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INVESTIGATION OF STEROID CONTENTS IN POULTRY CHICKEN AND FEEDS IN SELECTED POULTRY FARMS IN PORT HARCOURT, NIGERIA

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Abstract
Three different poultry feeds and chicken samples (broilers, old layers, and native) were screened for steroid hormones using Gas Chromatography-Mass Spectrometry. The target compounds were first extracted by ultrasonication (US EPA 1698); 10 g of the sample was homogenized with the same mass of anhydrous Na_2SO_4, and a 30 mL aliquot of a 1:1 dichloromethane (DCM)/n-hexane mixture was added to the homogenate. The mixture was ultrasonicated at 35 °C for 15 min. The process was repeated twice with fresh portions of the solvent mixture on the residue and finally analysed using gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis detected three (3) steroids in the poultry chicken samples, including cortisone, cortisol, and tetrahydrocorticosterone (THS). Cortisone showed concentration (µg/g) values of 0.74 ±0.2 and 0.89±0.01 in the broilers and layers chicken, whereas cortisol showed a mean concentration value of 0.92±0.2 in the broiler chicken only. THS was detected only in the layer chicken with a mean concentration of 0.23±0.21. Seven (7) steroids were detected in the feed samples. Steroids were not detected in the native chicken. The work proclaims that poultry feed is the source of the steroids detected in the meat and some may have been completely metabolised. The detectable steroid hormone levels in chicken were lower than the normal concentrations in humans and are not a consumer health risk as the steroids do not bioaccumulate in the body due to metabolism by the liver and kidney.

INTRODUCTION
In the modern period, poultry production has become one of the most important agricultural industries. Unquestionably, Nigeria's economy is significantly influenced by the chicken business, one of the fastest-growing

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segments of the agricultural industry (Burker and Saeed, 2014). According to Mahesar *et al*. (2010), poultry is an example of a reasonably priced, delicious, and nutrient-rich dietary protein. Broilers are chickens (*Gallus domesticus*) raised and bred specifically for meat production, while layers lay eggs (Kruchten, 2002). The behavioural repertoires of broiler chickens and other gallinaceous birds are similar because they belong to the same family as egg-laying hens. As an alternative source of animal protein, broiler chicks are currently the world's fastest-growing poultry (FAO 1994).

Oluyemi and Robert (1979) noted that broiler feeding has been recognized as providing a method for quickly transforming animal protein. Broilers are among the finest meat producers due to their capacity for rapid weight gain. One of the characteristic features of chicken agriculture that provides animal protein over human consumption is the quick cycle of broiler chick development (Edney *et al*., 2014). Furthermore, it is easy and practical to distribute broiler chicks throughout a wide area. The poultry industry experienced an astounding boom from the beginning of the 1960s to the mid-1980s. Nigeria supplied African blacks with a significant number of chickens in 1986 (Okunaiya, 1986). Poultry products are among the most important foods consumed by individuals of various racial backgrounds, religious beliefs, and socioeconomic classes in Nigeria. According to Afolayan and Afolayan (2008), over seventy percent of the cost of producing hens and broilers goes toward feeding. Therefore, attempts to increase productivity within the poultry industry should concentrate on improving feed quality.

Poultry feed refers to food for farm animals, such as chickens, ducks, and other domestic birds. Feed for chickens must include grains. The season, age, weight, and nutritional requirements of the poultry influence the quality of the feed. Nutritional quality significantly influences both the growth and performance of poultry chicks. Poultry production is an essential part of the food chain. Healthy poultry needs a suitable amount of protein, carbs and necessary vitamins and minerals in their diet, as well as water for maximal development, upkeep, and output (Addass *et al*., 2010). Rearing chickens incurs a high feed cost.

In many countries, growth-promoting steroids have been successfully used to hasten the growth of animals, especially cattle. The use of stilbenes was likewise determined to be prohibited by scientific consensus due to their potential to cause human malignancies. Although the EU has banned growth-stimulating hormones, consumers have not widely accepted most of these medications. Owing to this EU regulation, various countries now have a robust illicit market for hormone arrangements, including potentially hazardous synthetic steroids and corticoids. The increase in weight gain in steroid-treated rats was associated with net protein accumulation and retention, without obvious changes in the digestion of intake (Scarth *et al*., 2009). The main ingredients of poultry diets are a variety of feedstuffs, including cereal grains, soybean meal, animal by-product meals, lipids and premixes of vitamins and minerals (Longe, 1984; Alimon and Hair-Bejo, 1995). They are referred to as complete feed because they are full of all the nutrients needed for development, egg production, and overall body health (Bale *et al.*, 2002). A poultry diet should include enough energy as well as the three basic components of protein, vitamins, and minerals. (Larbeir and Leclercq, 1994). A high-energy-content diet promotes fast growth in broilers; therefore, their metabolized energy contents should generally not be less than 12.2 MJ/kg of feed (Whitehead *et al*., 1985). Broiler diet is the type of feed fed as a complete feed to meat-type birds. It may be in the form of crumbles or pellets. The diet is usually given to chickens within the first 2–3 weeks. To enhance the energy content of the diet, 3-5% extra fat may be added, and the amount of protein may be changed to preserve the ideal protein-to-calorie ratio. Because they include all the nutrients required for the growth, production, reproduction, and health of birds, poultry feeds are often referred to as balanced diets. Protein sources are the next most common item in poultry diets after energy sources. Today, genetically modified (GMO) soy and corn make up most of the crops farmed in the US. Scientists employed genetic engineering techniques to transfer genes between organisms, aiming to enhance resistance to pesticides, herbicides and diseases aa well as boost crop yields (Key *et al*., 2008; Kruft**,** 2001); however, this process has unintended consequence of altering the genetic make-up of the ingredients. Recently, the growing of these genetically modified plants has increased, with GMO soy accounting for 93% of domestic production and 77% of global production (GMO Compass, 2010); (USDA, 2013). According to the USDA (2013), 85% of the total corn grown in the United States is genetically engineered. The health and safety impacts of consuming genetically modified foods in human diets have been the subject of published studies. While a number of these investigations (Aeschbacher *et al*., 2005; Taylor *et al*., 2003; Taylor *et al*., 2007; Cromwell *et al*., 2002; Jennings *et al*., 2003) found no unanticipated harm or risk linked with eating foods made from GMO crops, others indicated some concerns (Nordlee *et al*., 1996; Vecchio *et al*., 2009).

Some poultry farms and/or feed producers enhance the chicken feed with hormones mixed as hormonal contraceptives to quickly boost the broiler and layer chicken growth and reproduction and make the chickens readily available. There is a huge demand for protein from chicken sources in Nigeria; therefore, there is a need to ensure that there are no adverse impacts of the chicken growth-boosting hormones on chicken meat consumers.

MATERIALS AND METHODS

Sample Collection and Preparation

Nine (9) chicken and nine (9) poultry feed samples were used in this analysis. Three broilers, three old-layer chickens, and their feed samples were purchased from three different poultry farms. The other three native fowl were purchased from three different markets in Port Harcourt, Rivers state, Nigeria. The nine (9) chicken samples were slaughtered and divided into three groups consisting of the broiler, layers, and native chicken groups. The broiler group had the thighs of the three broiler chickens were blended, mixed, and combined to give one sample. This procedure was repeated for the layer and native chicken. 10g of each chicken and feed samples were homogenised using 10g of anhydrous Na_2SO_4.

Steroid extraction

Using the US EPA Method 1698, 10 g of each sample was homogenised by stirring the solid samples in a container containing 10g of anhydrous Na_2SO_4. The homogenate was then added into a beaker containing a 30 mL volume of a 1:1 dichloromethane (DCM)/n-hexane mixture and placed in an ultrasonic bath, which was ultrasonically processed for 15 minutes at 35 °C. The procedure was performed twice, each time using new amounts of the mixture of solvents on the residue. The extracts were combined, passed through a 0.45 m glass microfibre filter, and then rotary evaporated to a concentration of 2 mL before being cleaned up in a silica gel (4.0 g) filled column. The steroids were extracted from the column using 30 mL of a 1:1 DCM/n-hexane combination, and the eluate was boiled down to 1 mL under a slowly running stream of nitrogen gas before running a GC-MS on it.

Chromatographic analysis GC–MS Analysis

A model 6890 gas chromatograph (GC) from Agilent Technologies, Inc. was used to examine the samples, together with a model 5975 mass analyzer (MS), a quadruple mass filter, and a model 7683 autosampler. The GC was fitted with an HP5MS 30 m 9 0.25 mm straight column from Agilent (USA), with an internal diameter of 0.25 mm, a stationary phase of 5% phenyl with 95% dimethyl polysiloxane, and a film thickness of 0.25 m. The oven temperature of the GC-MS was programmed as follows: 20 minutes at 120 ^{o}C, then a 15 ^{o}C/min ramp up to 250 ^{o}C, and a 5 ^{o}C/min climb to 300 ^{o}C which was maintained for 5 minutes. With an injection volume of 1.0 lL, the injector was operated in split-less mode at 300^{o}C. The carrier gas was helium (99.999%, INFRA), flowing at a steady rate of 1.0 mL/min. By using an ionizing source at 200^{o}C and electron impact (EI) at 70 eV, mass spectra were produced. The division and identification of chemicals were optimized using mass scanning in SCAN mode.

RESULTS AND DISCUSSION

Results

Figure 1 shows the results of the steroid analysis conducted on various types of chicken meat and feed. The samples include broilers meat (sample A), old-layer meat (sample B), and native chicken meat (sample C), as well as the egg-laying chicken feed (sample D), broiler chicken feed (sample E) and the growers chicken feed (sample F). The chart (Figure 1) shows that two (2) steroids were detected in samples A and B, whereas no steroid was detected in sample C (native chicken meat). In Sample D, E and F seven (7) steroids were each detected.

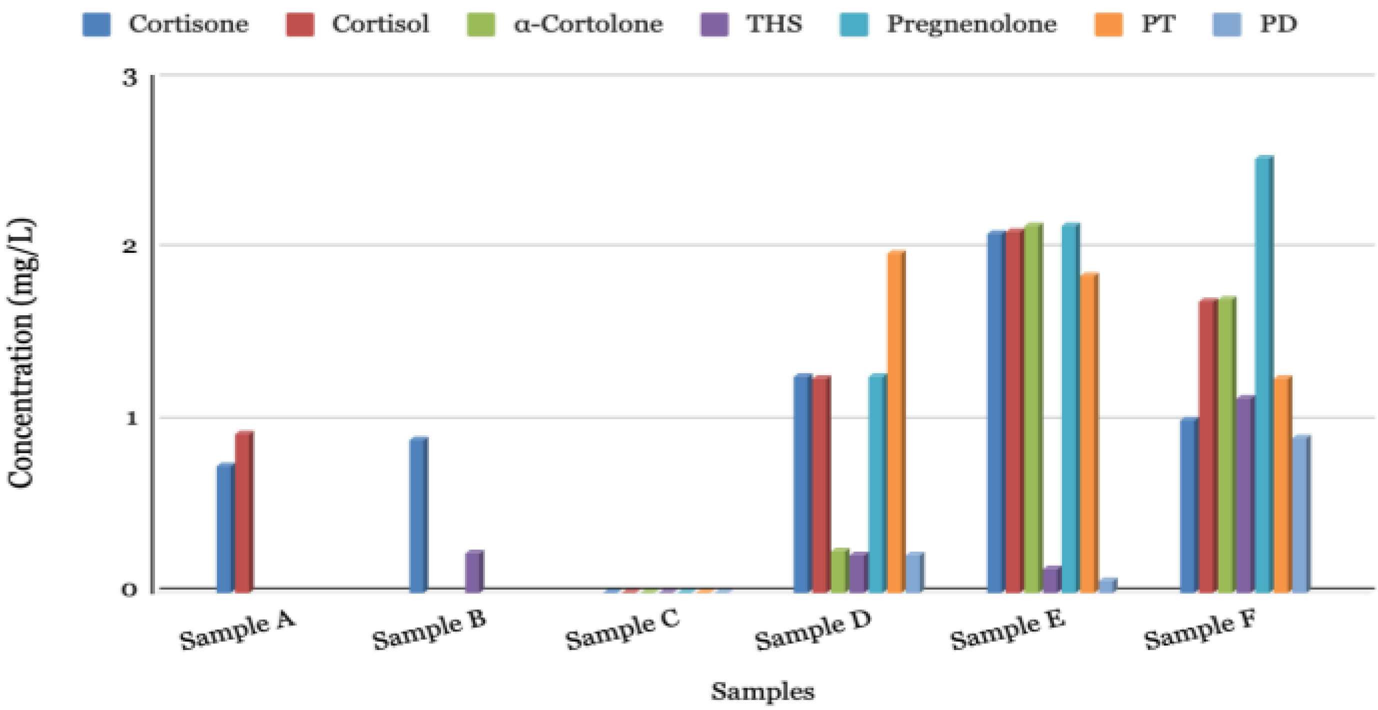

Figure 1: Concentrations of various steroid hormones present in different chicken meat and feed samples.

Discussion

Generally, fewer steroids were detected in the chicken samples than in the feed samples. This could be due to the steroid metabolism in the chickens. Seven steroids, namely cortisone, cortisole, α-cortolone, Tetrahydrocorticosterone (THS), pregnenolone, Progesterone (PT) and Prednisone (PD), were detected in each of the feeds at different concentrations. Broiler feed had the highest concentrations of cortisone (2.09 mg/L), cortisol (2.1 mg/L) and α-cortolone (2.19 mg/L) and the least concentration of THS and PD at 0.19 and 0.07 mg/L, respectively. It also registered a high concentration of pregnenolone and PT (2.19 mg/L and 1.85 mg/L) but a little lower than their respective highest records in growers (2.53 mg/L) and old-layer's (1.97 mg/L) feeds. The highest THS concentration was recorded in the grower feed (1.13 mg/L).

The average concentration of the hormones was highest in the broiler's feed, followed by the grower's and then the old-layer's feeds.

The chicken feed is the most likely source of steroids in chicken, as no steroids were detected in the locally reared native chickens. Two steroids each were detected in the broiler's meat cortisone (0.74mg/L) and cortisol (0.92mg/L) and in the old-layer's meat, cortisone (0.89mg/L) and THS (0.23mg/L). These concentrations are lower than the concentrations in their feeds, except for the THS, which is 0.22 mg/L in the old-layer's feed. As it is in their feeds, the combined average hormone content in the broiler meat is higher than in old-layer meat.

Some detected hormones are synthesised in the human biological system and are required for different body functions. Cortisol, for example, is usually produced and released by the adrenal glands and is responsible for the body's regulatory mechanisms for maintaining homeostasis in response to stress (de Kloet & Meijer, 2024). Generally, corticosteroids have been employed in the management of chronic pain (McEwen & Kalia 2010) and in recent times, used as immunosuppressants and anti-inflammatory drugs for the management of COVID-19 symptoms (Singh *et al*., 2020; Alexaki, & Henneicke, 2021).

In this work, the average concentrations of the hormones detected in the chicken, cortisone 0.83 mg/L and cortisol 0.89 mg/L, were lower than their respective natural reference concentrations in humans, 2.47 and 12.28 mg/L (Eisenhofer, 2017)

CONCLUSION

Steroid contents in chicken feed and meat were successfully analysed using Gas Chromatography-Mass Spectrometry. This research shows that poultry feeds contain hormones and are directly responsible for the hormones found in chicken meat. Because the concentrations of some of the steroids found in chicken meat are lower than their average natural concentration in humans, we ultimately conclude that detectable steroid hormone levels in poultry chicken and feeds were not a consumer health risk as the steroids do not bioaccumulate in the body due to metabolism by the liver and kidney.

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