IJBPAS, December, 2020, 9(12): 3544-3553

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EFFECT OF DIFFERENT BIOMARKERS ON HEALTH OF CROSS-BRED COWS DURING VARIOUS PREGNANCY STAGES

FARHAT R[1], NAEEM M[*1], REHMAN ZU[2]

1: Institute of Pure & Applied Biology (Zoology Division), Bahauddin Zakariya University, Multan, Pakistan

2: Institute of Pharmacy, Physiology & Pharmacology, University of Agriculture, Faisalabad, Pakistan

[*]**Corresponding Author: Muhammad Naeem: E Mail: dr_naeembzu@yahoo.com**

Received 17th Dec. 2019; Revised 19th Feb. 2020; Accepted 27th May 2020; Available online 1st Dec. 2020

https://doi.org/10.31032/IJBPAS/2020/9.12.5315

ABSTRACT

The aim of this study was to determine the biochemical profile, few serum enzymes and total antioxidant and oxidant status in pregnant crossbred cows at three different stages and in non-pregnant crossbred cows as well. Blood samples were collected from three different farms and analyzed for glucose, cholesterol, HDL-C, LDL-C, triglyceride, AST, ALT, GGT, total antioxidant status (TAS) and total oxidant status (TOS) by using commercially available kits. Significantly high values of LDL-C and AST were observed during mid pregnancy stage and then turned down at advance pregnancy stage, whereas gradual increase in cholesterol and triglycerides, and a gradual decline in TAS was observed from early to advance pregnancy in all animals in this study. Serum glucose, HDL-C, ALT, GGT and TOS were found non-significant. In conclusion, disturbance in food intake and rise in metabolic processes lift up oxidative stress in crossbred cows especially at advance pregnancy stage and can be determined by supervising blood alterations.

Keywords: Biochemical profile, Serum enzymes, Total antioxidant status (TAS), Total oxidant status (TOS)

INTRODUCTION

Biomarkers are actually indicators of some biological conditions and often can be measured and evaluated in order to examine the various biological, pathological and pharmacological processes to a remedial negotiation **[1]**. Changes in the pathological processes are liable of elevation in serum enzymes level. Biomarkers help in early diagnosis and understanding the health disorders as well as course of actions by finding the serum enzymes activity and protein in modest proportion in addition to other health related facts **[2]**. Finding of various biomarkers of oxidative stress (OS) due to many health disorders expansion in dairy cows has become research priority among scientists. It has been found experimentally that oxidative stress (OS) is also capable to cause different health disorders **[3, 4]**. The literature relevant to disorders caused by the changes in health biomarkers in dairy cows tissues is yet inadequate. Celi, and Gabai **[5]** encouraged other scientists to recognize biomarkers of protein oxidation used in veterinary medicine in order to understand role of oxidative stress that lead to decline in animal welfare and production. Provincial Government of Punjab in Pakistan has imported a flock of foreign species of purebred cows named Holstein Friesian and Jersey from USA in 1985 and kept at Research Institute of Physiology of Animal Reproduction, Pattoki, District Kasur **[6]**. Keeping in view the fast increase in human population in Pakistan, crossbreeding of indigenous dairy animals with exotic species is being practiced to improve the milk and meat production **[7]**. So, the present study was planned to examine the health biomarkers such as glucose, cholesterol, HDL-C, LDL-C, triglyceride, AST, ALT, GGT, total antioxidant status (TAS) and total oxidant status (TOS) in three different crossbred species during various stages of pregnancy.

MATERIALS AND METHODS

Selection of three different species of cross-bred cows (n=120) as Sahiwal x Jersey (S x J) grouped as Cross-I (n=40), Sahiwal x Holstein Friesian (S x HF) as Cross-II (n=40) and Cholistani x Holstein Friesian (C x HF) as Cross-III (n=40) aged between 3-7years was done at random from Buffalo Research Institute, Pattoki, District of Kasur; Dairy Farm, University of Agriculture, Faisalabad and Livestock Production Research Institute Bahadar Nagar, Okara. Each group was classified into further four stages such as non-pregnant (NP=10), early pregnant (EP=10), mid

pregnant (MP=10) and advance pregnant (AP=10). Early pregnant cows were taken ≥ 90 days of gestation, mid pregnant cows were ≥ 180 days of gestation and advanced pregnant ≥ 275 days of gestation period. Three expert veterinarians helped to collect the blood samples of all experimental animals and serum was preserved at -20 ^{0}C for the analysis.

Glucose (mg/dL), Choleterol (mg/dL), High-density Lipoprotein (HDL-Chol; mg/dL), Low Density Lipoprotein (LDL-Chol; mg/dL) and Triglycerides (TG; mg/dl) in the serum were determined by reagent kits. Concentration of glucose in serum was measured by using Fluitest Glu Biocon (Lot # H 265) and spectrophotometer (Screen master 35510). Reitman-Frankel colorimetric method was used to determine Aspartate Aminotransferase (AST; U/L) Alanine Aminotransferase (ALT; U/L) and Gamma-glutamyltransferase (GGT; U/L). The quantitative determination of AST was done by using Crescent Diagnostics Cat No. CZ 904C. The absorbance against water blank was recorded at A_{505} (BioSystems, BTS-330). Crescent Diagnostics Catalogue No. CZ 902 C (Commercially available kit) was used for the quantitative determination of ALT. GGT was determined by Szasz **[8]** method (Pishtaz Teb Zaman Diagnostics, Catalogue No. PT-GGT, Schulweg 8, 34516 Voehl/ Germany). Serum total antioxidant status (TAS; mmol trolox Equiv. /L) by adopting Erel **[9]** method and total oxidant status (TOS; µmol H_2O_2 Equiv./L) by Erel **[10]** method were measured respectively. Statistical analysis was done by two way ANOVA. DMR was used to find out the means of significance among different groups **[11]**.

RESULTS AND DISCUSSION

Analysis of variance of serum biochemistry of Cross-I (S x J), Cross-II (S x HF) and Cross-III (C x HF) cattle has been shown in **Table 1**. Between Crossbred groups at their different stages of pregnancy and into their interaction (groups x stages), values of cholesterol, LDL-C, Triglycerides, AST and TAS were significantly variable. Serum glucose, HDL-C, ALT, GGT and TOS among crossbred groups at different pregnancy stages showed significant difference while into their interaction (groups x stages) were found non-significant.

Mean ± SE values of different parameters of Cross-I, Cross-II and Cross-III at different pregnancy stages were shown in **Table 2** and **Table 3**. In this study, glucose concentration during various pregnancy stages of all crossbred cows increased

insignificantly at MP and decrease at AP **(Table 2)**. It has been reported in another study that decline in glucose concentration considered responsible of decreased reproductive performance in dairy cattle **[12]**. So, glucose administration should be provided to dairy cows in order to keep positive energy levels in balance during gestation and after calving **[13]** otherwise low glucose level may be the cause of stress for animals. There was also observed a significant increase ($P<0.01$) in cholesterol concentration from EP to AP stages in all pregnant crossbred cows **(Table 2)** that showed similarity to what observed by Ghanem *et al*. **[14]** and Pandupuspitasari *et al*. **[15]**. High values of HDL-C were seen in non-pregnant animals. A significant decrease in HDL-C concentrations was observed from early to advance pregnancy stages in all pregnant crossbred cows **(Table 2)** and these results were in evidence with the studies of Ghanem *et al*. **[14]**. In three crossbred cows, an LDL-C concentration was significantly increased from early to mid pregnancy. During mid pregnancy stages, LDL-C concentration was significantly high in all crossbred pregnant cows as compared to other stages of pregnancy. Serum LDL-C concentration was significantly declined in advance pregnancy in all cows. Kurpinska *et al*. **[16]** found the same results as in our study that tendencies of changes in the concentration of total cholesterol, HDL and LDL were found parallel in blood plasma in all the cows under experiment in the last month of pregnancy. Serum triglyceride level elevated significantly from early pregnancy to advance pregnancy in all three Crossbreds as shown in **Table 2**. At AP stage, all crossbred animals showed significantly high level of triglycerides. Ghanem *et al*. **[14]** found same results of high triglyceride concentration at pregnancy stage near to time of parturition in Friesian cows. The reasons for such commotion possibly are increase in triglycerides level in the liver **[17, 18]**.

In the present study as shown in **Table 3**, three crossbred cows showed significantly high AST concentration at MP as compared to other two stages (EP and AP) of pregnancy as well as in NP cattle. Insignificant variations in ALT values were seen in pregnancy stages among Cross-I, Cross-II and Cross-III cows in this study. AST may increase in concentration after laborious exertion and during physiological changes as pregnancy **[19, 20]**. Lowest AST activity just before parturition indicates high hepatic effort during advanced pregnancy. In our study the increase in ALT was small and

certainly not significant. Our results were in close agreement with the study presented by Abdulkareem **[21]** and Pandupuspitasari, *et al*. **[15]**. These changes in serum AST and ALT levels at advance pregnancy stages are normal physiological process for dietary metabolism, removal of destructive substances, blood proteins creation and performing other important physiological functions related with the fetus development **[22]**. According to our study, all the non pregnant animals showed significantly high serum GGT concentration while a gradual decrease in concentration was observed from EP to AP stages in pregnant crossbred cows. Djoković *et al.* **[23]** study on evaluation of metabolic status in dairy cows was found similar results of GGT and AST at advance pregnancy stage as in our study. GGT is regularly used as diagnostic marker to diagnose hepatic problems and bile ducts disorders and has the possible function as a biomarker in inflammations caused by other factors **[24]**.

Table 1: Analysis of variance of Glucose, Cholesterol, HDL-C, LDL-C, Triglycerides (mg/dL), AST, ALT, GGT, TAS and TOS of Cross-I, Cross-II and Cross-III during various stages of pregnancy

PARAMETERS (F-VALUE)	SOURCE OF VARITION		
	Groups	Stages	G x S
Glucose(mg/dL)	5.142^{*}	122.642^{**}	1.955^{NS}
Cholesterol(mg/dL)	29.648^{**}	449.149^{**}	8.842^{**}
HDL-C(mg/dL)	4.034^{*}	574.760^{**}	0.931^{NS}
LDL-C(mg/dL)	49.679^{**}	110.963^{**}	3.685^{**}
Triglycerides(mg/dL)	100.680^{**}	515.327^{**}	2.489^{*}
AST (u/L)	38.064^{**}	74.580^{**}	3.807^{**}
ALT (u/L)	1.017^{NS}	219.436^{**}	0.745^{NS}
GGT (u/L)	4.500^{*}	263.296^{**}	0.442^{NS}
TAS (mmol/L)	27.590^{**}	389.327^{**}	7.934^{**}
TOS(µmol H_2O_2equi/L)	8.988^{**}	346.735^{**}	1.613^{NS}

****Significant = P≤0.01 * Significant = 0.05 NS = Non-significant**

Table 2: Mean ± SE Glucose (mg/dL), Cholesterol (mg/dL), HDL-C (mg/dL), LDL-C (mg/dL) and Triglycerides (mg/dL) of Cross-I, Cross-II and Cross-III during various stages of pregnancy

PARAMETERS	STAGES				
	NP	EP	MP	AP	Overall Mean
Glucose					
Cross-I	43.80±1.58	45.41±0.66	52.80±0.80	50.40±0.78	$48.10±0.76^{A}$
Cross-II	39.70±0.47	44.00±0.60	52.80±0.74	48.80±0.83	$46.33±0.85^{B}$
Cross-III	40.00±0.49	45.70±0.24	52.20±0.76	50.10±0.80	$47.00±0.80^{A}$
Overall Mean	$41.17±0.65^{D}$	$45.04±0.33^{C}$	$52.60±0.43^{B}$	$49.77±0.46^{A}$	47.14±0.47
Cholesterol					
Cross-I	$32.10±0.74^{g}$	$44.30±0.92^{e}$	$62.08±0.91^{bc}$	$64.40±0.87^{ab}$	$50.72±2.16^{C}$
Cross-II	$40.80±0.74^{g}$	$57.20±0.68^{c}$	$60.50±0.54^{bc}$	$67.90±0.72^{a}$	$56.60±1.64^{A}$
Cross-III	$36.00±1.86f^{g}$	$50.80±1.87^{d}$	$62.90±0.8^{ab}$	$65.10±0.87^{ab}$	$53.70±1.98^{B}$
Overall Mean	$36.3±1.00^{D}$	$50.77±1.21^{C}$	$61.83±0.46^{B}$	$65.8±0.54^{A}$	53.67±1.13
HDL-C					
Cross-I	42.90±1.21	32.60±0.86	26.50±0.67	18.80±0.63	$30.20±1.47^{B}$
Cross-II	45.50±0.69	35.50±0.43	26.90±0.67	19.20±0.88	$31.78±1.6^{A}$
Cross-III	44.80±1.08	33.60±0.79	26.80±0.59	18.30±0.60	$30.88±1.60^{B}$
Overall Mean	$22.2±0.51^{D}$	$34.87±0.43^{A}$	$26.25±0.46^{C}$	$27.77±.62^{B}$	22.2±0.51

LDL-C					
Cross-I	**28.12±0.50^{g}**	**34.50±0.75^{def}**	**39.10±0.77^{bcd}**	**26.26±0.89^{g}**	**31.99±0.89C**
Cross-II	**30.80±0.49^{fg}**	**41.50±0.87^{bc}**	**46.60±0.97^{a}**	**37.20±0.79^{cde}**	**39.03±1.06A**
Cross-III	**28.70±0.67^{g}**	**36.60±1.13^{de}**	**43.50±1.34^{b}**	**33.80±1.93^{ef}**	**35.65±1.07B**
Overall Mean	**29.21±0.38^{D}**	**37.53±0.75^{B}**	**43.07±0.82^{A}**	**32.42±1.12^{C}**	**35.56±0.63**

A-D different capital letters on overall means in a row and column differ significantly at P≤0.01.
a-g different small letters on means differ significantly at P≤0.01.

Table 3: Mean ± SE of AST (u/L), ALT (u/L), GGT (u/L), TAS (mmol/L) and TOS (µmol H_2O_2equi/L) of Cross-I, Cross-II and Cross-III during various stages of pregnancy

PARAMETERS	STAGES				
	NP	EP	MP	AP	Overall Mean
AST	**18.80±0.55^{d}**	**23.00±0.26^{d}**	**47.88±0.71^{ab}**	**35.80±0.96^{c}**	**31.37±1.85^{B}**
Cross-I	**18.30±0.58^{d}**	**22.20±0.42^{d}**	**46.20±0.95^{ab}**	**35.80±0.94^{c}**	**30.63±1.81^{B}**
Cross-II	**36.10±4.54^{c}**	**39.50±4.72^{bc}**	**49.7±0.92^{a}**	**44.1±2.54^{abc}**	**42.35±1.89^{A}**
Cross-III	**24.4±2.14^{D}**	**28.23±2.13^{C}**	**49.7±0.55^{A}**	**38.57±1.18^{B}**	**34.78±1.17**
Overall Mean					
ALT	**19.40±0.58**	**24.70±0.47**	**34.30±0.65**	**26.60±0.76**	**26.25±0.91**
Cross-I	**19.20±0.66**	**24.70±0.47**	**34.70±0.73**	**25.90±0.77**	**26.13±0.95**
Cross-II	**21.30±1.09**	**24.40±0.54**	**34.70±0.83**	**26.80±0.76**	**26.80±0.89**
Cross-III	**19.97±0.48^{D}**	**24.6±0.82^{C}**	**34.57±0.41^{A}**	**26.43±0.43^{B}**	**26.39±0.52**
Overall Mean					
GGT	**25.40±0.34**	**19.80±0.68**	**15.70±0.34**	**14.20±0.44**	**18.78±0.73^{A}**
Cross-I	**24.80±0.51**	**20.00±0.71**	**16.10±0.38**	**14.30±0.45**	**18.80±0.69^{A}**
Cross-II	**24.70±0.3**	**18.40±0.78**	**15.10±0.6**	**13.10±0.48**	**17.83±0.76^{B}**
Cross-III	**24.97±0.23^{A}**	**19.4±0.43^{B}**	**15.63±0.26^{C}**	**13.87±0.27^{D}**	**18.47±0.42**
Overall Mean					
TAS	**1.93±0.02^{a}**	**1.80±0.02^{abc}**	**1.68±0.02^{c}**	**1.22±0.02^{ef}**	**1.66±0.04^{A}**
Cross-I	**1.85±0.01^{ab}**	**1.71±0.01^{c}**	**1.32±0.04^{e}**	**1.15±0.05^{fg}**	**1.51±0.05^{C}**
Cross-II	**1.92±0.02^{a}**	**1.73±0.02^{bc}**	**1.54±0.03^{d}**	**1.08±0.05^{g}**	**1.57±0.05^{B}**
Cross-III	**1.9±0.01^{A}**	**1.74±0.01^{B}**	**1.51±0.03^{C}**	**1.15±0.03^{D}**	**1.58±0.03**
Overall Mean					
TOS	**0.60±0.04**	**0.60±0.04**	**0.64±0.01**	**1.56±0.09**	**0.85±0.07^{A}**
Cross-I	**0.44±0.01**	**0.54±0.01**	**0.62±0.01**	**1.38±0.07**	**0.75±0.06^{B}**
Cross-II	**0.47±0.01**	**0.54±0.01**	**0.64±0.01**	**1.34±0.03**	**0.75±0.06^{B}**
Cross-III	**0.5±0.02^{D}**	**0.56±0.02^{C}**	**0.64±0.01^{B}**	**1.43±0.04^{A}**	**0.78±0.04**
Overall Mean					

A-D different capital letters on overall means in a row and column differ significantly at P≤0.01.
a-g different small letters on means differ significantly at P≤0.01

Non-pregnant animals showed much higher values of TAS as compared to pregnant animals in this study **(Table 3)**. A significant decrease in TAS concentration was also seen from EP to AP stage in all three breeds. Low TAS concentrations at AP stage in all crossbreds were in harmony with the values obtained in previous reports of cows at late pregnancy stage **[15]**. This lowest level of TAS concentrations at AP stage may be due to vitamins/minerals deficiencies. So, supplement of antioxidants exogenously may be very effective for animal health at dry period and during gestation **[25]**. Mohammed *et al*. **[26]** described a positive association between Total antioxidant capacity, total cholesterol and HDL-C. TOS concentration was non-significantly elevated from early to advance pregnancy in three Crossbred cows **(Table 3)**. At AP, high TOS concentration was seen in all pregnant animals. The oxidative stress

during gestation is due to nutrient metabolism and inflammatory problems and can be controlled by providing specific quantities of antioxidants in order to improve animal's health and reproductive performance **[27, 37-39]**. Rapid fetus growth occurs as pregnancy advances and ultimately metabolic process of mother and fetus increased **[28]** and an increase in the production of reactive oxygen species in dairy cows during late gestation occurs that ultimately causes oxidative stress **[29-36]**.

CONCLUSION

As pregnancy advances in crossbred cows, food intake dilemma and elevated metabolic processes may become causative agent of oxidative stress in them and can be determined by supervising blood alterations. By evaluating total antioxidant status (TAS), valuable biological information can be collected describing the effective relationship and balance between prooxidants and antioxidants in serum.

ACKNOWLEDGMENT

This article is the part of Ph.D thesis of 1st Author Ms. Rabia Farhat.

REFERENCES

[1] Strimbu, K. and T. Jorge (2010). "What are Biomarkers?" Curr. Opin. HIV. AIDS. 5: 463–466.

[2] Elazab, M.F.A. (2015). Evaluation of serum enzyme activities and protein fractions in Brucella-infected cows. Turk J. Vet. Anim. Sci. 39: 480-484.

[3] Serra, J.A., R.O. Domínguez, E.R. Marschoff, E.M. Guareschi, A.L. Famulari and A. Boveris (2009). Systemic oxidative stress associated with the neurological diseases of aging. Neurochem. Res. 34: 2122–2132.

[4] Puertas, M.C., J.M. Martínez-Martos, M.P. Cobo, M.P. Carrera, M.D. Mayas and M.J. Ramírez-Expósito (2012). Plasma oxidative stress parameters in men and women with early stage Alzheimer type dementia. Exp. Gerontol. 47: 625–630.

[5] Celi, P. and G. Gabai (2015). Oxidant/Antioxidant Balance in Animal Nutrition and Health: The Role of Protein Oxidation. Front. Vet. Sci. 2: 48-49.

[6] Anonymous (1988). Annual Report 1987-1988, Directorate of Research Institute for Physiology of Animal Reproduction, Pattoki, District Kasur, Punjab, Pakistan.

[7] Ahmad, M., J.H.J. Van der Werf and K. Javed (2001). Crossbreeding effects of Friesian, Jersey and Sahiwal crosses in Pakistan. Pak. Vet. J. 21: 180-183.

[8] Szasz, G. (1969). A kinetic photometric method for serum gama-glutamyltransferase. J. Clin. Chem. 15:124-136.

[9] Erel, O. (2004). A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin. Biochem*. **37**: 112-119.

[10] Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. Clin. Biochem. 38: 1103–1111.

[11] Duncan, D.B. 1955. Multiple rangeand multiple F-tests. *Biometries.* 11: 1-42.

[12] Van Knegsel, A.T., H. van den Brand, J. Dijkstra, S. Tamminga and B. Kemp (2005). Effect of dietary energy source on energy balanc, production, metabolic disorders and reproduction in lactating dairy cattle. Rep. Nutr. Dev. 45:665-88.

[13] Sreedhar, S., S.R. Kapa, J. Suresh, R. Patrapalle, S. Moorthy, and P.R. Velugoti (2013). Changes in haematocrit and some serum biochemical profile of Sahiwal and Jersey × Sahiwal cows in tropical environments. Vet. Arhiv. 83: 171-187.

[14] Ghanem, M.M., M.E. Mahmoud, Y.M. Abd El-Rauf, and H.M. El-Attar (2012). Metabolic profile test for monitoring the clinical heamatological and biochemical alteration in cattle during peripartuirent period. Ben.Vet. Med. J. 23 (2): 13-23.

[15] Pandupuspitasari, N.S., F.A. Khan, M. Sameeullah, Z.U. Rehman, A. Sudjatmogo, A. Muktiani, and J. Yi. (2016). Effects of diludine on the production, oxidative status, and biochemical parameters in transition cows. J. Environ. & Agri. Sci. 6:3-9.

[16] Kurpinska, A.K., A. Jarosz, M. Ożgo, and W.F. Skrzypczak (2015). Changes in lipid metabolism during last month of pregnancy and first two months of lactation in primiparous cows – analysis of apolipoprotein expression pattern and changes in concentration of total cholesterol, HDL, LDL, triglycerides. Pol. J. Vet. Sci. 18(2):291–298.

[17] Turk, R., D. Juretic, D. Geres, N. Turk, B. Rekic, V. Simeon-Rudolf, and A. Svetina (2004). Serum paraoxonase activity and lipid parameters in the early postpartum period of dairy cows. Res. Vet. Sci. 76: 57-61.

[18] Turk, R., D. Juretic, D. Geres, N. Turk, B. Rekic, V. Simeon-Rudolf, M. Robic, and A. Svetina (2005). Serum paraoxonase activity in dairy cows during pregnancy. Res. Vet. Sci. 79: 15–18.

[19] Pratt, D.S. (2010). Liver chemistry and function tests. In: Feldman M., Friedman, L.S., Brandt L.J., eds. Sleisenger and Fordtran's Gastrointestinal and liver disease. 9th ed. Philadelphia, Pa: Saunders Elsevier; chap 73.

[20] Berk, P. and K. Korenblat (2011). Approach to the jaundice or abnormal liver tests. In: Goldman, L., Schafer A.I., eds. Cecil Medicine. 24th ed. Philadelphia, Pa: Saunders Elsevier; chap 149.

[21] Abdulkareem, T.A. (2013). Some Hematological and Blood Biochemical Profile of Iraqi Riverine Buffaloes (Bubalus bubalis) During Different Gestation Periods. J. Buffalo Sci. 2: 78-84.

[22] Mili, B., S. Panditab, and M.S. Parmara (2015). Liver Functioning in Murrah Buffaloes (Bubalus bubalis) Supplemented with Vitamin E during Dry Period. Livestock Res. Internat.3: 43-46.

[23] Djoković, R., V. Kurćubić, R. Djoković, V. Kurćubić, Z. Ilić, M. Cincović, M. Petrović, N. Z. Ilić, M. Cincović, M. Petrović, N. Fratrić, B.F. Jašović, and B. Jašović (2013). Evaluation of metabolic status in Simmental dairy cows Evaluation of metabolic status in Simmental dairy cows during late pregnancy and early lactation during late pregnancy and early lactation. Vet. Arhiv. 83: 593-602.

[24] Lee, D.H., R. Blomhoff, and D.R. Jacobs (2004). Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic Res. 38:535–539.

[25] Sordillo, L.M. and W. Raphael (2013). Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. The Vet. Clin. North Am. Food Anim. Prac. 29: 267–278.

[26] Mohammed, G.A.E., E. M.Abd-Elnaser, and H. K. Elsayed (2015). Preliminary study on lipid profile with relation to total antioxidant capacity and some hematological and biochemical changes of pre-post-partum buffalo heifers at assiut city. Assiut Vet. Med. J. Vol. 61(144): 159-165.

[27] Sordillo, L.M. and V. Mavangira, (2014). The nexus between nutrient metabolism, oxidative stress and inflammation in transition cows. Anim. Prod. Sci. 54: 1204-1214.

[28] Aurousseau, B., D. Gruffat, and D. Durand (2006). Gestation linked radical

oxygen species fluxes and vitamins and trace mineral deficiencies in the ruminant. Rep. Nutr. Dev. 46(6):601–620.

[29] Castillo, C., J. Hernandez, A. Bravo, M. Lopez Alonso, M. Pereira, and J.L. Benedito (2005): Oxidative status during late pregnancy and early lactation in dairy cows. Vet. J. 169: 286–292.

[30] Danish, P., Ali, Q., Hafeez, M.M., Malik, A. Antifungal and antibacterial activity of aloe vera plant extract. *Biol. Clin. Sci. Res. J.*, 2020, Vol, 2020, p. e003.

[31] Masood, M., Ahsan, M., Sadaqat, H.A., Awan F., Screening of maize (*Zea mays* L.) inbred lines under water deficit conditions. *Biol. Clin. Sci. Res. J*, *2020*, Vol. 2020, p.e007.

[32] Nazir MI, Idrees I, Danish P, Ahmad S, Ali Q, Malik A. Potential of water hyacinth (*Eichhornia crassipes* L.) for phytoremediation of heavy metals from waste water . *Biol. Clin. Sci. Res. J*, *2020*, Vol. 2020, p.e006.

[33] Hayat S, Ramzan M, Zafarullah M, Ahmad I, Ali Q, Malik A. Conservation of an endangered carnivorous fish rita rita through induced breeding. *Biol. Clin. Sci. Res. J*, *2020*, Vol. 2020, p.e004.

[34] Khalil, R., Ali, Q., Hafeez, M.M., Malik, A. (2020). Phenolic acid profiling by RP-HPLC: evaluation of antibacterial and anticancer activities of *Conocarpus erectus* plant extracts. *Biol. Clin. Sci. Res. J.,* 2020*:* e010.

[35] Hayat, S., Naeem, M,. Ramzan, M., Zafarullah, M., Ahmad, I., Ali, Q., Malik, A. (2020). Study on zooplankton diversity during spring season in Chashma Lake, Pakistan. *Biol. Clin. Sci. Res. J., 2020:* e009.

[36] Khalil, R., Ali, Q., Hafeez, M.M., and Malik, A. (2020). Phytochemical activities of *Conocarpus erectus*: An overview. *Biol. Clin. Sci. Res. J.,* 2020: e008.

[37] Ali, Q., Khalil, R., Nadeem M., Azhar, M.M., Hafeez, M.M., Malik, A. (2020). Antibacterial, antioxidant activities and association among plant growth related traits of *Lepidium draba*. *Biol. Clin. Sci. Res. J.,* 2020*:* e011

[38] Ahmad, S., Hera, Z. Hanif MS., Syed AH (2020). Effects of carbosulfan on the biology of bird cherry oat aphid. *Biol. Clin. Sci. Res. J.,* 2020*:* e014.

[39] Iqra L, Rashid M, Ali Q, Latif I, Malik A. Genetic variability for salt tolerance in wheat. Biol Clin Sci Res J, (2020); 2020(e016).