2) (Mean ± SE)	S_iv (3) (Mean ± SE)	P (1 vs. 2)	P (1 vs. 3)	P (2 vs. 3)
OMD	58.4±0.59	57.8±0.95	58.4±0.87	0.233	0.984	0.091
ME	8.17±0.09	8.01±0.16	8.08±0.14	0.074	0.271	0.192

OMD: organic matter digestibility; ME: metabolizable energy; **F_iv:** formal *in vitro* technique of Goering and van Soest; **S_iv:** *in vitro* simplified reagents; **SE:** standard error; **P:** significant level.

Table 5: The linear relationships (n=24) between *in vivo* and *in vitro* results.

Regression between	Slope±SE	Intercept±SE	R^2	RSD	P
OMD and F_iv OMD	0.548±0.060	26.72±3.48	0.792	1.34	0.001
OMD and S_iv OMD	0.627±0.053	18.58±3.11	0.784	1.36	0.001
F_iv OMD and S_iv OMD	1.018±0.085	-1.68±4.97	0.868	1.73	0.001
ME and F_iv ME	0.508±0.052	4.10±0.42	0.811	0.19	0.001
ME and S_iv ME	0.551±0.059	3.71±0.49	0.796	0.20	0.001
F_iv ME and S_iv ME	1.022±0.083	-0.265 ±0.68	0.871	0.28	0.001

OMD: organic matter digestibility; **ME:** metabolizable energy; **F_iv:** formal *in vitro* technique of Goering and van Soest; **S_iv:** *in vitro* simplified reagents; **SE:** standard error; **R^2:** coefficient of determination; **RSD:** residual standard deviation; **P:** significant level.

In Vivo and *In Vitro* Relationships

Table 5 and Figure 1 illustrate the linear relationships between the *in vivo* and *in vitro* procedures used in this study. The linear regression study between the *in vivo* and S_iv procedure resulted in a coefficient of determination (R^2) of 0.784, a residual standard deviation (RSD) of 1.36, and a significant regression model (P<0.001). Based on dietary ME values (Table 5), the R^2 was 0.796, with an RSD of 0.20 and a P value < 0.001. There were close relationships (R^2 of 0.868-0.871, P<0.001) between the F_iv and S_iv procedure, as well as between the *in vivo* and F_iv procedure (R^2 of 0.792-0.811, P<0.001).

With observation number (n) = 24, this study obtained the R^2 values from 0.784 to 0.811 in linear regression analysis between the *in vivo* and *in vitro* techniques. Similarly, Forejtova *et al.* (2005) reported that the linear relationship between the *in vivo* and *in vitro* digestibility of hay and

silage was close, with an R^2 value of 0.75 and 0.76, respectively. Denek *et al.* (2006) stated that both rumen fluids of slaughtered animals could be used as inoculum for *in vitro* digestion and obtained an R^2 value of 0.80 for regression analysis between the *in vivo* and its. These results allow us to identify that the *in vitro* procedure proposed in this study has a high potential to evaluate the OMD and ME values for diets containing high roughage. The technique did not require fistulated animals, despite using the rumen liquor from slaughterhouses with unknown dietary histories. The medium of procedure could also remove some chemicals to better satisfy environmental and ethical concerns.

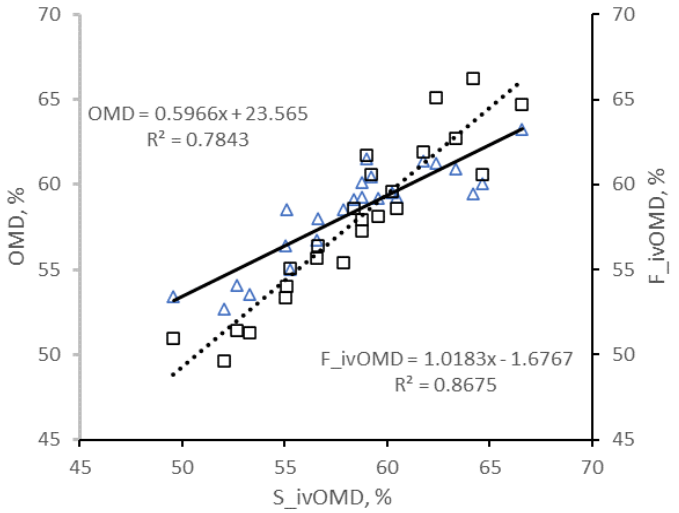

Figure 1: The linear relationships of the *in vitro* organic matter digestibility (S_ivOMD) of simplified medium to the *in vivo* (OMD, -Δ-, solid line) and formal *in vitro* (F_ivOMD, -□-, dot line).

This achievement agrees with Lutakome *et al.* (2017), who found that rumen liquor from slaughtered cattle of unknown dietary history can be used to derive *in vitro* gas production parameters. Wang *et al.* (2018) found that the *in vitro* test with rumen fluid from slaughtered cattle could be used to capture variation in methane emission potential between cattle types and with age. A finding by Beyihayo *et al.* (2015) also illustrated that the use of rumen fluid from slaughtered and fistulated cattle as an inoculum to measure the *in vitro* digestibility obtained results were similar. Mould *et al.* (2005) suggested that the *in vitro* medium could be simplified by removing micro-minerals, and trypticase, as well as reducing solution because these sources could satisfy ruminal microbes when the substrate and medium are doubled. In this study, in addition to the above components, macro-minerals were also removed and did not increase the medium and substrate. Instead, rumen fluid was increased because it is available at the slaughterhouse and contains ammonium, amino acids, and minerals similar to the medium (Mo and Thu, 2009).

CONCLUSIONS AND RECOMMENDATIONS

The OMD, TDN, and ME values of diets of RS basally for feeding cattle linearly increased with the rising level of the supplement of SF, but digestible fiber had a decreasing trend. The OMD and ME obtained from the simplified medium in *in vitro* technique were similar to and closely correlated with the conventional *in vivo* findings, allowing for an effective assessment of this effect. This *in vitro* procedure could use rumen fluid from slaughterhouses with unknown dietary histories, while eliminating certain chemicals (such as trypticase, macro- and micro-minerals, and reducing agents) in the medium, to better address concerns related to animal ethics and welfare, environmental impact, and cost.

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NOVELTY STATEMENT

In the case of cattle-fed basal rice straw, a supplement of legume foliage improved digestibility and energy values, and the *in vitro* results using a simpler medium and abattoir rumen fluid were quite similar to the *in vivo*. This *in vitro* technique promises to resolve concerns regarding animal ethics and welfare, finance, and the environment in digestibility studies.

AUTHOR'S CONTRIBUTIONS

The experiment's conception, design, and interpretation of data were by Danh Mo and Nguyen Van Thu. Acquisition of data, data analysis, writing, and final manuscript approval were done by Danh Mo.

Conflict of Interest

The author declares that there is no conflict of interest.

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