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 bicolor* 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; Ragae 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 In Africa, India and China, it is only superseded by rice and wheat as a cereal for human consumption (Elkhalifa and El-Tinay, 2002).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; McNaughton et al., 2007; Rama Rao et al., 2007; Yu et al., 2007).

Provision of dry diets containing cereals as the main ingredient is a feeding system that is used in many poultry industries. However, the feeding of dry diets can result in metabolic disorders in poultry birds due to the low fermentation properties of cereals (Huang et al., 2005). Therefore, sorghum and maize could be manipulated to provide appropriate fermentation properties for poultry feed in order to prevent metabolic disorders in birds. The present study was conducted to investigate the effect of particle size and micro-organism on fermentation of sorghum and maize for poultry feed.

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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 (Choc 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 (Choc 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 (Charalamopoulos 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 microorganism 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 Retsch 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 60Co by Becton and Dickinson, Plymouth, UK. Maize was obtained from Edwin Tucker and Sons, Ashburton, Devon while Sorghum was the white variety (Sorghum bicolor 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 Hoyberg et al. (2003). The mixture was inoculated with 0.01 ml of an overnight culture of De Man, Rogosa and Sharpe (MRS) broth containing one of two ca 108 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 Chromleon 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).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH</th>
<th>Total sugar*</th>
<th>Lactic acid</th>
<th>Acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.77a</td>
<td>227.67a</td>
<td>15.66a</td>
<td>2.64a</td>
</tr>
<tr>
<td><em>P. acidilactici (PA1)</em></td>
<td>3.71b</td>
<td>107.48b</td>
<td>167.57b</td>
<td>14.51b</td>
</tr>
<tr>
<td><em>L. plantarum (SLP)</em></td>
<td>3.56c</td>
<td>80.42c</td>
<td>206.17c</td>
<td>4.54a</td>
</tr>
<tr>
<td>SED</td>
<td>0.014</td>
<td>6.72</td>
<td>10.51</td>
<td>1.60</td>
</tr>
<tr>
<td>P-Value</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.003</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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.

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).

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; \( \mu \) is the overall mean; \( \alpha_i \) is the effect due to particle size; \( \beta_j \) is the effect due to lactobacilli used; \( (\alpha\beta)_{ij} \) is the interaction between particle size and lactobacilli used and \( \delta_{ij} \) 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.
Table 2. Effect of particle size and micro-organism used for maize fermentation on 0 hour pH, (n=3).

<table>
<thead>
<tr>
<th>Particle size</th>
<th>Control</th>
<th>PA1</th>
<th>SLP</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coarse</td>
<td>5.55</td>
<td>5.43</td>
<td>5.49</td>
<td>0.6366</td>
</tr>
<tr>
<td>Medium</td>
<td>5.62</td>
<td>5.55</td>
<td>5.51</td>
<td>0.6366</td>
</tr>
<tr>
<td>Fine</td>
<td>5.59</td>
<td>5.53</td>
<td>5.61</td>
<td>0.9946</td>
</tr>
<tr>
<td>Very fine</td>
<td>5.73</td>
<td>5.61</td>
<td>5.62</td>
<td>0.5211</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.08</td>
<td>0.12</td>
<td>0.48</td>
<td>0.524</td>
</tr>
</tbody>
</table>

*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).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Particle size</th>
<th>Control</th>
<th>PA1</th>
<th>SLP</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Coarse</td>
<td>5.73a</td>
<td>5.63</td>
<td>5.61</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>5.67bc</td>
<td>5.63</td>
<td>5.55</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>5.69ac</td>
<td>5.57</td>
<td>5.59</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Very fine</td>
<td>5.77b</td>
<td>5.59</td>
<td>5.53</td>
<td>0.41</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.003</td>
<td>0.94</td>
<td>0.15</td>
<td>0.322</td>
<td></td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Coarse</td>
<td>10.15</td>
<td>9.14</td>
<td>5.69a</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>8.07</td>
<td>9.42</td>
<td>8.80a</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>13.92</td>
<td>9.61</td>
<td>9.03a</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Very fine</td>
<td>13.25</td>
<td>9.58</td>
<td>20.05b</td>
<td>0.75</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.88</td>
<td>1.00</td>
<td>0.02</td>
<td>0.113</td>
<td></td>
</tr>
</tbody>
</table>

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 particle 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).

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

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).

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

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 (0.029), 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).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>pH</th>
<th>Total sugar*</th>
<th>Lactic acid</th>
<th>Acetic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.06a</td>
<td>167.19a</td>
<td>13.23a</td>
<td>5.42a</td>
</tr>
<tr>
<td>PA1</td>
<td>3.42c</td>
<td>46.81b</td>
<td>240.00b</td>
<td>33.07b</td>
</tr>
<tr>
<td>SLP</td>
<td>3.51b</td>
<td>33.95b</td>
<td>302.73c</td>
<td>10.62a</td>
</tr>
<tr>
<td>SED</td>
<td>0.04</td>
<td>7.28</td>
<td>8.64</td>
<td>4.92</td>
</tr>
<tr>
<td>P-Value</td>
<td>0.045</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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 ± 2.37 mmol/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
values between treatments or particle 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).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Particle size</th>
<th>Control</th>
<th>PA1</th>
<th>SLP</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Coarse</td>
<td>6.07a</td>
<td>5.25a</td>
<td>5.63a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>5.91a</td>
<td>5.08b</td>
<td>5.58a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>6.11a</td>
<td>5.33a</td>
<td>5.83b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Very fine</td>
<td>5.82b</td>
<td>5.09b</td>
<td>5.74b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>P-Value</td>
<td>0.03</td>
<td>0.04</td>
<td>0.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Coarse</td>
<td>5.34</td>
<td>29.26</td>
<td>19.00</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>5.08</td>
<td>27.95</td>
<td>15.57</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>6.11</td>
<td>38.28</td>
<td>18.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Very fine</td>
<td>14.77</td>
<td>90.89</td>
<td>32.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>P-Value</td>
<td>0.951</td>
<td>&lt;0.001</td>
<td>0.402</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Particle size</th>
<th>Control</th>
<th>PA1</th>
<th>SLP</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Coarse</td>
<td>6.04ab</td>
<td>3.49</td>
<td>3.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>5.94a</td>
<td>3.43</td>
<td>3.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>6.31b</td>
<td>3.50</td>
<td>3.58</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Very fine</td>
<td>5.94a</td>
<td>3.25</td>
<td>3.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>P-Value</td>
<td>0.002</td>
<td>0.085</td>
<td>0.10</td>
<td>0.009</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>Coarse</td>
<td>8.35</td>
<td>233.54a</td>
<td>272.46ab</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>8.92</td>
<td>197.08a</td>
<td>211.55a</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>12.09</td>
<td>216.21a</td>
<td>325.02b</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Very fine</td>
<td>23.55</td>
<td>313.15b</td>
<td>401.87c</td>
<td>&lt;0.002</td>
</tr>
<tr>
<td></td>
<td>P-Value</td>
<td>0.999</td>
<td>0.005</td>
<td>0.008</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>Coarse</td>
<td>3.70</td>
<td>25.64a</td>
<td>10.04</td>
<td>0.546</td>
</tr>
<tr>
<td></td>
<td>Medium</td>
<td>9.16</td>
<td>17.37a</td>
<td>9.25</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>2.61</td>
<td>62.80b</td>
<td>13.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Very fine</td>
<td>6.21</td>
<td>26.47a</td>
<td>9.43</td>
<td>0.654</td>
</tr>
<tr>
<td></td>
<td>P-Value</td>
<td>0.999</td>
<td>0.042</td>
<td>1.00</td>
<td>0.026</td>
</tr>
</tbody>
</table>

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-
incultated 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 con-
centration 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
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 reduc-
tions 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 fermente-
tion pH values for maize fermented with SLP were signi-
ficantly lower than the values for PA1 indicates that the
initial rapid lactic acid production from PA1 was not main-
tained until 24 h. Furthermore, lactic acid concentra-
tions in SLP fermentations for both grains were generally hig-
her than corresponding values for PA1 after 24 h fer-
mentation. The significant increase in acetic acid produc-
tion from PA1 compared with SLP in this study clearly
depicts a higher ratio of lactic to acetic acid concen-
trations for PA1. Charalampopoulos et al. (2002) indica-
ted that L. plantarum NCIMB 8826 isolated from human
saliva had a homoeofermative 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 concentra-
tion 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 concentra-
tion of 313.35 mM/L. The lack of a linear relationship be-
tween 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 be-
ween particle size and sugar availability has been high-
lighted by Anguita et al. (2006) who reported that reduc-
tion 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 metabo-
lites 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 follo-
wing 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 compara-
tible or higher lactic acid concentrations in most treat-
ments, 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
foraging (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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REFERENCES


