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Effects of Citric Acid on Growth Performance and Nutrient Retention of Broiler Chicken Fed Diets Having Two Levels of Non-Phytate Phosphorus and Rice Bran

Research Article

K.P. Wickramasinghe[1], N.S.B.M. Atapattu[1*] and R.T. Seresinhe[1]

[1] Department of Animal Science, Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka

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*Correspondence E-mail: nsbm@ansci.ruh.ac.lk

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ABSTRACT

Citric acid has been reported to increase utilization efficiency of dietary phytate-bound phosphorus and protein. Objective of this study was to determine the effects of citric acid on growth performance and nutrient retention of broiler chickens fed diets having two levels of non-phytate phosphorus and rice bran. Giving a completely randomize design in 2 × 2 × 2 factorial arrangement, 144 broiler chicks in 48 pens received one of the eight experimental diets containing two levels of dietary rice bran (20% or 30%), citric acid (0% or 2%) and non-phytate phosphorus (0.25% or 0.35%) *ad libitum* from day 21 to 42. Retention of nitrogen, phosphorus, mineral and dry matter was determined using a total collection trial. Feed intake, weight gain, feed conversion ratio and weights of feather, liver, gizzard and pancreas were not significantly (P>0.05) affected by the levels of dietary rice bran, citric acid, or non-phytate phosphorus levels. Retention of nitrogen, phosphorus or dry matter were not significantly affected (P>0.05) by the levels of dietary rice bran, citric acid, non-phytate phosphorus or their interactions. 2% citric acid improved the retention of mineral with 30% rice bran compared to diets with 20% of rice bran. There was no significant difference (P>0.05) in tibia ash when diet contained 20% or 30% rice bran. A significantly (P<0.05) higher tibia ash content was observed when diets had 2% of citric acid with 0.35% of non-phytate phosphorus. It is concluded that 30% rice bran had no adverse effects on growth performance of broiler chicken from day 21 to 42. 2% citric acid produces no beneficial effects at dietary rice bran levels of 20 or 30% or non-phytate phosphorus levels of 0.25 or 0.35%.

KEY WORDS broiler, citric acid, non-phytate phosphorus, nutrient retention, rice bran.

INTRODUCTION

Inclusion of locally available, relatively cheap feed ingredients such as rice bran (RB) at higher levels can reduce the cost of poultry feed formulations. *In vitro* nutritive value of RB is superior to or comparable with other cereal by products (Warren and Farrell, 1990a). However, use of more than 20% RB in broiler diets reduces the performance and mineral status of the birds while increasing the mineral excretion (Piyaratne *et al.* 2011). Presence of several anti-nutrients such as phytate (Puminn, 2003), fibre (Gallinger *et al.* 2004), lipases (Sharif, 2009) and anti-proteolytic substances (Mujahid *et al.* 2005; Ersin Samli *et al.* 2006) have reported as the reasons for poor *in vivo* nutritive value of RB. Selle *et al.* (2007) reported that, rice bran contains 17.8g kg^{-1} of total phosphorus (P) and 14.2g kg^{-1} phytate-P. Typically, poultry diets contain from 2.5 to 4.0g kg^{-1} phytate-P (Ravindran, 1995). Phytate-bound P is poorly utilized by monogastric animals, due to either insufficient quantity or a lack of intestinal phytase secretion (Rafacz-livingston *et al.* 2005; Mohammed *et al.* 2010). Phytic acid acts as a strong chelator, forming protein-mineral-phytic acid complexes and reduced protein and mineral bioavailability (Akande *et al.* 2010). On the other hand, excretion of undigested P creates environmental problems like eutrophication of water bodies (Selle *et al.* 2007). Consequently increased utilization efficiency of phytate bound P by poultry benefits both industry and environment. Even though the microbial phytase found to be effective in improving phytate degradation, supplementation of diets with phytase increased the litter moisture content (Puminn, 2003). Heat liability of phytase and cost are among the drawbacks of the use of phytase. Therefore alternative methods for increasing phytate degradation need to be developed. Several authors have reported positive effects of citric acid (CA) on phytate degradation (Boling *et al.* 2000; Liem *et al.* 2008; Connelly, 2011), digestibility of protein (Atapattu and Nelligaswatta, 2005; Ao *et al.* 2009) and growth performances (Chowdhury *et al.* 2009; Islam *et al.* 2012) in poultry. Atapattu and Nelligaswatta (2005) showed that CA have positive effects on growth performance and feed intake only when diets are low in available phosphorus (aP). Several other authors (Boling *et al.* 2000; Boling-Frankenbach *et al.* 2001; Snow *et al.* 2004) have reported that improved phytate-P utilization efficiency in broilers when the diets were deficient in non-phytate P (NPP). NRC (1994) reported a gradual reduction in dietary non-phytate-P requirement for broilers from 4.5g kg^{-1} (0-3 weeks), 3.5g kg^{-1} (3-6 weeks) to 3.0g kg^{-1} (6-8 weeks). If, CA degrades the phytates, one of the main anti-nutrients that lower the inclusion of RB in broiler diets, it is hypothesized that CA supplementation permits the use of higher levels of RB in broiler diets. Objective of this study was to determine the effects of citric acid on growth performance and nutrient retention of broiler chicken fed diets having two levels of non-phytate phosphorus and rice bran.

MATERIALS AND METHODS

Day old broiler chicks (mixed sex) were brooded for 7 days in a floor brooder. From day 0 to 20 post hatch, chicks were fed a nutritionally complete commercial broiler starter diet (CIC feeds (Pvt) Ltd, Sri Lanka) containing 22% crude protein (CP). On day 20, birds were weighted and allocated into 48 floor pens having paddy husk as the litter material. Each pen housed three birds and had a feeder and a drinker. Eight experimental diets were formulated (Table 1). The experiment followed a completely randomize design in a 2 × 2 × 2 factorial arrangement.

Main factors were two dietary non-phytate phosphorus (NPP) (0.25 and 0.35%), CA (0 and 2%) and RB (20 or 30%) levels. Pens were randomly allocated into eight dietary treatments.

Each treatment had six replicates. Experimental diets and water were given *ad libitum* for three weeks from day 21 to 42 and feed intake, and weights of birds were recorded. On day 35, birds were transferred to a raised-wire floor arranged in each pen. After three days of acclimatization period, a total collection trial was done for four days from day 38 to 41, to determine the nutrient retention. Excreta was dried at 105 ˚C to constant dry weight and pooled separately according to the experimental diets. CP, phosphorus (P), dry matter (DM), and ash content of feed and excreta were measured by standard methods (AOAC, 1980). On day 42 all the birds were weighted and 6 randomly selected birds from each treatment were killed by cervical dislocation. Weights of internal organ and pH of the crop and gizzard contents were recorded. Left tibia was removed to determine the fat free tibia ash content. Data were analyzed using the general linear models procedure of the SAS (1985). Pen means served as replicates in growth performance and digestibility data analysis. Individual bird served as the replicates in carcass data analysis. Effects were considered significant when $P<0.05$. Duncan mean separation procedure was used to compare the significant main effects. When interactions were significant LS means were used to compare treatments.

RESULTS AND DISCUSSION

Numerous workers (Warren and Farrell, 1990b; Farrell and Martin, 1998; Atapattu, 2005; Piyaratne *et al.* 2011) have shown that more than 20% of RB in the diets reduced the feed intake of broilers. However, in this experiment, a reduction in feed intake could be observed only up to day 35 (Data not shown), but not thereafter. The total feed intake was also not affected by the dietary RB levels. Gallinger *et al.* (2004) reported that birds given diets without RB consumed significantly more feed than birds fed diets with RB until 21 day of age and, from day 28 feed intakes of the birds fed 0, 20, 30 and 40% RB were not significantly different. These findings suggest that relatively mature broilers can tolerate higher levels of RB than younger counterparts. This argument is further supported by the findings of Batal and Parsons (2002) who reported that the metabolizable energy corrected for nitrogen (MEn) value and the digestibility of AA of corn-SBM and corn-canola meal diets increased with age of the chicks. Results of this experiment suggest that RB can be included in broiler finisher diets upto 30% without having negative effects on feed intake. In line with previous studies (Boling-Frankenbach *et al.* 2001; Atapattu and Nelligaswatta, 2005) dietary CA had no adverse effect on total feed intake (Table 2). Contrary, some other studies (Shellem and Angel, 2002; Brenes *et al.* 2003; Ao *et al.* 2009) have reported a reduction in feed intake due to CA.

Table 1 Ingredient composition and calculated nutrient compositions of the experimental diets

Rice bran (%)	20				30			
Citric acid (%)	0		2		0		2	
NPP (%)	0.25	0.35	0.25	0.35	0.25	0.35	0.25	0.35
	Ingredients (g/kg)							
Yellow maize meal	329.5	330.5	321.5	321.5	257.9	252.4	246.3	239.5
Rice bran	200	200	200	200	300	300	300	300
Soya oil meal	103.6	106.6	123.3	128.6	110.9	110.9	119	118.9
Coconut oil	83.3	83.3	87.3	87.3	81.5	83.5	87	89.1
Fish meal	57.4	57.4	57.4	57.4	57	57	58.3	58.9
Sesame oil meal	100	100	95	92	86.3	87	89.4	89.4
Coconut oil meal	110	103	79.4	74	90.3	90.3	64.9	65.9
Di-calcium phosphate	2.4	9.5	2.1	9.5	1.7	8.5	1	8.2
Calcium carbonate ($CaCO_3$)	8	4	8.3	4	8.8	4.8	8.9	4.8
L-Lysine	0.8	0.7	0.7	0.7	0.6	0.6	0.2	0.3
Salt	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Vitamin mineral mixture	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Citric acid	0	0	20	20	0	0	20	20
	Calculated nutrients composition (g/kg)							
Crude protein (analyzed)	200	200	200	200	200	200	200	200
ME (Kcal/kg)	3210	3209	3205	3202	3207	3208	3206	3205
Calcium	9.1	9.1	9.0	9.0	9.0	9.0	9.0	9.0
Non phytate phosphorus	2.52	3.53	2.51	3.55	2.55	3.50	2.50	3.51
Lysine	10.07	10.0	10.2	10.3	10.2	10.2	9.9	10.1
Methionine + cystein	7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.7
Methionine	4.5	4.5	4.4	4.4	4.4	4.4	4.4	4.5
Crude fiber (analyzed)	60.7	59.9	57.1	56.5	67.2	67.1	64.1	64.1

NPN: non-phytate phosphorus and ME: metabolize energy.

Table 2 Growth performances from day 21 to 42 and pH of the feeds and digesta of crop and gizzard of broiler chicken as affected by dietary rice bran, citric acid and non-phytate phosphorus levels

RB	CA	NPP	Growth performance			pH		
			Feed intake (g)	Weight gain (g)	FCR	Feed	Crop	Gizzard
20	0	0.25	2612	1209	2.17	5.46	4.53	3.70
		0.35	2466	1136	2.18	5.40	4.70	3.63
	2	0.25	2448	1170	2.11	4.80	4.60	3.71
		0.35	2617	1250	2.11	4.66	4.53	3.78
30	0	0.25	2387	1116	2.16	5.44	4.64	3.89
		0.35	2455	1106	2.22	5.43	4.65	3.60
	2	0.25	2410	1081	2.27	4.73	4.51	3.72
		0.35	2400	1126	2.16	4.71	4.48	3.63
Pooled SEM			255.9	193	0.197	0.08	0.14	0.31
Source of variation			Level of significance					
RB			NS	0.092	NS	NS	NS	NS
CA			NS	NS	NS	0.0001	0.037	NS
NPP			NS	NS	NS	0.056	NS	NS
RB × CA			NS	NS	NS	NS	NS	NS
RB × NPP			NS	NS	NS	NS	NS	NS
CA × NPP			NS	NS	NS	NS	NS	NS
RB × CA × NPP			NS	NS	NS	NS	NS	NS

RB: rice bran; CA: citric acid and NPP: non-phytate phosphorus.
SEM: standard error of the means.
NS: non significant.
FCR: feed conversion ratio.

Differences of the experimental conditions, particularly the level of CA, the age of the birds and dietary phytate and Ca contents may be the reasons for those discrepancies. Inclusion of CA significantly (P<0.05) reduced the pH levels of the feed and the digesta of the crop but not of the gizzard (Table 2).

Ao *et al.* (2009) have also reported similar findings. Boling-Frankenbach *et al.* (2001) reported CA, would not be expected to have a large effect on intestinal pH because it is an organic acid that is metabolized rapidly. Despite the fact that appetite controlling nerve endings of poultry are reported to be located in the crop, reduction in crop pH due to CA had no effect in feed intake. Interaction of RB, CA and NPP (RB×CA×NPP) was significant on feed intake, during the period of day 21 to 28, (P=0.017). Other than that, any interactions of dietary factors had no significant effect (P>0.05) on feed intake during the experimental period.

Weight gains followed more or less similar pattern to feed intake (Table 2). 30% dietary RB (P=0.0001), 0.25% NPP (P=0.039) significantly (P<0.05) reduced the weight gain from 21 to 28day. Dietary CA levels or any interactions of dietary factors had no significant effect (P>0.05) on weight gain from day 21 to 28. Contrary to a number of studies (Warren and Farrell, 1990b; Farrell and Martin, 1998; Gallinger *et al.* 2004; Atapattu, 2005) those who reported that high levels of dietary RB reduced the live weight and weight gain, 30% RB did not reduce weight gain in this experiment. Steyaert *et al.* (1989) also has suggested that RB up to 30% in mash form had no adverse effects on performance of broiler chicken. 2% of CA in broiler diets did not significantly reduce the weight gain from day 21 to 42. Ao *et al.* (2009) reported that CA significantly decreased the feed intake and weight gain of broiler chicks. Meanwhile, Rafacz-livingston *et al.* (2005) reported a linear (P<0.05) increase in weight gain and feed intake in chicks fed diets supplemented with up to 3% of CA. Above findings suggest that effects of CA on weight gain is mediated through its effects on feed intake. The absence of an effect of CA on FCR further supports this argument.

Feed conversion ratio (FCR) was not significantly (P>0.05) affected by dietary RB, CA, NPP or their interactions. However, Gallinger *et al.* (2004) has reported adverse effects of dietary RB over 20% on FCR. All the diets used in this experiment contained minimum of 0.25% NPP; the lower thresh hold limit suggested by Waldroup *et al.* (2000) for broiler finishers. On a one hand, Ao *et al.* (2009) observed reduced growth performance when broilers were fed CA supplemented diets with adequate levels of NPP (0.45%). On the other hand, Rafacz-livingston *et al.* (2005) reported positive effects of CA on growth performance when added to diets containing a low level of NPP (0.18%). Therefore, the lack of positive effects of CA on growth performance may be due to the use of marginally sufficient dietary NPP level. Results of the present experiment suggest that, 30% of RB does not impair the growth performance of broiler chicken and 2% CA has no beneficial effects either at 20 or 30% dietary RB levels if diets contain at least 0.25% NPP.

Effects of dietary RB, CA and NPP levels on the weights of internal organs of broiler finisher chicken are shown in Table 3. Weights of feather, liver, gizzard, and pancreas were not significantly affected by dietary levels of RB, CA, NPP or their interactions. Contrary to our findings Gallinger *et al.* (2004) have observed that higher pancreas weight when broilers were fed 40% dietary RB. They have suggested that negative effects of anti-proteolytic substances of RB are compensated by increased pancreatic secretions, which is reflected in increased pancreas weight. As in our experiment Gallinger *et al.* (2004) also reported no effects of RB on liver and stomach weights. Our findings in relation to carcass weights are in agreement with that of Puminn (2003). Interaction of dietary CA with NPP had significant effect (P<0.05) on weight of heart and dressing percentage. There is a trend (P=0.0627) to have higher weight of heart of broilers fed diets having 0.35% of NPP with 2% CA than those fed diets having 0.35% of NPP but without CA. On the other hand, significantly higher (P<0.05) heart weight was observed in the birds fed diets which contain 0.25% NPP without CA than the diets which contain 0.35% NPP without CA.

Dressing percentage without giblet was significantly low (P<0.05) when diets contain 2% of CA with 0.25% of NPP than the diets which contain 0.25% of NPP without CA. It can be suggested that use of 2% CA has no beneficial effect on dressing percentage when diets having sub optimum NPP level.

Significantly higher (P<0.05) dressing percentage was observed when CA free diet contained 0.25% NPP compared to a diet having 0.35% NPP.

Retention of N, P, or DM were not significantly affected (P>0.05) by the levels of dietary RB, CA, NPP or their interactions (Table 4). Contrary, several authors (Warren and Farrell, 1991; Farrell and Martin, 1998; Gallinger *et al.* 2004; Piyaratne *et al.* 2011) have reported inferior digestibility indices and/or mineral retention with increasing the level of RB in the diets.

Due mainly to the formation of less digestible phytate-protein / amino acid complexes with proteins and amino acids (Ravindran, 1995; Maenz, 2001) and increased endogenous amino acid losses, low protein digestibility values have been reported for the diets with high levels of RB. However, inclusion of 30% had no significant effect on nitrogen retention, compared to 20% RB. The retention of P and mineral was also not reduced significantly due to 30% RB. Puminn (2003) reported that, Percentage of tibia ash was significantly low in birds fed 25% defatted RB diets.

However, in this experiment, no such adverse effects were observed at 30% dietary RB. Gallinger *et al.* (2004) also observed no significant difference in tibia ash contents in broilers fed diet containing 20% or 30% RB. Therefore it is suggested that 30% RB in broiler finisher diets have no adverse effects on feed intake and the utilization efficiency of protein, P and other minerals and thereby giving comparable growth performance with 20% dietary RB.

Table 3 Effects of dietary rice bran, citric acid and non-phytate phosphorus levels on organ weights of broiler chickens from 21-42 day

RB	CA	NPP	Organ weights (%) relative to live weight at slaughtering					
			Feather	Carcass	Liver	Gizzard	Pancreas	Heart
20	0	0.25	6.46	71.6	3.05	1.39	0.26	0.56
		0.35	6.98	68.6	2.86	1.34	0.27	0.48
	2	0.25	6.16	68.8	2.73	1.30	0.26	0.51
		0.35	6.21	69.8	2.99	1.44	0.28	0.56
30	0	0.25	6.11	69.7	2.79	1.41	0.29	0.57
		0.35	6.00	68.0	3.13	1.45	0.23	0.51
	2	0.25	5.96	67.0	3.12	1.33	0.25	0.52
		0.35	6.48	68.3	2.98	1.36	0.28	0.55
Pooled SEM			1.38	2.98	0.56	0.18	0.06	0.10
Source of variation			Level of significance					
RB			NS	0.058	NS	NS	NS	NS
CA			NS	NS	NS	NS	NS	NS
NPP			NS	NS	NS	NS	NS	NS
RB × CA			NS	NS	NS	NS	NS	NS
RB × NPP			NS	NS	NS	NS	NS	NS
CA × NPP			NS	0.023	NS	NS	0.081	0.021
RB × CA × NPP			NS	NS	NS	NS	NS	NS

B: rice bran; CA: citric acid and NPP: non-phytate phosphorus.
SEM: standard error of the means.
NS: non significant.

Table 4 Effects of dietary rice bran, citric acid and non-phytate phosphorus levels on nutrient retention and tibia ash percentage of broiler chickens

RB	CA	NPP	Retention (g/100g body weight/day)				Tibia	
			Nitrogen	Phosphorus	Dry matter	Mineral	Ash %	Weight % / LW
20	0	0.25	0.56	0.065	13.03	1.40	39.45	0.27
		0.35	0.44	0.087	11.93	2.21	37.29	0.26
	2	0.25	0.44	0.083	10.80	1.55	37.92	0.27
		0.35	0.40	0.047	09.78	1.09	38.48	0.28
30	0	0.25	0.44	0.083	11.40	1.19	40.44	0.26
		0.35	0.45	0.065	10.00	1.17	37.51	0.26
	2	0.25	0.54	0.076	13.15	2.22	36.05	0.28
		0.35	0.45	0.053	10.78	2.05	43.01	0.26
Pooled SEM			0.22	0.034	05.51	0.68	4.92	0.11
Source of variation			Level of significance					
RB			NS	NS	NS	NS	NS	NS
CA			NS	NS	NS	NS	NS	NS
NPP			NS	NS	NS	NS	NS	NS
RB × CA			NS	NS	NS	0.006	NS	NS
RB × NPP			NS	NS	NS	NS	NS	NS
CA × NPP			NS	NS	NS	NS	0.014	NS
RB × CA × NPP			NS	NS	NS	NS	NS	NS

B: rice bran; CA: citric acid and NPP: non-phytate phosphorus.
SEM: standard error of the means.
NS: non significant.
LW: live weights of the broiler chickens.

Since RB contains more phytic phosphorus and total P and low phytase activity than soy and maiz (Eeckhout and De Paepe, 1994), it was expected that CA would be more effective in releasing more phytic phosphorus from a RB based diet than a corn / soybean diet. However, contrary to the reports that CA improved the phytate hydrolysis (Boling *et al.* 2000; Boling-Frankenbach *et al.* 2001; Rafacz-livingston *et al.* 2005; Liem *et al.* 2008) and N and DM digestibility (Ao *et al.* 2009), in this experiment CA had no significant effects on DM, N and P retention.

As discussed earlier, it is suggested that the dietary NPP levels used was not low enough to induce a positive effect. Interaction of RB with CA was significant (P<0.05) for mineral retention (MR). In the absence of CA, there was a trend (P=0.095) to reduce MR when diets contain 30% of RB compared to the diets having 20% of RB. Interestingly, 2% CA, significantly improved the mineral retention with 30% RB, compared to 20%. This observation suggests that 2% CA has beneficial effects on MR when added to a diet with higher level of (30%) RB.

Since CA did not increase the P retention, the beneficial effect of CA on MR at 30% RB, cannot be attributed to the increased phytate hydrolysis of CA. Furthermore, improved MR was not reflected by an increase in tibia ash percentage or weight of tibia, even though P is one of main fractions in tibia of poultry. One possible explanation would be that birds might have adopted mineral conserving adaptations when they were offered 30% dietary RB having a higher level of phytate. Alternatively, CA, may be due to physical effects, weakened the chemical bonds between phytic acid and other minerals thereby making them more available. Absence of negative effects on 30% RB on N or DM retention supports this argument. Interaction of dietary CA with NPP was significant (P<0.05) for tibia ash content. Brenes *et al.* (2003) found that, the addition of CA to the low NPP diets resulted in a significant increase in tibia ash. However CA with 0.25% NPP did not have beneficial effects on tibia ash. 2% CA significantly (P<0.05) increased the tibia ash content when added to a diet with 0.35% of NPP, than with 0.25% of NPP.

CONCLUSION

Overall results of this experiment conclude that RB can be included into broiler finisher diets up to 30%, without any adverse effects on growth performance and bone status. 2% CA produces no beneficial effects at dietary RB levels of 20 or 30% or NPP levels of 0.25 or 0.35%.

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