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Effect of micro-organism and particle size on fermentation of sorghum and maize for poultry feed

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A study was conducted to investigate the effect of particle size and micro-organism on fermentation of sorghum and maize for poultry feed. Sorghum (*Sorghum bicolour* L. Moench) and maize were milled in a hammer mill and separated into coarse, medium, fine and very fine particles sizes with a stack of sieves of apertures 2.5 mm, 850 µm and 500 µm, from the first to the last sieve and ending in a pan with the very fine particles. Samples were weighed into 100 g sachets and irradiated using ^{60}Co at 25 kGy γ-radiation. Grains were fermented with sterile distilled water for 24 h at a ratio of 1 feed:1.4 water and inoculated with 0.01 ml of an overnight culture of De Man, Rogosa and Sharpe (MRS) broth containing *Pediococcus acidilactici* (PA1) or *Lactobacillus plantarum* (SLP) (ca 10^9 cfu/ml). The medium was incubated at 30°C simultaneously with a control treatment without lactic acid bacteria (LAB). Sub-samples were collected aseptically at the beginning of the fermentation (0 h) and at 4, 8, 24 h after fermentation for pH, sugar and organic acids analysis. Significant reductions in the pH of maize and sorghum for LAB treatments (PA1 and SLP) were evident after 8 hours of fermentation. Twenty four hour lactic acid concentrations from coarse particle size fermentations were not significantly different from concentrations in the medium and fine particle size fermentations. The choice of LAB did not affect the concentration of lactic acid for any particle size. However, acetic acid production from fermentation with PA1 was significantly higher ($P<0.01$) than the concentration obtained with SLP. Results suggest that moderate grain processing may be enough to permit production of biosafe levels of lactic acid in fermented feed for poultry birds.

Key words: Fermentation, lactic acid bacteria, maize, particle size, sorghum.

INTRODUCTION

Grain sorghum is widely used as a food cereal in many parts of Africa, Asia and the semi-arid tropics world-wide (Elkhalifa and El-Tinay, 2002; Osman, 2004; Fombang et al., 2005; Ragaee et al., 2006). In Africa, India and China, it is only superseded by rice and wheat as a cereal for human consumption (Elkhalifa and El-Tinay, 2002). In addition to being a staple food for humans, it is also used as a feed for animals (Peiris et al., 1998; Elkhalifa and El-Tinay, 2002; Balogun et al., 2005; Huang et al., 2005) and as an industrial raw material (Elkhalifa and El-Tinay, 2002). In the semi-arid tropics it is more popular than maize because it grows well with limited water and under temperature stress (Osman, 2004). Maize on the other hand has been used in many parts of the world as a feed ingredient in poultry nutrition (example, Huang et al., 2005; McNaugthon et al., 2007; Rama Rao et al., 2007; Yu et al., 2007).

Provision of dry diets containing cereals as the main

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energy substrates has been the conventional feeding method used for chickens. However, it has been demonstrated that soaking the feed increases nutrient availability or, alternatively, reduces particle size with consequent increase in surface area of the diet for action of the animal's digestive enzymes (Choct et al., 2004a). Although the success of a feeding method such as liquid feeding is highly dependent on the manner in which the grains are initially processed (Choct et al., 2004b), addressing the nutrient requirement for physiological development of the animal is as important as the type of grain and the way in which the grain was processed, especially during milling.

Apart from the texture of the feed and its nutrient value, the ability of the feed to remain free of pathogens during short storage and handling for liquid feeds and to change gut microbial activity towards improved gut health is of critical importance to food and environmental safety. Consequently, striking a balance between the need for a good milled feed, cost of feed associated with extra milling and an appropriate concentration of organic acid (mainly lactic acid) in the feed is important for animal productivity, biosafety and economic reasons.

According to the study of Beal et al. (2002), to prevent the growth of *Salmonella typhimurium* DT104:30 in liquid feeds, a threshold lactic acid concentration of 75 mmol/L is required in the feed. However, due to the practical advantages of fermenting the carbohydrate-rich cereal component of the diet separately and combining it with the protein-rich components just before feeding (Beal et al., 2002, 2005; Moran et al., 2006; Canibe et al., 2007; Brooks, 2008), it is desirable to have a higher lactic acid concentration (ca >150 mmol/L) in the cereal component so as to minimize the dilution effect to the acid concentration of the feed and pH when mixed with the protein-rich component at feeding.

Several factors are thought to affect the production of lactic acid in cereal fermentation. Among these factors are, fermentation temperature (example Beal et al., 2002), cereal substrate type and strain of LAB (Charalampopoulos et al., 2002) and proportion of pre-fermented feed used in backslopping (Moran et al., 2006). With the same cereal substrate and LAB, a key factor that might influence lactic acid production is the particle sizes produced at milling, which could affect the amount of sugars available for microbial enzymatic fermentation. Furthermore, Anguita et al. (2006) concluded that technological processing of ingredients promotes higher starch hydrolysis in addition to increasing the amount of soluble non-starch polysaccharides and modifications in the physicochemical properties depending on the nature of the feed ingredients. According to the study of Williams et al. (2005) by assessing potential fermentability of a large number of ingredients, it is possible to make an informed choice as to which substrates are most suited for inclusion in a diet. This is true not only for the ingredients, but also for the fermenting microbes especially with the development of accelerated fermentation of animal feed substrates using lactic acid bacteria. With these points in mind, the aim of this study was to investigate the effect of particle size and micro-organism on fermentation of sorghum and maize for poultry feed.

MATERIALS AND METHODS

Experimental design

This study was conducted as a 4 x 3 x 4 factorial design with three factors: factor 1: particle size (coarse, medium, fine and very fine); factor 2: control treatments without LAB and LAB treatments (*Pediococcus acidilactici* (PA1) or *Lactobacillus plantarum* (SLP)); factor 3: incubation time (0, 4, 8, and 24 h). All treatments with both grains were replicated three times.

Particle size determination

Raw sorghum was milled in a hammer mill to pass through a 3 mm screen while equal quantities of raw maize were milled either through 6 or 3 mm screens and mixed manually to give a uniform mixture of particle sizes. Both grains were separated into coarse, medium, fine and very fine particles sizes using a Retisch flask shaker (Endecotts LTD London, England) with a stack of sieves. The sieve apertures were 2.5 mm, 850 µm, and 500 µm, from the first to the last sieve and ending in a pan with the very fine particles. Samples (185 ± 10 g) were placed on the sieves (diameter 200 mm) for each cycle and the sieving done for a period of 10 min at amplitude of 80. Samples of each particle size were weighed to 100 g sachets for subsequent irradiation. Irradiation of sorghum and maize was conducted with 25 kGy γ-radiation from ^{60}Co by Becton and Dickinson, Plymouth, UK. Maize was obtained from Edwin Tucker and Sons, Ashburton, Devon while Sorghum was the white variety (*Sorghum bicolour* L. Moench) acquired from the World Foods Shop, Plymouth.

Fermentation and sample collection

Feed samples were mixed with sterile distilled water at a ratio of 1:1.4 as recommended by Hojberg et al. (2003). The mixture was inoculated with 0.01 ml of an overnight culture of De Man, Rogosa and Sharpe (MRS) broth concentration containing one of two ca 10^9 cfu/ml LAB spp. (PA1 or SLP). These were incubated at 30°C simultaneously with a control treatment without lactic acid bacteria. Samples were stirred for 1 to 2 min and sub-samples removed aseptically from each beaker at the beginning of the fermentation (0 h) and at 4, 8 and 24 h after fermentation. The samples were used to measure the pH using a pH electrode (pH 213 microprocessor pH meter, Hanna instruments, Portugal) and 0.5 ml samples were collected for sugar and organic acid analysis and immediately frozen in Eppendorf tubes and kept at -20°C until analysis.

Analysis for short chain organic acids and sugars

Organic acids and simple sugars were analysed by high performance liquid chromatography (HPLC) according to the method of Niven et al. (2004) using a Varian metacarb 87H column (Serial N° 05524314, USA). Elution was performed using dilute sulphuric acid (5 mmol/L) at a flow rate of 0.5 ml/min. The volume of samples injected was 20 µL and analysis time was 30.5 min per sample. Detection of sugars and organic acids was conducted by refractometry and signals were recorded using the Chromeleon information management systems software version 6.20 SP2 Build 541 (Dionex corporation, UK).

Table 1. Effect of micro-organism used for maize fermentation on 24 hour pH, total sugars and organic acid concentrations (mmol/L), (n=12).

Parameter	pH	Total sugar*	Lactic acid	Acetic acid
Control	5.77[a]	227.67[a]	15.66[a]	2.64[a]
P. acidilactici (PA1)	3.71[b]	107.48[b]	167.57[b]	14.51[b]
L. plantarum (SLP)	3.56[c]	80.42[c]	206.17[c]	4.54[a]
SED	0.014	6.72	10.51	1.60
P-Value	<0.001	0.002	0.003	<0.001

abc significant difference between means bearing different letters in the same column. *Total sugars are the sums of maltose, glucose and fructose concentrations. n=number of observations per mean.

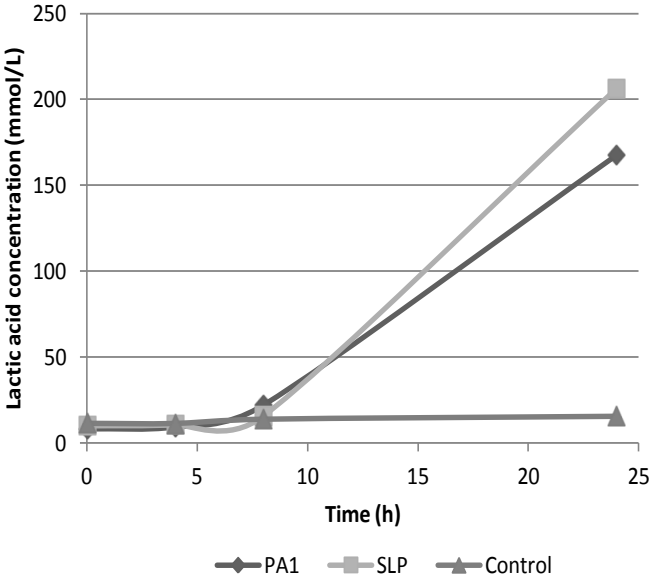

Figure 1. Effect of maize fermentation with *P. acidilactici* (PA1) or *L. plantarum* (SLP) or control without LAB on lactic acid concentration (mmol/L).

To each sample, 20 µL of 7 % (v/v) sulphuric acid was added to denature dissolved proteins and shift the acid dissociation equilibrium towards complete protonation of organic acids. Samples were mixed for 30 s using a vortex mixer and centrifuged at 13000 rpm for 10 min. The supernatant was extracted using 1 ml polypropylene disposable syringes and filtered through 0.45 µm NYL polypropylene syringe filters to eliminate any particulate material still present. Standards containing three concentrations of analytical grade lactic acid (300, 150 and 75 mmol/L), acetic acid (100, 50 and 25 mmol/L), maltose (100, 50 and 25 mmol/L), glucose (50, 25 and 12.5 mmol/L) and fructose (50, 25 and 12.5 mmol/L) were run before and after every six subsequent samples.

Data analysis

Data were analysed using the general linear model procedure (GLM) of analysis of variance using Minitab (release 15.0) according to the following general model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \delta_{ij}$$

Where, Y_{ij} is the observed dependent variable; μ is the overall mean; α_i is the effect due to particle size; β_j is the effect due to lactobacilli used; $(\alpha\beta)_{ij}$ is the interaction between particle size and lactobacilli used and δ_{ij} is the random error. Data for the different time periods and grains (maize or sorghum) were analysed separately and least square means with pooled standard error of the means (SEM) were obtained. Differences between means were determined using the Tukey's test (Zar, 1999). Probability values ≤ 0.05 were considered to be statistically significant.

RESULTS

Maize fermentation

After 24 h of fermentation, the pH had dropped significantly more (P<0.001) in the LAB fermentations (Table 1) than the control treatment. Fermentation of maize with SLP resulted in a significantly lower (P<0.001) pH (3.56) than fermentation with PA1 (pH 3.71). As expected, total fermentable sugars were significantly higher (P=0.002) in the control treatment than the LAB treatments. Maize fermented with SLP had a significantly lower (P=0.002) total fermentable sugar concentration than maize fermented with PA1. Lactic acid production from SLP fermented maize was significantly higher (P=0.003) than the production from PA1. However, acetic acid production from maize fermented with PA1 was significantly higher (P<0.001) than the concentration obtained with SLP.

Variation in lactic acid concentration with time

The initial rapid increase in lactic acid production resulting from fermentation with PA1 compared with SLP (Figure 1) for the first 8 h of fermentation was not maintained until 24 h fermentation. The concentration of lactic acid was consequently higher for SLP fermented maize (206.17 ± 7.43) (mean ± SEM) than fermentation with PA1 (167.57 ± 7.43).

Due to large differences in pH and organic acid concentrations between the different time periods especially

Table 2. Effect of particle size and micro-organism used for maize fermentation on 0 hour pH, (n=3).

Particle size	Control	PA1	SLP	P-Value
Coarse	5.55	5.43	5.49	0.6366
Medium	5.62	5.55	5.51	0.6366
Fine	5.59	5.53	5.61	0.9946
Very fine	5.73	5.61	5.62	0.5211
P-Value	0.08	0.12	0.48	0.524

*****Standard error of the difference - 0.054, n=number of observations per mean.

Table 3. Effect of particle size and micro-organism used for maize fermentation on 4 hour pH and lactic acid concentrations (mmol/L), (n=3).

Parameter	Particle size	Control	PA1	SLP	P-Value
pH	Coarse	$^{A}5.73^{a}$	$^{A}5.63$	$^{B}5.61$	0.02
	Medium	5.67^{bc}	5.63	5.55	1.00
	Fine	5.69^{ac}	5.57	5.59	0.59
	Very fine	5.77^{b}	5.59	5.53	0.41
	P-Value	0.003	0.94	0.15	0.322
Lactic acid	Coarse	10.15	9.14	5.69^{a}	0.98
	Medium	8.07	9.42	8.80^{a}	1.00
	Fine	13.92	9.61	9.03^{a}	0.96
	Very fine	13.25	9.58	20.05^{b}	0.75
	P-Value	0.88	1.00	0.02	0.113

^{abc} significant difference between means bearing different letters in the same column and parameter.
^{AB}significant difference between means bearing different letters in the same row. *****Standard error of the difference-pH (0.05) and Lactic acid (3.57), n=number of observations per mean.

between 0 and 24 h for the respective LAB and particle sizes, data were analyzed and are presented on a sampling time basis.

0 h

There were no significant interactions or between treatment effects in pH values of LAB treatments or particles sizes (Table 2).

4 h

Particle size x treatment interactions in pH and lactic acid production were not significant (Table 3). The presence of LAB had a linear effect on pH (P=0.02) for the coarse particle size in the SLP treatment. Particle size reduction also had a linear effect (P=0.02) on lactic acid concentration in this same treatment.

8 h

There were significant particle size x LAB treatment interactions for pH (P=0.002) and lactic acid concentrations (P<0.001) (Table 4). pH values in LAB treatments were all significantly lower than (P<0.04) values obtained with the control treatment. The pH of fine (5.04 ± 0.05) and very fine (4.96 ± 0.05) particles sizes in the PA1 treatment were significantly lower (P=0.033) than values obtained with the coarse (5.33 ± 0.05) and medium (5.29 ± 0.05). These values were also lower (P<0.04) than the mean pH values for all the particle sizes on the SLP treatment. Lactic acid concentrations for the particle sizes on the PA1 treatment were higher than the control treatment concentrations for the coarse (P=0.0138), medium (P=0.002) and fine particle sizes (P=0.0043). The concentrations of lactic acid in PA1 fermentations for the coarse (22.77 ± 2.07) and fine (27.47 ± 2.07) particle size, were significantly higher (P=0.0138 and P=0.0043) than corresponding fermentations (14.65 ± 2.07 and 14.08 ± 2.07) in the SLP treatment. Lactic acid production resulting from fermentation of very fine particles sizes with PA1 was significantly lower (P=0.0159) than the concentration obtained with the larger particle sizes.

24 h

Particle size x LAB treatments interactions in the pH (P=0.312) and acetic acid concentration (P=0.194) were not significant (Table 5). However, there was a significant interaction in the lactic acid concentration (P<0.001). All

Table 4. Effect of particle size and micro-organism used for maize fermentation on 8 hour pH and lactic acid (mmol/L), (n=3).

Parameter	Particle size	Control	PA1	SLP	P-Value
pH	Coarse	$^{A}5.76$	$^{B}5.33^{a}$	$^{B}5.51$	0.005
	Medium	$^{A}5.71$	$^{B}5.29^{a}$	$^{B}5.46$	0.03
	Fine	$^{A}5.75$	$^{C}4.96^{b}$	$^{B}5.36$	0.003
	Very fine	$^{A}5.78$	$^{C}5.04^{b}$	$^{B}5.29$	<0.04
	P-Value	0.999	0.033	0.11	0.002
Lactic acid	Coarse	$^{A}10.52^{ab}$	$^{B}22.77^{ab}$	$^{A}14.65$	0.0138
	Medium	$^{A}10.38^{a}$	$^{B}25.06^{b}$	$^{AB}15.01$	0.002
	Fine	$^{A}13.74^{ab}$	$^{B}27.47^{b}$	$^{A}14.08$	0.0043
	Very fine	21.05^{b}	12.98^{a}	19.70	0.2617
	P-Value	0.0469	0.0159	0.7393	<0.001

^{abc}Significant difference between means bearing different letters in the same column and parameter. ^{AB}significant difference between means bearing different letters in the same row. *Standard error of the difference-pH (0.07) and Lactic acid (2.93), n=number of observations per mean.

Table 5. Effect of particle size and micro-organism used for maize fermentation on 24 hour pH and organic acid concentrations (mmol/L), (n=3).

Parameter	Particle size	Control	PA1	SLP	P-Value
pH	Coarse	$^{A}5.90^{a}$	$^{B}3.80^{a}$	$^{C}3.64^{a}$	0.003
	Medium	$^{A}5.75^{b}$	$^{B}3.71^{ab}$	$^{C}3.55^{b}$	<0.004
	Fine	$^{A}5.70^{b}$	$^{B}3.67^{b}$	$^{C}3.54^{b}$	<0.0063
	Very fine	$^{A}5.71^{b}$	$^{B}3.66^{b}$	$^{C}3.50^{b}$	<0.004
	P-Value	0.0012	0.005	0.003	0.312
Lactic acid	Coarse	$^{A}7.82$	$^{B}175.89$	$^{B}245.09^{a}$	<0.001
	Medium	$^{A}13.37$	$^{B}132.72$	$^{B}200.49^{ab}$	<0.004
	Fine	$^{A}20.80$	$^{B}162.68$	$^{B}234.26^{a}$	<0.001
	Very fine	$^{A}20.66$	$^{B}199.00$	$^{B}144.85^{b}$	<0.003
	P-Value	1.00	0.1266	0.01	0.001
Acetic acid	Coarse	2.55	11.96	5.57	0.1879
	Medium	2.66	10.23	4.92	0.4618
	Fine	$^{A}3.49$	$^{C}21.56$	$^{B}5.07$	0.0014
	Very fine	$^{A}1.84$	$^{C}14.30$	$^{B}2.59$	<0.05
	P-Value	1.00	0.0573	0.998	0.194

^{abc} significant difference between means bearing different letters in the same column and parameter.
^{ABC}significant difference between means bearing different letters in the same row. *****Standard error of the difference-pH (0.029), Lactic acid (21.02) and Acetic acid (3.2), n=number of observations per mean.

LAB treatments had mean pH values ranging from 3.50 to 3.80 whilst the control treatment had values ranging from 5.70 to 5.90. The differences between LAB treatments and the control treatment were significant (P<0.004). The mean pH range of 3.50 to 3.64 observed in the PA1 treatment was lower (P<0.004) than the range of 3.66 to 3.80 obtained in the SLP treatment. Reduction in particle size tended to decrease the pH within all treatments (P≤0.005). LAB treatments all had significantly higher (P<0.004) lactic acid concentrations than the control treatment. The choice of LAB used in fermentation did not affect the concentration of lactic acid for any particle size. While there were no differences between particle sizes in lactic acid concentration within the control and PA1 treatments, coarse particles in SLP treatment produced significantly (P=0.01) more lactic acid (245.09 ± 14.86) than the very fine particles (144.85 ± 14.86). Acetic acid production from fine and very fine

Table 6. Effect of micro-organism used for sorghum fermentation on 24 hour pH, total sugars and organic acid concentrations (mmol/L), (n=12).

Parameter	pH	Total sugar*	Lactic acid	Acetic acid
Control	6.06[a]	167.19[a]	13.23[a]	5.42[a]
PA1	3.42[c]	46.81[b]	240.00[b]	33.07[b]
SLP	3.51[b]	33.95[b]	302.73[c]	10.62[a]
SED	0.04	7.28	8.64	4.92
P-Value	0.045	<0.001	<0.001	<0.001

[abc] significant difference between means bearing different letters in the same column. *Total sugars are the sums of maltose, glucose and fructose concentrations, n=number of observations per mean.

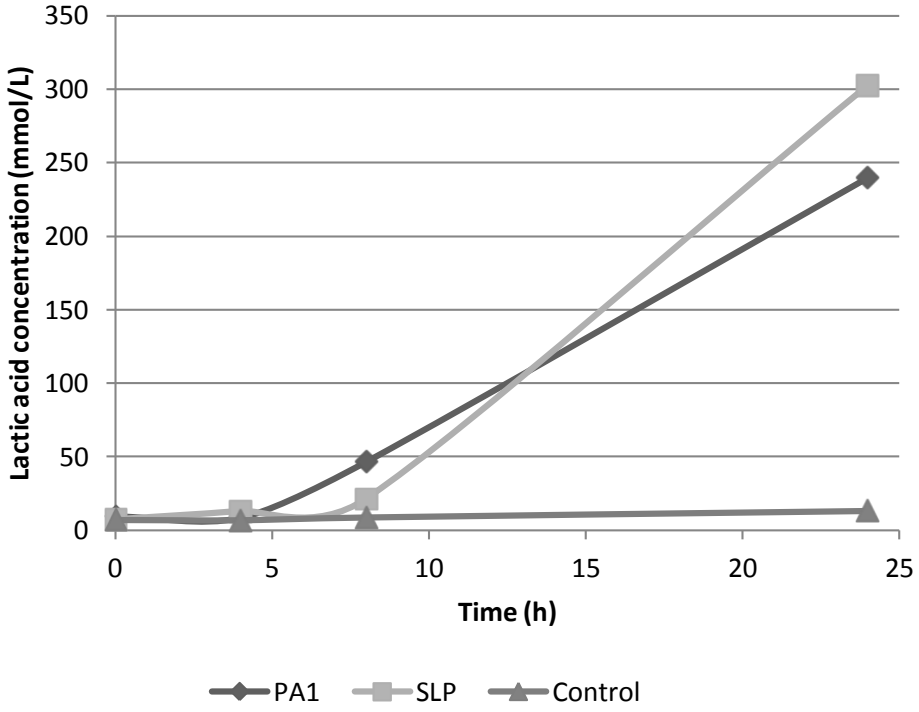

Figure 2. Effect of sorghum fermentation with *P. acidilactici* (PA1) or *L. plantarum* (SLP) or control without LAB on lactic acid concentrations (mmol/L).

particles sizes in the PA1 treatment were significantly higher (P<0.05) than the control and SLP treatments.

Sorghum fermentation

The pH after 24 h fermentation dropped significantly more (P=0.045) in the LAB treatments (Table 6) than the control treatment. Fermentation of sorghum with SLP resulted in a significantly higher pH than fermentation with PA1. As expected, total fermentable sugars was significantly higher (P<0.001) in the control than in the LAB treatments. There was no significant difference between the LAB treatments in the total fermentable sugar concentrations. Lactic acid production from SLP fermented sorghum was significantly higher (P<0.001) than the production from PA1. However, acetic acid production from sorghum fermented with PA1 was significantly higher (P<0.001) than the concentration obtained with SLP and the concentration in the control treatment.

Variation in lactic acid concentration with time

PA1 (Figure 2), produced more lactic acid within 8 h of fermentation (46.60 ± 2.37mmol/L) than SLP (21.38 ± 2.37 mmol/L). However, between 8 and 24 h fermentation, the increase in lactic acid production from SLP was higher (increase of 281 mmol/L) than from PA1 (increase of 193 mmol/L).

0 h

There were no significant quadratic or linear effects in pH

Table 7. Effect of particle size and micro-organism used for sorghum fermentation on 0 hour pH, (n=3).

Particle size	Control	PA1	SLP	P-Value
Coarse	6.02	6.06	6.13	0.843
Medium	5.94	5.98	6.02	0.971
Fine	6.12	6.03	6.01	0.866
Very fine	5.99	5.91	5.97	0.978
P-Value	0.236	0.446	0.385	0.264

*Standard error of the difference- 0.064, n=number of observations per mean.

Table 8. Effect of particle size and micro-organism used for sorghum fermentation on 4 hour pH and organic acid concentrations (mmol/L), (n=3).

Parameter	Particle size	Control	PA1	SLP	P-Value
pH	Coarse	6.04	6.07^{a}	6.12^{a}	0.9281
	Medium	5.94	5.92^{ab}	5.88^{b}	0.9904
	Fine	6.11	6.00^{ab}	5.98^{ab}	0.3899
	Very fine	5.92	5.87^{b}	5.89^{b}	0.9979
	P-Value	0.056	0.038	0.008	0.224
Lactic acid	Coarse	4.40	6.02	5.64	1.00
	Medium	4.72	7.61	6.90	0.999
	Fine	9.81	4.38	10.27	0.9655
	Very fine	8.43	16.01	14.49	0.8438
	P-Value	0.981	0.315	0.687	0.58

^{abc} significant difference between means bearing different letters in the same column and parameter
^{AB}significant difference between means bearing different letters in the same row. *Standard error of the difference-pH (0.054) and Lactic acid (4.42), n=number of observations per mean.

values between treatments or particles sizes (Table 7).

4 h

Particle size x LAB treatment interactions in pH and lactic acid production were not significant (Table 8). There were also no significant differences between treatments in pH and lactic acid concentrations. However, the presence of LAB in the fermenting medium had a significant effect (P<0.04) on particle size pH and particle size reduction had no effect on lactic acid concentrations.

8 h

There were significant particle size x treatment interactions in pH (P<0.001) and lactic acid concentrations (P<0.001) (Table 9). pH values in LAB treatments were all significantly lower (P<0.001) than values obtained in the control treatment. pH values ranging from 5.08 to 5.33 were obtained in the PA1 treatment and these were significantly higher (P<0.001) than the range of 5.58 to 5.74 observed in the SLP treatment. Treatments effects on pH were also reflected by higher lactic acid concentrations in the PA1 treatment especially for the fine (P=0.012) and very fine (P<0.001) particle sizes. Reduction in particle size increased lactic acid production significantly (P<0.001) in the PA1 treatment.

24 h

Significant particle size x treatment interactions in the pH (P=0.009), lactic (P<0.001) and acetic acid concentration (P=0.026) were also observed after 24 h fermentation (Table 10). All LAB treatments had pH values ranging from 3.25 to 3.63 that were significantly higher (P<0.001) than the range of 5.94 to 6.31 in the control treatment.

Treatment effects on pH were reflected by higher (P<0.002) lactic acid concentrations in LAB treatments (197.08 to 401.87 mmol/L) as opposed to the control treatment (8.35 to 23.55 mmol/L). Twenty four hour lactic acid concentrations from coarse particle size fermentations in LAB treatments were not significantly different from concentrations in the medium and fine particle size fermentations. Acetic acid production from the fine particle sizes in the PA1 treatment was significantly higher (P<0.001) than the control and SLP treatments.

Table 9. Effect of particle size and micro-organism used for sorghum fermentation on 8 hour pH and organic acid concentrations (mmol/L), (n=3).

Parameter	Particle size	Control	PA1	SLP	P-Value
pH	Coarse	$^{A}6.07^{a}$	$^{C}5.25^{a}$	$^{B}5.63^{a}$	<0.001
	Medium	$^{A}5.91^{a}$	$^{C}5.08^{b}$	$^{B}5.58^{a}$	<0.001
	Fine	$^{A}6.11^{a}$	$^{C}5.33^{a}$	$^{B}5.83^{b}$	<0.001
	Very fine	$^{A}5.82^{b}$	$^{C}5.09^{b}$	$^{B}5.74^{b}$	<0.001
	P-Value	0.03	0.04	0.05	<0.001
Lactic acid	Coarse	5.34	29.26^{a}	19.00	0.059
	Medium	5.08	27.95^{a}	15.57	0.082
	Fine	$^{A}9.81$	$^{B}38.28^{a}$	$^{A}18.83$	0.012
	Very fine	$^{A}14.77$	$^{B}90.89^{b}$	$^{A}32.12$	<0.001
	P-Value	0.951	<0.001	0.402	<0.001

^{abc} significant difference between means bearing different letters in the same column and sugar type. ^{AB}significant difference between means bearing different letters in the same row. *Standard error of the difference-pH (0.043) and Lactic acid (6.70), n=number of observations per mean.

Table 10. Effect of particle size and micro-organism used for sorghum fermentation on 24 h pH and organic acid concentrations (mmol/L), (n=3).

Parameter	Particle size	Control	PA1	SLP	P-Value
pH	Coarse	$^{A}6.04^{ab}$	$^{B}3.49$	$^{B}3.63$	<0.001
	Medium	$^{A}5.94^{a}$	$^{B}3.43$	$^{B}3.33$	<0.001
	Fine	$^{A}6.31^{b}$	$^{B}3.50$	$^{B}3.58$	<0.001
	Very fine	$^{A}5.94^{a}$	$^{B}3.25$	$^{B}3.50$	<0.001
	P-Value	0.002	0.085	0.10	0.009
Lactic acid	Coarse	$^{A}8.35$	$^{B}233.54^{a}$	$^{B}272.46^{ab}$	<0.001
	Medium	$^{A}8.92$	$^{B}197.08^{a}$	$^{B}211.55^{a}$	<0.001
	Fine	$^{A}12.09$	$^{B}216.21^{a}$	$^{C}325.02^{b}$	<0.001
	Very fine	$^{A}23.55$	$^{B}313.15^{b}$	$^{C}401.87^{c}$	<0.002
	P-Value	0.999	0.005	0.008	<0.001
Acetic acid	Coarse	3.70	25.64^{a}	10.04	0.546
	Medium	9.16	17.37^{a}	9.25	0.999
	Fine	$^{A}2.61$	$^{B}62.80^{b}$	$^{A}13.78$	<0.001
	Very fine	6.21	26.47^{a}	9.43	0.654
	P-Value	0.999	0.042	1.00	0.026

^{abc} significant difference between means bearing different letters in the same column and sugar type. ^{AB}significant difference between means bearing different letters in the same row. *Standard error of the difference-pH (0.074) Lactic acid (17.28) and Acetic acid (9.84), n=number of observations per mean.

DISCUSSION

Apart from high numbers of lactic acid bacteria, other desirable properties of fermented liquid feeds are low pH (3.5 to 4.5) (Geary et al., 1996; Scholten et al., 1999; Christensen et al., 2007) and a high lactic acid concentration (>150 mmol/L) (Geary et al., 1996). 24 h fermentation pH values for LAB treatments in this study in both grains were within this pH range. The pH values in this study are similar to those reported by Moran et al. (2006), who obtained pH values below 3.80 using backslopping with pre-fermented feed after 24 h fermentation. This is vital, as an important advantage of the pH of the feed lies in its ability to improve resistance to enteropathogenic contamination.

According to Brooks et al. (2001), coliforms and *Salmonella* will thrive when undesirable fermentation results in a pH greater than 4.5. Working on the effect of temperature on the growth and persistence of *Salmonella* in liquid pig feed, Beal et al. (2002), reported that the mic-

robial population initially increased more rapidly in co-inoculated feed incubated at 30°C compared with 20°C. They indicated that once the lactic acid concentrations reached ca. 75 mmol/L and the pH dropped below 4.5, the microbes were killed more rapidly.

In order for fermentation to achieve the > 75 mmol/L lactic acid concentration to resist *Salmonella* spp. growth as observed in pig feed (Beal et al., 2002), a higher concentration of ca > 150 mmol/L lactic acid in the fermented cereal-based component should be the goal. In the present study, this goal was achieved for all four particle sizes and LAB treatments by 24 h of fermentation for both cereals. Beal et al. (2005) stated that in liquid pig feed substrates where competing micro-organisms could involve enteropathogens, it is imperative to have a rapid build-up of lactic acid in the medium. According to the study of Moran et al. (2006), the duration of exposure of coliforms to low pH and/or high lactic acid concentrations needs to be recognized as an important factor in their exclusion from fermented feed. Earlier, Hansen (2004) indicated that if the risk of *Salmonella* infections in growing-finishing pigs is to be reduced, it is important to obtain a low gastrointestinal pH and a high concentration of organic acids as quickly as possible after intake of the feed. Therefore, the factors that might bring about a rapid drop in pH and/or rapid increase in lactic acid production within the feed are very important for the biosafety of the feed prior to and at feeding.

From the results of the current study, significant reductions in the pH of maize and sorghum for LAB treatments were evident after 8 h of fermentation. The reduction in particle size was related to a reduction in pH of maize although this trend was not observed with the 8 h lactic acid fermentation. A low pH is required for organic acids to remain in the undissociated form (Hansen, 2004). This undissociated form of the acid is required for the antimicrobial property of the feed.

PA1 was observed to produce lactic acid at a faster rate than SLP. However, the fact that the 24 h fermentation pH values for maize fermented with SLP were significantly lower than the values for PA1 indicates that the initial rapid lactic acid production from PA1 was not maintained until 24 h. Furthermore, lactic acid concentra-tions in SLP fermentations for both grains were generally higher than corresponding values for PA1 after 24 h fermentation. The significant increase in acetic acid production from PA1 compared with SLP in this study clearly depicts a higher ratio of lactic to acetic acid concentrations for PA1. Charalampopoulos et al. (2002) indicated that *L. plantarum* NCIMB 8826 isolated from human saliva had a homofermentative pattern for cereal-based substrates with significant depletion of glucose, fructose, maltose and sucrose. They also observed that the growth of *L. acidophilus* NCIMB 12116 was associated with the production of lactic acid and comparably significant amounts of acetic acid. However, high acetic acid in feed could have adverse effects on palatability and feed intake of chickens. Unpublished data in pigs (Moran and Brooks) demonstrates that acetic acid concentrations above 30 mM reduced feed intake particularly in young pigs.

The lactic acid concentration for 24 hour fermentation of sorghum obtained from this study with SLP is lower than the values of 312.3 and 313.65 mM reported by Niba et al. (2009) for red and white sorghum respectively with the same micro-organism. Corresponding values for fermentation with PA1 reported by the same authors were 203.67 and 264.07 mM respectively

The production of lactic acid from the coarse particle sizes was not significantly different from the smaller particle sizes (except the very fine particle sizes). Higher acid content did not always correspond to a lower pH value in this study. An overall mean total acid concentration of 273.07 mmol/L resulting from fermentation of sorghum with PA1 had a pH of 3.42 whilst fermentation with SLP had a pH of 3.51 for a total acid concentration of 313.35 mmol/L. The lack of a linear relationship between pH and acid concentration was also observed with the particle sizes in SLP fermentation of maize. The total acid concentration of the coarse particle size, though higher than any other particle size in the treatment, had a significantly higher ($P=0.003$) pH value. This observation could be related to the buffering capacity of the coarse particle size which could buffer the excess acid resulting in a resistance to drop in pH.

A reduction in size particle could increase the surface area for amylolytic enzyme action and result in a rapid fermentation of glucose and fructose. The relation between particle size and sugar availability has been highlighted by Anguita et al. (2006) who reported that reduction increased hydrolysis of starch especially for raw cereals. However, Tester et al. (2006) pointed out that whilst the size and shape of the starch granules is clearly a controlling factor in the hydrolysis of native starches with amylases, factors which control the accessibility of the enzyme to the interior of the granule also regulate hydrolysis. A rapid build-up of fermentation end metabolites will also depend on whether microbial fermentative capacity can handle immediate increases in fermentable sugars concentration resulting from hydrolysis of starch.

Based on the results of the current study and the following reasons, it is proposed that larger grain sizes could be better for fermentation and inclusion into moist poultry diets:

Coarse particle sizes in this study produced comparable or higher lactic acid concentrations in most treatments, suggesting that moderate grain processing may be enough to permit production of biosafe levels of lactic acid in fermented feed for chickens.

Secondly, Mai (2007) demonstrated that feeding wet and coarsely ground diets improved feed intake, feed conversion and growth rate in broilers. This effect was pronounced during the starter phase and was associated with improvements in the functional development of the

foregut (proventriculus-gizzard system).

Grain processing to small particle sizes of cereals like the fine and very fine sizes in this study could have important implications for both the diet and cost of feed for the farmer.

Increased particle size, feeding whole wheat or corn-based diets, reducing non-starch polysaccharides, and reducing levels of animal-based proteins in the diet seemed to help reduce the incidence of necrotic enteritis in broiler chickens (Dahiya et al., 2006).

Non-pelleted rolled barley or wheat increased both firmness and dry matter percentage of the stomach content of growing pigs compared with ground feed (Nielsen and Ingvartsen, 2000). A higher firmness of the stomach content coincided with a lower score of gastric lesions.

However, the use of coarse grains in fermentation for moist poultry diets may be more relevant in feeding programs where batch fermentation is practiced and cycles of 24 h feeding are strictly adhered to.

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