Journal of Applied Biosciences (2008), Vol. 4: 103 - 107.
ISSN 1997 – 5902: www.biosciences.elewa.org

DETERMINATION OF ANTINUTRITIONAL FACTORS AND PHYTOTOXIN CONTAMINATION OF POULTRY FEEDS

OKAFOR P.N.[1*], OKECHUKWU L.M.[1] and UZUKWU M.[2]
[1]Department of Biochemistry, Michael Okpara University of Agriculture, Umudike. PMB 7267 Umuahia, Abia State, Nigeria; [2]College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria.

*Corresponding author email: pnokafor@yahoo.com

Abstract
Objective: During late 2004 to early 2005, there were outbreaks of various disease conditions resulting in liver disorders and other non-specific pathological changes in the internal organs of poultry raised in Michael Okpara University of Agriculture, Umudike Poultry Farm. Differential elimination of all possible infectious diseases left the possibility of non-infectious diseases and toxicities associated with feeds. This study was carried out to investigate the non-infectious agents.
Methodology and results: Feed samples from the University Poultry Farm and from the major animal feed companies operating in Umuahia area were analysed for toxic constituents (phytotoxins). The results identified cyanogens, nitrate, nitrite and trypsin inhibitors as possible factors that contributed to the poor health of poultry at the University farm. Other anti-nutritional factors detected in the feeds included phytates, tannins and saponins.
Conclusion and application of findings: The presence of potentially toxic compounds and anti-nutritional factors in the feeds appear to have caused reduced feed availability, protein synthesis and compromised the immune system of the birds with concomitant predisposition of the birds to various disease conditions. These findings demonstrate the need to ensure that anti-nutritional factors in poultry feeds are reduced to non-lethal thresholds through adequate processing methods.

Keywords: antinutritional factors, poultry feeds, toxins, Nigeria.

Citation: Okafor PN, Okechukwu LM and Uzukwu M, 2008. Determination of antinutritional factors and phytotoxin contamination of poultry feeds. *Journal of Applied Biosciences* 4: 103 – 107.

INTRODUCTION
In the late 2004 up to early 2005, there were outbreaks of various disease conditions resulting in liver disorder and other non-specific pathological changes in internal organs of poultry at the Michael Okpara University of Agriculture, Umudike, Nigeria. Loses due to death of animals were significant. The main cause of the disease conditions could not be immediately ascertained, since most of the pathological changes observed when the internal organs of the dead birds were opened were non-specific. Death of poultry in semi intensive farms within the university area has been sporadic and in most cases, the causes are neither known nor investigated.

Intensive investigation and diagnosis revealed that malnutrition resulting from toxic constituents (phytotoxins) of poultry feeds was the most probable cause of the aberrant situation. In the presence of some of such toxic constituents or anti-nutritional factors, (Okafor and Okorie, 2006), nutrient availability to the birds would be highly reduced with concomitant predisposition of the birds to various kinds of disease conditions. Previously, an outbreak of liver disorder including cancer in various farm animals was reported in Norway, following ingestion of herring meal that had been preserved by addition of sodium nitrite. Nitrosamines formed from the reaction between dimethylamine and the added nitrite was determined to be the cause of the liver failure (Hotchikiss & Cassens, 1987). The adverse effects of some phytotoxins or anti-nutritional factors that are usually found in poultry feeds such as cyanogens of cyanophoric plants (Leuschuer *et al.,* 1995), trypsin inhibitors in soy beans and most legumes (Zhang & Parsons, 1993), saponins (Gerendi & Gippert, 1994), phytates and oxalates, are well know. The presence of all or some of these compounds in poultry feed in amounts above safe levels can affect the nutritional status of birds and predispose them to infections. In view of this knowledge, and considering the health status of the birds, an analysis was carried out on samples of poultry feeds from the University Poultry Farm and from four major animal feed companies that market their products in Umuahia area. All samples were analyzed for nitrate, nitrite, cyanogens, trypsin inhibitors, phytates and saponins, crude protein and tannins.

MATERIALS AND METHODS

Reagents: Cadmium metal, sulphanilic acid, N- (1-Naphthyl) ethylenediamine hydrochloride, sodium tungstate, orthophosphoric acid, potassium permanganate, sodium hydroxide, sulphuric acid, calcium chloride, methyl red, bromocresol green, BAPA, trypsin and potassium dihydrogen orthophosphate used in the analysis were of analytical grade.

Methods: Nitrate and nitrite were determined according to the method of Follett & Ratcliff (1963) and total cyanogens by the kit B2 for total cyanide content of cassava (Egan *et al., 1998)*. Tannin and crude protein contents were determined as described by Kirk & Sawyer (1998). Trypsin inhibitors activity was determined by the method of Artifield *et al.* (1988) and phytates by the method of Havy & Lantzsh (1983).

Sample collection: Samples of poultry feeds from four major animals feed companies in Umuahia and the University Farm were collected for analysis. The poultry feeds included broiler starter, broiler finisher, growers mash and layers mash. Five samples were collected for each of the different feeds from different sources. The materials were taken to the laboratory immediately after collection for analysis. Extracted samples that could not be analyzed immediately were stored in the refrigerator.

Data analysis: A one-way analysis of variance was used to analyze data (Duncan multiple range test).

RESULTS

There were variations in the concentrations of cyanogens, tannins, saponins, phytates, and trypsin inhibitor activity in the various poultry feeds that were analysed (Table 1). The total cyanide content of feeds from company A ranged from 5.12-20mg kg^{-1} with the highest amount occurring in growers mash (20.20mg kg^{-1}) and the least in layers mash (5.12mg kg^{-1}). Feed from company B had cyanide in the range of 3.00-22.18 mg kg^{-1} with the highest concentration in broiler finisher (22.18 mg kg^{-1}) and the least in broiler starters (3.0 mg kg^{-1}). Feed from company C had cyanide in the range of 2.8-19.00 mg kg^{-1} while cyanide in feed from Company D ranged 3.96-8.71 mg kg^{-1}. The feed samples from the University Farm had cyanide content between the range of 7.18-21.5 mg kg^{-1}. The levels of trypsin inhibitors were between 20-138 Tiu/g in feeds from Company A, 72-516 Tiu/g in feeds from Company B, 55-101 Tiu/g for those of Company C, 50-62 for company D and 30-142 Tiu/g for the University Farm feeds.

Variations were also detected in the concentrations of nitrate, nitrite and crude protein content of the different feed samples analyzed (Table 2). The concentrations of nitrite were between 28.97-63.71 mg NO_2-N kg^{-1} in feed from Company A, 4.61-60.22 mg NO_2- N kg^{-1} for company B feed, ND-28.97 mg NO_2- N kg^{-1} for Company C feeds, and 28.97-53.94

mg No_2-N kg^{-1} for Company D feed and 25.33-63.54 mg NO_2-N kg^{-1}in feeds from the University Farm. Total crude protein were between 39.90-45% in Company A feeds, 39.20-46.55% in Company B feeds, 39.55-46.55% in Company C feeds, 39.90-46.10% in Company D feeds and 39.80-44.10% in the University Farm feeds.

Table 1: Content of anti-nutritional factors in poultry feeds from five sampling sources in Umuahia, Nigeria.

	Feed Type	Cyanogens Mgkg-1	Trypsin Inhibitor Tui/g	Tanin %	Saponin %	Phytate
Company A	B/Starter	8.32	40.06	0.07	1.4	0.24
	B/finisher	14.26	138.0	0.14	2.5	0.40
	G/mash	20.20	20.0	0.06	1.4	0.32
	L/mash	5.12	84	0.03	3.25	0.44
Company B	B/Starter	3.00	159	0.07	3.8	0.35
	B/finisher	22.18	72	0.12	3.0	0.37
	G/mash	10.30	516	0.17	2.88	0.32
	L/mash	12.67	126	0.10	1.52	0.42
Company C	B/Starter	5.94	101	0.08	3.14	0.18
	B/finisher	10.70	65	0.09	1.90	0.25
	G/mash	2.50	55	0.08	2.52	0.35
	L/mash	19.00	87	0.07	1.66	0.40
Company D	B/Starter	7.52	50	0.05	1.44	0.36
	B/finisher	8.71	60	0.02	1.92	0.27
	G/mash	3.96	62	0.19	1.60	0.25
	L/mash	7.13	60	0.13	3.78	0.28
MOUAU	B/Starter	7.18	46	0.07	1.4	0.24
	B/Starter	21.5	142	0.13	2.2	0.41
	G/mash	10.32	30	0.06	1.2	0.32
	L/mash	14.33	82	0.04	2.24	0.44

B/starter= Broiler starter; B/finisher= Broiler finisher; G/mash = growers mash; L/mash = Layers mash; MOUAU= Michael Okpara University of Agriculture, Umudike.

Discussion

The results of this study indicated the presence of cyanogens in all the samples analyzed. The presence of cyanide in these feeds is not unexpected since cyanophoric plant materials such as cassava, sorghum, rubber seeds, and wheat offal are common components during poultry feed formulation in Nigeria (Okafor & Nwabuko, 2003). According to Leuscher *et al.* (1991), repeated daily intake of more than 10.8mg kg^{-1} CN equivalent Day^{-1} could result in chronic toxicity in broilers, with manifestation of anorexia, weakness, depression, stupor or excessive salivation. Laying hens can also be affected by levels as low as 2.5mg total cyanide kg^{-1} diet (Panigrahi *et al., 1996).*

Cyanogens in feeds could also result in depletion of sulphur-containing amino acids in the birds since these amino acids are needed to detoxify the CN, converting it to thiocyanate (Nagahara *et al*., 1999). This situation could further reduce protein synthesis and hence affect the immune system, leading to depressed growth. Moreover, cassava cyanide has been reported to affect the antioxidant status of animals particularly glutathione (Okafor *et al*., 2007), that play crucial roles in ameliorating the damaging effects of free radicals on the organs and tissues of the body. Thiocyanate formed could in turn act as a catalyst for the nitrosation of residual nitrite and secondary amines to form nitrosamines.

Nitrate and nitrite were detected in all the feed samples except in growers mash from Company C where nitrite was non-detectable. Reports on nitrate/nitrite toxicity in livestock are numerous especially in cattle, following consumption of nitrate rich food (Birnbreier, 1997). Symptoms of acute poisoning include methemoglobinaemia, vasodilatation and oxygen deficiency, while chronic nitrate/nitrite loading can disturbe iodine and vitamin metabolism, feed conversion and weight gain and liver damage (Brirnbreier, 1997). Since non-ruminants, e.g. poultry are poor in converting poisonous nitrite to ammonia, nitrite poisoning is much more likely to occur in them. In addition, breeding for semi-intensive and industrial scale farming results in breeds and hybrids that are capable of ever-higher meat and egg production. Due to their increased metabolic rates, these breeds and hybrids are less able to tolerate feed quality defects including phytotoxin contamination.

Table 2: Nitrate, nitrite and crude protein content in poultry feeds from five sources in Umuahia, Nigeria.

	Feed type	NO_2-N (mg kg^{-1})	NO_3-N (mg kg^{-1})	Crude protein %
Company A	B/starter	63.71±3.07*	146.21±12.10	39.90.
	B/finisher	29.30±2.27	124.02±12.31	44.10.
	Grower's mash	40.28±3.43	125.43±13.12	41.30
	Layer's mash	28.97±3.44	200.13±14.03	45.50
Company B	B/starter	4.61±0.73	124.65±13.32	39.29
	B/finisher	39.71±3.89	46.53±3.82	43.40
	Grower's mash	60.22±2.07	96.8 ±12.11	42.00
	Layer's mash	35.25±3.01	197.94±14.23	46.55
Company C	B/starter	28.97±1.93	70.33±7.31	39.55
	B/finisher	19.02±3.57	93.11±8.77	43.40
	Grower's mash	ND	79.40±3.44	41.65
	Layer's mash	8.43±2.71	73.22±6.73	46.55
Company D	B/starter	28.97±2.52	157.31±11.22	39.96
	B/finisher	43.56±3.61	197.53±14.22	44.10
	Grower's mash	29.43±2.33	93.70±9.03	41.30
	Layer's mash	53.94±3.12	91.96±7.32	46.10
MOUAU	B/starter	25.33±3.07	94.11±3.10	39.80
	B/finisher	37.22±2.88	102.32±10.47	43.10
	Grower's mash	56.24±3.44	86.43±8.77	41.20
	Layer's mash	63.54±5.02	99.45±8.37	44.10

*Each mean is based on three independent analyses.

Trypsin inhibitors were also found in all of the analyzed feeds. Feeding raw soybeans is well known to cause growth depression, poor feed efficiency, and pancreatic enlargement in young chickens and also small egg size in laying hens (Chubb, 1982). These effects are due to trypsin inhibitors in soybean that reduce digestibility of protein (Zhang & Parsons, 1993). Trypsin inhibitors inhibit the activities of the proteolytic enzymes that require trypsin for activation. Growth depression in chicks due to slow release of all essential amino acids by proteolytic enzymes in the presence of trypsin inhibitors has been reported (Chubb, 1982). Other antinutritional factors found in the feeds were saponins, tannins and phytates, all of which reduce feed availability to animals depending on concentration.

The analyzed feeds also contained considerable amounts of crude protein. Though the amounts of crude proteins in these feeds are quite high, it appears that the presence of these anti-nutritional factors or phytotoxins reduced their availability to the birds. The results from this study point to cyanogens, nitrate/nitrite and trypsin inhibitor as the most likely causes of the aberrant situation witnessed in the University Farm during late 2004 to early 2005. The presence of these compounds and other anti-nutritional factors identified in the feeds appear to have caused reduced feed availability and protein synthesis (including immune system) with concomitant predisposition of the animals to various disease conditions. Nitrosation of nitrite, probably from fishmeal, and secondary amines could also lead to the formation of N-nitrosamines, which are potent carcinogens as well as toxicants.

It is also likely that the poultry breed at the university farm has less capacity to tolerate feed quality defects including phytotoxin contamination.

REFERENCES

Arntfield SD, Ismond MAH and Murney ED, 1980. The fate of anti-nutritional factors during the preparation of fababean protein isolates using a micellization technique. Canadian Institute of Food science Technology Journal 8 (2): 137 – 143.

Birnbreier E, 1997. Evaluation of knowledge concerning the nitrate/nitrite loading of livestock to derive tolerance concentration in drinking water. 259 pp.

Chubb LG, 1982. Ant-nutritional factors in animal feed stuffs. Pages 21 – 37. In: Recent Advances in Animal Nutrition. Ed. William Harasign Butterworth.

Cierendai D and Gippert T, 1994. The effect of saponins content of alfalfa meal on the digestibility of nutrients and on the production traits of tetra-SL. Layers. Proceeding of 9th European Poultry. Conference, Glasgow. U.k. 2: 503 – 504.

Egan SV, Yeoh HH, Bradbury H, 1988. Simple picrate paper kit for determination of cyanogenic potential of cassava flour Journal of the Science of Food and Agriculture 75: 258 – 262.

Follet MJ and Ratcliff PW, 1963. Determination of nitrate and nitrite in meat products Journal of the Science of. Food and Agriculture 14: 138 – 144.

Havy W and Hans-Joachin Lantzsch, 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. Journal of the Science of Food and Agriulture 34 (12): 14 23-1426.

Hotchikiss JH and Canssens RG, 1978. Nitrate, nitrite and nitroso compounds in foods. Food Technology 41: 4.

Kirk RS and Sawyer R, 1998. Pearsons composition and analysis of foods. 9th edition pp. 360 – 361. Churchill Livingstone Edinburgh.

Leushuer J, Winkler A, Leushuer F, 1995. Toxicokinetic aspect of chronic cyanide exposure in rats. Toxicology Letters 57: 195 – 201.

Nagahara NT, Ito Minani M, 1999. Mercaptopyruvate sulphur transferase as a defence against cyanide intoxication: molecular properties and mode of detoxification. Histology and Histopathology 4: 1277 – 1286.

Okafor PN and Nwabuko CU, 2003 Cyanide, Nitrate and Nitrite Contents of Livestock feeds in Umuahia, Nigeria. Biokemistri 13 & 14: 57 – 63.

Okafor PN and Okorie CN, 2006. Determination of Nitrates and Nitrites of Poultry Feeds Marketed in Nigeria. Journal of Food Technology 4 (3): 225 - 2

Okafor PN, Anyanwu VO, Onyema HO, 2006. The effect of cassava cyanide on antioxidant (glutathione) status and some clinically important enzymes of rats. Journal of Pharmacology and Toxicology (New York) 1: (1) 40 - 46

Panigrahi S, Kurup GT, Palaniswami MS, Polty VP, Padmaja G, Kabeerathuma S, Pillai SV, 1996. A review of the potential for using cassava root meal in poultry diets. Tropical Tuber Crops; Problems, Prospects, and Future strategies. 1996: 416 - 428.

Zhang Y and Parsons CM, 1993. Effect of extrusion and expelling on the nutritional quality of conventional and kunitz trypsin inhibitor - free soybeans. Poultry Science 72: 2299 - 2303.