***In vitro* fermentation evaluation of selected agro-industrial by-products as alternative feed for ruminants**

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Abstract

In the tropic there is seasonal variation in the quality and quantity of forage which affects the sustainability of all year round ruminant production. There is need to source for cheaper and readily available feed to supply the nutrients required by the animals. Hence, this study was conducted to investigate the in vitro gas fermentation and its characteristics on selected agro-industrial by-products (AIBs) as alternative feed resources for ruminant animals. Corn bran (CB), soybean cheese waste (SBCW), shea nut cake (SNC) and Rice bran (RB) were incubated for 24hrs. The cumulative gas volume at the end of incubation was measured. Methane (ml/200mgDM) production of the AIBs was measured at the termination of the incubation with the introduction of 10ml NaOH into the syringes. The in-vitro fermentation characteristic, metabolizable energy (ME), organic matter digestibility (OMD) and short chain fatty acids (SCFA) were estimated. Proximate composition of the AIBs showed that crude protein (CP) ranged from 13.80% in RB to 30.12% in SBCW. Rice bran also had the highest ash content (17.20%) while the SNC recorded highest concentration of crude fiber. The SNC and SBCW had similar ether extract (EE) content. Gas volume statistically varied from 12.00ml/200mgDM to 27.67ml/200mgDM in rice bran and corn bran, respectively. The CB produced highest values of the fermentation characteristics. Methane production ranged from 2.5 to 5.5mLs in rice bran and corn bran, respectively. The calculated values of ME, SCFA and OMD were highest in SBCW. Overall, higher nutritive values found in the individual AIBs evaluated suggest that they are good alternative feed resources and when combined in the right proportion would sustain and improve ruminant productivity during the dry season.

Keywords: in vitro fermentation, agro-industrial by-products, nutritive value, supplement, dryseason

Introduction

The importance of livestock cannot be over emphasized. Indeed, it is a well-known fact that livestock is an essential aspect of human existence and a very essential means of improving living standard of smallholder farmers. Such animals are poultry, rabbits and ruminants. In tropical and sub-Saharan Africa, ruminants are kept under free range system where less attention is given to feeding as pasture is the major feed source. The pasture is poor in quality, of low digestibility and not available during off season of the year (Ogunbosoye and Babayemi, 2010). At

this period, there is weight loss, low milk yield, low reproduction, and high mortality due to various health challenges. The dry season spell has been recognized as one of the limiting factors to successful ruminant production enterprises in Nigeria and tropical countries in particular.

Feed supplements could be a good remedy to augment low quality roughage to enhance intake and digestibility and hence improved productivity. Susanti and Marhaeniyanto (2007) opined that feed supplement could be a means of mitigating feed insufficiency, thereby increasing digestive ability of rumen microbes for

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rumen metabolism. The use of conventional feed ingredients such as maize, soybean cake in ruminant feeding to augment production might be inappropriate because of their high cost and competition with both man and other farm animals. In light of this, non-conventional feed resources could be exploited for ruminant production in the tropics. These alternative feed resources should be cheaply available, nutritious, non-toxic and of high preference to animals and of little or no value to other animals and man. Agricultural by-products are good alternative feed for ruminants and are reported to be economical, viable and environmental concerns (Harjanti *et al.*, 2012, Rahman *et al.*, 2013). Agricultural production in many countries of Africa is now better organized as more and more priority is given to food production for domestic use. The increased mechanized farming has led to resultant increase in the number of agriculture-based industries. However, large quantities of these agro-industrial by-products and crop residues available in Africa could be used for animal feeding but some either go to waste or are underutilized. Soybean cheese waste (SBCW) is a by-product and a leftover when soy milk and beske (tofu) are prepared from soybeans. The SBCW is the main residue of soybean products considered as waste, and often dumped along the road where it is produced, causing health hazard. It is now being used as feed for farm animals like pigs and ruminants due to its nutritive quality and low cost. According to Dong *et al.* (2005), SBCW contains 11.2 MJ/kgME, 23.8% CP and 1.16% Ca. Shea nut cake (SNC) is the product after extracting the fat from shea butter seeds/nuts to produce shea butter. FAO (2007) reported that Nigeria is the leading producer of shea nuts and it is estimated that 355,000MT was produced in 1999 which amounted to 58% of the African production and 414,000MT in 2005 which amounted to 60.5% of the African

production. Shea butter production is on the increase in Nigeria, because of its medicinal value and its wide utilization for various functions such as cosmetics (Awolaye, 1995) and its dietary importance (ICRAF, 2000). The increase in the production of shea butter in Nigeria has led to progressive increase in shea nut cake. Shea nut cake cannot be consumed by any livestock in its natural form except it undergoes further processing which has led to its accumulation around the places of shea butter factory. It has been found to constitute a nuisance, causing a serious environmental pollution. Its incorporation into ruminant feed will be a big relieve to the environment, thereby creating a healthy environment to both man and even the animals. Corn bran is a well-known industrial by-products used as parts of compounded rations to both monogastric and ruminant animals but its inclusion level in the diets of non-ruminant animals is limited. Its high fibre content has hampered their utilization by mono-gastrics. Corn bran is a by-product of various maize processing industries, including starch and ethanol production, and the production of maize-based foods. It is largely produced locally because of the high production and processing of maize in the tropics, most especially in northern parts of Nigeria. Rice bran is the outer layer of rice grain which is removed during polishing. Rice bran is rich in vitamins and is widely used in the formulation of ruminants' diets (Akinyosoye, 1999; Gadzama *et al.*, 2016). It provides some key nutrients including fat, protein and phosphorus. According to Gadzama *et al.* (2016), rice bran has a crude protein content of about 7 to 9%, CF of 22.60% and NFE content of 38.70%. Feeding of rice bran alone may result in colic pain due to formation of ball inside the intestine (Sindhu *et al.*, 2002). Hence, it should always be mixed with other ingredients in the right proportion. It

contains 7% DCP, 65% TDN, 0.06% Ca, 1.12% P and oil content (13%) (Cheeke, 1991) and rich in vitamin B-complex. It may be used for feeding cattle, buffaloes, sheep and goat (Verma, 1997) if properly mixed. *In vitro* gas fermentation is a tool that has been used to estimate the digestibility patterns of feed in ruminant production (Sallam *et al.*, 2007). Its fastness, cheapness and the use of simple equipment has made it to gain more recognition as a technique to assess the nutritional status of feed. It is a laboratory estimation of degraded feeds which are basic in nutrition of animals. It has numerous advantages over other methods. It equally allows a large number of feed samples to be screened concurrently (Babayemi, 2007). The objective of this experiment is therefore to assess the fermentation characteristics of selected agro-industrial by-products as dry season feed in ruminant production.

Materials and methods

Four (4) agro-industrial by-products from different crops were obtained from the nearby processing factories as substrates for *in vitro* incubation: corn bran (CB), rice bran (RB), shea nut cake (SNC) and soybean cheese waste (SBCW). The feed samples were milled through a sieve of 1 mm using a hammer mill.

Chemical composition

The feed samples were collected and analyzed for dry matter (DM), crude protein (CP), crude fibre (CF), ether extract (EE), and ash as described by AOAC, (1990). NDF and ADL were determined as outlined by Van Soest *et al.* (1991).

In vitro gas fermentation

In vitro gas production was undertaken according to the procedure described by Menke and Steingass (1988). The samples were placed in a calibrated plastic syringe fitted with a rubber piston. The samples were incubated in an incubator at 39°C.

Rumen liquor was collected into the thermoflask before the morning feeding from three West African Dwarf (WAD) male goats which previously fed with *Panicum maximum* and wheat bran, sieved with a four layered cheese cloth mixed with a sodium buffer (9.8g NaHCO₃ + 2.77g (Na₂)HPO₄ + 0.57g KCl + 0.47g NaCl + 0.12g MgSO₄.7H₂O + CaCl₂.2H₂O per 1000ml) in a ratio 1:4v/v. 200mgDM weighed sample was put into the syringes and 30ml of rumen fluid and buffer were placed in each syringe and incubated in triplicate under continuous flushing with CO₂. A blank containing only rumen liquor and buffer sample was also incubated along with samples. At the end of the incubation, the reading of the blank was subtracted from the reading given by each of syringe while the standard sample was used as control to make sure the technique is acting correctly. The sample were shaking hourly before the reading started to have homogenous mixture. Gas production was recorded at 3, 6, 9, 12, 15, 18, 21 and 24 h and after 24 h of incubation. 4 ml of NaOH (10 M) was introduced into the incubated samples as reported (Fievez *et al.*, 2005) to obtain methane produced for each sample. The gas production characteristics were calculated using the equation $Y = a + b(1 - e^{-ct})$ as reported (Qrskov and McDonald, 1979), where Y = volume of gas produced at time 't', a = initial gas produced, b = gas produced from insoluble but degradable fraction, c = gas production rate constant for b, a+b = potential gas production, t = incubation time. Metabolizable energy (ME, MJ/kg DM) and organic matter digestibility (OMD %) were calculated from the volume of gas produced at the end of 24 h of incubation using the values obtained from the proximate composition of the samples as outlined (Menke and Steingass, 1988) while short chain fatty acid (SCFA, µmol) was also

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estimated as reported (Getachew *et al.*, 1999).

Statistical analysis

The means of all the data collected were analyzed by the analysis of variance (ANOVA) techniques using the General Linear model procedures of SAS (2010). Treatments means were compared using Duncan's Multiple range test of the same package.

Results and discussion

Presented in Table 1 is the proximate composition of the selected agro-industrial by-products. There were significant variations ($P<0.05$) among the evaluated feed samples. SBCW had the highest crude protein content while CB and SNC presented similar values. The RB produced the lowest value. The high concentrations of crude protein of the tested feed samples is an

indication that they could be used as feed supplements for ruminants on poor quality feed. Also, their low cost and availability at any period of the year, suggest that they are good feedstuffs to enhance productivity of animals during the dry season, thereby prevent weight lost and high mortality being experienced during this period. The crude fibre content of CB and SBCW were similar but the CF contents of SNC and RB were significantly different having 24.70g/100gDM and 32.30g/100gDM respectively. Ash concentration of CB and SNC were lower compared to RB and SBCW values. The crude protein concentrations of the AIBs were higher than the recommended value of 7-8% for ruminants to have active and health rumen environment (McDonald *et al.*, 2002) but similar to what was reported elsewhere when some agricultural by-products were evaluated (Mlay *et al.*, 2005).

Table 1: Chemical composition (g/100gDM) of some agro-industrial by-products (AIBs)

AIBs	Dry matter	Crude protein	Ash	Ether extract	Crude fibre	NDF	ADL
Corn bran (CB)	91.32	17.50 ^b	4.30 ^c	4.44 ^b	11.20 ^c	46.45 ^a	3.50 ^b
Soybean cheese waste (SBCW)	90.80	30.12 ^a	12.10 ^b	8.87 ^a	13.10 ^c	6.30 ^c	0.70 ^c
Rice bran (RB)	92.10	13.80 ^c	17.20 ^a	6.93 ^{ab}	24.70 ^b	13.80 ^b	1.50 ^c
Shea nut cake (SNC)	91.29	16.80 ^b	3.50 ^c	8.70 ^a	32.30 ^a	48.77 ^a	23.78 ^a
SEM	2.06	0.83	0.89	0.89	1.02	2.96	0.47

Fig 1: *In vitro* Gas Production of the Agro-industrial by-products

Table 2: Gas production characteristic and metabolizable energy (ME. MJ/kg DM), organic matter digestibility (OMD %) and short chain fatty acids (μmol) of the ingredients.

Treatment	a	b	a+b	c	t	Y	OMD	ME	SCFA
CB	12.67 ^a	15.00 ^a	27.67 ^a	0.110 ^b	10.00 ^a	21.67 ^a	47.63 ^a	6.95 ^a	0.47 ^b
SNC	6.00 ^b	6.00 ^c	12.00 ^c	0.1155 ^b	6.00 ^b	16.80 ^b	33.34 ^b	4.88 ^b	0.47 ^b
SBCW	12.33 ^a	9.67 ^b	22.00 ^b	0.260 ^a	7.00 ^b	20.33 ^a	48.78 ^a	6.99 ^a	0.60 ^a
RB	5.67 ^b	8.33 ^b	14.00 ^c	0.1708 ^a	11.00 ^a	12.33 ^b	33.34 ^b	4.96 ^b	0.27 ^c

^{a,b,c} means on the same column with different subscripts are significantly different ($P < 0.05$). Y = volume of gas produced (ml/200 mg DM) at time t, a = gas production (ml) from the soluble fraction, b = gas production (ml) from insoluble fraction, c = gas production rate (h^{-1}) constant from insoluble fraction b, a + b = potential gas production (ml), t = incubation time; CB= corn bran SNC= shea nut cake SBCW= soy bean cheese waste RB= rice bran

Fig 2: Methane production of the agro-industrial by-products (AIBs)

In vitro cumulative gas of the samples was statistically similar for SNC and RB but significant difference was observed in CB and SBCW (Figure 1). The highest rate of fermentation for CB may be due to the high concentration of fermentable carbohydrate in it which is a good substrate to rumen microbes for gas production (Kim *et al.*, 2013). The result was consistent with the report of Pereira *et al.* (2013) where some agro-industrial by-products were evaluated *in vitro*. Fermentation characteristics was significantly varied among the AIBs but corn bran is having the highest values in all except in gas production rate (c) indicating its fast degradability (Table 2). Both CB and

SBCW had higher cumulative gas volume and yet were also very high in protein content. The low degradability of RB and SNC may be due to the presence of lignin and theobromine, an anti-nutrient found in them which protects carbohydrates from attack by rumen microbes (Pereira *et al.*, 2013). This is an indication that CB and SBCW could enhance feed intake and digestibility if combined with other feed ingredients for better performance of animals. The calculated values of ME, OMD and SCFA also varied significantly among the feed samples (Table 2). The CB and SBCW presented the higher concentrations of these parameters while

RB and SNC gave the least values. Getachew *et al.* (2004) observed that cumulative gas volume produced at the end of fermentation is a reflection of the amount of SCFA produced, which is a source of energy to ruminant animals. The values obtained for ME and OMD were similar to the work of Sallam *et al.* (2007). Meanwhile, Menke *et al.* (1979) reported that the production of ME could be dependent on gas production during incubation and chemical composition of the samples. Methane volume production was highest in CB and statistically similar in other feedstuffs (Figure 2). The volume of methane produced from CB in this study was higher than the reported value of Kim *et al.* (2013) when compared with wheat bran and palm kernel. Methane production is of no use to ruminants but a big waste of energy. In fact, it contributes to greenhouse emission (Silivong *et al.*, 2013) in which its production to the atmosphere should be reduced. In other words, corn bran should not be fed alone to ruminants but in combination with any of the feed samples evaluated in this study.

Conclusion

The high nutritive content of the individual ingredients is an indication that the AIBs could be good feed resources for an improved ruminant production. It is therefore not suitable to feed any of these ingredients to the ruminants solely but probably in combination for efficient and increased production. It is evident from the study that it will not be ideal to feed corn bran alone to ruminant animals due to its high methane production that causes ozone layer problem. It is therefore recommended that ruminants be fed corn bran in combination with other agro-industrial by-products for enhanced animal productivity and promotion of healthy environment.

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