Sarcouncil Journal of Multidisciplinary

ISSN(Online): 2945-3445

Volume- 02| Issue- 01| 2022

Review Article **Received:** 06-01-2022 | **Accepted:** 15-01-2022 | **Published:** 26-01-2022

Pathological & Physical effects of Steroidal Hormones on Consumers Poultry

Usman Shahid[1], Huma Naz[2], Sidra Riaz[3], Omair Ali[4] and Faisal Arshad[5]

[1]*University of Sargodha, Pakistan*

[2]*GC University, Faisalabad, Pakistan*

[3]*Drug testing laboratories, Healthcare Department, Lahore*

[4]*University of Agriculture, Peshawar, Pakistan*

[5]*Abasyn University, Islamabad, Pakistan*

Abstract: The steroidal hormones have overall both good and negative impacts on the chickens as well as on the consumers. The steroidal hormones discussed under the article includes Vitamin D3, Boldenone, 17 beta-Estradiol, corticosterone, Testosterone, Corticotrophin, Vitamin E and Corticosterone. The vitamin D3 helps in mediating the immune response in broiler chickens. It was also seen to combat diseases in chickens by the help of b-defensin gene which was mainly increased by the VD3. IGF1 mRNA expression also upregulated in the group subjected to Vit D3. The tibia length was also seen to be longer in chickens treated with VD3. 25-hydroxycholecalciferol (25 (OH) D3) is a metabolite of VD3 and it proved to be more useful than the VD3. By the use of 25-hydroxycholecalciferol (25 (OH) D3), there is betterment in the hatchability of the eggs. It also improves pectoral muscle development. The next hormone is boldenone. Boldenone increases red blood cell production in the chickens. But on the other hand, it decreased leukocyte count when it was given to the chickens. It also increases agranulocytosis process which characterizes the inflammation. Liver and kidney was seen to be inflamed by its administration in the chickens. The increased value of ALT, ALP and AST in chickens administered with boldenone shows clear sign of liver damage. It also didn't prove beneficial for the kidneys because it increases the creatinine values which is a waste and it directly affects the kidneys. High dose of genistein, when given to chicks either by intraperitoneal injections or food supplements, shows reduced growth. Serum levels of albumin was also decreased. It also reduces villus height but cause increased abundance of pathogenic bacteria in ilium. Therefore, it effects hypothalamic RNA profile. Further, it also has influence on apoptosis, the immune process and synthesis of steroid hormones in the hypothalamus. The effects of 17B-estradiol are worse, cause's hepatocellular carcinoma and also effect lactating mother. It is given orally or in the form of I/M infusion. By using this hormone for enhancement purpose, it may lead to deposit of residues which, if ignored, causes serious threats to human. I/M infusion of this hormone causes highest concentration in kidneys followed by liver then muscles but can be reduced by oral organization. Parenteral injections of testosterone decrease the hatchability proportion when given to chickens. Testosterone is also responsible for increasing chicken weight. Due to testosterone there is also an increase in length of chicken. There is also an increase in plasma protein level, calcium and phosphorus and if they increased drastically, it may lead to death. Testosterone helps in regulation of ALP concentration. We can see a decrease in level of cholesterol as well. Ovulation rate, Eggshell weight, and eggshell density were extensively greater in those chicken in which testosterone is injected. Corticosterone has also produced effects on broiler performance, immune function, plasma biochemistry, related gene expressions and cell death morphology. When treated with corticosterone we saw a decrease in thyroid hormone and increase in metabolism, physique, body temperature and heart rate. The relative weights of thymus, bursa and spleen is lowered by means of the CORT therapy with low counts of Total white blood cells and decreased stimulation of T and B lymphocytes. This indicates an immunosuppressive role of corticosterone. There is a decrease in growth performance and increase in liver weight when corticosterone therapy is given.

Keywords: Steroidal Hormones, Vitamin D3, Boldenone, 17 beta-Estradiol, Testosterone.

INTRODUCTION

The use of steroidal hormones for obtaining a good quality meat from poultry chickens has been a common practice in recent years .The use of these hormones is almost prohibited world-wide but some infrerior countries where there is no check and balance for this activity, the poultry farmers use these hormones to benefit themselves economically and creates a disaster for local people. Most of the time these hormones are given parentrally by breeders to chickens without any prescription by veterinary doctor. The steroidal hormones like testosterone; Progestrone, Boldenone, etc. have been commonly used for beneficial purposes. These hormones increase the tenderness, juiciness and proportion of the meat but at the same they have savage effects on the biochemical and physical aspects of the chicken. Nutritional supplementation with Vit D3 is becoming popular in animal husbandry, using 25-hydroxycholecalciferol (25 (OH) D3), a metabolite of Vit D3, as its efficacy and cost are better than those of Vit D3. When in ovo 25 (OH) D3 was dissolved in vaccine diluent buffer and injected into broiler eggs, hatchability was improved, but no adverse effects on growth were seen. it was also showed that dietary supplementation of 25 (OH) D3 improved pectoral muscle development in broiler chicks. (Hayakawa. *et al*., 2019).Vitamin D3 is injected in broiler chickens indicated that VD3 is useful for mediating the immune response only when animals are exposed to immune stressors via synergistically upregulating chicken b-defensin gene expression to resist diseases in chickens. (Lu. *et al*., 2015)**.** The supranormal levels of RBC parameters (number, Hct and Hgb concentration) calculated by automated cell counter indicated that boldenone administration (Steroidal hormone) enhanced erythropoiesis

***Corresponding Author:** Usman Shahid

processes. It was also found that anabolic steroids stimulated erythropoiesis by increasing erythropoietin-stimulating factor. Total leuckocytic count decreased as a result of the decreased number of lymphocytes (lymphopenia), which constituted the major percentage of the differential count (Elmajdoub. *et al*., 2016). Chicks exposed to high-dose genistein by intraperitoneal injections and feed supplementation both showed a reduced body weight gain and feed intake in comparison with the control group. In comparison with the control, serum levels of albumin and total protein were decreased after high-dose genistein injection and diet supplementation (Zengpeng, L. *et al*., 2019). The massive meat production of broiler chickens make them continuously exposed to potential stressors that stimulate releasing of stress-related hormones like corticosterone (CORT) which is responsible for specific pathways in biological mechanisms and physiological activities. When broilers are treated with corticosterone injection there is a decrease in plasma concentration of T_3, which is a thyroid hormone. It affects almost every physiological process in the body, including growth and development, metabolism, body temperature, and heart rate (Gamal, M. K. *et al*., 2017). The detailed effects of steroidal hormones in chicken are discussed:

Vitamin D3

The VD3 only upregulates the expression of endogenous HDPs when the host was exposed to inflammatory challenge. Furthermore, VD3 was shown to inhibit the expression of inflammatory cytokines .In conclusion, when vitamin D3 injected in broiler chickens indicated that VD3 is useful for mediating the immune response only when animals are exposed to immune stressors via synergistically upregulating chicken b-defensin gene expression to resist diseases in chickens. Further studies are required to determine whether VD3 can modulate the expression of chicken b-defensin genes in vitro, as well as reveal the mechanisms of how VD3 modulates chicken b-defensin genes. (Lu. *et al*., 2015). Nutritional supplementation with Vit D3 is becoming popular in animal husbandry, using 25-hydroxycholecalciferol (25 (OH) D3), a metabolite of Vit D3, as its efficacy and cost are better than those of Vit D3. When in ovo 25 (OH) D3 was dissolved in vaccine diluent buffer and injected into broiler eggs, hatchability was improved, but no adverse effects on growth were seen .It reported that 25 (OH) D3 dissolved in vaccine diluent buffer, did not negatively affect growth performance after hatching, while the hatching rate in the 25 (OH) D3-treated group was higher than in the control group, suggesting the possibility of improving hatching rates by administration of 25 (OH) D3. In addition, it was also showed that dietary supplementation of 25 (OH) D3 improved pectoral muscle development in broiler chicks. There was no significant difference in body weight, liver weight, and pectoral muscle weight in the present study, though interactions were observed between treatments and sexes in tibia length. The tibia length of males in the Vit D3 with oil group was significantly longer than that of males in the control group ($P<0.05$). The effect of sexes was not observed in the results of hepatic IGF-1 mRNA expression, but differed significantly among treatments ($P<0.05$). IGF1 mRNA expression was significantly higher in the Vit D3 with oil group than in the control group ($P<0.05$). These changes in IGF-1 mRNA expression were therefore similar to the changes observed in tibia length in male chicks. The mRNA expression levels of IGF-1 receptor in the shallow pectoral muscle, which is an indicator of growth, increased only in females, while in the liver, the expression levels of IGF-1 mRNA increased in the area of Vit D3. Although multiple functions of Vit D3 are reported, the detailed mechanisms involved in this phenomenon remain unknown.From this, it is considered that sex steroid hormones may be responsible for sexual dimorphism in chickens.The vitamin D receptor is thought to be involved in all vitamin D functions.This receptor is regulated by 1,25-dihydroxy-vitamin D3 and 25 (OH) D 3, though some of the regulatory mechanisms which govern this are unclear (Hayakawa. *et al*., 2019).VD3 deficiency reduced egg production and egg quality. VD3 deficiency increased serum hormone (calcitonin, parathyroid hormone, estradiol, and progesterone) and cytokine (IL-6, IL-10) levels, the ratio of IFN-γ to IL-4, myeloperoxidase activity and total IgG content in the serum, and upregulated the blood CD3+ T cell population. Splenic retinoid X receptor (RXR), nuclear factor-κB (NF-κB), inducible nitric oxide synthase (iNOS), and polymeric immunoglobulin receptor (pIgR) gene mRNA levels were upregulated in VD3-deficienct hens. VD3 deficiency significantly reduced serum Follicle stimulating hormone (FSH) and Luteinizing hormone (LH) concentrations and the number of CD4+CD25+ T cells in the blood. These changes were completely normalized by VD3 sufficiency. LPS reduced serum LH concentration, splenic lysozyme, and pIgR gene

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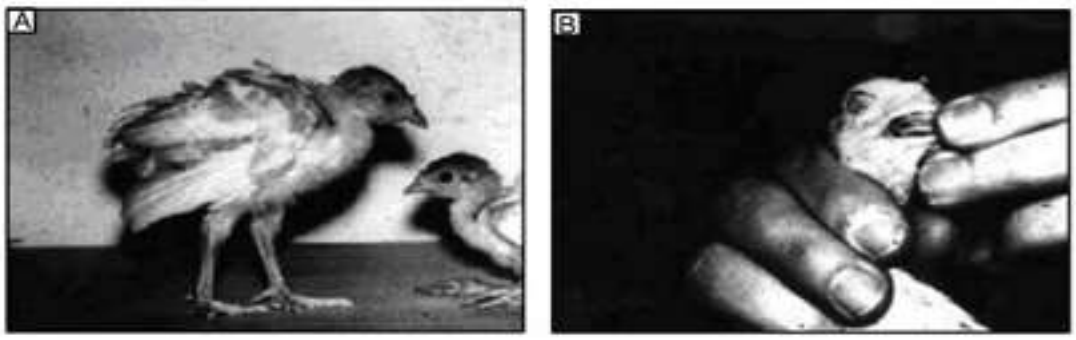
mRNA levels. LPS induced an increase in total serum IgM levels and the percentage of CD8+ T cells in the blood. The changes were completely reversed by VD3 addition. (Geng et *al*., 2018).



A: Poults received adequate vitamin D and exhibited normal beaks.
B: Beak is rubbery due to vitamin D deficiency.

Figure 1

Boldenone

The supranormal levels of RBC parameters (number, Hct and Hgb concentration) calculated by automated cell counter indicated that boldenone administration enhanced erythropoiesis processes. It was also found that anabolic steroids stimulated erythropoiesis by increasing erythropoietin-stimulating factor. Total leuckocytic count decreased as a result of the decreased number of lymphocytes (lymphopenia), which constituted the major percentage of the differential count. In contrast, the number of granulocytes exhibited significant increases (granulocytosis). Increased numbers of granulocytes are an indicator of inflammation caused by boldenone in the liver and kidney, as indicated by biomarkers, as well as histopathology. Decreased numbers of lymphocytes despite the increased globulin concentration in this study may have indicated that the of globulin concentration could be attributed to the increased levels of and b-fractions of globulins rather than the g-fractions.The former are biomarkers of inflammation, while the latter are biomarkers of immunity increment**.** ALP, ALT, and AST activity values at the tested dose of boldenone were significantly increased as compared to those of the controls. ALT is a cytoplasmic enzyme, and its increased level in plasma is an indication of mild injuries caused by the drug to the liver, while AST is a mitochondrial enzyme whose increased activity in plasma reflects severe hepatic-tissueinjury[. It should be noted that although ALP is formed mostly in the liver, it is nonspecific to hepatic injury, as it is also formed by other tissues, such as bone, kidney, skeletal muscle, and placenta.

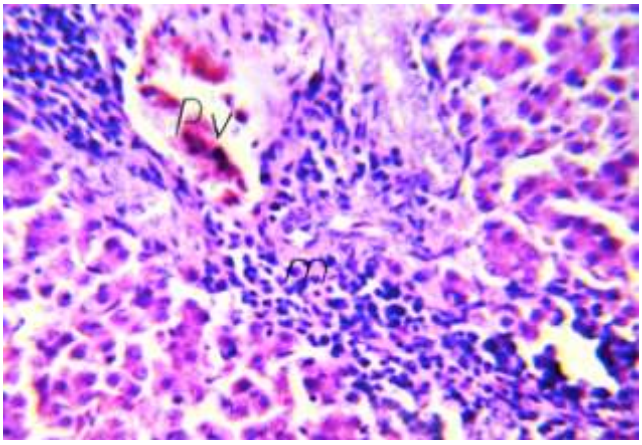


Figure 2: Effect of Boldenone administration on liver cells of broiler chicken

Degenerative changes in the liver tissue shown in may support hepatic injury caused by boldenone administration**.** Oral administration may be more stressful on the liver relative to injection due to the first-pass effect.The significant increase in urea and creatinine values are also observed after

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boldenone injection suggested that the drug might cause renal damage. This biochemical result confirms the histopathological alterations recorded in kidney samples taken from the tested group (Figure 3).

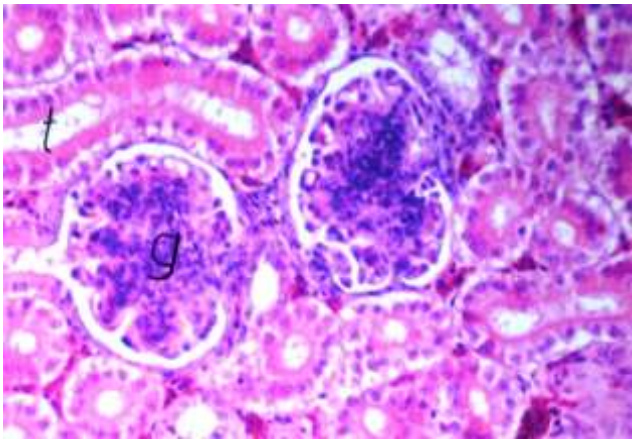

Figure 3: Kidney cells of broiler chicken administered with Boldenone

As urea is metabolized only in the liver, its elevation also indicates hepatic dysfunction, as discussed earlier. In normal birds, the pool of creatine from which creatinine is formed depends mainly upon muscle mass, and is mostly excreted before being converted to creatinine. Therefore, the level interval of creatinine in the blood of birds is 0.1e0.4 mg/dL, which is much lower than that of mammals.Although hypoproteinemia is an established finding in liver damage, given that the liver creates most plasmaprotein fractions, here, the total plasma protein and globulin concentrations were significantly increased after boldenone administration. These findings could be attributed to the nitrogen-retention capacity of the drug. Additionally, as mentioned earlier, the increment of globulins may be attributed to the increased a- and b-fractions rather than the g-fractions, including positive acute-phase proteins. Albumin, in contrast, is considered a negative acute-phase protein that is decreased in inflammation, especially hepatic. Although the calculated albumin/globulin ratio was decreased, albumin concentrations showed insignificant changes in the present study.Blood should be routinely cleared from supra-normal levels of Chol and Tag by a healthy liver with the help of lipoproteins and lipases. Chol is utilized in the synthesis of steroidal hormones and Tag is stored in fat cells for energy when needed. Glu should be converted to glycogen by the liver and muscles, as well. A diseased liver, therefore, results in what is called “Metabolic Syndrome”, as indicated by elevated Chol, Tag and Glu. Metabolic results in broilers recorded in the present study were consistent with this general rule, and with data reported by other investigators . The pH values of breast meat from the boldenone-treated group were significantly higher as compared to those of the control. Myofibrillar refraction contributed to differences in light scattering between pale, soft, exudative and dark, firm, dry chicken meat, as it does in pork and beef .This phenomenon was evident when comparing the meat quality of chicken breasts (Elmajdoub. *et al*., 2016)

17-B-Estradiol

17 β- Estradiol hormone used as growth promoter for poultry but it has great side effect on human who consume meat contain these hormone which made hepatocellular carcinoma and consider hepato tumorigenic agent. Also, it effect on gonadotrophins of lactating mother .17 B-estradiol usually used in chicken farms and given either orally as contraceptive tablets or intramuscular administration to increase the body weight in short time. Using hormones in broiler chicken farms for performance-enhancing purposes may lead to deposit of residues in their carcasses, particularly when the birds are slaughtered without the observance of withdrawal period of the hormones. Ignorance of observation of withdrawal period leads to a serious threat to human health upon exposure to these residues. I/M injection of 17- β estradiol to broilers was indicated the presence of high level of hormonal residues in different tissues with the highest concentration in kidneys (organ of excretion) followed by liver then muscles but these residues were lower by oral administration (Hemmat. *et al*., 2018)

Genistein

Genistein is abundant in animal feed. In this study, the side effects of high-dose genistein on intestinal health and hypothalamic RNA profile were

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evaluated. Chicks exposed to high-dose genistein by intraperitoneal injections and feed supplementation both showed a reduced body weight gain and feed intake in comparison with the control group. In comparison with the control, serum levels of albumin and total protein were decreased after high-dose genistein injection and diet supplementation . Interestingly, the genistein diet presented the chick hypothalamus with downregulated expression of bitter taste. Accordingly, high-dose dietary genistein reduced villus height and the abundance of Lactobacillus, along with the increased abundance of pathogenic bacteria in the ileum.Therefore, high-dose dietary genistein altered the hypothalamic RNA profile and signal processing. Cluster analysis further revealed that high-dose dietary genistein significantly influenced apoptosis, the immune process, and the whole synthesis of steroid hormones in the hypothalamus. In conclusion, high-dose dietary genistein altered the hypothalamic RNA profile and intestinal health of female chicks (Zengpeng, L. *et al*., 2019).

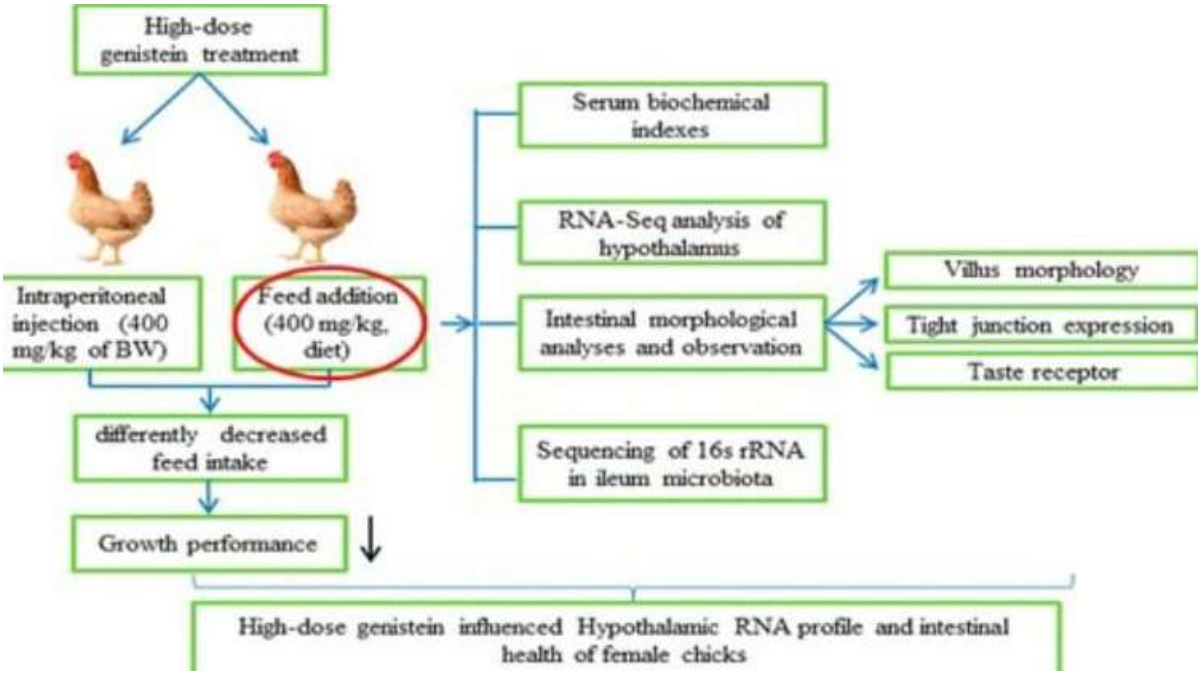

Figure 4: Effect of Genestein injected in Broilers

Corticotropin

In chicken embryos, intravenous injection of corticotropin-releasing hormone (CRH) causes the release of both corticosteroids and thyroid hormones. These hormones initiate and enhance the hatching process, raising the possibility that CRH treatment of the late chicken embryo could accelerate hatching and/or decrease the spread of hatching. We performed a series of exploratory tests to investigate whether in ovo delivery methods of CRH other than intravenous injection that are more practical in a commercial setting, affect hatching time in broilers. Corticotropin-releasing hormone was injected into the air cell, albumen, or amniotic fluid of broiler breeder eggs, in the last week of embryonic development. Average incubation duration was significantly decreased by 22 h when 2 μg of CRH was injected into the air cell on embryonic day 18 (E18) of Cobb eggs. Acceleration of hatching (but only by 8 h) was also seen for Ross chicks when CRH was injected daily into the albumen between E10 and E18. However, repeats of both experiments did not show consistent effects of CRH on hatching time; in most experiments performed, CRH did not affect hatching time. We speculate that the effectiveness of CRH uptake via these delivery methods and/or the duration and magnitude of the thyroxine and corticosterone response to CRH is not sufficient to have a substantial effect on hatching time. We therefore conclude that in ovo CRH treatment does not seem a feasible option as a practical tool to increase hatchery productivity or to investigate the effects of CRH agonists and antagonists on hatching (Yugo. *et al*., 2017)

Vitamin E

A study was conducted to evaluate the impact of vitamin E, C and/or probiotics to improve tolerance of broiler chicken to chronic heat stress (CHS).In this 1 day old Cobb-500 chickens were split into 7 test groups; Thermoneutral group was raised under thermoneutral conditions for days 25-

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42 of age.The other 6 were raised for 3 successive days a week at 36±2°C and 75-85% relative humidity for 7h daily: (1) heat stressed group, (2)vitamin E ,(3) vitamin C ,(4)Vit C+E , (5) probiotics (6)Vit C+E +probiotics .Exposure to CHS decreased body weight gain, feed Intake and abdominal fat. It had adverse effect of CHS on Feed Conversion Ratio, Packed Cell Volume, monocyte ,basophil, total protein and phagocytic activity but increased plasma cholesterol and aspartate amino transferase (ast) compared to thermoneutral group. Vitamin C,E or probiotics alone decreased adverse effects of CHS during experimental period. Vitamins C and E were both equally effective when alone but less than when combined. Vitamin E increased dressing percentage and abdominal fat but decreased AST while increasing Basophil ,monocyte and globulin compared to the heat stressed group. From this it can be concluded that supplementation of vitamins E, C and probiotics in different combinations of CHS and Vitamins E+ C + probiotics is the most effective for economic traits. (Youssef, A. A. *et al*., 2017)

Testosterone

This study was designed to evaluate the effects of testosterone on hatchability, chick measurements and some plasma metabolites in chickens during embryogenesis stage..The in ovo injection of testosterone produces a mark changes in embryonic motility and hatchability. When dose of testosterone is given parenterally it decreases a hatchability percentage but a significant increase in embryonic motility.The effect of injection of testosterone on the body weight of the hatchling was the main objective of the current studies. Testosterone increases the chick weight and egg weight as compared to those with non-injected.Due to testosterone the chick length is significantly increased. Generally, male chicken significantly increases more in chick length than females.Testosterone injections cause a significant increase in plasma protein levels. Plasma concentration of calcium and phosphorus is increased if it increase too much ot can be fatal. Alkaline phosphatase (ALP) is an enzyme that produce by the liver and the highest concentration of ALP is present in cell comprise bone and liver cell, any liver disease or bone disorders cause an increase in plasma levels of ALP. So, in ovo injection of testosterone ALP amount decreases. Cholesterol level along with triglycerides is also decreases due to injection of testosterone. This produces many biological changes in cell.Egg production performance is significantly increases due to testosterone injections as due to increase in ovulation rate. Feed intake is also increased due to hormones injections. Due to testosterone injections the diameter of Small white follicles (SWF) and Large white follicles(LWF) is also increased. From different experiments we have seen that egg mass is increasing in those chicken that are injected with testosterone. Eggshell weight, eggshell densities were significantly higher in those whom we give testosterone injections. (Hanaa, M. H. *et al*., 2018)

Corticosterone

The massive meat production of broiler chickens make them continuously exposed to potential stressors that stimulate releasing of stress-related hormones like corticosterone (CORT) which is responsible for specific pathways in biological mechanisms and physiological activities. Therefore, this research was conducted to evaluate a wide range of responses related to effect of corticosterone injections.The massive meat production of broiler chickens make them continuously exposed to potential stressors that stimulate releasing of stress-related hormones like corticosterone (CORT) which is responsible for specific pathways in biological mechanisms and physiological activities. Therefore, this research was conducted to evaluate a wide range of responses related to broiler performance, immune function, plasma biochemistry, related gene expressions and cell death morphology during and after a 7-day course of CORT injection. When broilers are treated with corticosterone injection there is a decrease in plasma concentration of T_3, which is a thyroid hormone. It affects almost every physiological process in the body, including growth and development, metabolism, body temperature, and heart rate. There is a decrease in plasma concentration of mRNA expression of hepatic Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is a hormone similar in molecular structure to insulin which plays an important role in childhood growth, and has anabolic effects in adults.The relative weights of thymus, bursa and spleen decreased by the CORT treatment with low counts of Total white blood cells and decreased stimulation of T and B lymphocytes while the H/L ratio(Measuring the ratio of heterophils and lymphocytes in response to different stressors is a standard tool for assessing long-term stress in laying hens.) is increased ;this indicates immunosuppressive effect of corticosterone treatment. Furthermore, high expression of caspase-9 gene occurred in the bursa of corticosterone treated chickens, however, it was associated with a high necrotic vs. low apoptotic

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cell death pathway in the spleen. Seven days after termination of the CORT treatment in broilers, most of these aspects remained negatively affected by CORT and did not recover to its normal status. Although apoptosis can occur as a normal and beneficial defense mechanism in most organisms, necrosis can also be seen as a part of cellular death during stressful stimulation but it is considered as an unnatural cell death process. The necrotic pathway form of cell death programs is accompanied by a rapid collapse of plasma membrane and it is independent of expression of new genes. The final body weight, daily weight gain , feed intake of broiler chickens during the week of the CORTICOSTERONE injection course significantly decrease because elevated corticosterone levels inhibits anabolic processes and suppress appetite. It was also reported that CORTICOSTERONE may suppress growth performance by reducing the absorption of feed through the small intestine to broiler performance, immune function, plasma biochemistry, related gene expressions and cell death morphology during and after a 7-day course of CORT injection. When broilers are treated with corticosterone injection there is a decrease in plasma concentration of T_3, which is a thyroid hormone. It affects almost every physiological process in the body, including growth and development, metabolism, body temperature, and heart rate. There is a decrease in plasma concentration of mRNA expression of hepatic Insulin-like growth factor 1 (IGF-1), also called somatomedin C, is a hormone similar in molecular structure to insulin which plays an important role in childhood growth, and has anabolic effects in adults. There is a significant increase in liver weight accompanied by an increase in plasma total proteins, AST and ALT was observed with CORT treatment, indicating an incidence of liver malfunction by CORT (Gamal, M. K. M. *et al*., 2017).

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Source of support: Nil; **Conflict of interest:** Nil.

Cite this article as:
Shahid, U., Naz, H., Riaz, S., Ali, O. and Arshad, F. "Pathological & Physical effects of Steroidal Hormones on Consumers Poultry." *Sarcouncil Journal of Multidisciplinary 2.1* (2022): pp 10-16

Publisher: SARC Publisher