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Effects of Consumption of Caged and Un-Caged Chicken Meat on Ovarian Health of Female Wistar Rats

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Authors' Contribution
IA was the principal investigator, SA and FA designed and conceived the experimental protocol, SA, ST and SM conducted the experiments and collected the data and ZB and SA analyzed the data and wrote the manuscript. SH critically analyzed the manuscript, TP and SBA provided financial contribution.

Key words
Caged chicken, Cholesterol, Polycystic ovaries (PCOs), Soybean, Spinach, Steroidal sex hormones.

ABSTRACT

The caged chicken meat consumption has peaked current days due to its incredible taste and low cost. However, to meet the increasing demand the chickens are reared commercially through feeds and battery cages allowing maximum weight gain and growth in considerably less time. The constituents that are fed to chickens concentrate in the flesh and bring deleterious health outcomes on the consumers. The ill effects account for hyper-lipidemias and imbalance in the steroidal sex hormones may result in the development of cysts in the ovaries with resultant difficulties in reproduction. The present study was done on 75 rats divided randomly in 5 equal groups fed with rat chow, caged chicken meat, uncaged chicken meat, raw spinach and soybean for a period of six weeks. The levels of plasma cholesterol, progestril, estradiol, and androgens were estimated at the end of the experiment. The ovaries of the rats were collected, weighed and histopathologically evaluated for the development of cysts. It was seen that the ovaries of the group treated with caged chicken meat showed increased cholesterol levels, imbalanced steroidal sex hormone levels, increased ovary weight and development of the cysts upon histopathological examination as compared to the rats of other groups in the study.

INTRODUCTION

Chicken meat delicacies are the most liked ones nowadays. This is due to the good taste and palatability, easy availability with low cost as compared to red meat (Ahmad *et al*., 2017). The chicken meat available in the markets is known as caged chicken meat or artificially grown chicken meat (Ahmad, 2017). Likewise, there are certain group of people especially vegetarian and people of low economic strata that regularly take soybean and spinach on the daily basis (Jiraungkoorskul, 2012). Soybeans are a good source of essential macro as well as micro nutrients. It provides ample amount of protein, dietary fiber, iron, manganese, phosphorus and several B vitamins, including folate. It is also known to be rich in high contents of natural anabolic steroids, vitamins, magnesium, zinc and potassium (Yoshioka *et al*., 2017). Soybean is not only consumed by humans but is also used for preparation of fodder for cattle and poultry (Rohe *et al*., 2017). Likewise, another vegetable consumed on a larger scale in south western part of our world that is Pakistan and India is spinach. Spinach is known to be a rich source of water and fat soluble vitamins, manganese, iron, anabolic steroids and folate (Roberts and Moreau, 2016).

The caged chickens are grown artificially in a hostile environment comprising of battery cages with the ready available feed recommended for their brisk growth and weight gain. Besides they are deprived of water ad libitum and exercise (Folorunso *et al*., 2014). All these factors enable the chickens to stay in a position and consume the fodder enabling them to gain weight quickly within the six weeks' time (Ahmad and Ahmed, 2015). This is not compatible with the natural course of chicken development

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as they need more than three weeks to develop to a size of around 1.5 kg when provided free access to grains as well as water and allowed to roam on the free ranges to digest the food (Ahmad and Ahmad, 2014). The feed provided to caged chickens are known as feed for broiler hens. The said feed is an amalgamation of macro and micronutrients. The mixture of wheat, maize and soybean provide the essential macronutrients like carbohydrates, fats and proteins to the chickens while the other additives like amino acids and vitamin premixes provide them with the micronutrients essential for the body to develop (Ahmad *et al*., 2017). But there is documented proof that the caged chicken feed also contains melamine, roxersone and pesticides. All these are to provide nitrogenous compounds to the growing hens so they may build their protein structure on these available nitrogenous groups (Ahmad *et al*., 2016). However, along with their activities in growth and development they also bring their deleterious effects on the body of the consumers by inflammatory changes to liver and other organs to development of cancers as in the case of inorganic arsenic roxersone, kidney stones and ovarian inflammation leading to reproductive disturbances, infertility and subfertility (Chovanec *et al*., 2010; Hu *et al*., 2017).

Previous research also indicated that the animals fed with the particular diet contain the remnants of the diet in their flesh (Oliveira *et al*., 2000). For the matter it is seen that the animal feed-production practices, the ingredients comprising chicken feed and the biological, chemical, and other agents that may have been detected in animal feed show effects on the human health (Silbergeld *et al*., 2008). In addition, it is also evaluated that current feeding practices may be associated with adverse human health impacts and address the data gaps that may prevent ample assessments of human health hazards associated with animal feed and meat production (Sapkota *et al*., 2007). The same findings were revealed in the animal model studies. The female rats were subjected to commercial chicken feed and commercial chicken meat and it was observed that the hormonal profile of the rats, got upheaval along with excessive weight gain and obesity in these rats (Ahmad *et al*., 2017). These findings are consistent with the effects of commercial animal produce on the human health based upon the way they are reared and fed (Ahmad *et al*., 2016).

The prevalence of polycystic ovaries (PCOs) is on a rise and been an alarming condition for the health practitioners (Konkel, 2016). This has been a main ground of obesity, hirsutism and infertility in young females of child bearing age (Fasano, 2017). To reduce this health concern, it is evident that the reasons including the dietary habits of consuming caged chickens that incur these health concerns must be curtailed and changed with the healthy natural eating options. The present study was therefore carried out to examine the effects of the artificially grown caged and uncaged chicken meat, spinach and soybean on the hormonal profile of the female Albino Wistar rats and their net effects on the ovaries of these rats.

MATERIALS AND METHODS

Animals

The experiment was carried out on 75 female rats of approximately 100 g each. Rats were purchased from Dow University of Health Science, OJHA campus, Karachi. Pakistan. Three rats were housed in one cage for 12 h day and night cycle with ambient room temperature of 22±2ºC. The experimental protocol was approved by the ethical committee of Baqai Medical University and executed in line with National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Assay kits

Plasma cholesterol and steroidal sex hormones including progestril, estradiol and androgen levels were assessed by using ELISA kits purchased from Biocheck, Inc. USA.

Experimental protocol

The uncaged chicken hatchlings were reared in the animal house of the Baqai Medical University, Karachi, Pakistan. The caged chickens were purchased between from the poultry farm proximate to the super-highway Karachi known for it supplies of chickens and eggs to the different suburbia of Karachi. The rats were randomly divided into five (equal) groups. These include group I standard rat chow treated rats, group II artificially grown caged chicken meat treated rats, group III uncaged chicken meat treated rats, group IV treated with raw spinach, group V treated with raw soybean. Cubes of caged and uncaged chicken meat were given in the raw form. Whereas, raw spinach and soybean was given in the form of pellet which was prepared by mixing 99% of crushed raw vegetable with 1% of wheat flour. All the rats were fed with provision of the specified diet and water *ad libitum*. Standard rat chow contained wheat flour (400 g), gram flour (171 g), barley flour (171 g), corn flour (100 g), vegetable oil (50 g), milk powder (100 g), vitamin mixture (2.5 g), iodized salt (NaCl; 5.5 g). At the end of procedure rats were scarified to collect the ovarian samples for morphological and histo-pathological examinations. Blood was also collected to assess the levels of cholesterol, progestril, estradiol and androgens using ELISA kit method.

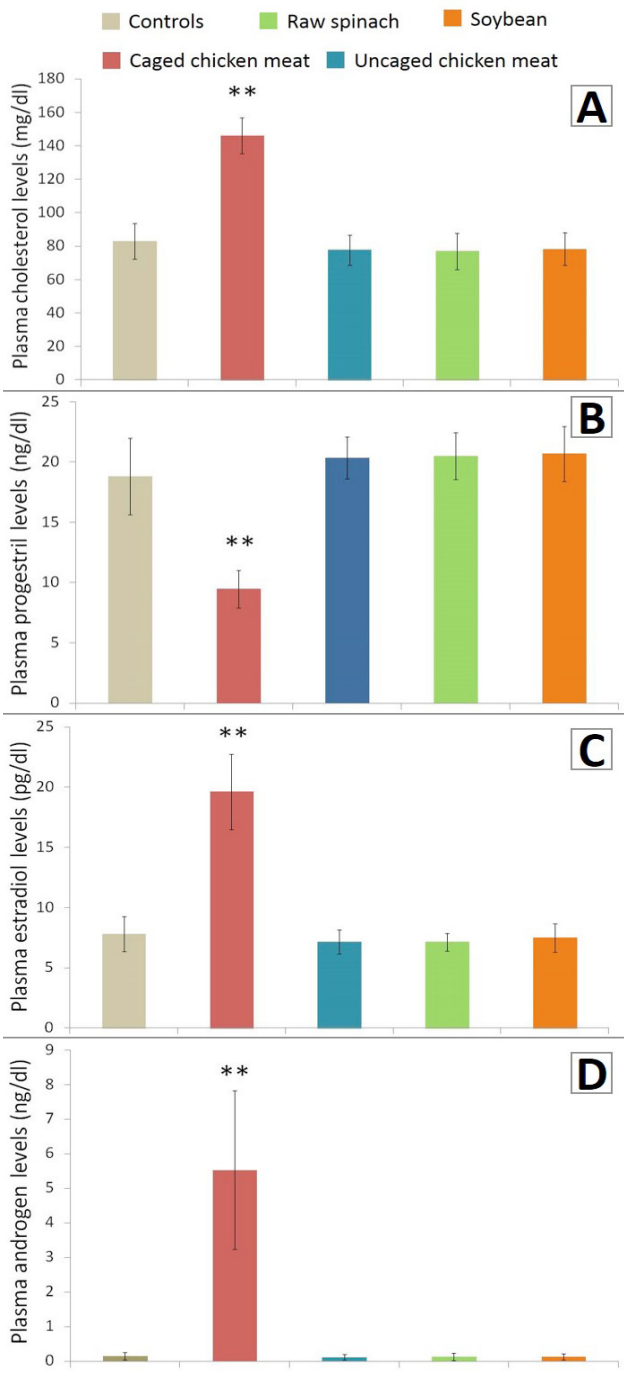

Fig. 1. Effects of different diets on plasma cholesterol (A), progestril (B), estradiol (C) and androgen (D) levels. Values are mean±SD; **p<0.01.

Histopathology of ovaries

Ovary samples were excised out and fixed in 10% formalin for 24 h. Dehydration was carried out with the help of alcohols. The specimens were then embedded in paraffin bees wax tissue blocks to prepare sections of 4 microns. Hematoxylin and eosin dyes were used to stain the sections for histopathological examination using the light microscope (Standish *et al*., 2006). Histopathological analysis was carried out to assess the presence of follicular cells and PCOs by a pathologist who was blinded to the grouping and treatment. The histopathology of ovaries was studied under light microscope model Olympus BX43 and the total magnification used was 400X.

Statistical analysis

Data was analyzed by one-way ANOVA followed by Tukey's post-hoc test using SPSS version 20.0. Values are expressed as mean±SD (n=15) with 95% confidence interval and p values less than 0.05 were considered as significant as compared to group I (control).

RESULTS

Total cholesterol levels

Data for cholesterol levels analyzed by one-way ANOVA revealed significant effects of treatment (F= 128.39, p<0.01). Post-hoc test showed significantly increased levels of cholesterol following the administration of caged chicken meat to female rats for six weeks as compared to that of controls (p<0.01). Whereas, levels of cholesterol in group III, IV and V were comparable to control rats (Fig. 1A).

Steroidal sex hormone levels

Results of progestril, estradiol and androgens also showed significant effects of different diet on levels of steroidal sex hormones (Fig. 1B, C, D). One-way ANOVA showed significant effects of treatment on progestril (F= 70.65, p<0.01), estradiol (F= 150.28, p<0.01) and androgen (F= 82.46, p<0.01) levels. Administration of caged chicken meat for six weeks resulted in significantly decreased levels of progestril (p<0.01). Whereas, levels of estradiol and androgens were significantly increased in group II as compared to that of control group. The levels of steroidal sex hormones in group III, IV and V were not significantly affected following the administration of respective diet to these groups as compared to that of controls (p>0.05).

Morphological and histopathological examination of ovaries

The gross morphology of the ovaries of the rats determined by evaluating weight of the ovaries. One-way ANOVA for the weight of ovaries revealed significant effects of treatment (F= 434.18, p<0.01). Significantly increased weight of ovaries was observed in group II as

compared to that of control rats (p<0.01). Whereas, group III, IV and V showed comparable ovary weight with controls (Fig. 2). The overall appearance of the ovaries of group II were swollen and cystic as compared to the normal ovoid shape of the ovaries of rest of all the other groups in the study. After gross observation, the ovaries underwent fixation and allied steps for histopathological evaluation. Thins sections of the ovaries were observed under the microscope for the presence of follicles and development of cyst or cysts in them. The present study showed the normal gross morphology of the rats of groups I, III, IV and V with the normal weight and the fat content attached to it. However, the weight of the ovaries of the group II rats significantly increased with the excessive presence of the fat content to it.

The histopathological evaluation of the ovaries of the groups I, III, IV and V showed the normal stroma of the ovaries with normal development of the follicles. There were areas with normal corpus albicans seen in the ovaries. However, on the other hand the ovaries of the group II rats upon histological evaluation showed the presence of small underdeveloped follicles also known as cysts along the rim of the ovaries. The number of the follicles was also calculated. It was determined that the ovaries containing one large follicle more than the size of 10 mm will be labelled as cystic ovary, ovaries with more than three cysts will be labeled as multicystic ovaries and more than seven cysts as polycystic ovaries. It was estimated that the rats in the group II developed more than ten cysts per ovary. The results showed the development of PCOs in the rats fed with the caged chicken meat (Fig. 3).

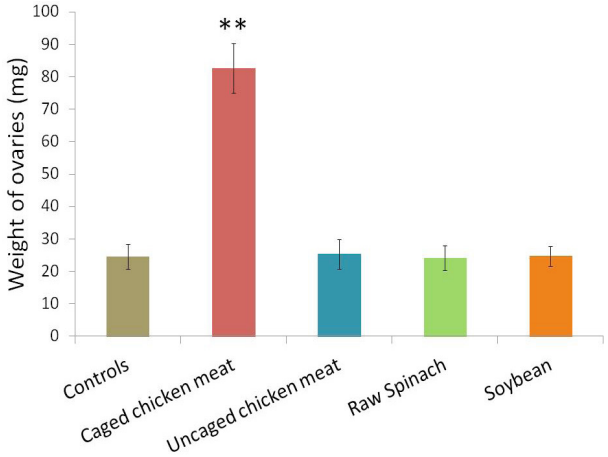

Fig. 2. Effects of different diets on weight of ovaries following six weeks of treatment. Values are mean±SD; **p<0.01.

DISCUSSION

Caged chicken meat as documented in previous research is raised artificially on the commercial fodder for the broiler that comprises whole grains and varied beneficial and non-beneficial additives (Ahmad *et al*., 2017). These components are known to not only to facilitate growth but also bring about harmful effects through imbalances in the steroidal sex hormones (Ahmad *et al*., 2017). On contrary, the uncaged chickens are reared in the open wilderness with whole grains provisions and no confinement to battery cages ensuring normal development (Ahmad *et al*., 2016).

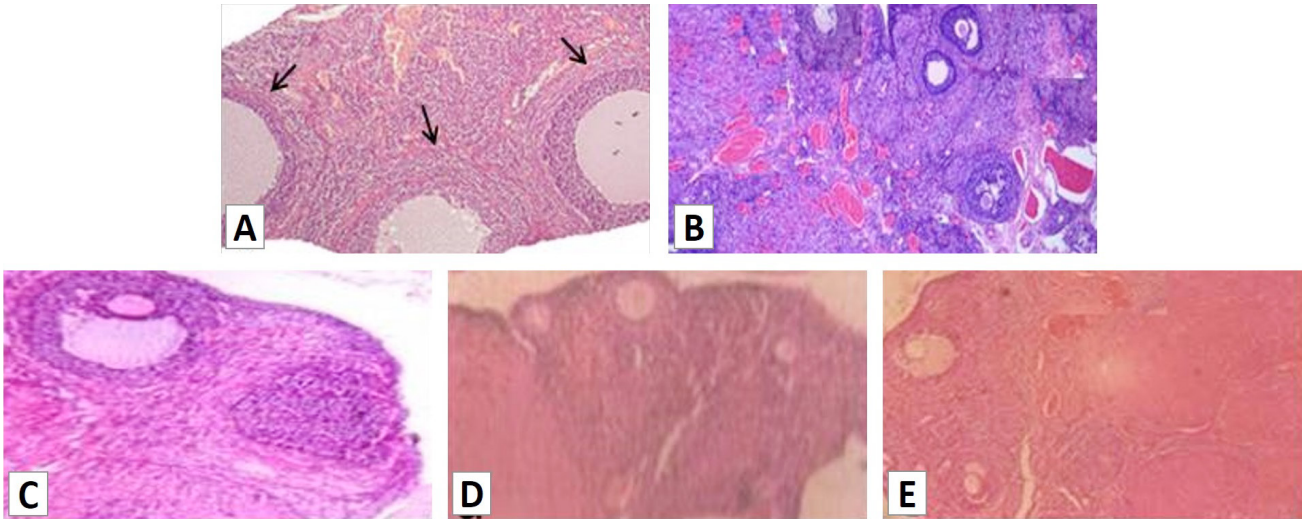

Fig. 3. Histopathological examination of ovary samples showing the presence of cysts in caged chicken meat treated animals (A), misrograph of ovary section of control (B), uncaged chicken meat treated rats (C), raw spinach treated rats (D) and soybean treated animals (E) showed normal ovarian cells.

The present study showed the abnormally high levels of the plasma cholesterol, estradiol and androgen levels while decreased plasma progestril levels in the groups II as compared to all the other groups. The abnormal imbalances in the cholesterol and hormones lead to the deleterious effects on the body (Sosic-Jurjevic *et al*., 2017). Research revealed that the high cholesterol levels not only results in the formation of atherosclerotic plaques in the body but also being the precursor of the steroidal sex hormones synthesizes them in the great amount (Mirmiran *et al*., 2017). These hormones in turn play havoc in the body when present in abnormal amounts. The cholesterol synthesizes the harmone progestril. The progestril then form androgens the male hormone and then in the presence of the aromatase enzyme androgens are converted to estradiol (Das *et al*., 2016). Soybean and spinach were also used in this study as both are documented to be a good source of anabolic steroids (Foyer *et al*., 2016). These steroids play vital role in the construction of the body muscles and organs and for the maintenance of the wear and tear of the body (Decloedt *et al*., 2017). Caged chicken meat is documented to be concentrated with the synthetic steroids that are injected to them (Ahmad, 2017). The difference between the natural and synthetic steroids is in terms of their effects on the body. The natural steroids can be converted or degraded to other components while the synthetic steroids can directly bring the deleterious effects on the body in terms of increased weight gain, obesity (Ashfaq and Farasat, 2017) and steroidal sex hormones synthesis (Ahmad *et al*., 2016). Levels of cholesterol and steroidal sex hormones in uncaged chicken meat, soybean and spinach treated rats were comparable to that of controls. This may be due to the fact that the respective diets given to groups III, IV and V did not contain any synthetic steroids or any additives that may increase the cholesterol levels and eventually the steroidal sex hormones.

The role of steroidal sex hormones is the synthesis of proteins and building of the muscle mass along with the maturation of the reproductive tract (Avilez *et al*., 2017). The progestril and estradiol are known to be female sex hormones. Their role is the maturation of the ovaries and initiation of the reproductive cycles in the body (Hebisha and Adel, 2017). Any abnormality or imbalance in their construction may lead to the abnormal stimulus to the pituitary gland with abnormal synthesis of the follicular stimulating hormone (FSH) and luteinizing hormone (LH) (Chen *et al*., 2017). The present study revealed the high plasma levels of cholesterol, estradiol and androgens while low progestril levels. Such imbalance in these hormones in turn have a negative impact on the generation of further estradiol and progestril from the ovaries and hence a vicious cycle ensues where the reproductive hormonal balance gets hay wired leading to small follicular generation not fit for the release of ovaries (Muth-Spurlock *et al*., 2017). These follicles in tiny size eventually become cysts in the ovaries making the ovaries cystic in nature. The cysts upon continuation of the hormonal imbalance keep on increasing in number forming multi-cystic to poly cystic ovaries. Besides development of the cysts the other regulatory mechanisms that the hormones control also are lost ensuing obesity, hirsutism and reproductive mayhems (Bani and Majdi, 2017). The PCOs were observed in the ovaries of female rats following the administration of caged chicken meat may be attributed to the contents that are concentrated in the chicken meat. The imbalance in the plasma cholesterol and steroidal sex hormone levels may be considered as the major cause of formation of PCOs in group II rats.

Speaking of the male pattern distribution the androgens play a vital role in this regard in the body. The increase in the androgens levels means the male pattern of hair distribution, more muscular body and have abnormal impact on the ovaries as well as they facilitate synthesis of estrogens through aromatase enzyme (Mirabolghasemi and Kamyab, 2017). The disturbed synthesis of estrogens will eventually affect the progestril and the FSH and LH (Krishan and Muthusami, 2017). The androgens are now documented to have profound effects on the ovaries when present in high quantity in the blood. They are also known to be the only causative factor of synthesis of cystic ovaries leading to multi-cystic and then poly cystic ovaries in the females (di Pietro *et al*., 2017). By doing so androgens estimation has also led the researchers to find the health and the fate of the reproductive tract. Being the intermediate of the progestril and estradiol, the androgens may manipulate the synthesis of both and itself will affect the ovarian health of the female individual (Palioura and Diamanti-Kandarakis, 2015). The present study directs the ill effects of diet that a human body is subjected to subsequent to consumption. This allows the understanding of the harmful effects that the caged chicken pose to the human consumers in terms of augmenting the cholesterol levels and bringing disturbance in the natural production of hormones. Once the hormonal level is upset, there is a vicious cycle that in turn abnormally stimulates the synthesis of the allied hormones in increased concentrations. Such increase in hormonal levels in turn affects the ovaries by causing development of cystic or polycystic ovaries that may eventually become a cause of sub fertility and even infertility.

CONCLUSIONS

Thus the present study showed the deleterious

effects of the consumption of the caged chicken meat on the ovaries and blood hormonal profile of the female rats which was not seen in the rats fed with uncaged chicken meat and the vegetables. This study therefore, emphasizes on the consumption of natural products as spinach or soy bean or those chickens that have been bred on the natural resources without any supplementation to reduce the risk of PCOs and to maintain the better health and quality of life in the human consumers.

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Statement of conflict of interest

Nothing to declare.

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