Short Communication

Effects of the Dietary Inclusion of Fish Meal, Rock Phosphate and Roxarsone on Arsenic Residues in Tissues of Broilers

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Abstract

The effect of dietary ingredients such as fish meal, rock phosphate and roxarsone on the accumulation of arsenic residues in breast meat, liver and heart was examined in Arbor Acre broilers from days 1-43 of age. Broilers receiving diets containing fish meal had a significantly greater amount of arsenic in breast muscle than other groups. There was a positive correlation between muscular arsenic concentration and dietary fish meal levels. Withdrawal of fish meal for a week prior to slaughter reduced the muscular deposition of arsenic. Chicks receiving roxarsone had significantly higher arsenic level in the liver compared to other groups and arsenic levels decreased after withdraw. Neither calcium sources such as rock phosphate nor arsenic-containing growth promoters affected the deposition of arsenic residue in meat. In conclusion, fish meal was the major factor resulting in the arsenic contamination of breast meat in broilers. Roxarsone and rock phosphate do not affect the arsenic level in meat.

Keywords : arsenic, broiler meat, roxarsone, fish meal, rock phosphate

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บทคัดย่อ

ผลของการเสริมปลาป่น ร็อคฟอสเฟต และ ร็อคซาโซน ในอาหารต่อสมรรถภาพการเจริญเติบโต และปริมาณสารหนูตกค้างในเนื้อเยื่อของไก่เนื้อ

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ศึกษาผลของการเสริมปลาป่นร็อคฟอสเฟต และร็อคซาโซน ในอาหารต่อปริมาณสารหนูตกค้างในเนื้ออก ตับและหัวใจของไก่เนื้อพันธุ์อาร์เบอร์ เอเคอร์อายุ 1-43 วัน ไก่เนื้อที่ได้รับอาหารผสมปลาป่น มีระดับสารหนูในเนื้ออกมากกว่ากลุ่มอื่นๆ เมื่อวิเคราะห์หาความสัมพันธ์ระหว่างระดับสารหนูในเนื้ออกและปริมาณปลาป่นที่ผสมในอาหาร พบว่ามีสหสัมพันธ์ในเชิงบวก ไก่ที่ได้รับอาหารที่ไม่มีปลาป่นในช่วง 1 สัปดาห์ก่อนส่งโรงฆ่าจะมีระดับสารหนูตกค้างลดลงในเนื้ออกอย่างชัดเจน ไก่ที่ได้รับร็อคซาโซนมีระดับสารหนูสะสมในตับสูงกว่ากลุ่มอื่นๆ และระดับสารหนูจะลดลงเมื่อถอนอาหารดังกล่าว 1 สัปดาห์ก่อนส่งโรงฆ่า ผลการทดลองสรุปได้ว่า ปลาป่นเป็นสาเหตุที่ทำให้มีการตกค้างของสารหนูในเนื้ออกไก่ การให้อาหารที่ไม่มีปลาป่นหรือมีระดับต่ำจะช่วยลดสารหนูตกค้างในเนื้ออกไก่ ร็อคฟอสเฟตและร็อคซาโซนไม่มีผลทำให้เกิดสารหนูตกค้างในเนื้ออกไก่

คำสำคัญ : สารหนู เนื้อไก่ ร็อคซาโซน ปลาป่น ร็อคฟอสเฟต

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Introduction

The production of good quality poultry meat is of the utmost importance in poultry farms worldwide. Concern over heavy metal contamination in poultry meat has become an issue of great importance in some countries. Arsenic is one of the elements contaminating the poultry production system in feed ingredients, water or growth promoters. Basically, the concentrations of arsenic in animal tissues depends mainly on the dietary concentrations of arsenic, absorption of arsenic and the homeostatic control mechanism of the body for arsenic (Doyle and Spaulding, 1978). Arsenic level can be used as an indicator of good quality chicken meat and is very important for those countries that export chicken meat. The maximum concentrations of total arsenic (ppm) allowed in frozen chickens in various countries showed that some countries, for instance Austria, France and the Netherlands do not allow the presence of arsenic residue in imported frozen chickens and some of them, such as Czech Republic condemn at a concentration greater than 0.1 ppm. This low level of arsenic residues will affect countries like Thailand, who export approximately 435,000 metric tons with a forecast 5% increase every year. The major markets of Thailand broiler production are the European Union and Japan. In most feed mills, the main feed ingredients used for broiler diets are corn, soybean meal and fish meal. Fish meal is known as a source of arsenic since fish acquires arsenic through the food chain in the sea. The level of arsenic in fish meal is approximately 2-20 ppm (Ammerman et al., 1973). Nonetheless, there are few studies that have reported the contribution and scale of contamination of arsenic in fish meal that will contaminate the meat. Other feed ingredients such as rock phosphate and growth promoters (roxarsone or 3-nitro-4-hydroxyphenylarsonic acid) are also known to contain some arsenic and their effects on meat deposition has not yet been clarified. Roxarsone in one of the phenylarsonic compounds used as feed additives in swine and poultry ration and has the least toxic (el Bahri and Ben Romdane, 1991). Therefore, the

objective of the present study was to examine the effect of fish meal, rock phosphate and roxarsone on the deposition of arsenic in the pectoral muscles, liver and heart of broilers.

Materials and Methods

Animals and diets

Two-hundred and ninety-seven, one day old, male and female Arbor Acre™ broiler chicks were randomly allocated into 11 treatments (3 replicates of 9 chicks in each treatment) based on similar body weights in each replicate. Each replicate of chicks was reared in a single-tier stainless steel cage. The room temperature was between 27-33°C and the relative humidity 60-80%. All chicks were vaccinated with Newcastle disease virus vaccine on day 10 of age. Water and feed were supplied *ad lib*. The corn-soybean meal based diets were formulated based on NRC (1994) and divided into 3 periods; period 1 from days 1-21, period 2 from days 22-35 and period 3 from 36-43 days. The lighting schedule was changed from Light: Dark (L:D) for 24:0 hr in the first two weeks of age to L:D for 16:8 hr for the rest of the experiment. The chicks were slaughtered at 43 days old. Chicks in treatment 1 were given the basal diets that contained imported dicalcium phosphate (India) as the phosphorus source. This group served as a negative control group. In treatment 2, chicks were given the basal diet plus fish meal and imported dicalcium phosphate. The level of arsenic in this diet was calculated to be approximately 0.5 ppm as shown in Table 2 and the diet was fed for all periods. Chicks in treatment 3 were given diet with the level of arsenic of 0.75 ppm for all three periods. The calculated level of dietary arsenic for chicks in treatment 4 was formulated to be 0.95 ppm and fed for three periods. Chicks in treatments 5 and 6 received diets similar to those given to chicks in treatments 3 and 4, respectively except that the diets were given in the first two periods. Diets free of fish meal (similar to treatment 1) were given in the third period. In treatment 7, chicks were given diet without fish meal and rock phosphate was used as the source of phosphorus while similar diets were given to chicks in treatment 8 except that the phosphorus source was monocalcium phosphate (Biofos™ MCP, USA). Chicks in treatments 9 and 10 were given diet without fish meal and dicalcium phosphate was used as the source of phosphorus. Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) (3-Nitro™, Alpharma, USA) was included in the diet at level 40 ppm (10% premix). The diets were fed for all three periods except in treatment 10 that the diet given in period 3 was diet without fish meal and roxarsone (as same as diet in treatment 1). In treatment 11, diet with fish meal, 40 ppm roxarsone and dicalcium phosphate were given to chicks for all periods. This group served as the positive control group.

Data collection

When chickens were 43 days old, they were tagged with plastic bands at the hock joint. Six chickens in each replicate weighing close to the group average were chosen and sent to the slaughter house. All chickens were fasted for 12 hr prior to slaughter process. The chickens were stunned by a procedure complied with the European Union directives and bled to death. Both sides of pectoral muscle were collected and kept frozen until analysis for arsenic concentration. The whole liver and heart were collected.

Determination of arsenic in feed and muscle

Arsenic in diets and feed ingredients were analyzed using methods in AOAC (1995). Feed samples were dried using dry ashing method. Magnesium nitrate was added onto the samples and dried using hot plate at temperature 375°C. The flask was then put on the oven temperature 450°C for 30 minutes so that the carbonate salt and excess magnesium nitrate will be oxidized. The residue was cool and then added with 2 ml of 8M HCl and 0.1 ml of KI to reduce As^{5+} to As^{3+}. The solution was analyzed using Hydride Generation Atomic Absorption Spectrophotometer. The standard solution of 0, 0.05, 0.10, 0.15, 0.20 and 0.25μg were analyzed and plotted as the standard curve. The sample was measured

together with reagent blank and was calculated followed the standard curve. The limit of detection (LOD) was 0.07 ng/kg and percent recovery of 90%.

For arsenic detection in breast meat, liver and heart, samples were digested with solution of 65% nitric acid and 30% hydrogen peroxide in microwave digester. Subsequently the digested samples were measured for arsenic using a hydride generation atomic absorption spectrophotometer at wavelength 193.7 nm. The limit of detection of this method was 0.01 ppm.

Statistical analysis

Data are presented as mean ± standard deviation. The data were analyzed using one-way analysis of variance (ANOVA) (Steel and Torrie 1982). Multiple comparisons of means were performed using Student Newman Keuls test with a significant level at $p<0.05$. The regression analysis and correlation between the dietary arsenic levels in groups 1-6 and amount of arsenic intake (average level of arsenic in each group x average feed intake (g/bird/day) in groups 1-6) and muscular level of arsenic were examined.

Table 1 Mean arsenic concentrations (ppm) determined in feed ingredients and tap water used in the experiment.

Ingredients	Mean arsenic concentrations (ppm)
Corn	0.035
Soybean meal	0.018
Full-fat soybean meal	0.037
Fish meal	12.77
Dicalcium phosphate (India)	0
Monocalcium phosphate (Biofos, USA)	4.70
Rock phosphate	23.6
Tap water	0.64×10^{-3}

Table 2 Mean dietary arsenic concentrations (ppm) and overall mean determined in diets used in each period of the experiment. There are three periods of diets fed, period 1 from 1-21 days, period 2 from 22-35 days and period 3 from 36-43 days post-hatching.

Group	Period 1	Period 2	Period 3	Weighted mean concentrations
1	0.215	0.153	0.211	0.194
2	0.459	0.536	0.447	0.483
3	0.751	0.748	0.766	0.753
4	0.853	0.927	1.290	0.951
5	0.778	0.773	0.425	0.718
6	0.934	1.170	0.273	0.903
7	0.572	0.581	0.552	0.572
8	0.420	0.415	0.439	0.422
9	10.50	10.60	10.40	10.52
10	10.80	10.40	0.182	8.90
11	10.70	11.60	10.40	10.47

Table 3 Mean arsenic level (ppm) in breast muscle, liver and heart of broiler at 43 days post-hatching.

Group	breast muscle	liver	heart
1	0.023±0.018[*d]	0.008±0.015[*d]	0.015±0.001[*d]
2	0.187±0.040[c]	0.131±0.074[c]	0.120±0.025[b]
3	0.327±0.058[a,b]	0.259±0.165[b]	0.212±0.071[a]
4	0.457±0.190[a]	0.239±0.174[b]	0.275±0.001[a]
5	0.183±0.060[c]	0.128±0.062[c]	0.133±0.019[b]
6	0.230±0.080[c]	0.131±0.056[c]	0.151±0.006[b]
7	0.020±0.010[d]	0.015±0.019[d]	0.015±0.013[c]
8	0.023±0.011[d]	0.014±0.022[d]	0.015±0.014[c]
9	0.053±0.020[d]	0.587±0.221[a]	0.075±0.022[b]
10	0.023±0.010[d]	0.158±0.064[c]	0.019±0.013[c]
11	0.463±0.090[a]	0.430±0.145[a]	0.305±0.074[a]

[*]Mean ± SD, n = 6

[a,b,c,d]Means in the same column with different superscripts differ significantly ($p < 0.05$)

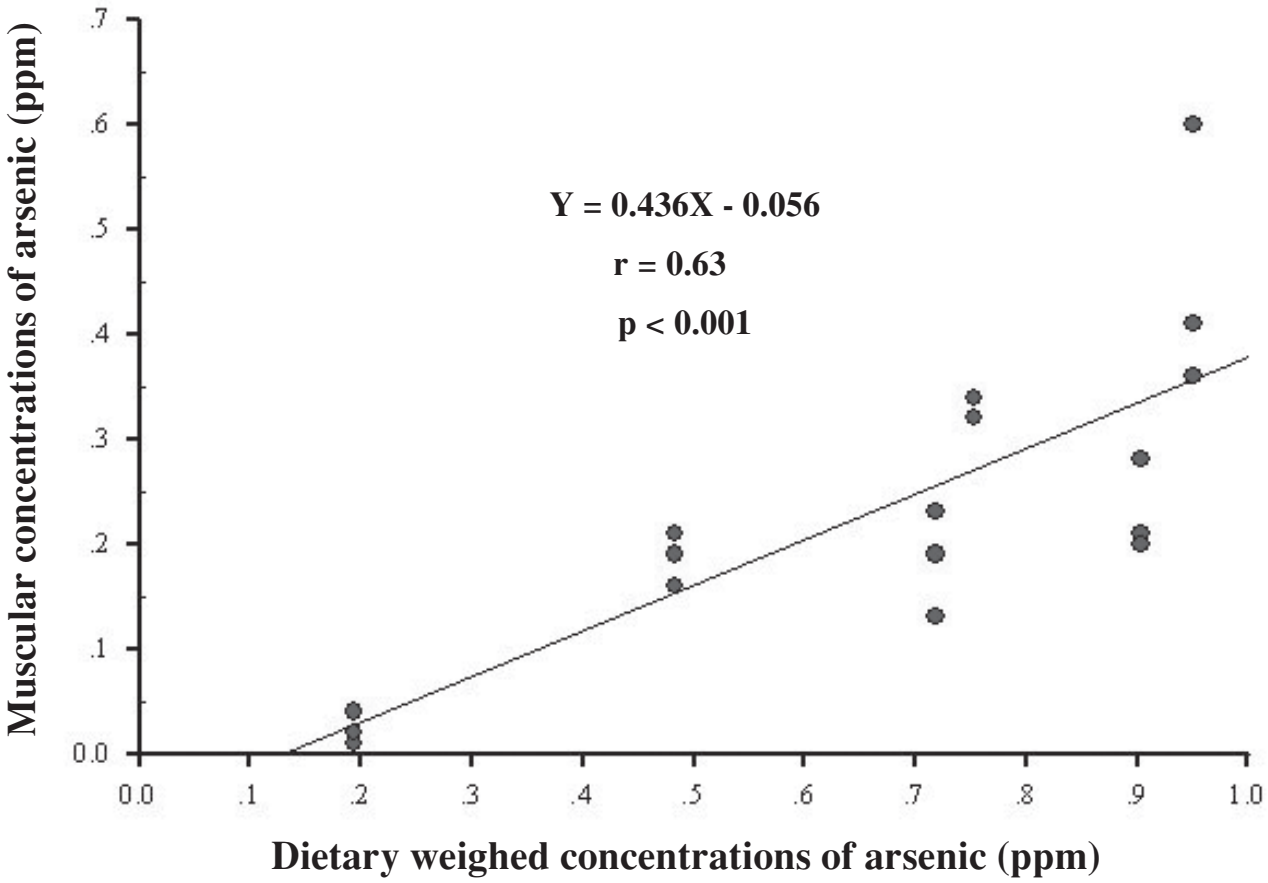

Figure 1 Regression analysis between dietary weighed concentrations of arsenic (ppm) and muscular concentration of arsenic (ppm)

Results and Discussion

Table 1 shows the level of arsenic in various feed ingredients used in the experiment. Rock phosphate and fish meal had the respectively high level of arsenic compared to other ingredients. Arsenic concentration in tap water was found to be very low (0.64 ppb).

Arsenic levels were different depending on the feed ingredients in each diet (Table 2). Effect of fish meal on arsenic contamination of meat was examined in groups 1-6 of the experiment. In group 1, the diets contained no fish meal and the mean dietary arsenic level of three feeding periods was 0.194 ppm. The arsenic level increased proportionally in groups 2, 3 and 4, respectively (0.483, 0.753 and 0.951 ppm). Increased level of arsenic in fish meal contributed to the enhanced arsenic level in these groups. When fish meal was withdrawn in period 3 of experiment as shown in groups 5 and 6, the mean dietary arsenic level decreased to 0.718 and 0.903 ppm, respectively. The effect of phosphorus source was studied in groups 7 and 8 which these diets comprised arsenic at 0.572 and 0.422 ppm, respectively. In groups 9 and 10, arsenic in the form of growth promoter (Roxarsone) was used. The arsenic concentration was 10.52 ppm in group 9 and the average level decreased to 8.90 ppm since roxarsone was withdrawn in period 3. The diet used in group 11 was similar to those used in the local broiler production. It was found that the average level of arsenic was 10.47 ppm (Table 2).

Average concentrations of arsenic in breast muscle, liver and heart are shown in Table 3. Chickens in groups 4 and 11 had the highest amount of arsenic deposit in the breast muscle. Both groups received the diet containing fish meal. When the dietary level of fish meal increased, the level of arsenic in breast muscle also enhanced proportionally as shown in groups 1-4 of the experiment (0.02, 0.19, 0.32 and 0.46 ppm). The withdrawal of fish meal in period 3 markedly reduced ($p<0.05$) the arsenic contamination as in groups 4 and 6 compared to corresponding groups 3 and 5. Previous studies on arsenic contamination on human food in Thailand were reported based on poultry meat sampling from the markets (Aroonskul et al., 1992; Thaveetiyanont, 1978). Data was varied due to unknown husbandry regimen including diets used. In this study, the chickens were in the same plane of nutrition and environment. Factors affecting the arsenic contamination were studied in the same population of chickens so that they can be compared. There are three possible factors influencing the contamination of arsenic in muscle tissues, arsenic in a form of growth promoter, arsenic in phosphorus sources and arsenic in protein sources such as fish meal. Contamination of arsenic in feeding water is negligible due to its low level as shown in Table 1. From the result, it was clear that fish meal was the most important factor that caused the highest level of arsenic residues in muscle. Feed devoid of fish meal as in group 1 resulted in very small amount of arsenic deposition in muscle. The arsenic accumulation was enhanced when the level of fish meal in diets increased. The regression analysis of the amount of arsenic deposit in muscle and mean weighed values of arsenic in diets used in groups 1-6 were calculated and shown in Figure 1. The increased level of arsenic in these diets was due to arsenic in fish meal since other feed ingredients such as corn, soybean meal and dicalcium phosphate had very low level of arsenic. It was found that the linear equation was $Y = 0.436X-0.056$ (Y is the level of arsenic in muscle and X is the level of arsenic in diets). The coefficient of correlation was statistically significant ($r=0.63$, $p<0.001$). From this regression equation, the highest level of fish meal inclusion in broiler diet should be less than 2.18% in order to minimize the level of arsenic in muscle to 0.10 ppm. Although fish meal was still included in the diet as a source of protein riches in sulfur containing amino acids, however, the utilization was reduced due to the effect of heavy metals contamination. The higher the arsenic in fish meal ingested, the more deposition was found in breast muscle. Feed ingredients such as fishes (Bennett, 1981; Pavelka and Sedicek, 1976), prawns (Edmonds and Francesconi, 1987) or shells (Shibata et al., 1992) have the respectively high levels of arsenic as they can accumulate arsenic through environmental contaminants. They can uptake arsenic in the sea in the form of inorganic arsenic for instance arsenate (Edmonds and Francesconi, 1987; Bennett, 1981) to organic arsenic in muscle (Penrose et al., 1977) in the form called arsenobetaine. Jongen et al. (1985) found that human subjects eaten seafood had significantly high level of arsenobetaine excreted in the urine. There was the distribution of ^{74}As-labelled arsenobetaine

throughout tissues of human volunteers whom were fed fishes labeled with ^{74}As arsenobetaine. Moreover, less than 1% of ingested arsenobetaine was detected in human 24 days after eating (Brown et al., 1990). It is speculated that the form of arsenic in breast muscle was likely to be arsenobetaine as those found in fish. Vahter et al. (1983) studied the metabolism of arsenobetaine in various species of animals. They found that arsenobetaine was highly absorbed in the intestine of mice and there was marked deposition of arsenobetaine in muscle of rabbits. Organic arsenic in the form of arsenobetaine did not have the biotransformation by animal body to other forms as found in inorganic arsenic but it excreted into urine. Organic arsenic in fish was difficult to transform in animal body (Lunde, 1977). In this study, chickens received fish meal for all three periods had substantially high level of arsenic in muscle and the level of arsenic significantly decreased when fish meal was withdrawn from the feed in period 3.

Chickens receiving various sources of phosphorus had very low level of arsenic in breast muscle, liver and heart. Despite high dietary arsenic concentrations, chickens receiving rock phosphate or monocalcium phosphate had very low deposition of arsenic in muscle compared to the control group. It was likely that the level of both ingredients used were low in the diets that made the total level lower than those from fish meal. Inorganic arsenic was found to be highly toxic when received in high amounts (Pershagen, 1981). This form of arsenic will be biotransformed by methylation process. However this process can be maximized when high concentration of inorganic arsenic such as As_2O_3 was ingested and this harmed the animals (Foa et al., 1984). The metabolites from the biotransformation will be excreted in urine. It is likely that there was little change in the form of inorganic to organic arsenic in chicken compared to marine animals which had high ability (Edmonds and Francesconi, 1987). Inorganic arsenic was well absorbed in chick intestine (Fullmer and Wasserman, 1985) but it was excreted effectively.

Chickens received diets containing roxarsone had low concentrations of arsenic in muscle (group 9). However, it is demonstrated that chicks receiving roxarsone throughout the period (group 9 and group 11) had significantly higher arsenic in the liver than other groups followed by those chicks receiving high dietary fish meal inclusion (groups 3 and 4). In heart muscle, arsenic level was highest in groups 3, 4 and 11. Chicks receiving roxarsone in both groups 9 and 10 had low levels of arsenic in the heart muscle. Those chicks receiving sources of phosphate had low arsenic levels in the liver and heart muscle. Withdrawal of roxarsone in period 3 reduced the level of arsenic in muscle (group 10) ($p>0.05$). Roxarsone is an organoarsenic compound used extensively in broiler industries as a growth promoter. The level of arsenic residues in breast muscle was as low as the control group when the roxarsone was withdrawn from the diet used in period 3. Garbarino et al. (2003) stated that nearly all the roxarsone in feed was excreted unchanged in the manure. Roxarsone can be metabolized by liver and kidney and excreted more easily than arsenic in the form of arsenobetaine that adhered to amino acids in muscle protein. Organic arsenic such as Roxarsone caused low tissue deposition of arsenic although chickens ate it every day. High accumulation of arsenic in chicken receiving roxarsone was in accordance with the result of Chiou et al. (1997) who found that arsenic contents in the liver significantly increased as level of roxarsone increased. Withdrawal of roxarsone in period 3 markedly decreased the arsenic level in the liver compared to those chicks fed roxarsone for the whole period. This finding was similar to the results in pig (Ferslew and Edds, 1979) and growing broilers (Proudfoot et al., 1991) that the arsenic contents in liver were lower after roxarsone was withdrawn from feed.

Conclusion

The present study showed that the arsenic residues in breast muscle of chickens resulted from the arsenic in fish meal. Inclusion of organic arsenic as a growth promoter resulted in little deposition of arsenic in muscle. Similar findings were observed when rock phosphate or monocalcium phosphate was used.

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