ORIGINAL ARTICLE

COMPARISON OF THE EFFECTS OF BROILER AND DOMESTIC CHICKEN MEAT ON SERUM TESTOSTERONE AND LUTEINIZING HORMONE LEVELS IN RATS

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Background: The aim of study is to evaluate and compare the effects of broiler chicken fed with commercially available feed and chicken fed with organic diet on serum testosterone and Luteinizing Hormone levels in Sprague Dawley rats. It was a randomized controlled trial conducted in Multi-Disciplinary Laboratory of Department of Biochemistry and Molecular Biology, Army Medical College, Rawalpindi, in collaboration with Quaid-e-Azam University, Islamabad from November 2017 to April 2019. Methods: Ninety male early weaned Sprague Dawley rats were randomly assorted into three groups (n=30). Group I control rats were nourished on standard pelleted diet. Group-II rats were nourished with organic chicken meat along with their standard pelleted diet. Group-III rats were nourished with broiler chicken meat along with their standard pelleted diet. Serum Testosterone and Luteinizing Hormone levels were estimated through ELISA. Weight gain and the % growth rate were also estimated. Mean±SD values of all variables were calculated. One-way ANOVA was applied to determine the significance among groups followed by Tuckey’s HSD post hoc test. p value ≤0.05 was considered significant.

Results: The current study demonstrated significant increase in serum Testosterone levels (p=0.002) and a significant decrease in serum Luteinizing Hormone levels (p=0.003) between control and broiler meat fed rats (Group-III). The study also showed significant increase in weight gain and % growth rate (p<0.001) in both experimental groups as compared to control group.

Conclusion: Based on the findings of our study we propose that broiler chicken meat consumption could be the potential cause of hormonal imbalance and out of proportion weight gain and growth in experimental rats.

Keywords: Broiler Chicken; Organic Chicken; Serum Testosterone; Luteinizing Hormone; Sprague Dawley Rats; Growth Rate

INTRODUCTION

The consumption of chicken as an indispensable source of animal protein has exhibited an immense upsurge globally over the passage of time and is being used as a staple food worldwide because of its easy accessibility, delightful taste, relatively reasonable price, nutritional benefits and acceptance by all echelons of society with miscellaneous backgrounds.¹ The growing worldwide population, shared with factors such as augmented pressure on the world’s resources, changing socio-demographics and amplified demand for animal-based protein, resulted in chicken meat as popular alternative source for protein.²

The consumption of global poultry meat in 2018 averaged 13.9 kg per capita.³ Consumption of chicken meat in Asia, increased from 6.6 kg to 9.5 kg per capita per year.¹ In Pakistan poultry consumption is 4.4 kg/capita³ in 2017. In 2025, the poultry consumption per capita in Pakistan is anticipated to approximately 4.68 kilograms per individual per annum.⁴ Presently revenue of Pakistan Poultry Industry is about 750 Billion rupees. Poultry division produces employment and profits for about 1.5 million people.⁵

The current sources of poultry in vogue are broiler and domestic. The former is the most affordable and accessible in local markets and is fed on commercially available feed.⁶ While the latter one is difficult to acquire due to high cost and scarcity. There is an inclination towards poultry meat instead of red meat because of delicious taste, diminutive price and easy accessibility. Modern broiler has 150 to 200 grams of fat per kg body of its weight⁷ which is 15–20% more fat than that of indigenous/organic chicken. Of this additional fat, >85% is not physiologically required.⁸ Increased fat consumption is linked to steroidal hormone imbalancing particularly testosterone, corticosterone, prolactin,
thyroid stimulating hormone, luteinizing hormone and melatonin. There is a hypothesis called “Endocrine Disruption Hypothesis” which proposes that exposure to endocrine disruptor during a life time may interfere with the production of reproductive hormones. This hypothesis also encompasses other endocrine abnormalities like altered glucose, TSH, corticosterone and prolactin levels.

There is also an association between diet especially chicken meat supplemented and hirsutism in females of reproductive age. Diet, especially fat enriched plays a very important role in hormonal imbalance, obesity and hyperlipidaemias. Synthetic oestrogens and many more compounds having androgenic and progestogenic properties have been in practice for growth promotion and to improve carcass quality in chicken. Endocrine disruptors are highly variable and exhibit unique properties. Most of the chemicals/drugs are only toxic at higher doses, but endocrine disruptors are detrimental to health even in very minute concentrations. They are ubiquitous in nature and present everywhere from our daily life household items to our diet to even animal litter. They are even more hazardous and lethal during developmental phase. The endocrine disruptors can cause diminished spermatogenic capacity and amplified incidence of male infertility. These effects can be transferred to new germ lines through the process of reproduction.

There is potential for natural or synthetic hormonal chemicals present in poultry litter and agricultural fertilizers to be transmitted to adjacent aquatic environments. Thus, altering endocrine function in exposed wildlife and the consumers of that water.

Along with them some essential ingredients included in poultry feed are steroids, antibiotics, minerals and arsenic which might be used decisively for the higher boom and overall performance of the chickens. The elements of the chicken feed are stated to impart higher flavour and increased growth rate and meat quantity in chickens and as a result more and more chicken is consumed on daily basis. As broiler chicken meat contains extra fat and augmented nitrogenous compounds so imparting humans with fat and proteins. The presence of the certain substances mainly oysters shells and roxersone from infected waters which are used in manufacturing chicken feed may be deleterious to the people consuming that chicken meat. Also, the antibiotics, fat from previous bird debris and hormones extracts inside the feed of the chicken might also deliver dangerous effects to the people ingesting the meat. By increasing the consumption of poultry, it is a growing myth in public that broiler chicken consumption may not be safe and is commonly believed to be an estrogenic, carcinogenic as well as growth retardant. It is a growing concern not in general public but also among the clinicians that broiler chicken consumption may lead to reproductive disorders such as precocious puberty, hirsutism, polycystic ovarian syndrome, early menarche and menopause. We have conducted a comparative study on the derangement of testosterone and luteinizing hormone in response to chicken fed with commercially available feed and chicken fed with organic diet.

**MATERIAL AND METHODS**

A total number of 90 early weaned Sprague Dawley rats weighing 45–60 grams and between ages of 3–4 weeks were arranged from inbred colony of animal house of Quaid-e-Azam University, Islamabad. Randomized control study was conducted in the Multidisciplinary laboratory, Biochemistry Department, Army Medical College, Rawalpindi, from November 2017 to April 2019. The Ethical Review Committee considered the ethical aspects of the project and was satisfied with the undertaking of the investigators and they were in line with the approvals of National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985). Non-probability convenience sampling was used to split rats into three groups, group I, II and III with 30 rats in each group. The rats in group I control were fed on standard pelleted rat diet and water ad libitum while group II were fed on standard diet supplemented with organic chicken meat and group III were fed on standard diet supplemented with broiler chicken meat for a period of ten weeks. At the end of the study rats were euthanized and serum Testosterone and Luteinizing Hormone levels were estimated by immunoassay techniques using, Awareness Technology Inc, Microplate Reader Model Reader- Statfax 2100. Bioassay Rat Kit Lot No. 180112 for LH and 1801007 for testosterone were used. Tests were performed following the manufacturer’s provided protocol.

The data analysis was performed using SPSS version 22. Quantitative data is presented in terms of mean and SD. Data distribution was checked using Shapiro Wilk test. The statistical significance among the groups was determined by applying one way ANOVA (data was found to be uniform or non-skewed). The difference was considered significant if p-value was found <0.05. Post Hoc test (Tukey HSD) was applied to find the group that significantly differed from others.
RESULTS

At the time of initiation of the study, age of all the rats was 21 days while at termination it was 70 days. Mean testosterone of the three groups (I, II, III) were 2.19 ng/ml, 2.24 ng/ml and 2.30 ng/ml respectively, while that of LH were 2.12 mIU/ml, 2.10 mIU/ml and 2.06 mIU/ml respectively. Similarly mean weight gain in group I, II and III are 190.27 g, 185.30 g and 229.17 g respectively while mean growth rate observed was 615.23 %, 617.07 % and 710.03% respectively.

We observed a decrease in levels of LH in both broiler and domestic chicken diets groups; however the significance level was more in broiler group while this trend was inverse in case of levels of testosterone, weight gain and rapidness in growth rate (Table-1).

The weight of the animals of all groups was also measured as an additional parameter before and after the experiment. The animals of group-I and II gained almost equal weight (187±17) during study period while the weight gain in animals of group-III was significantly higher (229±17) as compared to those of controls or group-II. Body weight gain with increasing age of animals in group III was significantly different while it was normal in rats of group I and II. Growth rate is also calculated and it is evident from the data that growth rate in group III is significantly higher as shown in table-1.

Comparison of testosterone levels by Post hoc Tukey’s HSD revealed that group III (2.30±0.17 ng/ml) rats displayed significantly higher (p=0.002) levels as compared to the group I rats (2.19±0.08 ng/ml). The testosterone levels in group I and II are not significantly raised (p=0.253). On comparison of LH levels by Post hoc Tukey’s HSD revealed that group-III (2.06±0.06 mIU/ml) rats displayed significantly lower (p=0.003) levels as compared to the group I rats (2.12±0.06 mIU/ml). The LH levels in group I and II are not significantly raised (p=0.347) as shown in table-2.

Table-1: Comparison of endocrine and anthropometric parameters in control and test groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group I (n=30)</th>
<th>Group II (n=30)</th>
<th>Group III (n=30)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Testosterone (ng/ml)</td>
<td>2.19±0.08</td>
<td>2.24±0.07</td>
<td>2.30±0.17</td>
<td>0.003*</td>
</tr>
<tr>
<td>Serum LH (mIU/ml)</td>
<td>2.12±0.06</td>
<td>2.10±0.06</td>
<td>2.06±0.06</td>
<td>0.004*</td>
</tr>
<tr>
<td>Weight Gain (Grams)</td>
<td>190.27±17.47</td>
<td>185.30±17.41</td>
<td>229.17±16.88</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Growth Rate (%age)</td>
<td>615.23±56.12</td>
<td>617.07±68.83</td>
<td>710.03±62.14</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*p-value <0.005 is significant.

Table-2: Intra-group association of study variables among the study groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group1</th>
<th>Group2</th>
<th>Group3</th>
<th>Group2</th>
<th>Group3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/ml)</td>
<td>0.253</td>
<td>0.002</td>
<td>0.138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteinizing Hormone (mIU/ml)</td>
<td>0.347</td>
<td>0.003</td>
<td>0.114</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight Gain (Grams)</td>
<td>0.509</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth Rate (%age)</td>
<td>0.993</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Significant Difference (p ≤ 0.05).

DISCUSSION

The equilibrium of dietary proteins is shifting from red meat and fish to chicken meat.24 The results of our study showed that consumption of broiler chicken significantly increases serum testosterone levels and decreases luteinizing hormone in male rats.25 Ahmad et al., 2017 also reported derangements of serum cholesterol, steroid hormones and FSH levels results in female Albino Wistar rats, while anthropometric parameters are similar to our study.26

Testosterone is a powerful androgen and excessive testosterone beyond its physiological limits is converted to oestrogen by an aromatase.28 Research shows that derangements in hormones like testosterone, progesterone and oestrogen synthesis may interrupt sexual development and maturity.27 This may be reflected as subfertility, genital malformations, precocious puberty and other gender related problems. These hormonal imbalances are also related to PCOS, hirsutism and menstrual irregularities in female.28 Our study also suggested that consumption of broiler chicken on daily basis may lead to oscillating levels of sex hormones. Studies conducted by Ahmad et al., 2017 in female wistar rats clearly discerned increased levels of oestrogen, progesterone and testosterone. Our study is consistent with this study but levels of testosterone in group III are clearly in excess of previous studies25 because of the gender differences.

Oestrogen as a pleiotropic hormone affects every system of the body and has profound effects on body metabolism.29 Studies show that excess of oestrogen is linked to disturbed metabolism which leads to inappropriate deposition of fats and adipose tissue. Sex hormones along with GH, leptin, adiponectin and insulin tamper with our appetite and may modulate our behaviour and preference over a particular diet and they also guide the distribution of fat. This chicken is reared on diets enriched with nutritional and non-nutritional ingredients.30 These
Ingredients accumulate in chicken meat and in the consumer and could be the cause of disturbed metabolism and obesity. Chicken feed causes more muscle mass and abdominal obesity in broilers and this may reflect in consumers as well.

The results of our study showed significant increases in weight gain in broiler chicken meat fed group as compared to control and organic meat fed groups. Previous studies reported the same results in female wistar rats. Growth rates are consistent with previous study but the weight gain is slightly tipped towards the higher side of the scale because of the gender differences. Growth, weight gain and fertility are said to be environmentally linked. But the reproductive ability of any organism is strongly bonded to fertility parameters and is influenced by genetic factors and sex hormones. Testosterone is one of the most important hormones with regards to male fertility. Group III rats showed mark increase in body weight and serum testosterone levels and is consistent with earlier reports and studies.

Their derangements are reflected in terms of precocious puberty, high weight and increased growth rates. This increment was however not seen in control and group II. The early growth spurt and obesity surge may be linked to increase in the rate of broiler chicken consumption in Pakistani population on daily basis and more general population in Pakistan is utilizing cholesterol and fats than proteins and hence imparting a major chunk to the burden of obesity and weight gain.

There is an important aspect called the endocrine disruption, the importance of which cannot be underscored. Tissues that seem to be at precise risk for developmental anomalies in progeny because of maternal vulnerability are those with receptors for steroidal hormones: in female foetuses this effects the fallopian tubes, uterus, mammary glands, vagina, and cervix, and in male foetuses it targets the seminal vesicles, prostate, testes and epididymis. In both sexes the brain, skeleton, liver, thyroid, external genitalia, immune system and kidneys are also targets for steroidal hormone action and are therefore possible targets for endocrine-disrupting compounds, even though these compounds may have diverse modes of action, in addition to acting as hormone agonists and antagonists, in different target tissues.

A foremost fear is the permanent effects after the exposure to endocrine disruptors on the future well-being of wildlife and humans in the critical periods of development while chronic exposure after adulthood can also present a health hazard. It is generally implicit that after maturity, contact with endocrine disruptors does not permanently modify the functioning of hormone-receptive tissues. However, investigational studies in animals have revealed permanent changes in vaginal epithelium, brain and prostate after administration of estrogenic compounds in adulthood. The likelihood thus exists that chronic, low-level exposure to estrogenic chemicals and endocrine disrupting chemicals in the atmosphere after maturity can have effects in humans analogous to those observed in experimental animals on oestrogen.

As mentioned earlier, there are important and potentially adverse ingredients which are added intentionally in the broiler chicken feed. Arsenic in the form of roxersone is added to increase the feed efficiency, higher boom and palatability of chicken meat. Arsenic is a well-known endocrine disruptor. It is detected in broiler chicken feed, meat and their droppings. Arsenic can disrupt retinoic acid receptor regulation which is a common receptor for intra nuclear acting ligands.

Some of the others EDCs detected in chicken feed and meat are melamine, organophosphates, DDT pesticide, phthalates and dioxin. They are lipid soluble so they get access to every nook and cranny of the body. Melamine is also a known nephrotoxic chemical and is a novel EDC which maliciously effects reproduction. Steroids are also found in chicken feed and meat. They get direct entry into the consumer, easily absorbed through GIT and may have ill effects. All the sex hormones are steroids in nature. In this way the overall balance of steroids hormones may not be in favour of an organism’s healthy reproductive profile.

CONCLUSION

Based on the findings of our study we propose that broiler chicken meat consumption could be the potential cause of hormonal imbalance and out of proportion weight gain and growth in experimental rats.

Conflict of interest: None.

Funding disclosure: Self.

AUTHORS’ CONTRIBUTION

HGK: Literature search, study design, data collection, data analysis, data interpretation, proof reading, lab work, provision of resources. AR: study design, data analysis, data interpretation, proof reading, and provision of resources. SAK: Data analysis, data interpretation, provision of resources. MJY: study design, data analysis, data interpretation, proof reading, and provision of resources. FA: data
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