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Nixtamalization Effects on the Contents of Phytic Acid in the Varieties of Maize and the Bioavailability of Iron in Nixtamalized Maize to Young Pigs

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Abstract: The present study was conducted to investigate the effect of nixtamalization process on the phytic acid loss of maize and the subsequent influence on iron bioavailability of the iron-deficient piglets. Two experiments were conducted to achieve the objective: in the first step, three types of maize varying in phytic acid contents were processed by five lime concentrations treatment (0, 0.6, 1.2, 1.8 and 2.4%), four cooking duration (0, 45, 60 and 75 min) and thereafter soaking for five periods (0, 2, 4, 6 and 8 h) to optimize the best nixtamalization process parameter based on the maximum loss of phytic acid. In the second study, weaning piglets suffered iron-deficient were fed two diets: either lime-cooked maize produced by optimal parameter (NTM) or non-nixtamalized maize as control (CON), to examine the effects of the nixtamalization on the iron bioavailability, iron status and growth performance. The results showed that: (1)The greatest phytic acid loss of three types of maize and their processing parameters (lime concentration, cooking duration and steeping duration) were 17.4% (1.2%, 75 min and 4 h), 14.9% (1.8%, 75 min and 4 h) and 27.5% (1.8%, 75 min and 4 h), respectively. (2) The hemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cell count (RBC) and Hemoglobin-Fe (Hb-Fe) content were improved in the NTM piglets compared with CON piglets ($p<0.05$). (3) The Average Daily Gain (ADG) and Average Daily Feed Intake (ADFI) at D 14 and D 28 were higher in NTM than CON group ($p<0.05$). These results demonstrated that nixtamalization process parameter should be varying according to the phytic acid contents of the maize to break down the maize phytic acid effectively. Above all, the lime-cooked maize could enhance the iron status and the growth performance of weaning piglets suffered iron-deficiency anemia, thus could be considered as an effective intervention protecting the consumer (including human and animal) from suffering iron deficiency.

Key words: Nixtamalization, maize, phytic acids, iron-deficiency, growth performance, piglets

INTRODUCTION

Iron deficiency is the most common nutritional disorder affecting more than 2 billion people over the world (WHO, 2003). Food fortification and meat supplements have been considered as the popular methods to conquer this problem in certain regions (Yip, 1997), but these interventions failed to reach the expected efficacy in a sustainable way. As staple food crops are the major dietary sources of iron for people in developing nations, enhancing iron bioavailability in those crops could be the most effective and sustainable strategy to reduce or prevent iron deficiency in these populations (Graham *et al.*, 2001).

The phytic acid in grain food is the major absorption obstacle and its over consumption would result in iron deficiency. Iron bioavailability of staple crops may be improved by removing this inhibitor of iron utilization by the way of nixtamalization, which refers to the lime cooking process of converting maize into a food known as tortillas, this processing could effectively degrade the phytic acid in corn and is popular in rural and urban households (Welch, 20002). Maize nixtamalization consists of two successive steps including cooking in an alkaline aqueous suspension of calcium hydroxide followed by steeping in the same solution for several hours after cooking and phytic acid could be broke down effectively after this process (Bressani, 1997, 2002, 2004). Nixtamalization process have been evaluated from technological, physical, chemical and nutritional points (Serna-Saldivar *et al.*, 1990), however, specific studies for different phytic acid-contained maize from varying regions to establish the best processing variables during nixtamalization on phytic acid loss are very few. In addition, there are few methods to evaluate the direct impact of the nixtamalized maize on iron availability due to ethics that not using human as a testing model.

Hence, we introduce young weaning piglets to evaluate the nixtamalization efficacy, because pig is an excellent model for the study of human nutrition due to the similar anatomy in gastrointestinal tracts, digestive physiology and diet composition between the two species (Miller and Ullrey, 1987). The iron status of young pigs can be readily manipulated by adjusting the dosage of the iron injections routinely shortly after birth and is easy to develop an iron-deficient state in a relatively short period of time, providing an appropriate physiological condition for lime-cooked maize to present its influence on iron bioavailability (Miller and Ullrey, 1987). Therefore, the objective of the present study was to select optimal nixtamalization process parameters on greatest phytic acid loss of different types of maize and to evaluate the subsequent effects of the nixamalized maize on the iron bioavailability and the growth performance of the pig suffered iron-deficient.

MATERIALS AND METHODS

Optimizing the nixtamalization parameters: Twenty five types of maize varying in phytic acid contents were purchased from markets of different region in China. Phytic acid was determined according to the method described by Haugh and Lantzsch (1983). Consequently, three types were used in the present study, of which phytic acid contents were defined as low (553.8±9.9 mg/100 g), medium (737.0±12.3 mg/100 g) and high (959.5±5.5 mg/100 g) and were stored in the laboratory at 4°C until used. This hybrid has a density of 1.30±0.01 g mL^{-1} and with a 1000 grain weight of 312.43 ±5.60 g.

Nixtamalization process involves two main operations, the effect of the processing variables on phytic acid loss was studied by stages. The first operation involves cooking the maize with lime solution, whereas the second operation is to steep the cooked maize with no additional heat.

For the lime-cooking operation, 200 g of each corn were chosen to be cooked for 0, 45, 60 and 75 min with 600 ml water by five lime solution concentrations at 0, 0.6, 1.2, 1.8 and 2.4% (lime weight/ maize weight) for each level of lime concentration at 96°C and were then washed 6-10 times to remove excess lime and the free pericarp of the cooked kernel followed by oven dry at 65°C.

The second stage is to evaluate the effect of steeping duration on the phytic acid loss of the cooked maize. Maize were cooked with 1.8% lime based on maize weight, in 3 volumes of water during 75 min at 94°C. After cooking, the maize samples were steeped in their own cooking liquor or in water for 0, 2, 4, 6 and 8 h at 25°C. After the applications of the treatments indicated, the samples were dried at 65°C and analyzed for phytic acid.

Raw and processed samples of each experiment dried as indicated above were analyzed in duplicate, for phytic acid according to the method of Haug and Lantzsch (1983). All data are expressed on an oven dry weight basis.

Animal model evaluation of the nixamalized maize: Twenty Duroc × Landrace × Yorkshire (DLY) weaning piglets, with initial average weight of 7.99 × 0.71 kg at 28 days old, were used for the iron bioavailability evaluation of nixtamalized corn. All the piglets were given 1/3 of the normal dosage of iron requirement in the form of iron dextran to induce severe iron deficiency (hemoglobin concentration below 70 g/L). Then the iron-deficient piglets were allocated to two diets formulated with either nixtamalized maize (NTM) or non-nixtamalized maize (CON) based on body weight, gender and the blood hemoglobin concentration. The piglets in NTM group were fed the diet formulated with nixtamalized maize under the optimized parameters, whereas piglets in the CON group were fed the maize substituted by maize with 0% lime concentration solution cooked. The diets were formulated according to NRC (1998), the other dietary ingredients were pure so that other factors were avoided to affect the iron bioavailability (Table 1). All experimental procedures were approved by Animal Care and Use Committee of Sichuan Agricultural University. Piglets were housed individually in pens in facility with temperature (22 to 25°C) totally controlled, all piglets were given free access to feed and water. The experiment lasted 4 weeks, Body Weight (BW), Average Daily Weight Gain (ADG), Average Daily Feed Intake (ADFI) and Feed Conversion rate (FC) of individual pigs were recorded weekly. Blood samples of all individual pigs (fasted overnight for 12 h) were collected weekly to determine hemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cell count (RBC), Hemoglobin Repletion Efficiency (HRE), hemoglobin-Fe (Hb-Fe) content. Specifically, Hemoglobin Repletion Efficiency (HRE) was determined using the following formula as described previously (Miller, 1982):

$$HRE = \frac{\text{Final total body Hb Fe, mg} - \text{Initial total body Hb Fe, mg}}{\text{Total Fe intake, mg}} \times 100$$

and total body Hb iron content was estimated using the following formula:

$$\text{Hb-Fe, mg} = [\text{body weight (g)} \times 0.067 \text{ mL blood/g BW}] \times (\text{Hb, g m/L}) \times (3.35 \text{ mg Fe/Hb, g}).$$

Table 1: Ingredient composition and nutrient content of experimental basal diets [a] (air-dry basis, %)

Ingredients	Percentage	Nutrients	Percentage
Corn	85.18	CP	17.19
Casein	11.00	DE (Mcal/kg)	3.37
L–lys·HCl	0.29	Ca	0.8
DL–Met	0.08	AP	0.41
L–Trp	0.06	Lys	1.19
L–Thr	0.14	Met+Cys	0.68
Salt	0.25	Trp	0.22
$CaCO_3$	1.18	Thr	0.74
$CaHPO_3$	1.15	Ile	0.65
Vit. premix[b]	0.05		
Chloride choline	0.1		
Mineral premixc	0.5		
Oxytetracycline	0.02		
Total	100		

[a]No additional iron was supplemented to the two diets. The analysed iron content in the control and treated diet were 55.30 ± 0.94 mg/kg and 57.05 ± 1.04 mg/kg, respectively, and the analysed phytic acid contents were 474.4 ± 8.7 mg/100g and 601.9 ± 10.2 mg/100g, respectively.
[b]Vitamin premix provided/kg diet: VA 7 000 IU; VD3 1 500 IU; VE 16 IU; VK3 2 mg; VB1 2 mg; VB2 3 mg; VB6 2 mg; VB12 20.5 μg; Ca-D-pantothenic acid 10 mg; niacin 15 mg; folic acid 0.3 mg; biotin 0.2 mg.
[c]Mineral premix provided/kg diet Cu 6 mg; Zn 100 mg; Mn 4 mg; I 0.14 mg; Se, 0.3 mg.

Table 2: Phytic acid changes in maize with respect to lime concentration and Cooking time during nixtamalization (means ± SEM, mg/100g)

Lime level (%)	cooking time raw	45 min	60 min	75 min	av
		low phytic acid content of maize			
0	553.8±9.9	550.9±9.6	542.6±7.5	532.9±5.6	545.0[a]
0.6	553.8±9.9	487.5±8.5	479.2±9.2	468.6±7.8	478.4[b]
1.2	553.8±9.9	481.8±13.2	469.6±12.8	457.5±6.6	472.3[b]
1.8	553.8±9.9	471.8±7.8	468.7±11.9	459.5±12.3	468.0[b]
2.4	553.8±9.9	472.2±8.9	462.4±12.1	461.3±11.4	465.3[b]
av	553.8[a]	492.9[b]	484.5[bc]	477.1[c]	
		medium phytic acid content of maize			
0	737.0±12.3	725.7±5.6	715.6±12.8	712.5±4.5	722.7[a]
0.6	737.0±12.3	657.9±7.5	651.7±5.9	637.1±10.4	648.9[b]
1.2	737.0±12.3	653.8±5.7	645.8±10.5	632.8±7.9	643.5[bc]
1.8	737.0±12.3	640.3±10.5	634.2±5.6	627.1±8.6	633.9[c]
2.4	737.0±12.3	637.1±10.3	632.1±6.4	628.3±9.3	632.4[c]
av	737.0[a]	662.3[b]	655.2[bc]	647.1[c]	
		high phytic acid content of maize			
0	959.5±5.5	944.6±11.4	935.1±7.9	929.5±9.8	942.2[a]
0.6	959.5±5.5	736.2±11.3	727.5±9.6	713.1±7.6	725.6[b]
1.2	959.5±5.5	729.8±4.7	717.7±10.6	702.5±8.9	716.7[bc]
1.8	959.5±5.5	718.6±10.5	711.5±11.8	694.9±9.6	708.3[c]
2.4	959.5±5.5	712.2±10.2	705.9±9.8	697.9±10.3	705.3[c]
av	959.5[a]	768.2[b]	759.5[bc]	747.6[c]	

The same letter means no significance (P>0.05), the different letter means having significance (P<0.05).

Statistical analyses: Effects of lime-cooked maizes on various measures were analyzed as a randomized block design using the Proc General Linear Models procedure of SAS (version 6.12, 1990). Each individually penned pig was used as the experimental unit, he Bonferroni/Dunn t-test was used to compare treatment means. And the significance level was set at $p \leq 0.05$ (Gill, 1986), values in the text are presented as means±SEM.

RESULTS

Nixtamalization parameter optimization: The effect of lime concentration variables on the phytic acid loss was shown in Table 2. Lime concentration over 0.6% degraded the phytic acid more effectively than the 0% (p<0.05). The phytic acid loss of low phytic acid-contained maize with lime concentration over 0.6% were similar (p>0.05), whereas for the medium and high phytic acid-contained maize, the phytic acid loss under the 1.8% and 2.4% lime concentration were higher than the 0.6% (p<0.05). Cooking duration affected the phytic acid loss which was lower for the maize cooking over 45 min than 0 min (p<0.05) and it was significantly lower after cooking for 75 min than 45 min (p<0.05), but cooking for 60 min did not differ from 75 min or 45 min (p>0.05). After nixtamalization, the greatest phytic acid loss and its parameter were 17.4% (with lime concentration of 1.2% and cooking for 75 min), 14.9% (with lime concentration of 1.8% and cooking for 75 min) and 27.5% (with lime concentration of 1.8% and cooking 75 min) for the low, medium and high phytic acid-contained maize, respectively.

As shown in Table 3, steeping treatment affected the phytic acid content. Steeping over 2 h degraded the phytic acid more effectively than 0 h in the types of maize (p<0.05), whereas steeping for 2, 4, 6 and 8 h did not contributed to the different phytic acid loss in the three phytic acid-contained maize (p>0.05). The greatest phytic acid loss were 4.9, 5.1 and 5.0% in the low, medium and high phytic acid maize after steeping for 4 h.

The iron bioavailability of lime-coooked maize to young pigs. The effect of limed-cooked maize on routine hematological values were shown in Table 4. Blood Hb concentration were not different between the NTM and CON groups at either D 0 or D 14 (p>0.05), but was higher in the NTM than CON groups (p<0.05) at D 28 or throughout the experiment. The blood PCV and PCV did not differ at D 0, D14 and D 28, whereas the total increase of blood PCV and RBC throughout the experiment were higher in the NTM than CON groups (p<0.05). Blood Hb-Fe concentration at the beginning, D14 or total Hb-Fe increment throughout the experiment were not different between the two groups (p>0.05), with the exception of the higher Hb-Fe content at D28 in the NTM group (p<0.05). There was no difference for HRE of the piglets suffered iron-deficient anemia between the two diets (p>0.05).

As shown in Table 5, ADG and ADFI were affected by the nixtamalizd maize compared with control. They were higher in the NTM than CON group at D 14 and D 28 of the experiment (p<0.05). No difference of feed conversion rate was observed between the two groups (p>0.05).

Table 3: Phytic acid changes in lime-cooked maize subjected to different steeping time (means ± SEM, mg/100g)

		Cooked maize steeping time (h)				
		0	2	4	6	8
Phytic acid	low	455.7±5.8[a]	438.4±8.2[b]	433.4±7.5[b]	435.1±9.7[b]	436.4±8.8[b]
	medium	629.3±9.7[a]	603.6±5.8[b]	598.9±9.3[b]	599.7±7.9[b]	601.3±6.2[b]
	high	697.4±10.2[a]	669.7±8.6[b]	664.8±7.5[b]	665.3±9.2[b]	667.3±7.2[b]

The same letter means no significance (P>0.05), the different letter means having significance (P<0.05).

Table 4: Effect of limed-cooked maize on routine hematological values and iron bioavailability of IDA piglets (means ± SEM, n=10)

		D 0[d]	D 14	D 28	IR
Hb a b (g/L)	NTM[c]	60.2±11.66	69.4±9.70	82.9±5.63*	43.59±11.64*
	CON	60.6±11.93	68.7±10.66	78.4±7.78	32.46±10.16
PCV (%)	NTM	14.67±1.97	17.35±2.07	28.17±3.78	97.80±18.71*
	CON	15.68±1.66	17.91±2.82	26.11±2.26	69.77±18.90
RBC (1012/L)	NTM	3.37±0.71	4.50±0.73	7.51±0.96	128.71±38.43*
	CON	3.95±0.83	4.65±0.97	7.33±1.03	89.77±30.07
Hb-Fe (mg)	NTM	107.38±18.77	173.48±8.91	313.14±12.79*	198.09±46.35
	CON	108.96±20.84	167.20±22.10	281.00±18.87	165.55±50.21
Iron intake (mg)	NTM	--	317.31±12.74	1061.37±79.38	--
	CON	--	311.81±38.93	1016.69±64.38	--
HRE (%)	NTM	--	20.74±1.29	19.37±0.86*	--
	CON	--	18.68±0.87	16.97±0.66	--

[a]Statistical significance, *means P<0.05, **means P<0.01. The same as below. [b]Hb: hemoglobin concentration, PCV: packed cell volume, RBC: red blood cell count, Hb-Fe: Hemoglobin Fe, HRE: Hemoglobin repletion efficiency. [c]NTM: nixtamalized maize group; CON: non-nixtamalized maize group. [d]D: days, IR: the increasing rate from D 0-28.

Table 5: The effects of limed-cooked maize on growth performances of IDA pigs (means ± SEM, n=10)

		D 7 d	D 14	D 21	D 28	IR
ADG ab (g)	NTM[c]	179.29±16.99	272.14±26.18*	355.00±39.42	457.14±23.77*	315.89±33.74
	CON	168.57±45.33	235.71±32.99	335.00±36.86	401.43±20.26	285.18±43.6
ADFI (g)	NTM	301.29±7.27	540.36±36.06*	779.29±35.27	1142.86±62.63*	685.46±51.27
	CON	288.29±8.08	492.50±45.56	747.93±13.23	1017.14±50.51	636.46±40.31
F/G	NTM	1.68±0.09	1.98±0.13	2.19±0.19	2.50±0.11	2.17±0.15
	CON	1.72±0.21	2.08±0.23	2.23±0.06	2.54±0.23	2.23±0.10.

[a]Statistical significance, * means P<0.05, ** means P<0.01. [b]ADG: average daily gain, ADFI: average daily feed intake, F/G: feed/gain. [c]NTM: nixtamalized maize group; CON: non-nixtamalized maize group. [d]D: days, IR: the increasing rate from D 0-28.

DISCUSSION

The nixtamalization process in Central America and Mexico improves iron availability, which could be attributed to the fact that nixtamalization treatment could soften and destroy the cellular wall to release the phytic acid. The nixtamalization process involves two main operations including cooking in lime solution for a certain period, and following by steeping in lime solution or water for another time and then removed the excess calcium hydroxide or other dopants. The phytic acid in maize was degraded by nixtamalization in present study, which was consistent with the study conducted by Bressani (1997, 2002, 2004), however, the greater phytic acid loss was found in the high phytic acid-contained corn, indicating that the lime concentration should be elevated if the maize contains higher content of phytic acid.

The iron bioavailability were observed to be decreased in the presence of dietary phytic acid or exogenous sodium phytate (Hallberg *et al*., 1989). In consistent with study on human conducted by Martinez *et al*. (1987), the present study found that the nixtamalized maize improved the iron bioavailability due to the phytic acid degradation, and the iron absorption was also improved as the decreasing phytic acid. Mendoza *et al*. (1998) also established that the iron absorption were increased by 50% if the maize loss 1/3 of phytic acid.

Blood iron content reflected the iron status whereas the Hb concentration is the determinant of anaemia status or not. NRC (1988) reported that the iron recommendation for normal growth and development of piglets was define as over 100 g/L with minimum requirement for normal growth as 90, 80 g/L as marginal deficiency and 70 g /L as severe iron deficient that would suppress the growth and development. Concurrently, PCV and RBC were associated with iron deficient anaemia. At the beginning of the experiment in the present study, the Hb concentration, PCV and RBC were about 60 g/L, 3.65×10^{12}/L and 15.18%, respectively, which were undoubtedly indicates of anaemia status. However, Hb concentration was over 80 g /L and the PCV, RBC, Hb-Fe and HRE approached to normal condition in the NTM group when the experiment proceeded the end, it was still below 80 g/L in the CON group, thus the growth performance, feed intake and average daily gain were improved in the NTM piglets. Based on these results conducted in the piglets, which is the best model for human iron nutrition, we concluded that the nixtamalized corn consumption could be used as an iron deficiency intervention to improve the iron status in the population suffered anaemia in poor regions.

Conclusion: Collectively, nixtamalization parameter should be varying according to the phytic acid content of the corn to breakdown the phytic acid more effectively. The nixtamalized corn could enhance the iron status and the growth performance of weaning piglets suffered anemia, thus could be considered as an effective measurement protecting the animal (including human) from suffering iron deficient anaemia.

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