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Fermentation of fibre rich ingredients exposed in vitro to the faecal inoculums of swine and turkeys

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Abstract

This study was focused on in vitro fermentation and in vitro dry matter (DM) digestibility of different fibre rich ingredients that can be used in diets of pigs and turkeys. In vitro DM digestibility was determined by Daisy system via using faecal or excreta fluid of swine/turkeys as a source of inoculum. The ingredients used as substrates were commercial swine or turkey diet, dried beet pulp, wheat bran, hay, straw and two types of lignocelluloses (A and B). Marked differences between the ingredients were found and the values were: dried beet pulp (80%), swine/turkey diet (75%), wheat bran (60%), hay (40%) and straw (10%-20%). Of special interest are the data on the two lignocellulose products, the in vitro DM digestibility of product A was in the range of 20% (in turkeys) up to 30% (in pigs), whereas the product B had values of <5%. Moreover, the inoculums were incubated with the same substrates for 24h using gas measuring technique. Consecutively, commercial swine or turkey diet, dried beet pulp, wheat bran and hay produced high amounts of gas and volatile fatty acids. Lignocellulose A and straw provided lower and equal amounts of gases and fatty acids. However, lignocellulose B showed very little fermentation compared to the product A. In conclusions, faecal or excreta can be used as a source of microbial activity to determine the in vitro DM digestibility or fermentation of feeds. Comparing lignocellulose products with traditional fibre sources, the DM digestibility of lignocellulose A was greater than straw but its fermentation rate seems to be equal to straw. Thus, lignocellulose A can be used as a new source of fibre in diets of monogastric animals to optimize the gut health and improving the faeces or excreta quality.

KEYWORDS

digestibility, faecal liquor, fibre sources, lignocellulose, swine, turkeys

1 | INTRODUCTION

Dietary fibre is necessary to regulate digestion in monogastric animals (Grosse Liesner, Taube, Leonhard-Marek, Beineke, & Kamphues, 2009; Johnston, Noll, Renteria, & Shurson, 2003). The non-fermentable and fermentable constituents of dietary fibre are differentially degraded

by intestinal bacteria and, therefore, have different modes of action (Govers, Gannon, Dunshea, Gibson, & Muir, 1999). The non-fermentable fibre fraction is minimally degraded by the intestinal microbes and has primarily physical effects. It physically organizes digesta passage rate, affects faecal quality and switches fermentation to the large intestine (Krieg, Martienssen, & Zentek, 2012; Krieg,

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Schüle, & Dohms, 2008). But, the fermentable fibre fraction is digested by the microflora in the large intestine, where lactate and the volatile fatty acids (acetate, butyrate and propionate) are produced (Bach Knudsen & Jorgensen, 2001). Recently, special dietary fibre products derived from lignocellulose are being used more and more in animal nutrition due to their positive impacts on digestive processes and gut health status with low inclusion rates (Sarandan, Neufeld, & Neufeld, 2008; Youssef & Kamphues, 2018). Lignocellulose is a product made from fresh wood and has been used as a high quality fibre source in livestock animal diets. Compared to traditional fibre sources, lignocellulose is characterized by high crude fibre (>55%) and high lignin (25%-30%) contents. During the last years, new lignocellulose products have become commercially available. These products contain a blend of non-fermentable and fermentable fibre fractions. It had been tested in several field studies in weaning pigs, fattening pigs, broilers and rabbits at different dietary concentrations showing improvement in performance and gut health (Neufeld & Leibetseder, 2008; Zeitz et al., 2019). There is an increasing interest in using lignocellulose feeds in the diets of monogastric animals, especially with increasing the price of commonly used fibre sources. However, a little is known about the fermentation rate of these ingredients in swine and poultry.

The in vitro dry matter digestibility (IVDMD) has been used widely to assess the nutritive value of the feeds (Marten & Barnes, 1980). It can be determined by using Daisy^{II} apparatus (ANKOM Technology Corp.; Mabjeesh, Cohen, & Arieli, 2000). The Daisy^{II} system is more labour efficient than traditional methods in analyses of feeds (Holden, 1999). Moreover, the gas production technique is a useful method to determine the rate and extent of feed degradation (Cone, Gelder, & Driehuis, 1997; Groot, Cone, Williams, Debersaques, & Lantinga, 1996). Gas production can be used as a basic indicator of feed fermentation (France et al., 1993). A number of different in vitro gas production techniques has been developed (Getachew, Blümmel, Makkar, & Becker, 1998; Stern, Bach, & Calsamiglia, 1997). Glass syringes or pressure transducers were used for monitoring accumulated gases during fermentation of substrates (Cone, Gelder, Visscher, & Oudshoorn, 1996; Menke & Steingass, 1988; Theodorou, Williams, Dhanoa, McAllan, & France, 1994; Varadyova, Baran, & Zelenak, 2005).

Use of fresh faeces from ruminants, rather than rumen fluid, has been tested as an alternative inoculum (El-Meadaway, Mir, Mir, Zaman, & Yanke, 1998; Nsahlai & Umunna, 1996; Omed, Lovet, & Axford, 2000) and its potential in the in vitro gas production technique has been determined by some researchers (Cone, Gelder, & Bachmann, 2002; Harris, Barlet, & Chamberlain, 1995; Mauricio et al., 2001). The same findings were reported using faeces of horses as an inoculum (Plumhoff, 2004). Moreover, Cappai, Wolf, Rust, Pinna, and Kamphues (2013) found that the faeces quality can be used as valuable indicators of the gut health and digestive process in pigs. However, there is a lack in information about using fresh faecal matter of swine and poultry as sources of inocula in gas production procedures.

Dietary fibre sources are essential to maintain gut health, welfare and reproductive performance. The present study attempted

to provide information about the microbial degradability of these ingredients in the hind gut. Therefore, this research was conducted to investigate the in vitro fermentation patterns of feeds using fresh faeces of swine or turkeys, as a source of inoculum, in Daisy^{II} incubator and gas measuring technique. Furthermore, the fermentation of new fibre rich ingredients (especially, lignocellulose products) in the hindgut of swine and turkeys using faeces/excreta was evaluated and compared with traditional fibre sources that can be used in diets of these animals.

2 | MATERIALS AND METHODS

Six swine (about 50 kg BW) and three turkeys (15 kg BW) were used in this experiment as faeces or excreta donor. The animals were fed commercial diets alone or supplemented with tested fibre ingredients at a rate of 2.0%. The investigated substrates were commercial swine or turkey diet (P6), dried beet pulp, wheat bran, hay, straw and lignocellulose A and B. These ingredients were chemically analysed and its composition is shown in Table 1. Swine were fed individually; each animal was fed one tested diet, while turkeys were fed together on the same diet in order to obtain enough amounts of excreta which are needed for an inoculum preparation. Moreover, the turkey birds were fed on the experimental diets consecutively, one after each other. Wheat bran was fed to turkeys instead of hay. The experimental diets were offered to animals for about 5 days (turkeys) to 10 days (swine) before collection of excreta or faeces, to adapt the animals on these diets. Then, the faeces or excreta was collected and prepared as an inoculum to be used in the Daisy incubator or in the gas measuring technique.

2.1 | IVDMD determination using Daisy system

The complete unit of Daisy II incubator consisted of four incubation vessels with a capacity of 2,000 ml each. Each vessel contained 1,600 ml of buffer solution, 400 ml of faecal inoculum, and 20 nylon bags.

Samples of the tested ingredients were ground to pass a 0.75-mm screen. Substrates (0.25 g) were weighed into nylon filter bags (Ankom F57, Ankom Technology), three bags for each substrate, then heat-sealed. These bags were previously rinsed with acetone, air dried and then dried at 100°C for 24 hr, after which their weight was recorded. Bags containing the different ingredients/feeds were incubated in Daisy vessel with 400 ml of faecal inoculum and 1,600 ml of buffer solution for 48 hr at 39°C.

The microbial inoculum was prepared by collecting fresh faeces (swine) or excreta (turkeys). Faecal samples were put into an air-tight freezer bag to maintain an anaerobic environment and transported to the laboratory in a cooler containing warm (39°C) water. Once in the laboratory, a 50-g sample of faeces was placed in a blender with 450 ml of warm distilled water (10:1). Samples were mixed for 2 min while being gassed with CO_2 , then strained through a sieve

TABLE 1 Chemical composition of ingredients (g/kg) on fresh matter basis

	Swine diet	Turkey diet (P6)	Lignocellulose A	Lignocellulose B	Beet pulp	Hay	Straw	Wheat bran
DM	898	876	910	917	907	930	890	885
Ash	51.6	52.1	37.9	34.7	60.9	42.1	31.9	56.0
Crude protein	175	156	32.0	7.95	90.9	90.9	26.6	132
Crude fat	44.2	72.5	13.1	2.65	8.46	14.5	21.1	35.1
Crude fibre	38.2	21.4	560	612	130	307	389	132
NfE	589	574	267	260	617	475	422	530
ADF	70	52	698	715	167	367	420	138
NDF	185	143	872	855	297	648	668	432
Ca	9.80	6.91	4.48	1.16	8.04	3.84	1.80	1.20
Р	5.70	4.55	0.45	0.66	0.79	2.49	0.42	11.1
Mg	2.10	1.20	0.92	0.08	1.31	1.61	0.54	3.50
Na	2.50	1.21	0.08	0.09	2.99	1.24	0.70	0.50
K	8.30	4.80	3.44	0.08	13.9	13.4	0.20	8.50

Abbreviation: ADF: acid detergent fiber; DM, dry matter; NfE, nitrogen free extract; NDF, neutral detergent fiber.

(diameter: 200 μ m) into the prewarmed incubation jars (Daisy system) or glass beakers (gas production technique).

Buffers consisted of two solutions that were combined in prewarmed incubation jars or glass beakers immediately before the incubation. Buffer solution A (KH $_2$ PO $_4$, 10.0 g/L; MgSO $_4$ ·7 H $_2$ O, 0.5 g/L; NaCl, 0.5 g/L; CaCl $_2$ ·2 H $_2$ O, 0.1 g/L; and urea, 0.5 g/L) was added at 1,330–266 ml of buffer solution B (Na $_2$ CO $_3$, 15.0 g/L and Na $_2$ S·7 H $_2$ O, 1.0 g/L) to obtain a final pH of 6.8.

The faecal inoculum was then added to each fermentation jar, after which the jars were purged with ${\rm CO_2}$ for 30 s and then sealed. The sealed jars were placed into the prewarmed Daisy II incubator. The incubator maintained a constant temperature of 39°C throughout the incubation, and the jars were continuously agitated. The jars were removed after 48 hr, and the filter bags were immediately rinsed for 30 min with cold water to stop microbial activity, then dried at 100°C and the weight was recorded. Then, the IVDMD of the samples was calculated.

2.2 | Gas production and fermentation determination

To measure the generated gas amount and gas pressure during incubation of the test feed, glass bottles (120 ml capacity) were prepared. Four bottles were used for each substrate; in each bottle exactly 1 g of the tested feed was weighed. Further four glass bottles per animal were used without feed as control. All bottles were sealed with aluminum foil and stored at room temperature.

Before incubation, 100 ml of produced faecal suspension was mixed in a warmed glass beaker with 400 ml of warmed buffer (ratio of suspension to buffer [mixture of buffers A and B] is 1:5). After filling the prepared glass bottles with 100 ml of the diluted suspension (ratio of feed to faecal suspension is 1:101) and a magnetic stirrer, the bottles were sealed with rubber stoppers and lids so that a piercing

of the rubber stopper with cannulas for measurement remained possible. The incubation was carried out on a magnetic stir plate in 39°C warm water bath at about 400 rotations per minute for 24 hr.

In this experiment, both gas pressure and gas volume produced during the incubation of the corresponding experimental feed in glass bottles were determined. These measurements were taken after 24-hr incubation. For this purpose, the gas pressure was measured in the glass bottle by means of a pressure metre (Tracker 200 Series, DATA TRACK PROCESS INSTRUMENTS), which stated the prevailing pressure in the glass bottle in mV. This measuring device was connected via a three-way valve with a cannula. After reading the displayed 'zero value' (= atmospheric pressure), this cannula was inserted through the rubber stopper into the bottle. This three-way valve is only the connection between the pressure metre and the glass bottle, so that the prevailing pressure in the bottle could be read in voltage. From this voltage displayed the 'zero' value was subtracted. For subsequent measurement of the gas volume in the bottle, 5, 10, or 50 ml plastic disposable syringe was connected to the valve. After turning the three-way valve, the gas volume in the bottle was aspirated by syringe at once until the initially measured prevailing pressure outside the bottle 'zero value' on the display appeared. Then, the amount of gas in the syringe was read off and recorded.

After measuring the amount of gas produced in each bottle, the pH of the fermented substrate was monitored using pH metre. Furthermore, the concentrations of volatile fatty acids (VFAs) and L-lactate produced in each glass bottle were also determined by using gas chromatography and photometer, respectively.

2.3 | Statistical analyses

The statistical analyses were performed using SAS statistical program (SAS Institute, 2002). The data were evaluated using the General Linear Models procedure for analysis of variance. The

results were subjected to one-way ANOVA accompanied by Tukey test to detect the differences between the treatments. Differences were considered to be significant when p < .05. Values are presented as arithmetical means with standard deviation (mean \pm SD).

3 | RESULTS

It was found that the type of diets fed to swine or turkeys did not affect the IVDMD of different substrates. This is manifested by similar results of tested substrates among the animals. Therefore, the DM digestibility of each ingredient was calculated collectively as shown in Table 2. The values were higher with the following substrates: dried beet pulp (80%), swine/turkey diet (75%), wheat bran (60%), hay (40%) and straw (10%–20%). Of special interest are the data on the two lignocellulose products, the DM digestibility of product A was in the range of 20% (turkeys) up to 30% (pigs) while the product B had values of about 2% in swine to 5% in turkeys.

The data of gas production and fermentation products in the glass bottles revealed that no influence of the diet type on fermentation of different substrates. In swine, regardless the kind of diet, the amount of gas produced was higher in commercial swine diets (29.1 ml/g), followed by dried beet pulp (23.5 ml/g) and hay (8.50 ml/g; Table 3). The gas amount provided by straw and lignocellulose A was low and nearly identical (1.0 ml/g). However, no gas was determined in case of lignocellulose B. Moreover, the pH values of the substrates after 24 hr fermentation were a reflection of the gas production. It was observed that the higher gas produced yielded lower pH values. As expected, commercial swine diet followed by dried beet pulp and hay resulted in high amounts of VFAs produced. Lignocellulose A and straw produced considerable amounts of acetic and propionic acids, with nearly equal values (about 3.25 mmol/kg for acetic and 0.75 for propionic). However, lignocellulose B generated very low amounts of VFAs in spite of no gas production. Also, the concentration of total VFAs was higher

TABLE 2 In vitro dry matter digestibility (%) of different ingredients using Daisy incubator and faeces/excreta of swine/turkeys as inoculums, regardless the consumed diets

Substrate	Swine (n = 18) ¹	Turkeys (n = 18) ²
Normal diet ³	74.3 ± 5.55 ^b	75.1 ± 2.40 ^a
Dried beet pulp	87.1 ± 6.07^{a}	71.8 ± 6.55^{b}
Wheat bran	_	58.6 ± 3.22 ^c
Hay	42.5 ± 10.1 ^c	_
Straw	20.5 ± 10.2 ^e	10.2 ± 1.25 ^e
Lignocellulose A	28.9 ± 9.30 ^d	23.8 ± 3.80^d
Lignocellulose B	2.16 ± 2.84 ^f	4.57 ± 2.46 ^f

 $^{^{}a,b,..}$ Means within the same column with different superscripts are significantly different (p < .05).

in commercial swine diet (38.9 mmol/kg), followed by dried beet pulp (34.2 mmol/kg), hay (23.7 mmol/kg), and at the end both of straw and lignocellulose A (4.13 mmol/kg).

In turkey birds, all substrates used in swine experiment was tested, but wheat bran and commercial turkey diet (P6) were used instead of hay and commercial swine diet. As observed in swine, no effect of the kind of diet on the fermentation using turkey excreta as an inoculum. The gas amount produced was higher in commercial turkey diet (P6), wheat bran and dried beet pulp and the values were about 31.5, 18.0, and 12.6 ml/kg, respectively (Table 3). The pH values of these substrates were coincided with the amount of gas output and the values were 5.36, 5.92 and 6.02, respectively. Lignocellulose A and straw produced low amounts of gases (about 1.0 ml/g) and the pH values averaged about 6.68. However, lignocellulose B did not produce any gases. Moreover, commercial turkey diet (P6), followed by wheat bran and dried beet pulp, and ultimately straw showed marked increase in production of total and individual VFAs. Nevertheless, lignocellulose A produced lower amounts of VFAs than straw, but higher than lignocellulose B.

Concerning L-lactate produced in the substrates, in swine, lignocellulose A was found to produce high amount of L-lactate (0.05 mmol/kg) which was nearly similar to that of straw (Table 4). Nevertheless, commercial swine diet had a lower concentration of L-lactate (0.03 mmol/kg) compared to that of lignocellulose A and straw. Moreover, lignocellulose B, hay and dried beet pulp produced very low amounts of L-lactate (0.01 mmol/kg). In turkeys, it was observed a higher concentration of L-lactate in case of lignocellulose A substrate (0.05 mmol/kg). However, other substrates provided very low amounts of L-lactate (0.01 mmol/kg), except commercial turkey diet (P6) which produced a considerable amount (0.03 mmol/kg).

4 | DISCUSSION

Faeces have been identified as readily available source of microorganisms. It can be used as an alternative to ruminal fluid in ruminants (Akhter, Owen, Theodorou, Butler, & Minson, 1999). Also, it is a suitable inoculum source for in vitro digestibility and gas production studies in horses (Lattimer, Cooper, Freeman, & Lalman, 2007; Lowman, Theodorou, Hyslop, Dhanoa, & Cuddeford, 1999). There was very little research on using the swine faeces or poultry excreta in in vitro digestion or fermentation assessments.

The IVDMD was higher in commercial swine or turkey diet, dried beet pulp, wheat bran and hay compared to straw and lignocellulose products. This could be due to lower fibre contents and higher organic compounds (protein and NfE) in these ingredients. Moreover, the DM digestibility of lignocellulose A was better than that of straw. This finding could be attributed to its higher content of protein (32.0 vs. 26.6 g/kg) and NDF (872 vs. 668 g/kg). However, the IVDMD of lignocellulose B was the lowest among the tested substrates. This is due to lower digestible nutrients content and

¹6 animals × 3 samples per substrate.

²6 trials (each one was done on 3 birds together) × 3 samples per substrate.

³Normal diet: commercial diets of swine and turkeys.

TABLE 3 Gas amount (ml/g), pH of the fermented substrate and volatile fatty acids (mmol/L or kg) produced after incubation of the different substrates with buffer and faeces/excreta of swine/turkeys for 24 hr, regardless the consumed diets

	Swine $(n = 24)^1$)1						Turkeys $(n = 24)^2$	4)2					
Substrate Gas	Gas	Hd	ΣVFA	C ₂	رع	Ω δ	ر ک	Gas	Hd	ΣVFA	C ₂	ິບ	C ₄	C _s
Normal diet ³	29.1 ± 9.99ª	5.74 ± 0.07^{d} 38.9 ± 4.47^{a}	38.9 ± 4.47 ^a	22.6 ± 3.72^{a}	9.88 ± 1.28 ^a	4.55 ± 1.83 ^a	9.88 ± 1.28^{a} 4.55 ± 1.83^{a} 1.87 ± 1.48^{a} 31.5 ± 6.19^{a}	31.5 ± 6.19^{a}	5.36 ± 0.19^{d}	40.2 ± 3.83 ^a	23.0 ± 2.03^{a}	12.7 ± 1.50^{a}	$40.2 \pm 3.83^{\text{a}} 23.0 \pm 2.03^{\text{a}} 12.7 \pm 1.50^{\text{a}} 2.57 \pm 2.60^{\text{a}} 1.99 \pm 1.20^{\text{a}}$	1.99 ± 1.20^{a}
Dried beet pulp	23.5 ± 8.95 ^b	5.93 ± 0.25 ^d	23.5 ± 8.95^{b} 5.93 ± 0.25^{d} 34.21 ± 5.58^{b} 22.0 ± 4.10^{a}		9.13 ± 1.72 ^a	2.24 ± 0.78 ^b	0.84 ± 0.55 ^b	9.13 ± 1.72^{a} 2.24 ± 0.78^{b} 0.84 ± 0.55^{b} 12.6 ± 3.60^{c} 6.02 ± 0.12^{c} 30.8 ± 4.95^{b} 18.3 ± 2.66^{b} 10.2 ± 1.32^{b} 1.56 ± 1.78^{b} 0.71 ± 0.55^{b} 1.56 ± 1.78^{b} 1.56 ± 1.78^{b	6.02 ± 0.12°	30.8 ± 4.95 ^b	18.3 ± 2.66 ^b	10.2 ± 1.32^{b}	1.56 ± 1.78^{b}	0.71 ± 0.55 ^b
Wheat bran	I	I	I	I	1	I	I	18.0 ± 3.41^{b}	$5.92 \pm 0.17^{c} 31.9 \pm 4.04^{b} 18.4 \pm 2.62^{b} 10.7 \pm 1.54^{b} 1.88 \pm 2.29^{b} 0.91 \pm 0.28^{b}$	31.9 ± 4.04^{b}	18.4 ± 2.62^{b}	10.7 ± 1.54^{b}	1.88 ± 2.29^{b}	0.91 ± 0.28 ^b
Нау	$8.50 \pm 3.71^{\circ}$	$6.35 \pm 0.06^{\circ}$	$8.50 \pm 3.71^{\circ}$ $6.35 \pm 0.06^{\circ}$ $23.7 \pm 2.72^{\circ}$	$14.96 \pm 2.06^b - 6.77 \pm 1.64^b - 1.38 \pm 0.71^c - 0.56 \pm 0.51^c$	6.77 ± 1.64^b	$1.38 \pm 0.71^{\circ}$	0.56 ± 0.51^{c}	1	ı	1	ı	ı	1	ı
Straw	1.09 ± 0.25^{d}	6.64 ± 0.06^{b}	$1.09 \pm 0.25^{d} + 6.64 \pm 0.06^{b} + 4.16 \pm 1.93^{d} + 3.19 \pm 1.46^{c} + 0.79 \pm 0.42^{c} + 0.15 \pm 0.13^{d} + 0.03 \pm 0.05^{d} + 1.20 \pm 0.31^{d} + 6.69 \pm 0.18^{b} + 7.70 \pm 4.34^{c} + 4.46 \pm 2.39^{c} + 2.58 \pm 2.51^{c} + 0.55 \pm 0.50^{c} + 0.10 \pm 0.06^{c} + 0.00 \pm 0.00^{d} + 0.00 \pm 0.0$	$3.19 \pm 1.46^{\circ}$	0.79 ± 0.42^{c}	$0.15\pm0.13^{\rm d}$	0.03 ± 0.05^{d}	1.20 ± 0.31^{d}	6.69 ± 0.18^{b}	7.70 ± 4.34°	4.46 ± 2.39°	$2.58 \pm 2.51^{\circ}$	$0.55 \pm 0.50^{\circ}$	0.10 ± 0.06 ^c
Lign. A	1.01 ± 0.16^{d}	6.64 ± 0.04^{b}	$1.01 \pm 0.16^d 6.64 \pm 0.04^b 4.10 \pm 1.91^d 3.32 \pm 1.45^c$	$3.32 \pm 1.45^{\circ}$	0.71 ± 0.47^{c}	$0.05 \pm 0.06^{\rm e}$	0.02 ± 0.02^{d}	$0.71 \pm 0.47^{c} 0.05 \pm 0.06^{e} 0.02 \pm 0.02^{d} 1.0 \pm 0.20^{d} 6.68 \pm 0.05^{b} 2.90 \pm 1.26^{d} 1.48 \pm 0.95^{d} 1.22 \pm 1.01^{d} 0.12 \pm 0.17^{d} 0.08 \pm 0.02^{ce} 0.08 \pm 0.02^{ce} 0.08 \pm 0.02^{d} 0.08 \pm 0.$	6.68 ± 0.05 ^b	2.90 ± 1.26^{d}	$1.48\pm0.95^{\rm d}$	$1.22\pm1.01^{\rm d}$	0.12 ± 0.17^{d}	0.08 ± 0.02 ^{ce}
Lign. B	ı	6.99 ± 0.04 ^a	6.99 ± 0.04^{a} 0.99 ± 0.66^{e} 0.70 ± 0.48^{d}		0.21 ± 0.24^{d} 0.08 ± 0.06^{e}	0.08 ± 0.06 ^e	ı	ı	6.75 ± 0.09 ^a	$6.75 \pm 0.09^{a} - 1.43 \pm 0.95^{e} - 0.77 \pm 0.49^{e} - 0.55 \pm 0.59^{e} - 0.05 \pm 0.04^{e} - 0.06 \pm 0.05^{e}$	0.77 ± 0.49 ^e	$0.55 \pm 0.59^{\rm e}$	0.05 ± 0.04 ^e	0.06 ± 0.05 ^e

Abbreviations: C_2 , acetic; C_3 , propionic; C_4 , butyric; C_5 , valeric.

 $^{\mathrm{a,b..}}$ Means within the same column with different superscripts are significantly different (p<.05).

 1 6 animals × 4 samples per substrate.

 26 trials (each one was done on 3 birds together) imes 4 samples per substrate.

 $^3\mbox{Normal diet:}$ commercial diets of swine and turkeys.

TABLE 4 L-lactate amounts (mmol/L or kg) *produced* after incubation of the substrates with buffer and *faeces/excreta* of swine/turkeys for 24 hr, regardless the consumed diets

Substrate	Swine (n = 24)	Turkeys (n = 24)
Normal diet ¹	0.03 ± 0.01^{b}	0.03 ± 0.02^{b}
Dried beet pulp	0.01 ± 0.01 ^c	0.01 ± 0.01 ^c
Wheat bran	_	0.01 ± 0.01 ^c
Hay	0.01 ± 0.00^{c}	_
Straw	0.05 ± 0.03^{a}	0.04 ± 0.02^{ab}
Lignocellulose A	0.05 ± 0.03 ^a	0.05 ± 0.02^{a}
Lignocellulose B	0.01 ± 0.01^{c}	0.01 ± 0.00^{c}

 $^{^{}a,b,..}$ Means within the same column with different superscripts are significantly different (p < .05).

higher ADF in this product compared to other ingredients. The same findings for lignocellulose products were reported by Youssef and Kamphues (2018). Cappai et al. (2013) found a low digestibility of organic matter and starch in pigs fed high proportions of acorn hulls due to high amounts of non-fermentable crude fibre in the diet.

The amounts of gas and VFAs produced were higher in commercial swine or turkey diet, dried beet pulp, wheat bran and hay. This could be due to its higher contents of fermentable nutrients and easily digestible fibres. However, lower amounts of gas and VFAs were produced by straw and lignocellulose A. This is due to high, relatively less digestible, fibre contents in these substrates. In spite of no gas produced in lignocellulose B, very little fermentation occurred as demonstrated by the presence of few amounts of VFAs. The pH values was highly correlated (r = .91) with amounts of gas and VFAs produced, so it can be used as indicators of the fermentation process in the substrates (McDonald et al., 2010). The findings of gas and VFAs production in substrates with swine inocula were nearly coordinated with that of turkey inoculums. Nevertheless, only VFAs formed in lignocellulos A with swine inoculum was similar to that of straw, but was lower than straw when incubated with turkey inoculum. Moreover, Youssef and Kamphues (2018) found that lignocellulose A can be used as source of fibre in diets of monogastric animals as well as the digestibility rates of its DM, organic matter and crude fibre were about 50%, 47.0% and 48.0%, respectively. Zeitz et al. (2019) reported that supplementation of lignocellulose to broilers' diets could affect the gut bacterial population and bacterial fermentation, have anti-inflammatory activities, and increase mucin formation in the intestine, and consequently, improve broiler performance.

The amount of L-lactate formed in lignocellulose A and straw was higher compared to other substrates. This could be due to higher cellulose content in these ingredients, which can be splitted by bacteria into lactate. The same result for lignocelluloses was obtained by Youssef and Kamphues (2018). The results of L-lactate in swine were consistent with that of turkeys.

The obtained findings revealed that the data of employing swine faeces as an inoculum were comparable with that of using turkey

excreta—inoculum. Furthermore, faeces can be used as a source of microbial enzymes for estimating digestibility and fermentation rates of feeds (Omed et al., 2000; Youssef & Kamphues, 2018). Moreover, the present study tried to provide new information about the fermentation of not only lignocellulose products, but also to other fibre rich ingredients that can be used in diets of monogastric animals.

5 | CONCLUSIONS

The obtained results indicate that the faeces or excreta inoculum can be used for in vitro studies, as an indicator of the potential microbial degradation of ingredients in the gastrointestinal tract. Comparing lignocellulose products with traditional fibre sources, the DM digestibility of lignocellulose A was greater than straw but its fermentation rate seems to be equal to straw. Thus, lignocellulose A can be used as a new source of fibre in diets of monogastric animals, especially when higher dietary fibre levels are intended for different reasons (such as animal welfare, gut health, ...etc.).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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REFERENCES

- Akhter, S., Owen, E., Theodorou, M. K., Butler, E. A., & Minson, D. J. (1999). Bovine faeces as source of microorganisms for the in vitro digestibility assay of forages. *Grass Forage Science*, *54*, 219–226. https://doi.org/10.1046/j.1365-2494.1999.00174.x
- Bach Knudsen, K. E., & Jorgensen, H. (2001). Intestinal degradation of dietary carbohydrates From birth to maturity. In J. E. Lindberg & B. Ogle (Eds.), *Digestive physiology of pigs* (p. 109). New York: CABI Publishing.
- Cappai, M. G., Wolf, P., Rust, P., Pinna, W., & Kamphues, J. (2013). Raw hulled shredded acorns from Downy Oak (*Quercus pubescens*) in the diet of pigs: Effects on digestibility and faeces characteristics. *Journal of Animal Physiology and Animal Nutrition*, 97, 1–5. https://doi.org/10.1111/jpn.12065
- Cone, J. W., Van Gelder, A. H., & Bachmann, H. (2002). Influence of inoculum source on gas production profiles. *Animal Feed Science and Technology*, 99, 221–231. https://doi.org/10.1016/S0377-8401(02)00073-1
- Cone, J. W., Van Gelder, A. H., & Driehuis, F. (1997). Description of gas production profiles with a three-phasic model. *Animal Feed Science and Technology*, 66, 31–45. https://doi.org/10.1016/S0377-8401(96)01147-9
- Cone, J. W., Van Gelder, A. H., Visscher, G. J. W., & Oudshoorn, L. (1996). Influence of rumen fluid and substrate concentration on fermentation kinetics measured with a fully automated time related gas production apparatus. *Animal Feed Science and Technology*, 61, 113–128. https://doi.org/10.1016/0377-8401(96)00950-9
- El-Meadaway, A., Mir, Z., Mir, P. S., Zaman, M. S., & Yanke, L. J. (1998). Relative efficacy of inocula from rumen fluid or faecal solution for determining in vitro digestibility and gas production. *Canadian Journal of Animal Science*, 78, 673–679. https://doi.org/10.4141/A97-109

¹Normal diet: commercial diets of swine and turkeys.

- France, J., Dhanoa, M. S., Theodorou, M. K., Lister, S. J., Davies, D. R., & Isac, D. (1993). A model to interpret gas accumulation profiles associated with in vitro degradation of ruminant feeds. *Journal of Theoretical Biology*, 163, 99-111. https://doi.org/10.1006/jtbi.1993.1109
- Getachew, G., Blümmel, M., Makkar, H., & Becker, K. (1998). In vitro gas measuring techniques for assessment of nutritional quality of feeds: A review. *Animal Feed Science and Technology*, 72, 261–281. https://doi.org/10.1016/S0377-8401(97)00189-2
- Govers, M. J., Gannon, N. J., Dunshea, F. R., Gibson, P. R., & Muir, J. G. (1999). Wheat bran affects the site of fermentation of resistant starch and luminal indexes related to colon cancer risk: A study in pigs. *Gut*, 45(6), 840–847. https://doi.org/10.1136/gut.45.6.840
- Groot, J. C. J., Cone, J. W., Williams, B. A., Debersaques, F. M. A., & Lantinga, E. A. (1996). Multiphasic analysis of gas production kinetics for in vitro fermentation of ruminant feeds. *Animal Feed Science and Technology*, 64, 77–89. https://doi.org/10.1016/S0377-8401(96)01012-7
- Grosse Liesner, V., Taube, V., Leonhard-Marek, S., Beineke, A., & Kamphues, J. (2009). Integrity of gastric mucosa in reared piglets Effects of physical form of diets (meal/pellets), pre-processing grinding (coarse/fine) and addition of lignocellulose (0/2.5 %). Journal of Animal Physiology and Animal Nutrition, 93(3), 373–380. https://doi.org/10.1111/j.1439-0396.2008.00871.x
- Harris, D. M., Barlet, A., & Chamberlain, A. T. (1995). The use of dairy cow faeces rather than rumen liquor in the gas pressure transducer technique for assessing digestion kinetics in vitro. *Animal Science*, 60, 541.
- Holden, L. A. (1999). Comparison of methods of in vitro dry matter digestibility for ten feeds. *Journal of Dairy Science*, 82, 1791–1794. https://doi.org/10.3168/jds.S0022-0302(99)75409-3
- Johnston, L. J., Noll, S., Renteria, A., & Shurson, J. (2003). Feeding by-products high in concentration of fiber to nonruminants. The Third National Symposium on Alternative Feeds for Livestock and Poultry held in Kansas City, MO on November 4, 2003.
- Krieg, R., Martienssen, M., & Zentek, J. (2012). Effect of the ratio lignin to cellulose (ADF-ADL) on caecal fermentation, gut morphology and performance of rabbits around weaning. Proceedings 10th World Rabbit Congress September 3–6, 2012– Sharm El- Sheikh –Egypt (pp. 685–689).
- Krieg, R., Schüle, S., & Dohms, J. (2008). Lignocellulose als sichere Rohfaserquelle zur Leistungsstabilisierung bei Häsinnen und Jungtieren in der Kaninchenzucht. In: 7.BOKU- Symposium Tierernährung. Wien, Austria (pp. 136–145).
- Lattimer, J. M., Cooper, S. R., Freeman, D. W., & Lalman, D. L. (2007). Effect of yeast culture on in vitro fermentation of a high concentrate or high-fiber diet using equine fecal inoculums in a Daisy^{II} incubator. *Journal of Animal Science*, 85, 2484–2491. https://doi.org/10.2527/ jas.2006-655
- Lowman, R. S., Theodorou, M. K., Hyslop, J. J., Dhanoa, M. S., & Cuddeford, D. (1999). Evaluation of an in vitro batch culture technique for estimating the in vivo digestibility and digestible energy content of equine feeds using equine faeces as the source of microbial inoculum. *Animal Feed Science and Technology*, 80, 11–27. https://doi.org/10.1016/s0377-8401(99)00039-5
- Mabjeesh, S. J., Cohen, M., & Arieli, A. (2000). In vitro methods for measuring the dry matter digestibility of ruminant feedstuffs: Comparison of methodand inoculum source. *Journal* of *Dairy Science*, 83, 2289–2294. https://doi.org/10.3168/jds. s0022-0302(00)75115-0
- Marten, G. C., & Barnes, R. F. (1980). Prediction of energy digestibility of forages with in vitro rumen fermentation and fungal enzyme systems. In W. J. Pigden, C. C. Balch, & M. Graham (Eds.), Standardization of analytical methodology for feeds (pp. 61–128). Ottawa, Canada: Int. Dev. Res. Center.

- Mauricio, R. M., Owen, E., Mould, F. L., Givens, I., Theodorou, M. K., France, J., ... Dhanoa, M. S. (2001). Comparison of bovine rumen liquor and bovine faeces as inoculum for in vitro gas production technique for evaluating forages. *Animal Feed Science and Technology*, 89, 33–48. https://doi.org/10.1016/s0377-8401(00)00234-0
- McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A., & Wilkinson, R. G. (2010). *Animal nutrition* (7th ed.). New York, USA: Longman Scientific and Technical, John Wiley & Sons Inc.
- 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 and Development*, 28, 7–55.
- Neufeld, K., & Leibetseder, J. (2008). Nahrungsfaser in Der Nutztierernährung. Feed Magazine, 5-6, 21-27.
- Nsahlai, I. V., & Umunna, N. N. (1996). Comparison between reconstituted sheep faeces and rumen fluid inocula and between in vitro and in sacco digestibility methods as predictors of intake and in vivo digestibility. *Journal of Agriculture Science Cambridge*, 126, 235–248. https://doi.org/10.1017/s0021859600073196
- Omed, H. M., Lovet, D. K., & Axford, R. F. E. (2000). Faeces as a source of microbial enzymes for estimating digestibility. In D. I. Givens, E. Owen, H. M. Omed, & R. F. E. Axford (Eds.), Forage evaluation in ruminant nutrition (pp. 135-154). Wallingford, UK: CAB International.
- Plumhoff, M. (2004).Bildung (Menge und Dynamik) von Fermentationsprodukten von Futtermitteln mit unterschiedlichen Gehalten an fermentierbarenKohlenhydraten in einem in-vitro System mit Faeces von Pferden. Dissertation, Institut für Tierernährung, TierärztlicheHochschule Hannover.
- Sarandan, H., Neufeld, N., & Neufeld, K. (2008). Effects of a new lignocellulose product for fibre supplementation on MMA symptoms, reproductive performance and faecal quality in a pig farm with evident MMA problems in Romania. Lucrări Stiinłifice Medicină Veterinară Vol. XIi, 2008, Timisoara (pp. 851–858).
- SAS Institute (2002). SAS^{\oplus} user's guide: Statistics. Version 9.1. Cary, NC: SAS Institute.
- Stern, M. D., Bach, A., & Calsamiglia, S. (1997). Alternative techniques for measuring nutrient digestion in ruminants. *Journal of Animal Science*, 75, 2256–2276. https://doi.org/10.2527/1997.7582256x
- Theodorou, M. K., Williams, B. A., Dhanoa, M. S., McAllan, A. B., & France, J. (1994). A new gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. *Animal Feed Science and Technology*, 48, 185–197.
- Varadyova, Z., Baran, M., & Zelenak, I. (2005). Comparison of two in vitro fermentation gas production methods using both rumen fluid and faecal inoculum from sheep. Animal Feed Science and Technology, 123–124, 81–94. https://doi.org/10.1016/j.anifeedsci.2005.04.030
- Youssef, I. M. I., & Kamphues, J. (2018). Fermentation of lignocellulose ingredients in vivo and in vitro via using fecal and caecal inoculums of monogastric animals (swine/turkeys). Beni-Suef University Journal of Basic and Applied Sciences, 7(4), 407–413. https://doi.org/10.1016/j. bjbas.2017.05.006
- Zeitz, J. O., Neufeld, K., Potthast, C., Kroismayr, A., Most, E., & Eder, K. (2019). Effects of dietary supplementation of the lignocelluloses FibreCell and OptiCell on performance, expression of inflammation-related genes and the gut microbiome of broilers. *Poultry Science*, 98(1), 287–297. https://doi.org/10.3382/ps/pey345

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