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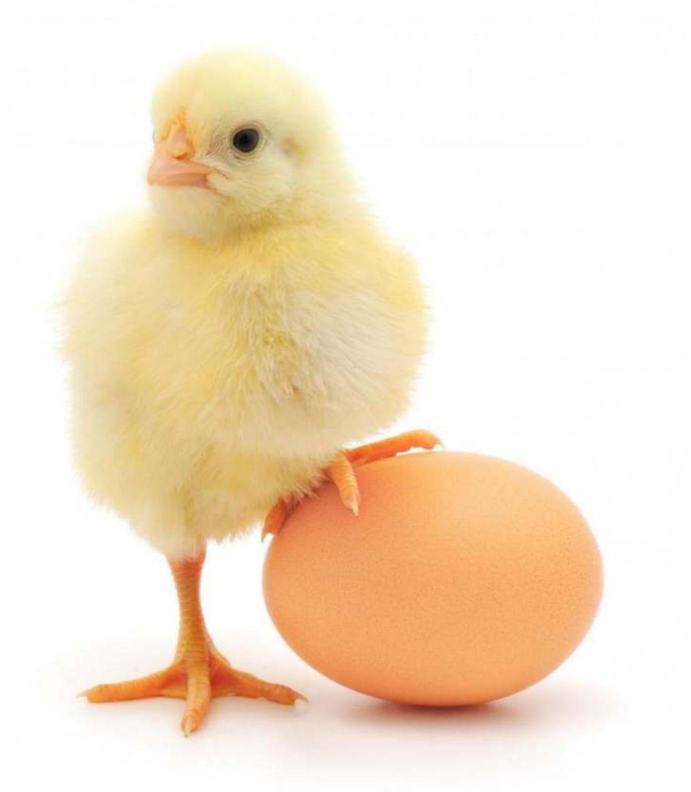

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Effects of Citric Acid on the Performance and the Utilization of Phosphorous and Crude Protein in Broiler Chickens Fed on Rice By-Products Based Diets

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Abstract: An experiment was conducted to study the effects of two levels of citric acid (CA) on the performance and the utilization of phosphorous and crude protein in broiler chickens fed on rice by-products based diets. Growth performance and feed conversion ratio (FCR) were not significantly affected by the inclusion of citric acid. pH of the contents of the crop was 5.1, 4.8 and 4 in control, 1% citric and 2% citric diets fed birds, respectively. Though not significant, 2% dietary citric acid increased the feed intake and thus resulted in poor FCR. Toe ash percentage was significantly ($p<0.05$) increased by 2% citric acid. Though not significant, mineral retention was also improved by 2% dietary citric acid. Orthogonal contrast between control and citric acid given diets showed that dietary citric acid increased the digestibility of crud protein and crud fibre and dressing percentage. It is hypothesized that citric acid serves as proton donor for phytic acid and thus prevents the formation of insoluble complexes. Being an organic acid, citric acid may weaken the structure of CF thus making CP and phytate associated with them more susceptible for enzymatic digestion.

Key words: Citric acid, poultry, rice bran, phytate

Introduction

The major ingredients used in poultry feeds are of plant origin. About two third of the phosphorous (P) in these feedstuff is present as phytate P, which is poorly utilized by poultry. The inability of poultry to utilize phytate P causes both economic and environmental problems. Run off of unutilized P in poultry excreta to water bodies causes eutrophication. Inclusion of inorganic P to poultry diets greatly increases the cost of rations. Phytates are associated with other cations such as Ca, Mn, and Zn (Maenz, 2000), and proteins, amino acids (Selle *et al.*, 2000) making them also less available to the animal. Consequently, apparent metabolizable energy value is also reduced. Thus any attempt to improve the utilization of phytate could reduce the feed cost and environmental pollution. Phytates are associated with other cations such as Ca, Mn, and Zn, and proteins, amino acids making them also less available to the animal.

Physical methods such as soaking, drying, germination (Jongbloed *et al.*, 1991), supplementation of diets with exogenous microbial phytase (Kornegey, 2001) and Vitamin D (Mitchel and Edwards, 1996) have found to be effective in increasing phytate hydrolysis. Several authors (Bolling *et al.*, 1998; Bolling *et al.*, 2000; Bolling *et al.*, 2001; Brenes *et al.*, 2003) have found that citric acid alone or in combination with phytase increased the phytate hydrolysis in chicken. It is hypothesized that citric acid complex with Ca and reduces the formation of more stable Ca-phytate complexes. Alternatively, citric acid may change the intestinal pH for better phytase activity. Rice bran is a major component of the poultry diets in Asian region, mainly due to its low cost and high availability. Rice bran is rich with P, but the availability is as low as 16%. And the intrinsic phytase activity is also reported to be low in rice bran (Ravindran *et al.*, 1995). In addition, high fibre content may also reduce the degradation of both phytate as well as other nutrients (Martin *et al.*, 1998). Only a few studies have aimed at finding the methods of improving phytate hydrolysis of rice bran. The objectives of the present experiment was to study the effects of two levels of citric acid on the performance and the utilization of phosphorous and crude protein in broiler chickens fed on rice by products based diets.

Materials and Methods

Day old chicks were purchased from a commercial hatchery and were brooded for three weeks on a floor brooder. During the first three weeks birds were fed a commercial starter ration (Preema Feeds, Sri Lanka) (CP 21%, MEn 3200 Kcal/Kg, Ca 1%, available P 0.5%, LYS 1.25%, MET+CYS0.88%). On day 20 birds were weighed and put into 9 wire-meshed cages (3ft * 2 ft * 2.5ft) so that between cage weight variation was minimum. Each cage had three birds. Three treatments with three replicates were employed. Three treatments were employed in a completely randomize design with three replicates. Three treatments were;

C_0 Control diet (0.03% available P without citric acid)
C_1 C_0 + 1% citric acid
C_2 C_0 + 2% citric acid

All diets were isocaloric (assuming citric acid provides no energy).

Ingredient composition and the calculated nutrient composition are given in the Table 1.

Table 1: Ingredient composition and the calculated nutrient composition

Ingredient %	C0	C1	C2
YMM	6	6.29	8
RP	35	36	33.4
BR	17	17	15
coconut oil	6.6	6.84	7.4
COM	9.62	8	8
GOM	8.7	8	10
SOM	9	9	9
FISH(denish)	6.3	6	5.4
salt	0.25	0.25	0.25
shell powder	0.675	0.72	0.62
Shell grit	0	0	0
Bone meal	0	0	0
Dical PO_4	0.44	0.45	0.52
COCC			
Four-F-Twin	0.2	0.2	0.2
Dl methionine	0.1	0.1	0.1
L lysine	0.1	0.1	0.156
DOT	0.05	0.05	0.05
CA	0	1	2
Furozol	0	0	0
	100	100	100
Calculated composition			
CP(%)	20	20	20
K cal/kg	3175	3180	3175
TotP	0.9	0.9	0.9
Ca	0.74	0.74	0.74
aP	0.30	0.30	0.30
LYS	1.0	1	1
M+C	0.8	0.8	0.8

Each cage had a feeder and a drinker. Birds were fed one of above diets for three weeks. Per cage feed intake and weight gain were recorded weekly. Faeces were collected during last four days to determine the CP, CF digestibility and mineral retention, using total collection method. Mineral retention was defined as follow;

Ash intake with diet during last four days- ash excretion with faeces during last four days/ash intake with diet during last four days*100

CP digestibility was determined by using Kjeldhal method. CF digestibility was determined by using fibre Tech apparatus. Birds were killed by cervical dislocation and digesta were collect from crop, gizzard, ileum and caecum to determine the pH. Visceral organ weights were also recorded. Cloacal fat was manually separated and weighed. Right middle toe of every bird was severed. Toe samples were dried at 100 C for 16 hours and then ashed in a muffle furner at 600^{0}C for 4 hours. Toe ash % was determined in relation to the dried toe weight. Data were subjected to analysis of variance (SAS institute, 1989) and orthogonal contrasts were done where appropriate.

Results and Discussion

Effects of dietary citric acid on performance and toe ash are given in Table 2.

Growth performance in general was inferior across all three treatments. This may mainly be due to the high dietary RB levels. Presence of high levels of phytates, fibre and tripsin inhibitor, and low amino acid digestibility in RB have found to responsible for the poor growth when included more than 20% of the diets (Martin *et al.*, 1998). In general growth performance parameters were not significantly affected by dietary citric acid. Weight gain and feed:gain were not significantly affected by supplementing diets having adequate available P (aP) (Bolling *et al.*, 2000) or diets having inadequate aP (Brenes *et al.*, 2003) with 2% citric acid. Our findings support the thesis given by Bolling *et al.* (2000, 2001) that citric acid could have a positive effects on growth performance and feed intake only when diets are low in aP and high Ca:aP ratio. When Ca:aP ratio is low, the release of additional P causes Ca deficiency by creating unfavorable Ca:aP ratio. The aP level used in this experiment was 0.3% and the Ca:aP ratio was 2:4. Diets used in this experiment were not severely aP deficient and the Ca:aP was 2.4. On a one hand, birds given C_0 diets might have got marginally sufficient aP levels which do not reduce the feed intake or growth. On the other hand, inclusion of CA does not seem to have increased the P level that alter the Ca:aP ratio to a level that interferes with Ca and/or P absorption. Bolling *et al.* (2001) found that 4% dietary citric acid was required to increase the dietary aP level by 0.1%. If this is valid for RB based diets, 2% dietary CA might have increased the aP level to 0.35% which is the NRC (1994) recommendation. Increased toe ash % when 2% CA supplemented diets supports this argument.

Bolling *et al.* (2001) suggested that 4% citric acid could reduce the dietary aP requirement level by 0.1% units in corn soy bean meal based broiler diets. Since rice bran contains more phytic P and total P, and low phytase activity than soy and maize, it can be speculate that citric acid could release more phytic P from rice bran than maize and soy bean.

Though not significant, 2% citric acid increased the daily feed intake. Bolling *et al.* (2000) have also reported similar results. They speculated that this may be due to the dilution of energy in the diet due to the inclusion of citric acid. However, diets of the present experiment were isocaloric. Even if it is assumed that citric acid provides some energy, it should lead to a reduction of feed intake in citric acid supplemented diet which was not the case. Therefore the reason for increased intake cannot be the low energy levels of treated diets. Inclusion of citric acid reduced the pH levels of the feeds

Table 2: Effects of dietary citric acid on growth performance, cloacal fat and toe ash of broiler chicken fed rice by products based diets from 3-6 weeks

	C_0	C_1	C_2	P value
Defeather weight	1194+13	1142+16	1226+20	0.53
Weight gain (g/d)	37.87+3.7	33.83+2.6	36.79+1.3	0.25
Dressing %	73.32+3.3	77.44+0.94	77.59+1.5	0.09
Feed intake (g/d)	106.53+10.7	100.29+10.3	112.27+4.6	0.33
FCR	2.81+0.07	2.96+0.12	3.05+0.19	0.18
Cloacal fat %	1.34+0.3	1.35+0.23	0.94+0.2	0.12
Toe ash %	10.38+0.02	10.71+0.11	10.89+0.05	0.008

Table 3: pH of the diets and digesta collected at the different regions of the digestive tract of the broiler chicken as affected by dietary CA

Site	C_0	C_1	C_2	P
Feed	5+0.1	4.36+0.11	3.86	0.001
Crop	5.1+0.08	4.8+0.41	4+0.26	0.23
Gizzard	3.13+0.57	2.73	3.33+0.47	0.41
Illeum	5.36+0.23	5.3+0.17	5.1+0.08	0.21
Ceacum	6.23+0.11	6.03+0.75	6+0.2	0.31
Rectum	5.45+0.31	5.36+0.55	5.93+0.37	0.28

and digesta of the crop and gizzard (Table 3). Appetite control nerve endings are located in the crop of the birds. Therefore, it can assume that citric acid it self and/or low pH environment created by it stimulate the feed intake. Two percent citric acid significantly increased the toe ash percentage. It has been shown that citric acid increased the phytate P utilization efficiency as determined by the tibia ash content in poultry (Brenes *et al*., 2003; Bolling *et al*., 2000; Bolling *et al*., 2001) and in pigs (Bolling *et al*., 2000; Omogbenigum *et al*., 2003). However, this is the first such experiment in which the effects of citric acid are studied in rice by products based diets.

Increased toe ash content in citric acid supplemented diets fed birds suggests that an additional amount of minerals has been released from the diets. Bolling *et al.* (2001) showed that citric acid didn't improve Ca availability. Therefore, it can reasonably be assumed that citric acid hydrolyzed phytates and released available P. Mineral retention was also higher in citric acid treated birds indicating low excretion of P. Possible effects of citric acid could be higher when rice bran based diets are supplemented with citric acid due mainly to two reasons; 1) rice bran contains a very high amount of P of which the aP fraction is as low as 16% and b). Intrinsic phytase activity is very low in rice bran. Therefore, it is worthwhile to test lower aP levels with various levels of citric acid.

The mechanism/s by which citric acid increases phytate hydrolysis is/are not clear. One possible mechanism is that it combines with dietary Ca and thus reduces the formation of highly indigestible Ca phytate complexes. Maenz (2000) suggested that one or more weak phosphate groups of the phytic acid have a higher affinity for protons than Ca^{++} and Mg^{++}. Inclusion of citric acid reduced the pH in the crop (Table 3). At this acidic condition citric acid may serve as a proton donor and thus make phytic acid partially protonized and prevents the formation of insoluble Ca phytate complexes. However, since rice bran contains virtually no phytase, how and where actually phytate hydrolysis takes place is of a question. We assume that hydrolysis of phytate should have taken place in the small intestine due to intestinal phytases. Maenz and Clansen (1998) and Applegate *et al.* (2003) found that a significant intestinal phytase activity in broilers. Interestingly, the latter authors found that intestinal phytase could increase the phytate hydrolysis up to 72% when diets are low in Ca. Interestingly, Ca levels used In this experiment were sub optimal according to NRC (1994) recommendations and so were in other experiments cited earlier. Recently, Rama Rao *et al.* (2003) suggested that broilers require around 0.75% dietary Ca when diets contain 0.35% non phytate phosphorus. Further researches are needed to test whether CA increases the phytate hydrolysis only when dietary Ca levels are low.

Second possible mechanism could be that citric may alter the pH profile of the GIT making a more favorable environment for both plant and intestinal phytase. Our findings suggests that citric acid doesn't alter the pH of the GIT after gizzard. And as discussed earlier since our diet contained very low phytase activity, this mechanism can not explain the effects of citric acid. However physical effects of citric acid on the chemical bonds between phytatic acid with fibre, amino acids and proteins can be helpful to make them more accessible to endogenous enzymes. Digestibility values of CP and CF support this possibility.

There was a trend to increase CP digestibility (P=0.092) at 2% citric acid level. Martin *et al.* (1998) suggested that rice bran increased the endogenous AA losses and thus reduced the AA digestibility. In the light of above findings, increased CP digestibility found in this experiment becomes more important. Orthogonal contrast between treated and control diets showed that citric acid significantly increased (P= 0.05) the CF digestibility as well. Two of the main disadvantage of rice bran is that its low CP digestibility and the high fibre content. Much of the AA s in rice bran is associated with fibre. This reduces the digestibility of AA while increasing endogenous AA losses. Hydrolysis of phytate has found to increase the CP digestibility (reviewed by Sella *et al*., 2000). To our knowledge no study has found the beneficial effects of phytate hydrolysis on fibre digestibility. Supplementation of RB based diets with citric acid could be helpful to address the three main nutritional problems associated with RB; high phytate and fiber levels and low amino acid digestibility. It was concluded that citric acid could be useful in hydrolyzing phytate P and reducing faecal P excretion.

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