

# Effect of season on the some blood hormones and enzymes of Iraqi bull buffalo

## Abstract

The present study was conducted to investigate some aspects of the reproductive system in Iraqi bull buffaloes and the effects of seasonal changes on physiological parameters. 96 blood samples and male reproductive system of buffalo's bull were collected from the slaughter house during August 2013 to August 2014. Blood samples were analyzed to study the effect of season on the blood hormones and enzymes during the different seasons of the year, the results of the hormonal assays showed that the highest level of the Follicular Stimulation Hormone (FSH) and Lutenizing Hormone (LH) record in autumn 0.66ml U/ML, 0.91,μ/ml, respectively, while it was 0.46μ/ml, 0.23ml U/ML in winter and 0.40ml U/ML, 0.18,μ/ml in spring, while the lowest level showed in summer 0.32ml U/ML, 0.16,μ/ml, respectively. The results of the of the hormones testosterone and estradiol-17β showed that in autumn were 2.732m.mol/L, 108.77p.mol/L, respectively, in winter were 2.339m.mol/L, 70.69p.mol/L and in spring were 1.675m.mol, 40,80p.mol, while the lowest rate were in summer 1.516m.mol, 23.37p.mol/L.

Study results showed that the levels of enzymes to the privation of significant difference between the different seasons in the enzyme lactic Dehydrogenase and has the highest rate recorded in autumn 1552.85IU/L and 1510.33IU/L in winter and then spring and summer 1471.66IU/L, 1379.36IU/L. While there was a significant difference  $p(<0.05)$  during the seasons (autumn, Spring, Summer) for the enzyme acid phosphatase and the highest level was in autumn 2.732U/L, while the lowest level was in summer 1.496U/L. The difference between the seasons of the year in the enzyme alkaline phosphatase, the highest rate has been recorded in autumn, 224U/L and then winter, spring and summer, respectively (211.66, 210.75, 198.66U/L). The results of the study also showed that there is no significant differences between the seasons of the year in the amylase enzyme and the highest rate were showed in autumn and winter 11.62, 13.5U/L while the lowest rate in spring and summer 11.4U/L, 8.6U/L respectively.

**Keywords:** bull buffaloes, hormones, enzymes, season

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## Introduction

The water buffalo has been associated with people since prehistoric times. It is one of the oldest species of domesticated livestock and continues to be used as a source of milk and meat, and as a draft animal. Water buffaloes have been classified into the river and swamp types.<sup>1</sup> The river type is larger, used for milk, wallows in fresh-water and originates, the swamp type is smaller, used for draft and meat, wallows in muddy water and is indigenous to most Asian countries.<sup>1</sup>

The Iraqi Buffalo contribute in supplying the local market with high nutritional value and to fill part of the shortfall in the case of dairy products in scarce during some months of the year, noting that most of the dairy products in the local markets is of buffalo milk during a period of drought, with cows male as well as the superiority of some local animals in the amount of milk production and content of fat.<sup>2</sup> The environmental factors associated with heat stress which affects the physiological systems governing thermal regulation and the maintenance of positive heat loss, high ambient temperature is the major constraint on animal productivity.<sup>3</sup> Therefore, care must be taken because it represents animal the Twenty-first century for being resistant to diseases and producer of milk and has the ability fattening outweigh all other farm animals.

For these reasons and the fact that male buffalo being responsible for raise reproductive efficiency by selecting the right season for the breeding to reduce the economic losses, especially since the female buffalo characterized silent estrus, weak estrus signs, short period of ovulation,<sup>4</sup> and the problem of reduced libido in male during the non-breeding season. So through the study, we will determine the appropriate season for pollination by studying the level of hormone and differences between the gonadotropin hormones, enzymes in mature male buffalo, and by this study we can raise the reproductive efficiency of female buffaloes and thus raise production and increased income for owner and increase development and economic activity of the country and prevent import from abroad.

## Materials and methods

Ninety six blood samples of healthy bulls buffalo, aged (3-5) years, were obtained from the slaughterhouse from August 2013 to August 2014, 3visits/week before slaughter immediately, (8 samples) for each month from the (Jugular vein) then empties in test tube (Gel tube) size (8ml) contains Gelatin substance which help to isolate the serum from the blood, then transported to the lab, the samples have been centrifuged at 3000/rpm for 10minutes for determination the levels of hormones (estrogen, testosterone, follicle stimulating

hormone, luteinizing hormone) and enzymes (lactic dehydrogenase, acid phosphates, alkaline phosphatase, and amylase) with specific kits and according to the instructions of the company.

### Statistical analyses

Data were analyzed statistically by SPSS program, version 17 software 2010. Testing method used include one way ANOVA for comparisons among season followed by least significant differences (LSD) test for comparison between two groups. P value of  $p < 0.05$  were considered to record statistical significances.

## Results

### Testosterone hormone

The results showed that the mean of the level of the hormone testosterone in blood serum in autumn ( $2.732 \pm 0.323$ ) m.mol/L, winter ( $2.339 \pm 0.199$ ) m.mol/L, spring ( $1.675 \pm 0.249$ ) m.mol/L and summer ( $1.516 \pm 0.122$ ) m.mol/L. The statistical analysis showed significant variation ( $p < 0.05$ ) between the level of the hormone through the different seasons of the year autumn, summer and that the highest level of the testosterone in the autumn, no significant variation between winter, spring Table 1 & Figure 1.

### Estradiol hormone

The mean level of estradiol hormone in blood serum in autumn ( $108.77 \pm 17.23$ ) p.mol/L, winter ( $70.69 \pm 7.21$ ) p.mol/L, spring ( $40.8 \pm 8.37$ ) p.mol/L and summer ( $23.37 \pm 3.27$ ) p.mol/L. The statistical analysis showed significant variation ( $p < 0.05$ ) between the level of the hormone through the different seasons of the year (autumn, winter, summer) that the highest level of the estradiol in the autumn, there are no significant variation between winter, spring. (Table 1 & Figure 2).

### Follicular stimulation hormone

The results showed that the level of hormone FSH in blood serum in autumn was ( $0.66 \pm 0.08$ ) m.l U/ML, winter ( $0.46 \pm 0.07$ ) m.l U/ML,

Spring ( $0.40 \pm 0.04$ ) m.l U/ML and summer ( $0.32 \pm 0.03$ ) m.l U/ML. The statistical analysis showed significant variation ( $p < 0.05$ ) between the level of the hormone in autumn and the other of season (winter, spring, summer). And that the highest level of the FSH in the autumn, there are no significant variation between winter, spring and summer Table 1 & Figure 3.

### Luteinizing hormone

The mean level of LH hormone in blood serum in autumn was ( $0.91 \pm 0.26$ ) m.u/ml, winter ( $0.23 \pm 0.06$ ) m.u/ml, spring ( $0.18 \pm 0.02$ ) m.u/ml and summer ( $0.16 \pm 0.02$ ) m.u/ml. The statistical analysis showed significant variation ( $p < 0.05$ ) between the level of the hormone in autumn and the other of season winter, spring, summer that the highest level of the LH in the autumn, there are no significant variation between winter, spring and summer. (Table 1 & Figure 4).

### Lactic dehydrogenase

In Table 2 & Figure 5 showed the level of Lactic dehydrogenase in blood serum in autumn was ( $1552.85 \pm 82.55$ ) U/L, winter ( $1510.33 \pm 140.39$ ) U/L, spring ( $1471.66 \pm 146$ ) U/L and summer ( $1379.36 \pm 57.9$ ) U/L. The statistical analysis showed no significant variation between the level of the lactic dehydrogenase through the different seasons of the year and that the highest level of the lactic dehydrogenase was in autumn, summer and followed by spring and the lowest level was in winter.

### Acid phosphatase

In Table 2 & Figure 6 showed the level of Acid Phosphatase in blood serum in autumn was ( $2.732 \pm 0.323$ ) U/L, winter ( $2.337 \pm 0.195$ ) U/L, spring ( $1.533 \pm 0.185$ ) U/L and summer ( $1.496 \pm 0.132$ ) U/L. The statistical analysis showed significant variation ( $p < 0.05$ ) between the level of the Acid Phosphatase through different seasons of the year (autumn, spring, summer) and that the highest level of the Acid Phosphatase was in spring, autumn and followed by winter and the lowest level was in the summer.

**Table 1** The effect of season on hormones in blood of Iraqi buffalo bull

Hormonal	Autumn mean $\pm$ SE	Winter mean $\pm$ SE	Spring mean $\pm$ SE	Summer mean $\pm$ SE
Testosterone m.mol	2.732 $\pm$ 0.323a	2.339 $\pm$ 0.199 ab	1.675 $\pm$ 0.249 bc	1.516 $\pm$ 0.122c
Estradiol p.mol/L	108.77 $\pm$ 17.23a	70.69 $\pm$ 7.21 b	40.8 $\pm$ 8.37bc	23.37 $\pm$ 3.27c
Follicular Stimulation Hormone m.l U/ML	0.66 $\pm$ 0.08a	0.46 $\pm$ 0.07 b	0.40 $\pm$ 0.04b	0.32 $\pm$ 0.03b
Luteinizing Hormone m.u/ml	0.91 $\pm$ 0.26a	0.23 $\pm$ 0.06 b	0.18 $\pm$ 0.02b	0.16 $\pm$ 0.02b

\*The similar letters refers to the non-significant differences among months while different letters refers to the significant differences at ( $p < 0.05$ )

**Table 2** Effect of season on enzyme in blood of Iraqi buffalo bull

Hormonal	Autumn mean $\pm$ SE	Winter mean $\pm$ SE	Spring mean $\pm$ SE	Summer mean $\pm$ SE
Lactic dehydrogenase IU/L	1552.85 $\pm$ 82.55a	1510.33 $\pm$ 140.39 a	1471.66 $\pm$ 146a	1379.36 $\pm$ 57.9a
Acidphosphatase U/L	2.732 $\pm$ 0.323a	2.337 $\pm$ 0.195 ab	1.533 $\pm$ 0.185 bc	1.496 $\pm$ 0.132c
Alkaline phosphatase U/L	224 $\pm$ 35.52a	211.66 $\pm$ 36.95a	210.75 $\pm$ 16.69a	198.66 $\pm$ 24.4966a
Amylase U/L	13.5 $\pm$ 1.95	11.62 $\pm$ 1.66	11.4 $\pm$ 1.42	8.6 $\pm$ 3.2

\*The similar letters refers to the non-significant differences among months while different letters refers to the significant differences at ( $p < 0.05$ )

### Alkaline phosphatase

In Table 2 & Figure 7 showed the level of Alkaline Phosphatase in blood serum in autumn was  $(224 \pm 35.52)$ U/L, winter  $(211.66 \pm 36.95)$  U/L, spring  $(210.75 \pm 16.69)$ U/L and summer  $(198.6 \pm 24.49)$ U/L. The statistical analysis showed no significant variation between the level of the Alkaline Phosphatase through the different seasons of the year and that the highest level of the Alkaline Phosphatase was in summer, spring and followed by winter and the lowest level was in the autumn.

### Amylase

In Table 2 & Figure 8 showed the level of Amylase in blood serum in autumn was  $(13.5 \pm 1.95)$ U/L, winter  $(11.62 \pm 1.66)$ U/L, spring  $(11.4 \pm 1.42)$ U/L and summer  $(8.6 \pm 3.2)$ U/L. The statistical analysis showed no significant variation between the level of the amylase through the different seasons of the year and that the highest level of the amylase was in autumn and the lowest in summer.

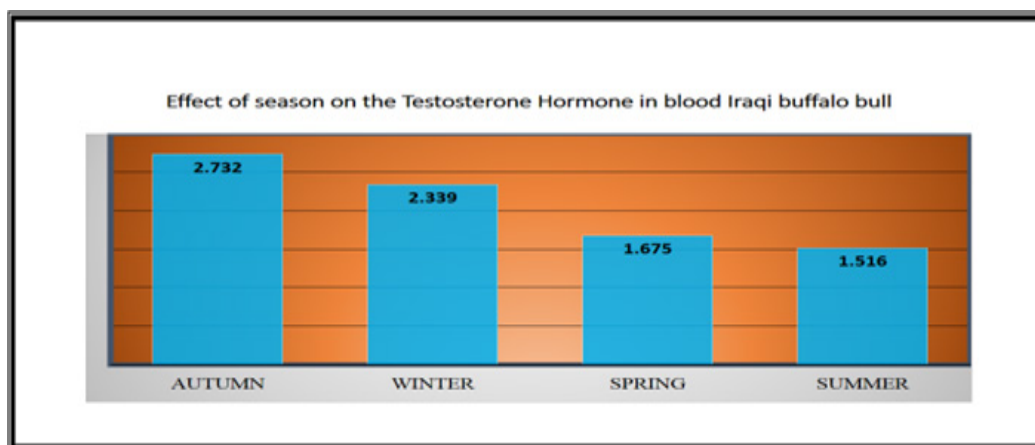


Figure 1 The effect of season on testosterone hormone level.

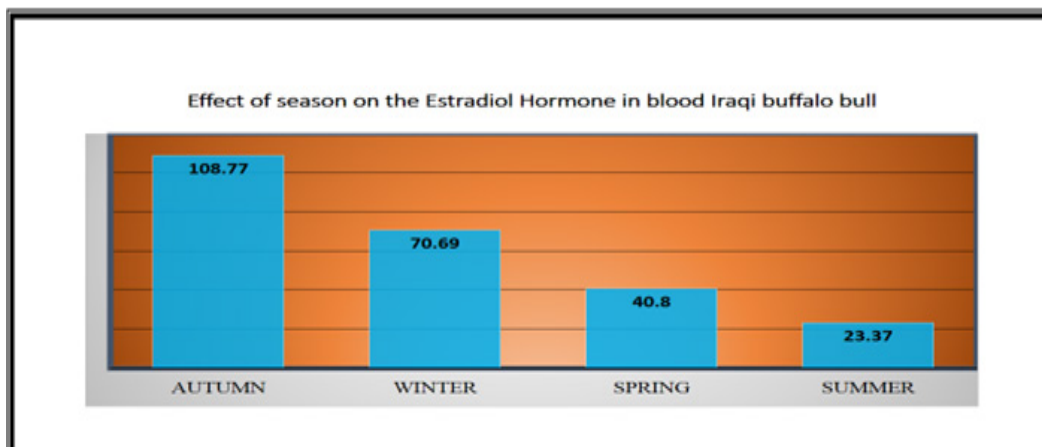


Figure 2 The effect of season on estradiol hormone level.

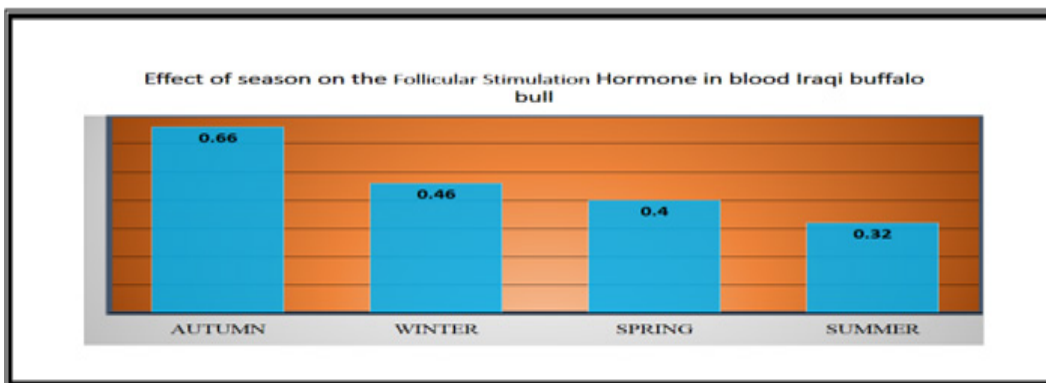


Figure 3 The effect of season on follicular stimulation hormone level.

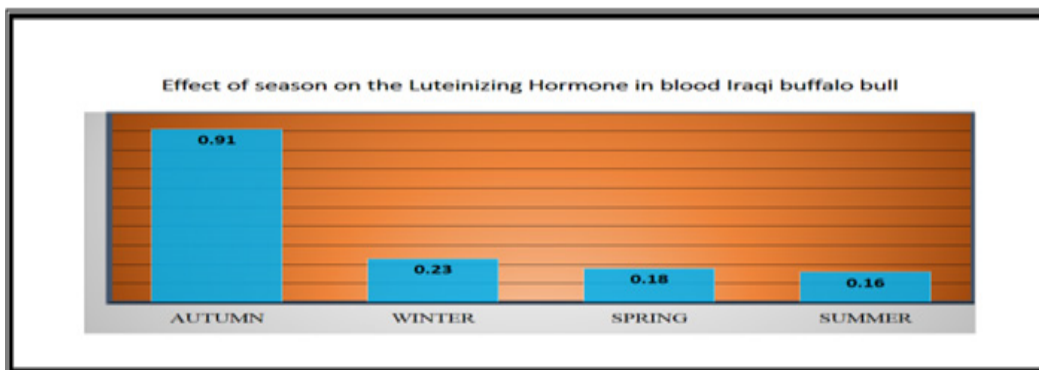


Figure 4 The effect of season on luteinizing hormone level.

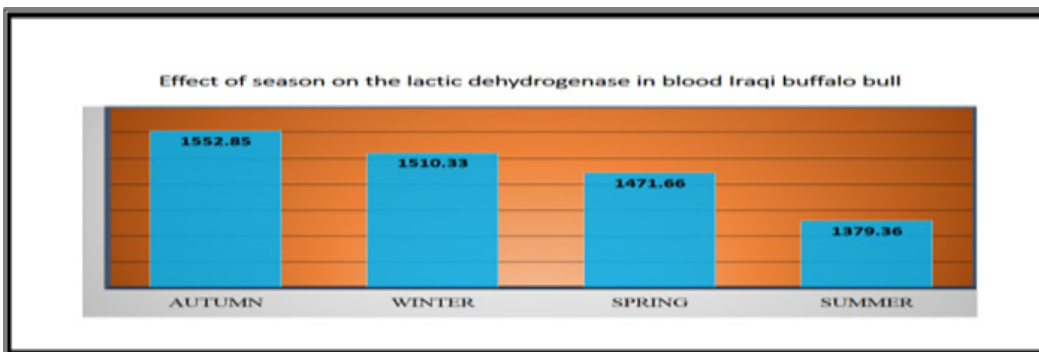


Figure 5 Effect of the season on lactic dehydrogenase in blood Iraqi buffalo bull.

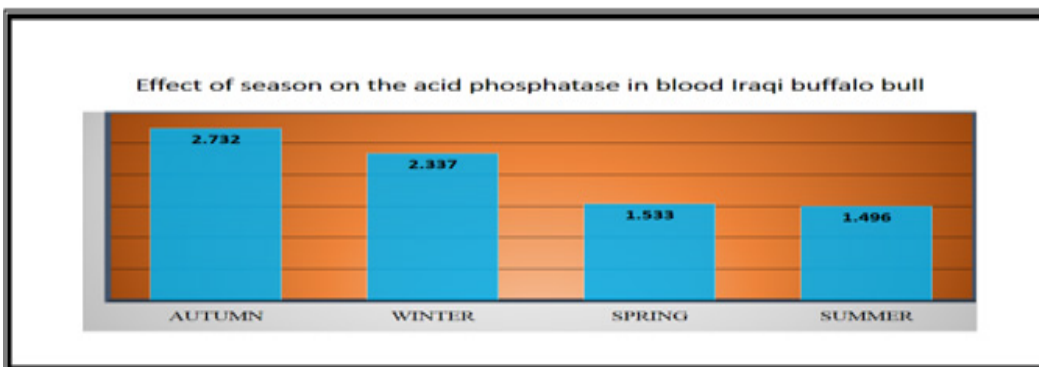


Figure 6 Effect of the season on acid phosphatase in blood Iraqi buffalo bull.

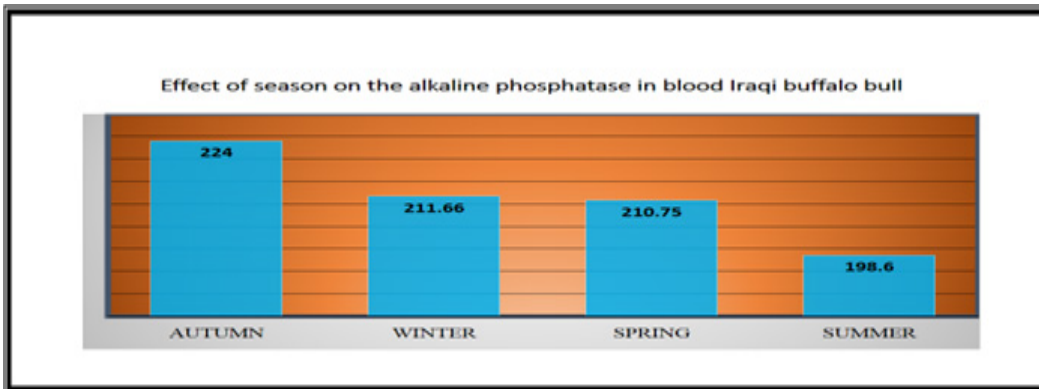


Figure 7 Effect of the season on alkaline phosphatase in blood Iraqi buffalo bull.

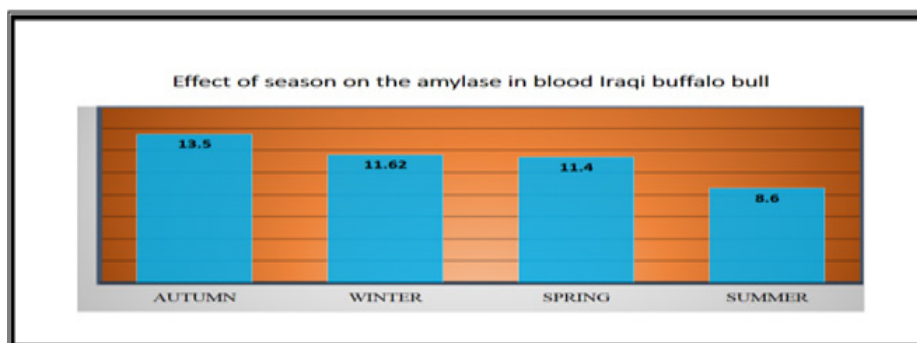


Figure 8 Effect of the season on amylase in blood Iraqi buffalo bull.

## Discussion

There were no available references of the hormonal and enzymes levels in the blood bull buffalo during different seasons except for the seminal fluid.

### The Hormonal study

The present study showed the mean of hormone (Testosterone, Estradiol, Follicular stimulation hormone, Luteinizing hormone) was (2.732±0.323m.mol/L, 108.77±17.23p.mol/L, 0.66±0.08m.IU/ML, 0.91±0.26m.u/ml) respectively in autumn, and in winter were (2.339±0.199m.mol/L, 70.69±7.21p.mol/L, 0.46±0.07m.IU/ML, 0.23±0.06m.u/ml) while in spring and summer was (1.675±0.249m.mol/L, 40.8±8.37p.mol/L, 0.40±0.04m.IU/ML, 0.18±0.02m.u/ml) and (1.516±0.122m.mol/L, 23.37±3.27p.mol/L, 0.32±0.03m.IU/ML, 0.16±0.02m.u/ml) respectively Table 1. This results agreement with<sup>5</sup> which indicated that an initial rise in Follicle Stimulating Hormone (FSH) results in a proliferation of Sertoli cells, a lengthening of the seminiferous tubules and an increase in tubule diameter. At the same time, there is a rise in Luteinizing Hormone (LH) secretion resulting in increased testosterone production by the Leydig cells and agree with as show by Simanainen et al.,<sup>6</sup> which indicate that the weight of the testis was one of the markers of a possible alteration in androgen status; A decrease in testicular weight is most likely due to a decrease in the level of serum testosterone.

The results of present study agree with Tekepety et al.<sup>7</sup> which shown that the Increase in the activity of the testes and epididymis in moderate and cold season and these activities are regulated by the increases testosterone hormone levels in these seasons, that increased in mating seasons and decreased in non-mating seasons as shown by Arrighi et al.,<sup>8</sup> and in bull.<sup>9</sup> while Wu et al.<sup>10</sup> Al-Sahaf et al.<sup>11</sup> refer that the testes are affected by the increase of temperature lead to decreasing the number of the receptors of Luteinizing Hormone (LH) that presents on Leydig cells and then decreasing in testosterone hormone and activity of the testes in hot season, Al-Sahaf et al.<sup>11</sup> indicated to an increase in testosterone, FSH and LH hormones caused an increase in diameter of testicular.

This results of present study similar with<sup>12</sup> which shown that the level of hormone testosterone and LH-receptors were higher in the breeding season (autumn, winter) compared to those in the low breeding season (spring, summer), and agree with Hochereau-de-Rievers et al.<sup>13</sup> The increase in the measures of epididymis in moderate and cold months as compared with hot months is a result of an increase in length and diameter of the seminiferous tubules.

### Enzymatic study

**Alkaline phosphatase and acid phosphatase:** The Enzymatic study showed that the means of alkaline phosphatase and acid phosphatase in autumn were (224±35.52) U/L, (2.732±0.323)U/L and in winter (211.66±36.95)U/L, (2.337±0.195)U/L, also in spring and summer were (210.75±16.69)U/L, (1.533±0.185)U/L and (198.66±24.49)U/L, (1.496±0.132)U/L respectively. This result supports with Gundogun et al.<sup>14</sup> refer to presence of the enzyme activities follows reproductive seasonality, Abdel-Samee et al.<sup>15</sup> indicated that the liver function may be partially affected by heat stress that not agreement with present study. Kataria et al.<sup>16</sup> found that ALP and ACP were significantly higher during extremely hot (May - June) than in extreme cold (December-January) conditions, in India not supported present study, and also not agree with Litwack.<sup>17</sup> The increase in ALP activity in summer and winter may be due to increase secretion adrenocorticotrophic hormone (ACTH) due to environmental stress. Perhaps to different the breed of buffalo in Indian or by the environment condition in this region, the possible source of these enzymes is thought to be the testes or epididymitis because they show a positive correlation with sperm concentration and a negative correlation with semen volume.<sup>18</sup>

**Amylase:** In Table 2 & Figure 8 showed that the means level of amylase in different season of the year's were 13.5±1.95 U/L, winter 11.62±1.66 U/L, spring 11.4±1.42U/L and summer 8.6±3.2U/L respectively, this study supports by Hafs HD et al.,<sup>19</sup> have reported separate trials, both of which showed that crude preparations of  $\alpha$ -amylase (50-100units/mg), when added to extenders for bull semen at 10 $\mu$ g/ml significantly increased conception rates. The former trial also showed that the addition of  $\beta$ -amylase increased of conception rates,  $\alpha$ -amylase, and enzymes involved in the degradation glycogen, sperm capacitation.<sup>20</sup> Increase amylase leads to increase enrage which effected on reproduction and growth of animals.

**Lactic dehydrogenase:** In present study showed Increase in LDH in autumn was 1552.85±82.55IU/L, winter 1510.33±140.39 IU/L, spring 1471.66±146 IU/L and summer 1379.36±57.9IU/L respectively. The present study agree with Duan et al.<sup>21</sup> which shows that the Lactic dehydrogenase plays an important metabolic role in sperm capacitation and fertilization, the results of present study supported by Karoskoski et al.,<sup>18</sup> which shows that the source of enzyme Lactic dehydrogenase is thought to be the testes or epididymides because they show a positive correlation with sperm concentration and a negative correlation with semen volume, and the present study agree with Gundogun et al.,<sup>14</sup> found presence of enzyme activities follows reproductive seasonality, Jones et al.<sup>22</sup> indicate reduced Lactic dehydrogenase activity in seminal plasma might indicate disturbed spermatozoal

function and metabolism, may be due to hot conditions changes in the biological functions that include depression in feed intake, efficiency and utilization, disturbances in metabolism of water, energy and enzymatic reactions. Such changes result in impairment of reproduction and production performances. The effect of heat stress is aggravated when heat stress is accompanied by high ambient humidity,<sup>23,24</sup> which lead to decrease lactic dehydrogenase in summer.

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## Conflict of interest

Author declares that there is no conflict of interest.

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