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Determination Ofcyanide, Nitrate and Nitrite, Contents of Poultry Feeds of Four Top Animal Feed Companies in Nigeria

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Abstract: The levels of total cyanogens, nitrate and nitrite which many cause deleterious effects in poultry animals were determined in poultry feeds of four major poultry feed companies in Nigeria using enzymatic and spectrophotometric methods. Nitrite was determined spectrophotometrically and nitrate after reduction to nitrite with cadmium. Enzymatic analysis of the feed samples indicated mean total cyanide level 1.98±0.13-25.74±2.03, 1.58±0.15-18.22±1.97, 5.94±0.58-13.86±1.89, 3.56±0. 22–78.01±3.95 mg CN^- equivalent kg^{-1} DM for broiler finisher, growers mash, layers mash and broiler starter feeds respectively. The mean values of nitrite determined ranged from 22.02±3.5.7-48.65 ±5.67, ND-60.22±4.07, 9.34±2.84-53. 94±2.13 NO_2-N kg^{-1} Dm following the order above. The mean concentration of nitrate ranged 47.48± 5.82-194.60±13.14, 83.40±11.08-127.44± 12.33, 94 76.45±10.34-199.21±9.76 and 70.71 ±8.17-154.51±10.11 mg NO_3-N kgg^{-1} DM, for broiler finishers, growers mash, layers mash and broiler starter respectively. The results are discussed from nutritional and toxicological points of view.

Key words: Cyanide, nitrate, nitrite, poultry feeds

INTRODUCTION

The importance of increased livestock production in developing countries like Nigeria cannot be over emphasized. The human need for foods such as meat and eggs and the possibility of covering them in this part of the world has become so necessary with increasing population and disease conditions. In spite of the great demand for protein of light biological value for human consumption, pigs and poultry production in the tropics (developing countries) is scanty and inefficient as a rule[1]. The number of cattle, sheep, goats, pigs and chickens found in developing countries in 1991 far out numbered those in developed countries but the quantity of meat and milk produced in developing countries was far higher than that produced in developing countries[2]. The underlying reason for this situation is the system of management and feeding strategies in the developing countries[3].

Poultry feeds in Nigeria contain components of plant origin at high ration with some of them containing naturally occurring compounds that significantly reduce their nutritional value (toxic constituents). Therefore, occasionally phytotoxin contamination of these feed constituents may have some serious consequences such as animal death or decreased production. The implications of cyanogenic glycosides of cassava and cassava by-products, sorghum, rubber seeds[4,5,6] and millet all of which are poultry feed resources are known. In addition, there has been concerns over the presence of nitrates and nitrites in both human foods and animal feeds as they can be metabolized to potentially cancinogenic N-nitrosamines[7]. An outbreak of liver disorder including cancer in various farm animals following ingestion of herring meal which has been preserved by addition of sodium nitrite has been reported in Norway. N-nitrosamine formed from the reaction between dimethylamine and added nitrite was determined to be the cause of the liver failure[8]. Fishmeal is also a source of protein in Nigerian poultry feeds. Furthermore, increasing evidence has shown that thiocyanate, the main cyanide metabolite can lead to the promotion of N-nitrosamine formation *in vivo* which enhances carcinogenesis[9,10].

The present study was carried out to assess the potential toxic substances mainly cyanogens, nitrate and nitrite in poultry feeds, produced by four Top Animal Feed Companies in Nigerian. Death of poultry animals as well as decreased production (especially eggs) have been common in many semi-intensive and industrial poultry farms. In most of these cases, dietary cyanide exposure from cassava has been implicated without determining the amount of cyanogens in the material,[11]. Currently, poultry feed constitute more than 90% of all commercial rations produced by millers in Nigeria[3]. Thus, poultry feeds serve as good representative of commercial ration for analysis for these anti-nutritional factors.

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MATERIALS AND METHODS

Reagents: All chemicals sulphanilic acid, N- (1-Naphlthyl) ethylenediamine hydrochloride and cadmium were analytical grades. Kit B2 for total cyanide was a gift from Howard Bradbury of Australian National University.

Sample collection: Samples of poultry feeds from four top animals feed companies in Nigeria were purchased from re-tail outlets in some major Nigerian markets and were brought for analysis. The poultry feeds included broiler starter, broiler finisher, growers mash and layers mash. Three samples of each feed were obtained from ten different Marketers for each of the four companies. Samples of each feed from each company were mixed together and treated as one sample followed by triplicate analysis.

Nitrate and nitrite determination: Nitrite was determined by the spectrophotometric methods described by Follet and Ratclif[12]. Nitrate was determined after reduction to nitrite using cadmium column[12]. Coloured samples were cleared with animal charcoal. For colour formation, nitrite was reacted with sulphanilic acid and N(1-naphlthyl) ethylenediamine hydrochloride and the purple colour developed after 20 minutes was read spectrophotometrically at 520 nm .

Cyanide determination: The cyanide content of the feed was determined spectrophotometircally using kit B2 for total cyanide determinations according to Bradbuy *et al.*,[13].

RESULTS

Cyanide: The amount of total cyanide measured in the poultry feeds are presented in Table 1. Cyanide was found in all the feed samples produced by the Top Four Animals Feed Companies. For broiler finishers, layers mash and broiler starters, the highest level of cyanide was measured in the feed samples produced by company B, being 25.74±2.13 , 13.86±1.89 and 78. 01±3.95 mg CN-kg-^{1} of feed samples, respectively.

Growers mash from company. A has the highest cyanide concentration (18.22±1.97mg CN^{-1} feed sample) among this feed group while company D feed has the least amount of cyanide (1.58±0.15 mg CN^{-1} kg^{-1} feed sample). The highest amount of cyanide measured among all these feed samples was in broiler starter from company B while the least cyanide level was found in growers mash from company D.

Nitrate and nitrite: The concentration of nitrate and nitrite found in these poultry feeds are presented in Table 2. Nitrate and nitrite were detected in all the feed samples at varying concentrations except for growers mash from company A where non detectable amount of nitrite was measured. Broiler finishers from Company D has the highest concentration of nitrate (194.60±13.14 mg NO_3-N kg feed sample) among this feed group, while growers mash and layers mash from company C have the highest nitrate concentration, (127.44±12.33 and 199.21±9.76 mg NO_3-N) for growers and layers respectively. For broiler starters the highest nitrate concentration was found in company D feed being 154.51± 10.11 mg NO_3-N kg^{-1} feed samples. The varying concentrations of nitrite measured in these feeds are also as presented in Table 2. The mean concentrations ranged from 22.01±3.57-48.65±5.67 mg NO_2-Nkg^{-1} for broiler finishers, ND-60.22±4.07 mg No-N kg^{-1} for grower mash, 9.34-53.94±2.13mg NO_2-N kg^{-1} for layers mash and 4.61±1.77-63.71±4.01 mg No_2 –N kg^{-1} feed samples for broiler starters.

DISCUSSION

The results of this study clearly indicated that the poultry feed produced by these Four Top Animal Feed Companies contain varying concentrations of cyanogens, nitrate and nitrite. The determined level of these compounds in the feeds would not cause any acute toxic effects, but they have some obvious implications.

The cyanogens levels of these feeds were below 50 mg HCN equivalent kg-1 feed (except for broiler starter from company B) recommended to be the safe level to be incorporated into animal feeds. The earliest guide for cassava safety i.e Bolhius[14] guide, gave a level below 50 mg HCN equivalent kg^{-1} as safe, 50-100 mg HCN equivalent kg^{-1} as moderately toxic and above 100 mg HCN equivalent kg^{-1} as dangerously toxic. The most consistent report on the levels of dietary cyanogens tolerated in animal feed is that from European Community

Table 1: Cyanide content of poultry feeds of four top nigerian poultry feed companies

Feed type	Company A (CN mg kg^{-1})	Company B (CN mg kg^{-1})	Company C (CN mg kg^{-1})	Company D (CN mg kg^{-1})
Broiler finisher	19.80± 2.05	25.74± 2.13	1.98± 0.13	14.65±2.33
Growers Mash	18.22±1.97	13.07± 2.09	7.92± 0.73	1.58± 0.15
Layers Mash	5.94±0.58	13.86±1.89	12.28±2.11	7.92±0.83
Broiler Starter	11.88±1.07	78.01± 3.95	24.95±2.95	3.56±0.22

Each is an average of three determinations

Table 2: Nitrate and nitrite content of poultry feeds of four top nigerian poultry feed companies

Feed type	Company A (NO_3-N mg kg^{-1}DM)	Company B (NO_3-N mg kg^{-1}DM) (NO_3-N mg kg^{-1}DM)	Company C	Company D (NO_3-N mg kg^{-1}DM)
Broiler finisher	93.85± 10.17	47.48± 5.82	120.49± 10.74	194.60±13.14
Growers mash	83.40±11.08	93.83±4.93	127.44± 12.33	97.30± 10.03
Layers mash	76.45±10.34	196.94±12.17	199.21± 9.76	94.96±8.08
Broiler starters	70.71± 8.17	121.65±11.32	148.29±11.18	154.51± 10.11
	NO_2-N mg kg^{-1}DM	NO_2-N mg kg^{-1}DM	NO_2-N mg kg^{-1}DM	NO_2-N mg kg^{-1}DM
Broiler finishers	22.0 2± 3.57	41.70± 2.89	31.30± 3.30	48.65±5.67
Growers mash	ND	60.22±4.07	39.37±2.73	32.41± 3.76
Layers mash	9.34±2.84	38.25±3.21	28.97±3.14	53.94±2.11
Broiler starters	28.97±3.93	4.61±1.77	63.71±4.01	28.97±1.88

Each is an average of three determinations

Countries where cassava has been used in livestock rations for more than two decades now. Wood (1992) stated that a level of 100 mg HCN equivalent kg^{-1} was the highest which could be tolerate in cassava chips imported into European Community countries. Panigrahi *et al.*,[15] also reported that cassava root meal may be incorporated into nutritionally balanced poultry diet without reduction in weight gain or egg production and that dietary cyanide content in excess of 100 mg HCN equivalent kg^{-1} diet appears to adversely affect broiler performance.

According to Leuschuer *et al.*,[16] repeated daily intake of more than 10.8 mg kg^{-1} Day^{-1} cyanide could result in chronic toxicity in broilers, with manifestations of anorexia, weakness, depression, stupor or excessive salivation. It is interesting to note that 62.5% of the analyzed feeds contained cyanide level above 10.8 mg kg^{-1} feed sample. Laying hens have also been reported to be affected by levels as low as 2.5 mg total cyanide kg^{-1} diet(Panigrahi *et al.*,[15]). Depressed growth and small eggs production among others were observed at Michael Okpara University of Agriculture, Umudike, Nigeria where some of these feeds were used in raising poultry birds between late 2004 and early 2005.

The presence of cyanogens in these feeds could also result in depletion of sulphur- containing amino acids of the poultry birds, since they are needed to detoxity the CN converting it to thiocyanate (SCN). This situation can further result in reduced protein synthesis and hence depressed growth since adequate amount of amino acids is one of the prerequisite for protein synthesis. Thiocyanate formed could in turn act as a catalyst for the nitrosation of residual nitrite and secondary amines endogenous in the feeds to form toxic N-nitrosamines as earlier reported[8]. The need to supplement cassava ration with methionine and cystein (sulphur- containing amino acids) has been demonstrated for monogastric species[17] and the role of these amino acids in cyanide detoxification for ruminates has also been demonstrated[18].

The mean levels of nitrate and nitrite in these feed samples are presented in Table 2. The relatively large variability in nitrate, nitrite and cyanide levels of these feeds was due to differences among the different production lots. Reports on nitrate/nitrite in livestock are numerous especially in cattle following consumption of nitrate rich food[19]. Symptoms of acute poisoning include methemoglobinaemia, vassodilation, oxygen deficiency etc. while chronic nitrate/nitrite loading can cause disturbances in iodine and vitamin metabolism, reduced weight gain and feed conversion and liver damage[19]. Nitrate/Nitrite intoxication in sheep in Mosul, Iraq was reported in the summer of 1995[20]. The lethal dose of sodium nitrate for pigs is 88mg kg^{-1} body weight[21] and doses of 48-77 mg kg^{-1} cause moderate to severe but total methe moglobinaemia[22]. Since non-ruminant animals such as poultry birds are poor in converting poisonous nitrite to ammonia, nitrite poisoning will be much more likely to occur in them. On the other hand simple stomach animals such as swine and poultry do not have microorganisms which can make rapid conversion of nitrate to nitrite, thus they are not susceptible to nitrate toxicity. In addition, in most semi-intensive and industrial poultry species, genetic progress yields breeds and hybrids capable of ever-higher meat and egg production. Due to their increased metabolic rate these breeds and hybrids are less able to tolerate feed quality defects including phytotoxin contamination.

Nitrite is known to interact with haemoglobin forming methemoglobin by oxidation of ferrous iron to ferric state, preventing or reducing the ability of blood to transport oxygen a condition known as methemoglobinaemia[23,24]. Nitrite is also involved in the formation of toxic and carcinogenic N-nitrosamines[25]. However, species difference in handling nitrate and nitrite should be considered when interpreting the risks posed to animals by these compounds.

In this study evidence for the contamination of Nigeria poultry feeds with nitrate, nitrite and cyanogens has been presented and discussed from nutritional and toxicological points of view. The findings of this study are of immerse importance since the neutralization of anti-nutritive factors or phytotoxin such as these compounds by feed processing or by efficient diet manipulation requires prior knowledge of their level or concentrations in the feed resources as well as the physiological and biochemical basis of their action.

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