**Revista de Chimie**
*https://revistadechimie.ro*
*https://doi.org/10.37358/Rev.Chim.1949*

# Nutritional Imbalance, Toxicology and Deficiency Potential of Livestock

**SALAMA A. OUF[1*], JAHANZAIB RASHEED[2], KAFEEL AHMAD[2], ZAFAR IQBAL KHAN[2], KINZA WAJID[2], MUHAMMAD NADEEM[3], SHAHWANA TEHREEM[3], TASAWAR ABBAS[4], HUMAYUN BASHIR[2], ASMA ASHFAQ[2], MUDASRA MUNIR[2], IFRA SALEEM MALIK[2], HAFSA MEMOONA[6], IJAZ RASOOL NOORKA[7], SHAHZADI MAHPARA[8], OSAMA B. MOHAMED[9]**

[1]Botany and Microbiology Department, Faculty of Science, Cairo University, Giza 1263, Egypt
[2]Department of Botany, University of Sargodha, University Road, Sargodha, Punjab, 40100 Pakistan
[3]Institute of Food Science and Nutrition, University of Sargodha, University Road, Sargodha, Punjab 40100, Pakistan
[4]Department of Earth Sciences, University of Sargodha, University Road, Sargodha, Punjab 40100 Pakistan
[5]Department of Botany, University of Gujrat, Jalalpur Road, Gujrat, Pakistan
[6]Department of Zoology, Lahore College for Women University, Jail Road, Lahore, Pakistan
[7]Plant Breeding and Genetics, Agriculture College, University of Sargodha, University Road, Sargodha, Punjab 40100, Pakistan
[8]Department of Plant Breeding and Genetics, Ghazi University, Dera Ghazi Khan, Punjab 32200, Pakistan
[9]Department of Zoology, College of Science, King Saud University, P.O Box 2455, Riyadh 11451, Saudi Arabia

***Abstract.*** *This study investigated the changes in the enzymatic and biochemical profiles of lactating, non-lactating, and young buffaloes during different sampling. In the present research thirty buffaloes (Nili-Ravi) were selected and divided into three categories lactating, non-lactating, and young. Four samplings were performed in different seasons (summer, autumn, winter, and spring), and 10 blood serum samples were collected from each category of Nili-Ravi buffaloes during each sampling period. Higher glucose, urea, creatinine, and mycotoxins (AFB1, ZEA, OTA) values were found during summer sampling season, higher SGPT (ALT) and SGOT (AST) values were found during the autumn season, higher cholesterol, alkaline phosphatase and uric acid values were found during the winter season.*

***Keywords:*** *blood serum, buffalo, enzymatic profile, biochemistry, Pakistan*

## 1.Introduction

The role of livestock is important to convert crop residues, agricultural by-products, and wastes into milk, meat, wool, hair etc. In this regard, especially buffalo, can efficiently convert poor roughages into valuable products, like meat and milk. Otherwise, these by-products and wastes would lead to an increase environmental pollution, which is the most severe issue at present [1].

Among livestock buffaloes playing a major role in Pakistan's economy, there are many breeds of buffaloes in Pakistan. However, Nili-Ravi (*Bubalus bubalis*) is the best performing animal, producing more milk than the other breeds of the world. Milk yield of this breed is 1800-2500 L/day with a 6.5% fat. Given the increasing demand for milk and meat, more and more emphases are being placed in the improvement of the health of this species. In spite of having good production potential, the buffaloes are vulnerable to various fatal diseases, and thus the farmers face heavy economic losses. These fatal diseases such as the late age of maturity, long calving interval and silent heat etc. are very common.

Assessment of the nutritional and health status of animals is invaluable in present-day animal husbandry. The metabolic depiction is forsooth a complete deposit of blood hematological, enzymatic, and biochemical characterization, which gives a full evaluation of the health status of livestock and also help researchers to treat their metabolic disorders [2].

In buffaloes, during late pregnancy, blood serum lipids depiction is distinguished by a higher concentration of total cholesterol, triglycerides, and lipoproteins. The physiological changes are due to

**email:salama@sci.cu.edu.eg*

the deprecated responsiveness of target tissues respecting insulin that, basically related to higher mobilization of fatty acids from adipose tissue. Disparity in blood cholesterol content has been noticed throughout estrus and pregnancy, as progenitor of the steroid hormones.

Blood biochemical variables like total protein, triglycerides, free fatty acids and urea are prime index of the metabolic activity in lactating animals [3]. In the course of pregnancy, maternal tissues take part in supplying energy for reproduction processes, that may change the blood serum chemistry values, change may also by some other factors as a breed, age, malnutrition, fetal growth, or season [4]. Moreover, seasonal variations in mineral concentration affect the biochemical parameters (urea, AST, ALT, ALP, bilirubin, cholesterol, triglycerides) in the blood of Ruminants [5].

In this direction, this study critically investigated the changes in the biochemical profiles of lactating, non-lactating, and young buffaloes during different sampling seasons (summer, autumn, winter, and spring).

## 2.Materials and methods

### Study site

Livestock Experiment Station Chak No. 61/Mb, Khushab was allocated for the current exploration. Khushab is a district of Punjab province of Pakistan (Supplementary Figure S1). Khushab district has Natural Uranium Research Reactor and area to the Heavy water, for Pakistan crucial ammunition Program censorious segment, which is warmly examined**.** Khushab is located in Pakistan with 102,793 population and 100-miles (or 160 km) South-West of Islamabad. Khushab District coordinates 32.2883° N, 72.2831° E.

### Blood samples

The Nili-Ravi buffaloes were maintained under the existing field conditions comprised of the study animals. In the current research, thirty buffaloes (Nili-Ravi) were selected and divided into three categories lactating, non-lactating, and young. Four samplings were done in different seasons (summer, autumn, winter, and spring), and 10 blood plasma samples were collected from each category of Nili-Ravi buffaloes during each sampling period. With the disinfected needle, blood samples of buffaloes were taken in a standing position from the jugular vein. Na-Citrate voiles were placed in the heparin, so clotting should be dodged. Through centrifugation, serum was separated from plasma. The serum was kept in a freezer at -20$^{o}$C in small voiles with labeling to be used for biochemical tests.

### Statistical analysis

Information for various credits was exposed to a factual investigation utilizing the SPSS (Statistical Package for Social Sciences) software and one-way analysis of variance (ANOVA). As demonstrated by Steel et al. in 2006, he tried at 0.05, 0.01, and 0.001 proportions of the likelihood of amongst the mean of accurate criticalness, as suggested by Steel et al. [6].

### Biochemical tests

### Determination of cholesterol

Measurement of cholesterol was determined to detecting hypercholesterolemia, lipid and lipoprotein digestion issues. Rule free cholesterol and cholesterol discharged from its esters are oxidized after enzymatic hydrolysis. The pointer quinoneimine was shaped from hydrogen peroxide and 4-amino-antipyrine within sight of phenol and peroxide [7].

We ascertain the cholesterol level in the examples as pursues:

$$Cholesterol = \frac{Absorbance\ (TEST) \times Concentration\ of\ standard\ (\mu mol/L)}{Absorbance\ (STANDARD)}$$

**Determination of glucose**

Reagent kits were employed for the quantitative determination of glucose concentration in the serum.

**Enzymatic colorimetric method (GOD/POD/PAP)**

Determination of glucose concentration is important in the diagnosis and treatment of disorders of carbohydrate metabolism. Values higher or lower than the reference are of diagnostic significance. The levels are increased in diabetes mellitus, hyperthyroidism and in the hyperactivity of the pituitary gland. Decreased levels are observed in cases of overproduction of insulin by the pancreas, of pancreas tumors, as well as with hypofunction of the organs involved in glucose synthesis, glycogen biodegradation, and another carbohydrate metabolism.

**Determination of AST, ALT, and total serum protein**

Serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) was determined by the colorimetric method using commercially available kits (BioMérieux, France and Spin react, Spain).

**Determination of alkaline phosphatase**

Alkaline phosphatase was determined by spectrophotometry technique [8].

**Determination of urea and uric acid**

Uric acid was determined by using the urease enzyme to form hydrogen peroxide. Hydrogen peroxide reacts with a chromogenic dye using peroxidase. The concentration of urea was calculated by sample absorbance and their standard solution [9].

**Determination of creatinine**

The creatinine procedure is a kinetic modification of the Jaffe procedure, 2 in which the response of creatinine with picric acid at basic *p*H shapes a yellow-orange complex [10]. Creatinine is released during the metabolism of creatine phosphate and is excreted by the kidneys. Creatinine concentration in blood and urine represents a primary indicator for renal function, especially glomerular filtration. Increased levels are associated with acute renal impairment, chronic nephritis, obstruction of the urinary tract, strong physical overloading. Low creatinine concentrations are found in conditions with juvenile diabetes mellitus, pregnancy, and muscular dystrophy.

**Mycotoxins determination**

The collected blood has been tested for Aflatoxin B1 (AFB1), Zearalenone (ZEA), and Ochratoxin (OTA) production. The toxin was extracted from the sample based on the method reported by D'Arco et al., 2008) [11]. Five replicates were investigated for each treatment. OTA was extracted with 15 mL ethyl acetate after acidifying with 10 $\mu$L concentrated HCl. After shaking for 30 min, the supernatants were combined and evaporated to dryness in a rotary evaporator. OTA and FB2 were measured quantitatively using enzyme-linked immunosorbent assay (ELISA) test strips, which were examined using the rapid one-step assay (ROSA) system (Charm Biosciences Inc., Lawrence, MA, USA). This system provides results equivalent to those of commercial HPLC methods [12]. The strip is a quantitative lateral flow immunoassay with a sensitivity range of 0-150 µg/kg and a limit of detection of 1 µg/kg. One hundred µL of the extracted mycotoxin was diluted with 1 mL of mycotoxin dilution buffer, and 300 µL of this solution was pipetted onto the strip and incubated for 10 min before removing the strip. The strip was then read on a ROSA-M reader within 2 min. Each sample was analyzed in triplicate.

# 3.Results and discussions

## Biochemical compounds

### Cholesterol

According to the analysis of variance, in lactating and young buffaloes, the cholesterol from blood serum fundamentally ($p<0.001$) influenced by inspecting seasons but it was non-significantly ($p>0.05$) affected by the sampling seasons in dry buffaloes (Table 1). The mean concentrations of cholesterol in blood serum of lactating buffaloes were 148.90 mg/dL (summer), 109.67 mg/dL (autumn), 154.70 mg/dL (winter), and 154 mg/dL (spring). The mean values of cholesterol in blood of dry buffaloes were 108.83 mg/dL (summer), 99.43 mg/dL (autumn), 106.40 mg/dL (winter) and 106.73 mg/dL (spring). The average contents of cholesterol in blood of young buffaloes were 133 mg/dL (summer), 105.40 mg/dL (autumn), 140 mg/dL (winter), and 138.7 mg/dL (spring). The higher mean cholesterol concentrations were observed in lactating buffaloes during winter season, and the lower mean cholesterol contents were noticed in dry buffaloes in the autumn sampling season (Figure 1a). The detected orders of cholesterol contents were winter>spring>summer>autumn in lactating buffaloes, summer>spring> winter>autumn in dry buffaloes and winter > spring > summer > autumn in young buffaloes.

**Table 1.** Analysis of variance for biochemical parameters in blood of buffaloes influenced by different seasons

| Source of Variation (SOV) | | Degrees of Freedom | Mean Squares | | |
|---|---|---|---|---|---|
| | | | Lactating Buffaloes | Dry Buffaloes | Young Buffaloes |
| Cholesterol | Sampling Season | 3 | 4660.762*** | 167.133$^{ns}$ | 2737.292*** |
| | Error | 36 | 129.836 | 236.782 | 53.183 |
| Glucose | Sampling Season | 3 | 16.596 $^{ns}$ | 277.609*** | 15.255 $^{ns}$ |
| | Error | 36 | 10.798 | 10.290 | 11.923 |
| SGPT (ALT) | Sampling Season | 3 | 143.532* | 13.001 $^{ns}$ | 2144.144*** |
| | Error | 36 | 23.906 | 49.590 | 42.532 |
| SGOT (AST) | Sampling Season | 3 | 1176.594 $^{ns}$ | 6426.085*** | 24170.367*** |
| | Error | 36 | 262.913 | 454.253 | 423.496 |
| Alkaline Phosphatase | Sampling Season | 3 | 72036.576*** | 11586.958*** | 69134.517*** |
| | Error | 36 | 369.989 | 964.632 | 938.972 |
| Urea | Sampling Season | 3 | 20.895 $^{ns}$ | 57.928*** | 23.714 $^{ns}$ |
| | Error | 36 | 18.633 | 5.739 | 10.020 |
| Creatinine | Sampling Season | 3 | 0.534*** | 0.075 $^{ns}$ | 0.234*** |
| | Error | 36 | 0.060 | 0.038 | 0.026 |
| Uric acid | Sampling Season | 3 | 1.277*** | 0.188 $^{ns}$ | 0.528* |
| | Error | 36 | 0.071 | 0.103 | 0.145 |

All calculated values of serum cholesterol were higher than the reference range (34.92-76.82 mg/dL), which are earlier reported by Clerc and Solberg in 1987 [13]. It was noticed that lactating buffaloes, contrary to dry pregnant buffaloes, have decreasing serum cholesterol level. The serum total cholesterol content was minimum following calving and got build up as the lactation progresses [14]. To meet the lactation need a higher level of cholesterol with advancement of lactation was a physical modification. The observed blood cholesterol contents are higher than the investigations of Maurya *et al*. in 2015 [15].

## Glucose

In lactating and young buffaloes, the glucose concentration in blood serum fundamentally ($p<0.001$) was influenced by sampling seasons, but it was non-significantly ($p>0.05$) affected by the sampling seasons in dry buffaloes (Table 1). The mean glucose values in blood serum of lactating buffaloes were 29.77 mg/dL (summer), 32.10 mg/dL (autumn), 32.71 mg/dL (winter) and 31.97 mg/dL (spring). The mean glucose concentrations in blood serum of dry buffaloes were 34.10 mg/dL (summer), 32.63 mg/dL (autumn), 24.74 mg/dL (winter) and 23.90 mg/dL (spring). The average glucose contents in blood serum of young buffaloes were 31.28 mg/dL (summer), 31.10 mg/dL (autumn), 32.03 mg/dL (winter) and 33.80 mg/dL (spring). The higher mean glucose concentrations were observed in dry buffaloes during the summer season, and the lower mean glucose contents were noticed in dry buffaloes in the winter sampling season (Figure 1b). The detected orders of glucose contents were winter>autumn>spring> summer in lactating buffaloes, summer>autumn>spring>winter in dry buffaloes, and spring>winter> summer>autumn young buffaloes.

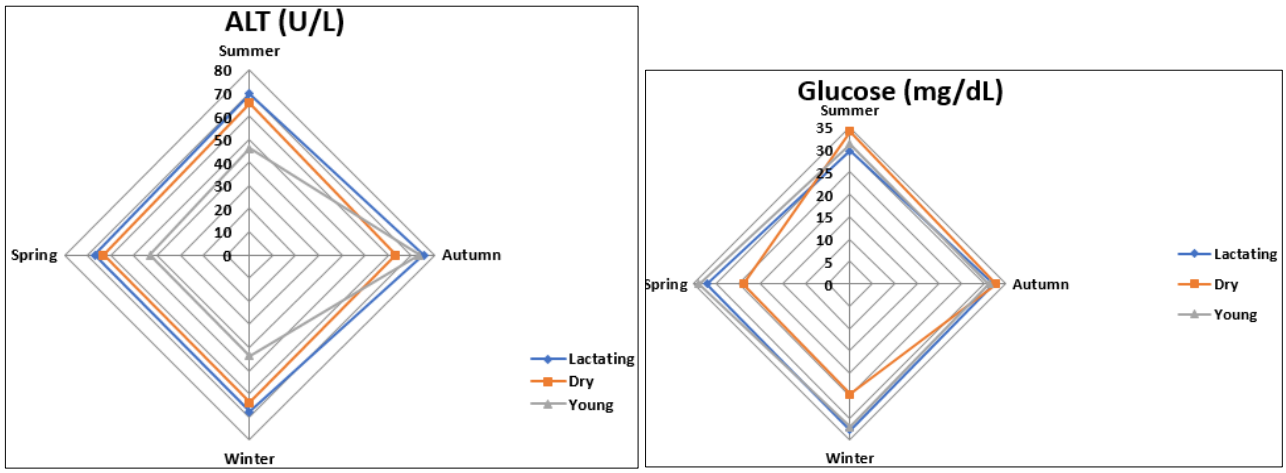


**Figure 1ab.** Fluctuations in some biochemical compounds and electrolytes in blood of buffaloes during different seasons. a-cholesterol value (ALT); b-glucose

The present findings of blood glucose level are lower than the reference range (36-52 mg/dL) determined by Jain and Lasmanis in 1978 [16]. The physiological serum glucose level in dairy cattle should go somewhere in the range of 2.2 and 4.5 mmol/L [17]. On account of numerous maladies (e.g. respiratory acidosis, ketosis) it is either expanded or lessened [18]. One of the biochemical markers is blood glucose level body vitality supply results are related to blood glucose level [19, 20]). In dairy animals, glucose in the serum is basic to create lactose it's lower fixation following a more massive request of the mammary organ for this sugar may result in calving [21].

Qureshi et al. [22] reported higher glucose values compared to the presented glucose values in this study. Mandali et al. [23] and Prajapati *et al*. [24] described lower values of glucose than the present observations. Present findings were lower than the reported range of 72.00 to 75.60 mg/dL by Borghese [25]. It was evident that circulating glucose levels depend on nutritional status [26, 27]). The destination of glucose is regulated by different hormones such as insulin, cortisol, glucagon, somatotropin, and adrenalin.

## Liver functioning test (LFT)

### ALT (SGPT)

The blood serum ALT (SGPT) concentration significantly ($p<0.001$) affected by sampling seasons in lactating and young buffaloes but it was non-significantly ($p>0.05$) affected by the sampling seasons in dry buffaloes (Table 1). The mean values of ALT in blood of lactating buffaloes were 69.53 U/L (summer), 75.33 U/L (autumn), 67.80 mg/ U/L (winter) and 66.90 U/L (spring). The mean

concentrations of ALT in the blood of dry buffaloes were 65.57 U/L (summer), 63.10 U/L (autumn), 63.70 U/L (winter), and 63.23 U/L (spring). The average ALT contents in the blood of young buffaloes were 46.07 U/L (summer), 73.33 U/L (autumn), 43.63 U/L (winter), and 42.83 U/L (spring). The high mean ALT concentrations were observed in lactating buffaloes during the autumn season and the low mean ALT contents were noticed in young buffaloes in the spring sampling season (Figure 2b). The detected order of ALT contents was autumn>summer>winter>spring in lactating buffaloes, summer> winter>spring>autumn in dry buffaloes, and autumn>winter>summer>spring in young buffaloes.

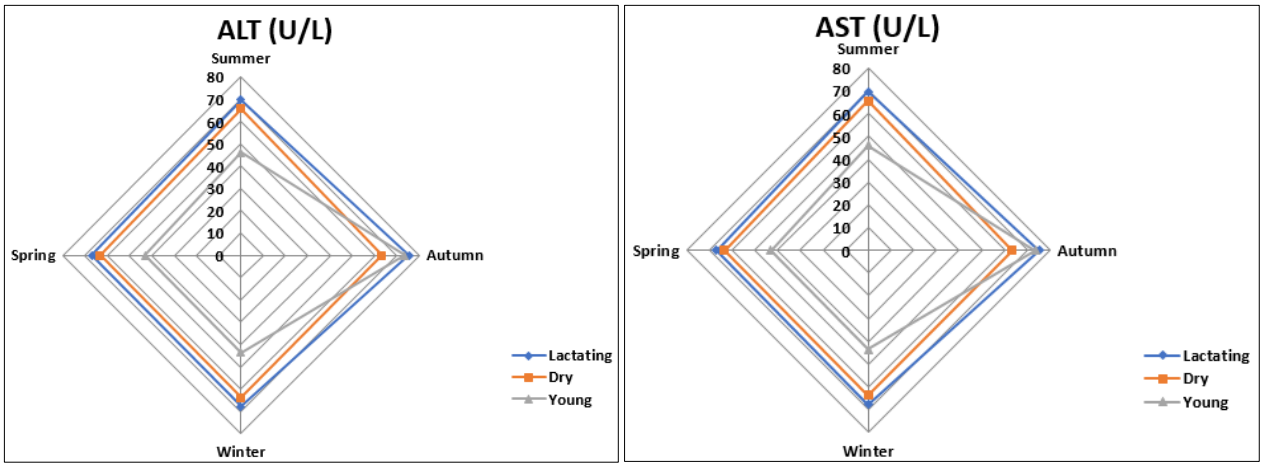


**Figure 2ab.** Fluctuations in liver functions in blood of buffaloes during different seasons a-ALT(SGPT) values, b-AST (SGOT) values

The available ALT contents in this study are in conformation with the reference range (29-74 U/L) proposed by Jain and Lasmanis [16]. The ANT estimations watched surpassed the physical standards serum of the animals [17]. Assurance of enzymatic movement (ALT) is seen by numerous creators in the serum as a valuable apparatus to analyze ailments of tissues and organs [28]. Eventually, to discover its fitting and safe minerals supply of these compounds, act attempts were made [29, 21]).

**AST (SGOT)**

In dry and young buffaloes, the blood serum AST (SGOT) congregation fundamentally ($p<0.001$) was influenced by sampling seasons in dry and young buffaloes while non-significant results were noticed in lactating buffaloes (Table 1). The mean AST values in blood of lactating buffaloes were 206.43 U/L (summer), 231.03 U/L (autumn), 212 mg/ U/L (winter) and 211.33 U/L (spring). The mean AST concentrations in the blood of dry buffaloes were 216.37 U/L (summer), 213.3 U/L (autumn), 171.47 U/L (winter), and 170.5 U/L, (spring). The average AST contents in the blood of young buffaloes were 137.53 U/L (summer), 234.7 U/L(autumn), 136.3 U/L (winter), and 135.4 U/L (spring). The maximum mean AST concentrations were observed in young buffaloes during the autumn season, and the minimum mean AST contents were noticed in young buffaloes in the spring sampling season (Figure 2b). The detected orders of AST contents were summer>autumn>winter>spring in lactating buffaloes, summer>autumn>winter>spring in dry buffaloes, and autumn>summer>winter>spring in young buffaloes.

The calculated AST values higher than the reference range (56-165 U/L) were reported by Jain and Lasmanis in 1978 [16]. Physical standards watched surpassed in the serum of the animals by AST determination was demonstrated by Winnicka in 2004 [17]. To analyze the ailments of organs and tissues, the assurance of enzymatic movement (AST) in the serum is seen by numerous creators as a helpful instrument [28]. To decide the activity of these catalysts endeavors was made, in this manner, to assess on the off chance that it is fitting and safe to supply minerals. To evaluate the appropriate and safe to provide minerals, trials were done on the activity of these enzymes [21, 29]).

## Alkaline phosphatase

The blood serum soluble phosphatase is fundamentally ($p<0.001$) influenced by inspecting seasons in all classes of buffaloes (Table 1). In blood serum of lactating buffaloes the mean alkaline phosphatase values were 375.4 U/L (summer), 218.3 U/L (autumn), 393.6 mg/ U/L (winter) and 392.8 U/L (spring). The mean concentrations of alkaline phosphatase in blood serum of dry buffaloes were 210 U/L (summer), 189.4 U/L (autumn), 257.2 U/L (winter), and 256.4 U/L (spring). The average alkaline phosphatase contents in the blood of young buffaloes were 372.2 U/L (summer), 204.63 U/L (autumn), 372.03 U/L (winter), and 368.4 U/L (spring). The higher mean basic phosphatase concentrations were spotted in lactating buffaloes during the winter season, and the lower mean basic phosphatase contents were noticed in dry buffaloes in the autumn sampling season (Figure 2c). The detected orders of the alkaline phosphatase contents were winter>spring>summer>autumn in lactating buffaloes, winter> spring>summer>autumn for dry buffaloes, and summer>winter>spring>autumn in young buffaloes.

c

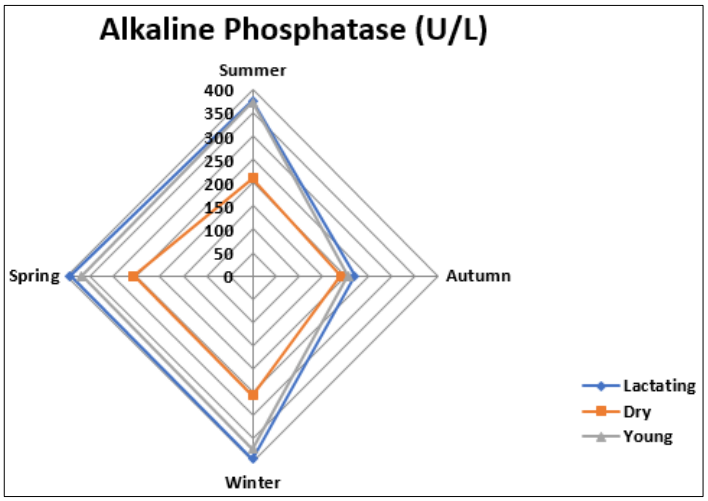


**Figure 2c.** Fluctuations in liver functions in blood of buffaloes during different seasons c-alkaline phosphatase values

In the serum of lactating dairy animals, the substance of the antacid phosphatase movement surpassed the ordinary upper points of confinement. In the serum of lactating cows, the content of basic phosphatase activity has been found to exceed the upper normal parameters [17]. While, in dry, cows enzyme activity values expected upper than physical parameters (61.16-81.00 U/L), however, the blood serum alkaline phosphatase values were found significantly higher than the previous findings determined by Sharma and Sridhar [30]. It is suggested that liver illnesses may lead to expanded movement of soluble phosphatase. The worse digestion of P and Ca (e.g. calcium and phosphorus insufficiencies) may vary due to many creators concerning the start of the post-delivery period [31]. The current investigation consequences uncovered a lack of supply of phosphorus to animals [32]; for this condition, it was noticed that the ALP level was a garbed demonstrative mark.

## Renal functioning test

### Urea

The urea concentration in blood serum notably ($p<0.001$) altered by sampling seasons in dry buffaloes but in lactating and young buffaloes, it was non-considerably ($p>0.05$) altered by the sampling seasons (Table 1). The mean urea values in blood serum of lactating buffaloes were 35.1 mmol/L (summer), 32.4 mmol/L (autumn), 35.5 mmol/L (winter) and 35.1 mmol/L (spring). The mean urea concentrations in blood serum of dry buffaloes were 31.5 mmol/L (summer), 32.4 mmol/L (autumn), 28 mmol/L (winter) and 27.7 mmol/L (spring). The average urea content in blood serum of young buffaloes was 37.4 mmol/L (summer), 33.6 mmol/L (autumn), 35.12 mmol/L (winter) and 35.07 mmol/L (spring). The higher mean urea concentrations were observed in young buffaloes during the summer season, and the lower mean urea contents were noticed in dry buffaloes in the spring sampling season (Figure 3a). The detected orders of urea contents were winter>spring>summer>autumn in lactating buffaloes,

autumn>summer>winter>spring in dry buffaloes, and summer>winter>spring>autumn in young buffaloes.

The investigated urea concentration being higher than the reference range (6-27 mmol/L) was reported by Radostits et al. [33]. The urea contents fall off as the animals near its cyclicity. This finding is following Zaman et al. [34] and Butler [35]. Impaired fertility in cows was led by the amplified urea association on the microenvironment of the uterus. The initial action of progesterone retains urea value in higher blood, causing the suboptimal conditions for embryo development [35]. Significantly lower values of urea were described by Anthony et al. [36] as compared to the present results.

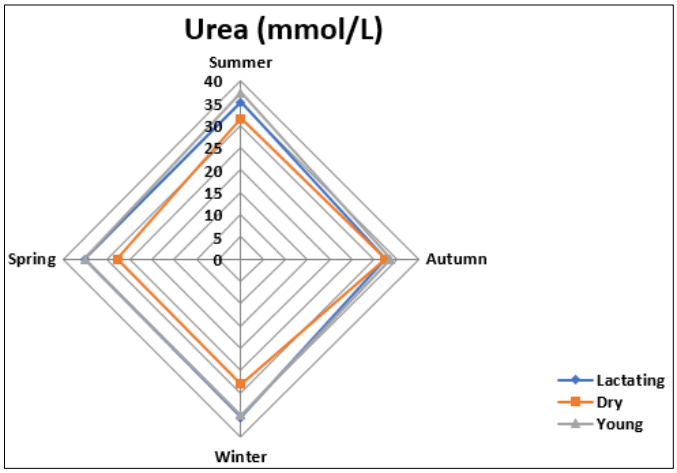


**Figure 3a**. Fluctuations in renal functions in blood of buffaloes during different seasons - urea values

## Creatinine

In lactating and young buffaloes, the creatinine concentration in blood seum notably ($p<0.001$) changed by sampling seasons, but in dry buffaloes, it was non-significantly ($p>0.05$) affected by the sampling seasons (Table 1). The mean creatinine values in blood serum of lactating buffaloes were 1.26 mg/dL (summer), 1.66 mg/dL (autumn), 1.64 mg/dL (winter) and 1.24mg/dL (spring). The mean creatinine concentrations in blood of dry buffaloes were 1.63 mg/dL (summer), 1.6 mg/dL (autumn), 1.49 mg/dL (winter) and 1.45 mg/dL (spring). The average contents of creatinine in blood of young buffaloes were 1.77 mg/dL (summer), 1.6 mg/dL (autumn), 1.48 mg/dL (winter) and 1.43 mg/dL (spring). The higher mean creatinine concentrations were observed in young buffaloes during the summer season, and the lower mean creatinine contents were noticed in lactating buffaloes in the spring sampling season (Figure 3b). The detected orders of creatinine contents were autumn>winter>summer> spring in lactating buffaloes, summer>autumn>winter>spring in dry buffaloes and summer>autumn> winter>spring in young buffaloes. The present creatinine contents are in accordance with the reference range (1.2-1.93 mg/dL) reported by Jain and Lasmanis [15].

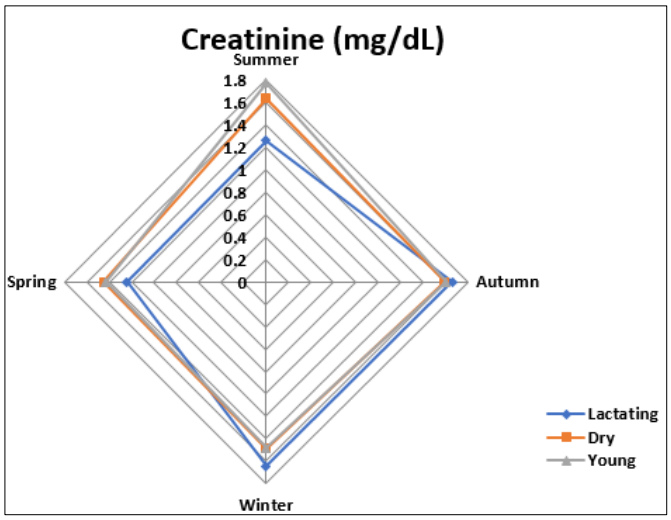


**Figure 3b.** Fluctuations in renal functions in blood of buffaloes during different seasons - creatinine values

## Uric Acid

In all categories of buffaloes (lactating, dry, and young) the uric acid concentration in blood serum fundamentally ($p<0.001$) is influenced by sampling seasons (Table 1). The uric acid mean values in blood of lactating buffaloes were 1.34 µmol/L (summer), 0.82 µmol/L (autumn), 1.69 µmol/L (winter) and 1.29 µmol/L (spring). The mean uric acid concentrations in blood serum of dry buffaloes were 1.06 µmol/L (summer), 1.06 µmol/L (autumn), 1.31 µmol/L (winter) and 1.28 µmol/L (spring). The average content of uric acid in the blood of young buffaloes was 1.8 µmol/L (summer), 1.29 µmol/L (autumn), 1.75 µmol/L (winter), and 1.7 µmol/L (spring). The higher mean uric acid concentrations were observed in young buffaloes during the winter season, and the lower mean uric acid contents were noticed in lactating buffaloes in the autumn sampling season (Figure 3c). The detected orders of uric acid contents were winter>summer>spring>autumn in lactating buffaloes, summer>spring>winter>autumn in dry buffaloes, and winter>spring> summer>autumn in young buffaloes. The calculated uric acid values were lower than the reference range (2.30-2.45 µmol/L) determined by Clerc and Solberg [13].

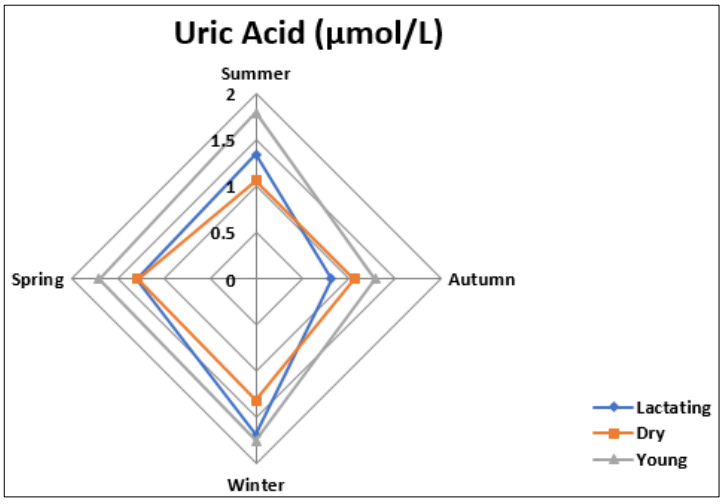


**Figure 3c.** Fluctuations in renal functions in blood of buffaloes during different seasons - uric acid

Uric acid is a powerful antioxidant and has been proposed to protect against cardiovascular disease and some cancers [37]. In animals, the gene for urease or urate oxidase (which is expressed most in the kidney and liver [38] is a non-functioning pseudogene. The absence of a functional unit disables this locus and results in uniquely high levels of serum urate, with about 5-25% of humans having impaired renal excretion and, ultimately, hyper uricaemia. The relative fitness advantages gained from the antioxidant properties of uric acid have been suggested to explain why the genetic precondition for such levels persists [39].

### Mycotoxins

The assessment of three types of common mycotoxins in the blood of buffaloes in different seasons revealed a significant increase in summer compared to the other seasons reaching 73, 52, and 42 µg/L for ochratoxins and 16, 10 and 6 in µg/L for AFB1 in the case of lactating, non-lactating and young buffaloes, respectively (Table 2). The zearalenone was significantly detected in lower values in spring and summer ad did not detect in buffaloes' blood at all in winter and in autumn. Curtui *et al*. [40] has shown that approximately 90% of aflatoxins present in the cow blood are found afterward in the milk and urine. The relative lower values of the toxins in the blood may be related to the lower fungal contamination of the feed or the passive absorption of the toxins, in unionized form, at the digestive tube level, especially at the level of the short intestine level or due to their rapid metabolism depending on the animal species [41]. Moreover, OTA has the most potent inhibitor effect on animal growth, determining the excessive accumulation of glycogen in the liver of distressed animals [42].

**Table 2.** Fluctuations of some mycotoxins in blood of buffaloes during different seasons

| Season | Mycotoxins (µ/L) in blood of buffaloes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Lactating | | | Dry | | | Young | | |
| | AFB1* | ZEA* | OTA* | AFB1 | ZEA | OTA | AFB1 | ZEA | OTA |
| Spring | 8±1 | 13±1 | 40±3 | 6±9 | 8±0.6 | 21±2 | 5±0.4 | 3±0.3 | 10±1 |
| Summer | 16±2 | 10±1 | 73±41 | 10±1 | 7±0.5 | 52±4 | 6±0.7 | 5±0.3 | 42±3 |
| Autumn | 12±1 | 0 | 33±33 | 8±1 | 0 | 0 | 4±0.4 | 0 | 0 |
| Winter | 6±1 | 0 | 0 | 4±0.5 | 0 | 0 | 1±0.2 | 0 | 0 |

## 4.Conclusions

It is concluded from present investigation that enzymatic and biochemical profiles of lactating, non-lactating and young buffaloes during different sampling seasons (summer, autumn, winter and spring) considerably changed as indicated by change in cholesterol level, alkaline phosphatase activity, and uric acid values during different seasons.

**Acknowledgment**.The authors extend their appreciation to the Researchers supporting project number (RSP-2020/94) King Saud University, Riyadh, Saudi Arabia.

### References

1. BILAL, M. Q., SULEMAN, M., RAZIQ, A. (2006). Buffalo: Black gold of Pakistan. *Livestock Research for Rural Development. Volume 18, Article #128.* Retrieved May 11, 2020, from http://www.lrrd.org/lrrd18/9/bila18128.htm
2. SAMANC, H., KIROVSKI, D., STOJI, V., STOJANOVI, D., VUJANAC, I., PRODANOVI, R. (2011) Application of the metabolic profile test in the prediction and diagnosis of fatty liver in Holstein cows, *Acta Vet* (Beograd), 61: 5-6, 543-53.
3.KARAPEHLIVAN, M., ATAKISI, E., ATAKISI, O., YUCAYURT, R., & PANCARCI, S. M. (2007). Blood biochemical parameters during the lactation and dry period in Tuj ewes. *Small Ruminant Research*, *73*(1-3), 267-271.
4. YOKUS, B., CAKIR, D.U., KANAY, Z., GULTEN, T. UYSAL, E. (2006). Effects of seasonal and physiological variations on the serum chemistry, vitamins and thyroid hormone concentrations in sheep. *Journal of Veterinary Medicine* 53: 271-276.
5.KOVACIK, A., ARVAY, J., TUSIMOVA, E., HARANGOZO, L., TVRDA, E., ZBYNOVSKA, K., CUPKA, P. ANDRASCIKOVA, S., TOMAS, J., MASSANYI, P. (2017). Seasonal variations in the blood concentration of selected heavy metals in sheep and their effects on the biochemical and hematological parameters. *Chemosphere* 168:365-371.
6. STEEL, P., KÖNIG, C.J. (2006). Integrating theories of motivation. *Academy of management review*, 31(4): 889-913.

7.MOSHIDES, J.S., (1988). Enzymic determination of the free cholesterol fraction of high-density lipoprotein in plasma with use of 2,4,6-tribromo-3-hydroxybenzoic acid., *Clinical Chemistry*, 34 (9): 1799–1804.
8. WANG, J. H., WANG, K., BARTLING, B., AND LIU, C. C. (2009). The detection of alkaline phosphatase using an electrochemical biosensor in a single-step approach. *Sensors (Basel, Switzerland)*, *9*(11), 8709–8721. https://doi.org/10.3390/s91108709
9.YUNSHENG, Z., XIAOYAN, Y., WEI, L., HONG, L., FEI, L. (2008). Uricase based methods for determination of uric acid in serum (English). *Microchimica Acta* 164(1):1-6.
10. TOORA, B.D., RAJAGOPAL, G. (2002). Measurement of creatinine by Jaffe's reaction--determination of concentration of sodium hydroxide required for maximum color development in standard, urine and protein free filtrate of serum. *Indian J Exp Biol*. 40(3):352-4.
11. D'ARCO, G, FERNÁNDEZ-FRANZÓN, M., FONT, G., DAMIANI, P., MAÑES, J. (2008). Analysis of fumonisins B1, B2 and B3 in corn-based baby food by pressurized liquid extraction and liquid chromatography/ tandem mass spectrometry. J. Chromatogr A 1209:188-194.
12. BAINES, D., ERB, S., LOWE, R., TURKINGTON, K., KULDAU, G. (2011). Moldy feed, mycotoxins and shiga toxin-producing *Escherichia coli* colonization associated with Jejunal hemorrhage syndrome in beef cattle. *BMC Vet. Res.*, 7: 24.
13. CLERC, C., H.E. SOLBERG. (1987). Approved recommendation on the theory of reference values. Part 2. Selection of individuals for the production of reference values, *Clinical Chemistry Acta* 170: S1–S11. http://dx.doi.org/10.1016/0009-8981 (87)90150-1.
14. ROWLANDS, G.J., MANSTON, M. RITA, POCOCK, M.D. SALLY. (1975). Relationships between stage of lactation and pregnancy and blood composition in a herd of dairy cows and the influences of seasonal changes in management on these relationships. *Journal of Dairy Research*, 42: 349-362.
15. MAURYA, S. K., B. KUMAR, B.L. KUMAWAT, A. SAXENA, R. SAGAR, M. H. JAN, JAI SINGH, VANDANA, DAS, G. K., PERUMAL, P. (2015). Mineral Supplementation on Hormonal, Mineral and Biochemical Profile of Buffalo Under Rural Managemental Condition of India. *Journal of Cell and Tissue Research*, 15(1): 4839-4842
16. JAIN, N.C., J. LASMANIS (1978) Leucocytic changes in cows given intravenous injections of *Escherichia coli* Endotoxin. *Research Veterinary Science* 24: 386-387.
17. WINNICKA, A. (2004). Reference values of basal veterinary laboratory examinations Šin Polish SGGW, *Warszawa*, 17-35: 97-108
18. BOMBIK, T., A. BOMBIK AND L. SABA (2002). Effects of an herb extract on the level of selected biochemicalindicators in the blood of calves Šin Polish. *Med Weter*, 58(6): 464-6.
19. GOFF, J.P., R.L. HORST, F.J. MUELLER, J.K. MILLER, G.A. KIESS, H.H. DOWLEN. (1991). Addition of chloride to aprepartal diet high in cations increases 1, 25 - dihydroxyvitamin dresponse to hypocalcaemia preventing milk fever. *Journal of Dairy Science*, 74: 3863-71.
20. KUPCZYNSKI, R., B. CHUDOBA-DROZDOWSKA (2002). Values of selected biochemical parameters of cows blood during their drying-off and the beginning of lactation. *EJPAU Ser Vet Med*,5,1
21. DARUL, K., H. KRUCZYNSKA. (2005). Changes in some blood constituents of dairy cows. Association with pregnancy and lactation in Polish, *Acta Scientiarum Polonorum-Medicina Veterinaria*, 4 (1), 73-86.
22.QURESHI, M.S., G. HABIB, H.A. SAMAD, M. MOHSIN, N.A. SIDDIQUEE, M. SYED (2002). Reproduction-nutrition relationship in dairy buffaloes I. Effect of intake of protein, energy and blood metabolites levels. *Asian-Australian Journal of Animal Science 15*(3):330-339.
23.MANDALI, G. C., PATEL, P. R., DHAM A. J. I, RAWAL, S. K., CHRISTI, K. S. (2002). Biochemical profile in buffaloes with periparturient reproductive and metabolic disorders. *Indian Journal of Animal Reproduction*, 23(2): 130-134.
24. PRAJAPATI, S. B., D. J. GHODASARA, B. P. JOSHI, K. S. PRAJAPATI, V. R. JANI. (2005). Etio-pathological study of Endometritis in repeat breeder buffaloes. *Buffalo Journal*, 2: 145-165.

25. BORGHESE, A. (2005). Buffalo production and research.REU technical series 67. FAO, United Nations, Rome.
26. CAMPANILE, G., R.D. PALOAND A. D'ANGELO. (1997). Profile of metabolic buffalo. *Bubalus Bubalis*, (4): 236-249.
27. MONTEMURRO, N., PACELLI, C., BORGHESE, A. (1997). Blood metabolites change in milking buffalo cows. Bubalus Bubalis 3: 69-78.
28. RAMIN, A.G., HASHEMI, A., ASRI-REZAIE, S., BATEBI, E., TAMADON, A., RAMIN, S. (2011). Prediction of traumatic pericarditis in cows using some serum biochemical and enzyme parameters. *ActaVet* (Beograd) 61:4,383-90.
29. MARTYNA, J., WNUK, W., SABA, L., BIS-WENCEL, H., POLONIS, A., TRAWINSKA, B. (2006). The effect of feed supplementation a mineral mixture on micro- and macro elements content in blood serum of cows from the Zulawy Region Šin Polish[1], *Ann UMCS Sect EE*, 24: 6, 39-45.
30. SHARMA, V., SRIDHAR, S. (2007). Evaluation of some liver function tests in clinical cases of hepatic insufficiency in buffaloes, Italian Journal of Animal sciences. Sci.vol. 6, (Suppl. 2), 984-987, 2007.Sharma, M.C. 2004. *Livestock International. 8*(5), 5-10.
31. SABA, L., NOWAKOWICZ-DEBEK, B., STENZEL, R., TYMCZYNA, L., HOLODA, E. (1999). Effect of mineral and herbal mixtures on Ca, P, Mg and total protein levels and activity of someenzymatic indices in blood serum of calves Šin Polish, *Ann UMCS Sect EE*, 17, 44: 339-45.
32.GÓRSKI, K., SABA, I. (2012). Changes in the level of selected hematological and biochemical parameters in the blood of dairy cows in central-eastern Poland. *Acta Veterinaria (Beograd)*,62(4): 421-428.
33. RADOSTITS, O. M., GAY, C.C.K., HINCHCLIFF, W., CONSTABLE, P.D. (2007). Veterinary Medicine. 10th ed., *Saunders Publications, London*, pp. 2047-2050.
34. ZAMAN, M.S., ALI, C.S., AHMED, K.M. (1985). Comparative study of blood glucose, cholesterol, protein and urea contents in cyclic, non-cyclic and sub estrus lactating buffaloes. *Pakistan Veterinary Journal* 5: 72-75.
35. BUTLER, W.R. (2000). Nutritional interactions with reproductive performance in dairy cattle. *Animal Reproduction Science*, 2: 449-457.
36. ANTHONY, S., KUMAR, V.G., NANDI, S., MURTHY, V.C. (2012). Blood hematological and biochemical parameters in normal cycling, pregnant and repeat breeding buffaloes (*Bubalus bubalis*) maintained in exothermic and is nutritional conditions. *Asian Pacific Journal of Reproduction*, 1(2): 117-119.
37. AMES, B.N., CATHCART, R., SCHWIERS, E., HOCHSTEIN, R. (1981). Uric acid provides an antioxidant defense in humans against oxidant and radical caused aging and cancer a hypothesis. *Proceedings of the National Academy of Sciences*, 78:6858–6862.
38. PHAY, J.E., HUSSAIN, H.B., MOLEY, J.F. (2000). Cloning and expression analysis of a novel member of the facilitative glucose transporter family, SLC2A9 (GLUT9) Genomics. 66(2):217–220.
39. VITART, V., RUDAN, I., HAYWARD, C., et al. (2008). SLC2A9 is a newly identified urate transporter influencing serum urate concentration, urate excretion and gout. Nat Genet.40(4):437–442.
40. CURTUI, V., USLEBER, E., DIETRICH, R., LEPSCHY, J., MÄRTLBAUER, E. A. (1998). survey on the occurrence of mycotoxins in wheat and maize from western Romania. Mycopathologia. 143:97-103.
41. NAZARIZADEH, H., POURREZA, J. (2019). Evaluation of three mycotoxin binders to prevent the adverse effects of aflatoxin B1 in growing broilers. Journal of Applied Animal Research 47 (1): 135-139.
42. RUHLAND M, ENGELHARDT G, SCHAFER W, WALLNOFER PR. (1996). Transformation of the mycotoxin ochratoxin A in plants: 1. Isolation and identification of metabolites formed in cell suspension cultures of wheat and maize. Natural Toxins, 4(6):254-260

Manuscript received: 12.05.2020