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IN VITRO ASSESSMENT FOR PREBIOTIC POTENTIALS OF SOME CARBOHYDRATE/FIBROUS FEEDSTUFFS FED IN BROILER DIETS.

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INTRODUCTION

The use of indigestible fermentable carbohydrates as prebiotics is founded on "colonization resistance" (Van der Waaij, 1989) where short chain fatty acids (SCFAs) are produced via saccharolytic fermentation in the hindgut lowering the pH of the environment, improving mineral absorption, inhibiting acid sensitive pathogens (Apajalahti, 2005) and inducing proliferation of indigenous microbiota in the hindgut. The fermentation of poorly digestible proteins present in the hindgut on the other hand (putrefactive fermentation) results in branched chain fatty acids (BCFAs), amines, phenols, indoles and ammonia which have toxic effects.

It is therefore a cause of concern when ingredients are added to diets on the assumption that because they are "fibre" or "soluble" they will also be fermentable and therefore have positive effects on gut health (Williams *et al*., 2005).This has led to a need to screen locally available fibres for possible use as prebiotics ingredients using the following criteria as enumerated by Gibson *et al*., 2004.

1. Resistance to gastric acidity, enzymatic hydrolysis and gastrointestinal absorption
2. Fermentation by intestinal microbial
3. Selective stimulation of the growth/activity of intestinal bacteria that contribute to health and well-being.

MATERIALS AND METHODS

SAMPLE PREPARATION AND CHEMICAL ANALYSIS

Ten feed ingredients; maize bran, wheat offal, rice bran, distillers dried grain, cassava root peel, cassava root sieviette, cassava starch, cassava starch residue, palm kernel cake and sweet potato flour were selected based on their high fibre/low protein content. Samples were air dried and ground, and their proximate composition and fibre assay were determined according to methods of AOAC, 1990, Van Soest, 1973 and Van Soest *et al*., 1991.

In vitro determination of resistance to gastric acidity, enzymatic hydrolysis and gastrointestinal absorption

Samples were pre-digested with pepsin and pancreatin to simulate digestion in the foregut by a modification of the dialysis bag method (Gauthier *et al*., 1986, Savoie and Gauthier, 1986). Crude protein content of the dialysate and residues were determined.

In vitro determination of fermentation by intestinal microbiota using the cumulative gas production technique *Inoculum preparation*

This phase of the experiment was run in three batches using broiler birds at 4, 6 and 8 weeks. Fresh caecal content of broiler birds fed a standard diet free of the test ingredients, antibiotics and copper (Lan *et al*., 2005) were pooled, weighed and mixed with 150ml buffer to obtain slurry. Slurry was filtered through a double layer cheese cloth to obtain the inoculum which was then made up to require volume with the buffering media. The anaerobic medium was nitrogen free and a modification of William *et al*., 2005.

Gas production incubations Approximately 100mg of "pre-digested" test ingredients were weighed into 50ml syringes fitted with a silicon tube to which 20mls of the "buffered inoculums" were added. The syringes were secured with metal clips to eliminate gas escape. Samples were run in quadruplicate and incubated at 39± 1^0C for 72 hours or till gas production ceased. Gas production readings were taken 3 hourly for the first 24hours and 6 hourly thereafter till experiment was terminated.

Gas production kinetics and post fermentation analysis. Gas production data was fitted to the monophasic model as described by Groot *et al*., 1996. Post fermentation, pH of each fermentation vessel was determined, substrates were analyzed for dry matter and ash content to calculate organic matter loss and filtrates were analyzed for ammonia by the steam distillation method. All data collected were subjected to analysis of variance and means separated using Duncan's multiple range test (SAS, 1999)

RESULTS AND DISCUSSION

In vitro determination of resistance to gastric acidity, enzymatic hydrolysis and gastrointestinal absorption.

In the feed samples, crude protein ranged from 24.1g/kgDM in cassava starch to 264.5g/kgDM in distillers' dried grain. Pre-digestion with pepsin and pancreatin (Table 1) resulted in DM digestion ranging from 168 g/kgDM for rice bran to 544.4 g/kgDM for maize bran. This guarantees that the carbohydrates investigated will resist gastric acidity and enzymatic hydrolysis up to 45-83 % *in vivo*. All carbohydrates tested, therefore met the first criteria for classification of any feedstuff as a potential prebiotic (Roberfroid, 2007).

In vitro determination of fermentation by intestinal microbiota using the cumulative gas production technique.

Since a nitrogen-free medium was used in the incubations, all carbohydrate and protein involved in the fermentation were assumed to be furnished by the test samples. Table 4 shows the fermentation kinetics profiles of the test samples, time lag, pH, ammonia and organic matter loss.

Of all carbohydrates tested, only four were fermentable by intestinal microbiota of broiler birds at all ages tested. This trend can be explained as being due to solubility of their constituent NSP fractions (Nyman *et al.,* 1986, Bach Knudsen and Hansen, 1991), degree of lignification (Bach Knudsen and Hansen, 1991), origin/source of NSPs (Chebeauti *et al*., 1991), processing methods, and presence of anti-nutritional factors (Getachew *et al*., 2004).

Kinetics of fermentation is a tool to measure feed fermentability *in vivo*. The rate at which different chemical constituents are fermented is a reflection of microbial growth and accessibility of the feed to microbial enzymes. The fermentation kinetics parameters; gas production per 100g DM sample (DMCV), maximum rate of gas production, R_{max} and time of occurrence of maximum rate of gas production, T_{max} were used to rank fermentation times for each substrate, inferring , that *in vivo*, cassava starch and sweet potato flour will ferment in the same segment of the large intestine at week 4, the different substrates will ferment in different segments of the large intestine at week6 and substrates will tend to ferment in an overlapping fashion in the large intestine at week 8.

Time lag which represents the time lapse between commencement of incubation and gas production, reduced significantly with age of birds owing to increased microbial diversity in the caecum. It also differed significantly among substrates owing to their varied chemical compositions.

Ammonia which estimates the measure of putrefactive fermentation occurring ,noted no significant difference among substrates at week 4 and 6, however, significant differences were recorded amongst substrates at week 8 and an obvious decline in ammonia profile was recorded among batches (age). This trend was attributed to poor digestion and absorption of proteins in younger birds resulting in more protein forms arriving the caecum thus reflecting in higher ammonia production.

Also, a strong relationship between pH and ammonia production (Cahn *et al*., 1998) was

confirmed as a minor change in pH observed at different ages of the birds, produced a large effect on ammonia production. More acidic pH was recorded at week 8 for all substrates indicating that more short chain fatty acid (SCFA) production is supported by older birds.

Table 1: Crude protein content of samples, dry matter digested after incubation with pepsin and pancreatin enzymes, crude protein content of the residue and crude protein digestibility.

	CP content (g/kg DM)	DM digested (g/kg DM)	CP in residue (g/kg DM)	CP digestibility (%)
Maize Bran	115.3	544.4	104.9	9.0
Wheat Offal	184.1	457.0	155.1	15.8
Rice Bran	96.3	168.0	42.6	55.8
Distillers Dried grain	264.5	251.8	262.7	0.7
Cassava Root Peel	113.8	368.9	76.1	33.1
Cassava Root Sievette	41.6	346.8	20.2	51.4
Cassava Starch	24.1	344.0	15.8	34.4
Cassava Starch Residue	43.8	389.4	11.7	73.3
Palm Kernel Cake (Solvent extracted)	224.3	192.7	147.3	34.3
Sweet Potato Flour	28.4	372.9	24.7	13.0

Table 2: Fermentation kinetics and end product characteristics of carbohydrate samples using caecal inoculum from broiler birds at 4, 6 and 8 weeks of age.

Age of birds		Lag time **(h)**	**DMCV**	**T_{max}**	**R_{max}**	**NH_3**	**pH**	**OMloss**
Week 4	RS	41.25^{a}	1.20^{c}	41.19^{a}	1.07^{c}	249.48	6.68^{a}	47.57^{d}
	CS	24.00^{c}	9.18^{a}	23.87^{b}	6.82^{a}	429.79	6.59^{c}	84.36^{a}
	SP	25.88^{c}	4.75^{b}	25.75^{b}	3.36^{b}	290.30	6.64^{b}	55.75^{c}
	SR	35.50^{b}	9.22^{a}	19.30^{c}	5.94^{a}	299.49	6.45^{d}	75.54^{b}
	SEM	0.44	0.49	0.65	0.19	40.60	0.00	0.21
Week 6	RS	6.00^{c}	5.48^{d}	11.35^{d}	0.73^{c}	49.90	6.78^{a}	30.09^{c}
	CS	10.00^{b}	14.49^{a}	18.28^{c}	1.97^{a}	53.23	6.58^{c}	86.16^{a}
	SP	21.00^{a}	7.50^{c}	32.52^{a}	1.21^{b}	39.97	6.76^{a}	57.05^{b}
	SR	9.00^{b}	11.13^{b}	23.41^{b}	1.06^{b}	28.92	6.63^{b}	60.71^{b}
	SEM	0.29	0.22	3.55	0.06	4.77	0.01	2.56
Week 8	RS	6.00^{c}	7.83^{b}	18.74^{bc}	6.00^{b}	11.91^{ab}	6.19^{a}	33.47^{d}
	CS	18.00^{a}	10.00^{a}	23.52^{ab}	7.75^{a}	7.21^{b}	5.61^{b}	91.91^{a}
	SP	18.00^{a}	9.95^{a}	28.82^{a}	7.07^{ab}	13.61^{a}	6.19^{a}	65.68^{c}
	SR	12.00^{b}	9.95^{a}	14.18^{c}	5.86^{b}	5.95^{b}	5.75^{b}	79.34^{b}
	SEM	-	0.04	0.21	0.21	1.03	0.02	0.85

R_{max} = Maximal rate of gas production (ml/h); T_{max}= Time at occurrence of maximal rate of gas production (h); DMCV= Dry matter cumulative gas production (ml/100mgDM); SEM= Standard error of mean; SEM= Standard error of mean. RS= Cassava root sieviette; CS= Cassava starch; SP=Sweet potato flour; SR= Cassava starch residue. Means with different superscripts in each column differ significantly ($p<0.05$).

Conclusion.

Ten carbohydrate feedstuffs were investigated for prebiotic potentials. Though all test feedstuffs showed a resistance to gastric acidity and hydrolysis by digestive enzymes in varying degrees, only four showed fermentative activity by intestinal microflora evidenced in gas

production. The four test feedstuffs showed significant effects on pH and ammonia production confirming a shift in microbial load (a proliferation of saccharolytic microbes to the detriment of putrefactive microbes).

Their fermentation kinetics profile also showed that the substrates if used as prebiotics in combination will tend to maintain saccharolytic fermentation and its attendant acidic pH throughout the length of the large intestine. Further studies to establish that these feedstuffs can be used to manipulate intestinal microflora populations *in vivo* are in progress

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