



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2017; 5(6): 1876-1878

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Received: 16-09-2017

Accepted: 19-10-2017

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## Micrometrical changes in the histomorphological architecture of the broiler adrenal gland due to fasting stress

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**Abstract**

The adrenal gland of 24 broiler chickens (*Gallus domesticus*), divided into four groups (each group consisting of 6 birds) exposed to different hours of fasting, were observed to find out different micrometrical changes of various elements of the gland at different hours of fasting. The groups were designated according to duration of fasting stress as; a (control), B (exposed to 12 hours of fasting), C (exposed to 24 hours of fasting), D (exposed to 48 hours of fasting) and Clinically healthy samples of adrenal gland of broiler chicken were collected immediately after sacrifice following fasting stress. Paraffin sections stained with Haematoxylin and Eosin were used for micrometric studies. The measurements included capsule thickness, cellular and nuclear diameters of cortical and medullary tissue and size of medullary sinuses. Various micrometric measurements were recorded by using image analyzing 'video test 4' software. The average thickness of the capsule of adrenal gland was  $20.82 \pm 0.492 \mu\text{m}$  which showed non significant changes among different experimental groups. The average cortical cell diameter in, Peripheral Zone (PZ) and Inner Zone (IZ) of adrenal gland of the control group were  $9.15 \pm 0.209 \mu\text{m}$  and  $6.19 \pm 0.14 \mu\text{m}$  respectively, while as the nuclear diameter of these cells in the corresponding zones of the same group were  $4.48 \pm 0.18 \mu\text{m}$  and  $4.50 \pm 0.218 \mu\text{m}$ . The average medullary cell diameter in Subcapsular (SZ), Peripheral Zone (PZ) and Inner Zone (IZ) of adrenal gland of control group were  $5.92 \pm 0.288 \mu\text{m}$ ,  $9.13 \pm 0.256 \mu\text{m}$  and  $7.12 \pm 0.136 \mu\text{m}$  respectively, while as the nuclei diameter of the cells in the corresponding zones were  $4.36 \pm 0.240 \mu\text{m}$ ,  $4.18 \pm 0.116 \mu\text{m}$  and  $4.49 \pm 0 \mu\text{m}$ . The average diameter of medullary sinuses in SZ, PZ and IZ in the adrenal gland of control group was,  $29.26 \pm 1.476 \mu\text{m}$ ,  $53.22 \pm 1.47 \mu\text{m}$  and  $64.86 \pm 1.49 \mu\text{m}$ . There was a significant difference in various micrometrical parameters like cellular and nuclear diameters of cortical and medullary tissue and size of medullary sinuses in different zones between the control and among the fasting groups. Results showed that fasting, caused significant adrenal gland changes, suggesting a possible role for the study of adrenal gland morphology as an indicator of chronic welfare problems in broiler

**Keywords:** Micrometry, cellular elements, adrenal gland, broiler chicken, fasting

**1. Introduction**

In avian species, a noteworthy interest has been excited by the involvement of poultry adrenal gland in stress reaction [1, 2]. Form and structure of endocrine glands are altered by various environmental stress factors like transportation, withdrawal of feed and water which directly or indirectly is reflected in the growth and development of an organism. In the glandular system of the body, the adrenal gland shows substantial variation both structurally and functionally because of the specific type of the hormones produced under various stress conditions. These hormones perform the vital functions of an organism such as metabolism; homeostasis [3]. Any disturbance of homeostasis result in structural changes within the adrenal gland which in turn bring changes in the concentration of large number of different hormones that have a crucial role in the regulation of functioning of the immune system. Changes in structure of adrenal gland in poultry due to different stress factors indicate that adrenal glands are involved in stress reaction [1, 2]. Since the adrenal gland is very sensitive and its function in animals including the poultry is altered by many environmental factors, the detailed knowledge about its micrometrical changes in broiler birds becomes imperative to study the effects of fasting stress on the micrometry of the gland. Paucity of literature on this aspect prompted this present study.

## 2. Materials and Methods

The present study was conducted in the laboratory of the Division of Veterinary Anatomy F.V.Sc& A.H. SKUAST-K, Shuhama, Srinagar. The birds were sacrificed (after taking due permission from the institutional animal ethical committee), dissected and the topography of adrenal glands were recorded. The specimen were collected and preserved in specific fixatives. viz., Neutral buffered formalin and Bouin's fluid. Specimens from each group were fixed separately in the above mentioned fixatives, for the routine H & E studies [3]. Paraffin sections stained with Haematoxylin and Eosin were used for micrometric studies. The various measurements, as detailed below, were recorded by using image analyzer 'video test 4' software.

- Thickness of capsule
- Cortical and medullary cell diameter in different zones.
- Cortical and medullary cell nuclei diameter in different zones.
- Width of medullary sinuses in different zones.

The data were analyzed using standard statistical methods as provided by [5]

## 3. Results and Discussion

### Capsule

The average thickness of the capsule of adrenal gland was  $20.82 \pm 0.492 \mu\text{m}$ . The capsular architecture of experimental groups, showed some histomorphological changes. These changes might be a compensatory mechanism to overcome the stress reaction, which was further supported by Cockrem [6]. However no significant changes were observed in average thickness of the capsule of different experimental groups Table 1.

### Cortical (interrenal) tissue

The cortical cell and nuclear diameter were measured in the different zones of the chicken adrenal gland. The average cortical cell diameter in, PZ and IZ of the adrenal gland of the control group were  $9.15 \pm 0.209 \mu\text{m}$  and  $6.19 \pm 0.14 \mu\text{m}$  respectively, while as the nuclear diameter of these cells in the corresponding zones of the same group were  $4.48 \pm 0.18 \mu\text{m}$  and  $4.50 \pm 0.218 \mu\text{m}$ . Significant variation ( $p < 0.05$ ) was observed in the nuclear and cell diameter of cortical cells in all three zones of the experimental groups (Table 2 and Table 3). There were variations in the diameters of cells and nuclei within the PZ and IZ. The PZ cells were characterized for their larger diameters of cell nuclei compared to that of the cells nuclei of IZ. However, there might have certain structural differences between the cortical cells in PZ and IZ of the chicken adrenal gland. To the best of our knowledge, little information was available regarding the cellular and nuclear diameter. However similar findings were observed related to the cortical cell nuclei diameter in chicken by Kober *et al* [7]. The functional significance of these few variations in the diameters of cell

and nuclei in chicken adrenal glands could be due to the different stages of the secretory activity of the cortical cells. However, on average, nuclear diameter of cortical cells was less than those observed in ostrich chicks ( $5-7 \mu\text{m}$ ) by Tang *et al* [8] and in domestic fowl ( $5 \mu\text{m}$ ) by Hodges [9].

### Medullary tissue

The average medullary cell diameter in SZ, PZ and IZ of adrenal gland of the control group were  $5.92 \pm 0.288 \mu\text{m}$ ,  $9.13 \pm 0.256 \mu\text{m}$  and  $7.12 \pm 0.136 \mu\text{m}$  respectively, while as the nuclei diameter of the cells in the corresponding zones were  $4.36 \pm 0.240 \mu\text{m}$ ,  $4.18 \pm 0.116 \mu\text{m}$  and  $4.49 \pm 0 \mu\text{m}$ . The average cell and nuclei diameter of these cells in different zones at different fasting intervals is depicted in Table 4 Table 5. The medullary cell and nuclear diameter showed a variation among the different zones of the adrenal gland which could be due to various secretory phases of the cells. However similar findings were observed in white leg horn, related to the medullary cell nuclei diameter by Kober *et al* [7]. However, on average, nuclear diameter of medullary was inconsistent with those of Tang *et al* [8] and Hodges [9].

### Medullary sinuses

The sinusoids were more prominent within the inner zone where they often create larger sinus venosi. The average diameters of the medullary sinuses in SZ, PZ and IZ in the adrenal gland of control group were,  $29.26 \pm 1.476 \mu\text{m}$ ,  $53.22 \pm 1.47 \mu\text{m}$  and  $64.86 \pm 1.49 \mu\text{m}$ . The average diameter of medullary sinuses in different zones of various experimental groups is given in Table 6. The present study not only allowed identification of the important effects of fasting stress in cortical and medullary tissue, but they also showed an increased percentage of blood vessels and medullary sinuses, often seen dilated and extensively filled with blood cells (hyperaemia) with increasing hours of fasting stress. Similar results in different stresses were observed in chicken by Muller *et al*. [10] and Zikic *et al*. [11]

## 4. Conclusion

Changes in adrenal gland morphology and cellular cum nuclear diameters of the cortical and medullary cells reduced at different levels of fasting stress as compared to normal in broiler chickens suggesting a possible role for the study of adrenal gland morphology as an indicator of chronic welfare problems in broiler.

**Table 1:** Mean values ( $\pm$ SE) of capsule thickness of the chicken adrenal gland at different fasting intervals

Fasting level	Capsule Thickness
Normal	$20.82 \pm 0.492^a$
12 hour	$21.54 \pm 0.484^a$
24 hour	$22.15 \pm 0.612^a$
48 hour	$22.25 \pm 0.796^a$
Over all mean	$21.62 \pm 0.303^a$

**Table 2:** Mean values ( $\pm$ SE) of cortical cell diameter in various zones of the chicken adrenal gland at different fasting intervals

Parameter	Fasting level				
	Normal	12 hours	24 hours	48 hours	Overall Mean $\pm$ SE
Cortical cell diameter					
Peripheral zone	$9.15 \pm 0.209^c$	$5.50 \pm 0.231^a$	$7.42 \pm 0.323^b$	$5.21 \pm 0.181^a$	$6.82 \pm 0.350$
Inner zone	$6.19 \pm 0.147^a$	$5.38 \pm 0.256^b$	$6.32 \pm 0.240^a$	$6.07 \pm 0.140^a$	$5.99 \pm 0.121$

Mean  $\pm$ SE values having different superscripts 24 hour 48 hour column wise differ significantly

**Table 3:** Mean values ( $\pm$ SE) of cortical cell nuclei diameter in various zones of the chicken adrenal gland at different fasting intervals

Parameter	Fasting level				
	Normal	12 hours	24 hours	48 hours	Overall Mean $\pm$ SE
Cortical cell nuclei diameter					
Peripheral zone	4.48 $\pm$ 0.181 <sup>a</sup>	3.18 $\pm$ 0.168 <sup>b</sup>	3.40 $\pm$ 0.163 <sup>b</sup>	3.51 $\pm$ 0.051 <sup>b</sup>	3.64 $\pm$ 0.125
Inner zone	4.50 $\pm$ 0.218 <sup>a</sup>	3.47 $\pm$ 0.142 <sup>b</sup>	3.44 $\pm$ 0.138 <sup>b</sup>	3.43 $\pm$ 0.154 <sup>b</sup>	3.71 $\pm$ 0.122

Mean  $\pm$ SE values having different superscripts row wise differ significantly

**Table 4:** Mean values ( $\pm$ SE) of medullary cell diameter in various zones of the chicken adrenal gland at different fasting intervals

Parameter	Fasting level				
	Normal	12 hours	24 hours	48 hours	Overall Mean $\pm$ SE
Medullary cell diameter					
Sub capsular zone	5.92 $\pm$ 0.288 <sup>a</sup>	5.23 $\pm$ 0.107 <sup>b</sup>	5.24 $\pm$ 0.089 <sup>b</sup>	5.41 $\pm$ 0.196 <sup>b</sup>	5.45 $\pm$ 0.105
Peripheral zone	9.13 $\pm$ 0.256 <sup>c</sup>	5.27 $\pm$ 0.179 <sup>ab</sup>	5.54 $\pm$ 0.232 <sup>b</sup>	5.67 $\pm$ 0.272 <sup>a</sup>	6.40 $\pm$ 0.348
Central zone	7.12 $\pm$ 0.136 <sup>a</sup>	5.15 $\pm$ 0.148 <sup>b</sup>	5.80 $\pm$ 0.267 <sup>b</sup>	5.69 $\pm$ 0.268 <sup>b</sup>	5.94 $\pm$ 0.181

Mean  $\pm$ SE values having different superscripts row wise differ significantly

**Table 5:** Mean values ( $\pm$ SE) of medullary cell nuclei diameter in various zones of the chicken adrenal gland at different fasting intervals

Parameter	Fasting level				
	Normal	12 hours	24 hours	48 hours	Overall Mean $\pm$ SE
Medullary cell nuclei diameter					
Sub capsular zone	4.36 $\pm$ 0.240 <sup>b</sup>	3.47 $\pm$ 0.152 <sup>a</sup>	3.40 $\pm$ 0.127 <sup>a</sup>	3.12 $\pm$ 0.038 <sup>ab</sup>	3.59 $\pm$ 0.121
Peripheral zone	4.18 $\pm$ 0.116 <sup>b</sup>	3.52 $\pm$ 0.169 <sup>a</sup>	3.63 $\pm$ 0.092 <sup>a</sup>	3.19 $\pm$ 0.067 <sup>a</sup>	3.63 $\pm$ 0.092
Inner zone	4.49 $\pm$ 0.155 <sup>b</sup>	3.49 $\pm$ 0.112 <sup>a</sup>	3.29 $\pm$ 0.103 <sup>a</sup>	3.14 $\pm$ 0.069 <sup>a</sup>	3.60 $\pm$ 0.122

Mean  $\pm$ SE values having different superscripts row wise differ significantly

**Table 6:** Mean values ( $\pm$ SE) of length of medullary sinus in different zones the chicken adrenal gland at different fasting intervals

Parameter	Fasting level				
	Normal	12 hours	24 hours	48 hours	Overall Mean $\pm$ SE
Medullary sinus					
Sub capsular zone	29.26 $\pm$ 1.47 <sup>a</sup>	30.08 $\pm$ 1.67 <sup>a</sup>	32.42 $\pm$ 2.1 <sup>ab</sup>	35.48 $\pm$ 1.50 <sup>b</sup>	31.81 $\pm$ 0.942
Peripheral zone	53.22 $\pm$ 1.47 <sup>a</sup>	53.13 $\pm$ 0.13 <sup>a</sup>	53.82 $\pm$ 1.47 <sup>a</sup>	54.70 $\pm$ 0.133 <sup>a</sup>	53.72 $\pm$ 0.674
Inner zone	64.86 $\pm$ 1.49 <sup>a</sup>	65.14 $\pm$ 1.26 <sup>a</sup>	65.58 $\pm$ 1.18 <sup>a</sup>	65.56 $\pm$ 1.17 <sup>a</sup>	65.54 $\pm$ 0.614

Mean  $\pm$ SE values having different superscripts row wise differ significantly

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