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Acute phase proteins in cattle as markers of chronic exposure to environmental pollutants at El Salam canal region, North Sinai, Egypt.

DONIA, G. R.

Animal and Poultry Health Department, Animal and Poultry Production Division, Desert Research Center, Mataria, Cairo, Egypt.

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ABSTRACT

This study was conducted to assess the changes in acute phase proteins including haptoglobin (Hp) and serum amyloid A (SAA) induced by chronic exposure of the adult cattle to environmental pollutants at El-Tina Plain which depends on El Salam Canal water. Blood samples were collected from the jugular vein of thirty apparently healthy cattle reared at two different regions: G1, the first group reared at non polluted area (control group) and G2, the second group reared at polluted area. The Hp concentration was measured by using an Hp hemoglobin binding assay and SAA was measured by a solid phase sandwich ELISA. Hp and SAA means as serum concentrations of G2 were significantly (P < 0.01) higher than concentrations in the control group. Moreover, albumin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in addition to urea and creatinine were determined. In conclusion, the stress resulting from environmental pollutants caused the elevation of serum HP and SAA which are markers for evaluating the health status of a herd.

1. INTRODUCTION

El-Tina Plain of East Suez Canal of Port Said Governorate depend on El Salam Canal water which originates from mixing the River Nile water with drainage water (El-Serw and Hadous drainage water) by a ratio of about 1:1. High amount of organic pollutants disposed into the Canal from El Serw drain with high amount of sewage disposed into the canal from Bahr Hadous drain. Total dissolved solids (TDS), total suspended solids (TSS) and heavy metals concentrations along the Canal were found above the standard limits. Increase of phosphorus level at Hadous drain was attributed to the agricultural runoff that contains phosphate fertilizers as well as domestic wastewater containing detergents, most of wastewaters are dumped straight into canals, lakes and estuaries without any treatment and the major agro-industrial effluents of sugarcane and starch industries companies pose a serious threat to surface waters so El-Salam Canal water is subjected to sewage and agricultural pollution and suffering from chemical and biological contamination (Yousry, 2009; Donia, 2010; Yehia, and Sabae, 2011; Donia, 2011; Badawy *et al.*, 2013).

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Contamination of the environment is one of the major stressors that can be defined as the biological response elicited after a threat to homeostasis. Stress is an important factor in the animal industry since it has been directly linked to growth, reproduction, meat quality. animal welfare, and disease susceptibility, thereby giving it the potential for making a substantial economic impact (Gruys et al., 2005). Chronic exposure to environmental pollutants is a type of stress (Duffy et al., 1996).

The acute phase response (APR) is a non-specific reaction by an individual to different types of tissue damage (Gruys et al., 1994). It includes a series of complex physiological events occurring in the host after a tissue injury, an infection or chronic stress. One of the main phenomena during the APR is the hepatic production of acute phase proteins (APPs), which play a role in the defense response of the host (Nazif et al., 2010). Although the acute phase proteins (APPs) have been extensively investigated in various inflammatory and non-inflammatory conditions in cattle, knowledge of the behavior of APPs in certain physiological and disease conditions is still limited (Orro, 2008). APPs are a group of blood proteins that change in concentration in animals subjected to external or internal challenges such as infection, inflammation, surgical trauma or stress and they are sensitive factors allow the early detection that of inflammation in ruminants (Kent, 1992).

Acute phase proteins represent an appropriate assessment of animal health over the last few years as they have become biomarkers of inflammation and infection for diagnostic and prognostic purposes in both farm and companion animals. They are produced in response to a variety of disease conditions stimulated bv the proinflammatory cytokines and in response to infection, inflammation, surgical trauma and stress. They consist of negative and positive proteins that show a decrease and an increase in concentration respectively, in response to challenge (Eckersall & Bell, 2010 and Sharifiyazdia *et al.*, 2012).

In cattle and other ruminants, Hp has been one of the APPs most commonly monitored as a marker of inflammation in cattle and it is a major bovine APP that shows a high relative increase during acute phase response (APR). SAA is an APP in cattle as well as in humans and in fact in the majority of animal species (Petersen et al., 2004). It has been shown to be increased during the course of infection or endotoxaemia (Jacobsen et al., 2004). Alsemgeest et al. (1995) reported higher SAA concentrations in calves housed on a slippery floor and suggested that SAA could be a marker of stress. Recently, this suggestion was supported by Saco et al. found (2007)who increased SAA concentrations in a group of cows living in stressful conditions. In both of these studies, Hp concentrations were not different, indicating that SAA is a more sensitive APP in bovines, measuring circulating levels of these proteins thus provides valuable quantifiable information about the ongoing APR and can be used as a non-specific disease marker (Petersen et al., 2004).

2. MATERIALS AND METHODS

This study was carried out at two sites, the first was at El-Tina Plain of East Suez Canal of Port Said Governorate, it depends on El Salam Canal water which suffers from many pollutant as high levels of minerals, heavy metals, organic matters, residues of pesticides, herbicides as well as microbial contamination according to many previous studies (Donia, 2010; Donia, 2011; Shaban et al., 2012; Hamed, et al., 2013 and Badawy et al., 2013) and the second site was Balooza at North Sinai Governorate which depends on potable water which is carried to Al-Areesh across Al-Qantara water station, that is the main source of Nile water to Sinai, cattle from this site was the control group. The climates of the investigated areas are typically Mediterranean that belongs to the

arid region with long hot rainless summer, mild winter and low amount of rainfall (73 mm) (Badawy *et al.*, 2013). Blood samples were collected from two groups (fifteen in each group) of apparently healthy adult cattle from the two sites. They were taken from jugular vein into vacutainers, serum was separated by centrifugation at 4000 rpm for 15 min and stored at -20 °C until used.

Acute phase proteins (Hp and SAA) were determined by commercial kits of the manufacturer (Tridelta Development Plc, Wicklow, Ireland). Haptoglobin level in serum was measured according to prevention of the peroxidase activity of hemoglobin, which is directly proportional to the amount of Hp and determined by the haemoglobin binding method, using micro-titre plates and SAA was measured by sandwich ELISA, using phase SAA kits in ELISA reader according to the manufacturer's instructions.

Albumin (Alb), (AST), (ALT), urea and creatinine were determined by using

Diamond kits. Data on content of biochemical analysis were subjected to the General Linear Model (GLM) procedure of SAS, Statistical package (SAS, 2002).

3. RESULTS AND DISCUSSION

In the present study, analysis of cattle serum included positive acute phase proteins (Hp & SAA), albumin (Alb) as a negative acute phase protein, ALT, AST, urea and creatinine as liver and kidney functions in the two groups of adult cattle from El-Tina Plain and Balooza, North Sinai Governorate, Egypt. This study compared between cattle from two sites, cattle from the first site are suffering from chronic exposure to environmental pollutants and the second was the control group. Data recorded in Table (1) and Figs. (1-7) as means and means of standard error (means \pm SEM), in the same column, means in a certain item having the same small letter do not differ significantly.

Table 1: Means ± SE of haptoglobin (HP), serum amyloid A (SAA), albumin (Alb), ALT, AST, urea and

Parameter Groups	HP (g/l)	SAA (mg/l)	Alb (mg/dl)	ALT (Iu/l)	AST (Iu/l)	Urea (mg/dl)	Creat. (mg/dl)
G1	0.135 ^a	21.94 ^a	3.61 ^a	7.49 ^a	51.21 ^a	27.1 ^a	0.823 ^a
(n=15)	(±0.0059)	(±2.681)	(± 0.132)	(±1.592)	(±2.632)	(±2.604)	(± 0.064)
G2	0.197 ^b	70.55 ^b	2.71 ^b	22.05 ^b	58.51 ^a	32.2 ^a	1.061^b
(n=15)	(±0.0059)	(±2.681)	(±0.132)	(±1.592)	(±2.632)	(±2.604)	(± 0.064)

cattle.

G1: cattle from unpolluted area selected as the control group.

G2: cattle from polluted area.

In the same column, means in a certain item having the same small letter do not differ significantly. Values followed by different letters are significantly different at $P \le 0.01$.

The mean concentrations of the two positive acute phase proteins (Hp and SAA) were $0.135 \pm 0.0059 \text{ g/l} \& 0.197 \pm 0.0059 \text{ g/l}$ and $21.94 \pm 2.681\& 70.55 \pm 2.681 \text{mg/l}$ for group 1 and 2 respectively as shown in Table

(1) and Figs. 1 and 2. Statistical analyses of results between the two tested groups showed significant differences in measured serum concentration of Hp and SAA (p < 0.01) significantly higher serum HP and

SAA concentrations in the second group relative to the control group.

These results agree with that of Murata (2007) who mentioned that APPs could have potential use not only in identifying animals suffering from severe stress. but also in general welfare management, i.e. high APPs levels indicate underlying problems whether these are hygiene or disease related. Water is the major nutritional factors determining the growth, feed efficiency and body composition of cattle. In addition, the supply of minerals, trace elements and vitamins are important to ensure undisturbed growth. Shortages in water supply and in feed, as well as poor quality water and feed can be the cause of severe stress for the animals and result in various metabolic disorders (Koenen et al., 2012). This may explain the elevation of Hp and SAA in serum of group two which was reared at El Tina Plain that is suffering from considerable problems as soil salinity, arid climate and poor water quality as it depends on El Salam Canal water which is suffering from several pollutants with poor quality water (Donia, 2010; Donia, 2011; Yehia and Sabae, 2011; Shaban et al., 2012 and Badawy et al., 2013).

The above authors reported that there were high levels of pollution loads such as large particles, nutrients, metals, organics and toxic compounds and these pollutants were above the safe limits recommended by WHO and FAO standards. the Also microbial analysis of El Salam Canal water samples showed microbial contamination with coliform bacteria and presence of different pathogenic microbes like: E. coli, Salmonella sp., Proteus, Shigella and Klebsiella (Badawy et al., 2013). This might be explained by the effect of domestic and agricultural wastes discharged from the urbanized surrounding area.

APPs represent appropriate analyses for assessment of animal health, whereas they represent non-specific markers as biological effect reactants they can be used for assessing nutritional deficits and reactive processes, especially when positive and

negative acute phase variables are combined in an index (Gruys et al., 2005). The effect of stress on the serum concentration of APP remains controversial, since it is difficult to distinguish the effect of stress from the effect trauma or subclinical infections of (Alsemgeest et al., 1995). The detection of haptoglobin and serum amyloid A in the serum of both the control and chronic exposure indicated that an acute phase response had occurred and these proteins may have the potential to be used as diagnostic tests supporting previous observations which have suggested that haptoglobin could be a useful aid to the diagnosis of the disease (Eckersall et al., 2001).

Although early research indicated that SAA in dairy cows is increased more during acute rather than chronic inflammatory conditions (Horadagoda, 1999), recent research shows that SAA is also increased during chronic conditions (Chan, 2010). The latter author indicated that concentrations of SAA in the serum of cows affected by metritis reached peak values on 4-7 day after calving $(85 \pm 23 \text{ mg/ml})$ compared to healthy cows (48 \pm 20 mg/ml) and remained above the baseline values for 2 months after parturition. Also concentrations of SAA in the serum of normal cows were 3.6-11.0 mg/ml, in those with mild mastitis 5.4-142 mg/ml, and those with moderate mastitis 5.9-141 mg/ml (Eckersall, 2001). Tourlomoussis et al. (2004) reported Hp concentration of 110 ug/ml in the plasma of healthy beef cattle; however, cattle under different pathological conditions have average plasma Hp values of approximately 270 ug/ml. Smith al., (2010)showed that et concentrations of Hp in the serum of healthy cows, free of lameness, were below the detection limit of <1.0 mg/dl. Lame cows, with infectious or non-infectious claw disorders, were found to have either increased serum Hp of more than 1.0 mg/dl, or found with concentrations lower than 1.0 mg/dl. Cows tested positive for Hp had concentrations ranging between 37 and more than 100 mg/dl. Additionally, Orro et al., (2008) showed that Hp in the plasma of the newborn calves between 0-21 d were between 100-350 mg/ml. Horadagoda *et al.* (1999) found that SAA showed the highest sensitivity, while Hp had the highest specificity.

Biochemical analyses of blood serum are very useful to get insight in the metabolic and health status of animals. During diagnostic procedure it is very useful to compare the values obtained from ill animals with normal values in healthy animal (Jezek et al., 2006). Biochemical parameters responsible for impairment of various body functions might induce structural and physiological abnormalities. It is well known that variations existing in biochemical constituents can be due to sampling procedure, analytical techniques, physical environmental conditions factors, or variations in breed (Mcdowell, 1985).

Concentrations of serum total proteins, albumin, globulin, urea, creatinine and metabolic enzymes are useful indicators of health status of cattle. Despite their importance, reference values for these protein metabolites in cattle breeds raised on communal rangelands in the semi-arid areas are not available, making it difficult to draft suitable supplementary feeding and disease prevention and control strategies (Agenas *et al.*, 2006; Ndlovu *et al.*, 2009).

Data recorded in Table (1) and Figure (3) show that, the serum albumin levels (3.61 \pm 0.132 and 2.71 \pm 0.132 mg/dl) were significantly (p < 0.01) reduced in cattle from the first site than the second. This decrease in albumin values was reported in the group which depends on El Salam Canal water, which may be as a result of damage in the liver tissue of these animals as a result of increased pollution in such region. The present result agrees with Donia (2010) on goats. Hypo-albuminemia is a common phenomenon in the farm animals due to malnutrition, maldigestion or malabsorption (Johnson et al., 1982). It is a common terminal feature of chronic liver disease, occurring when the functional hepatic mass has been reduced to 20 per cent or less (Dunn, 1993). Sevinc *et al.* (2001) recorded that albumin concentrations were 3.4 ± 0.1 and 2.1 ± 0.1 mg/dl for healthy and severe fatty liver cows respectively. Singh *et al.*, (2002) studied the changes in some blood constituents of adult cross-bred cattle fed different levels of extracted rice bran and found that albumin concentrations were 2.63 ± 0.07 and 2.07 ± 0.6 mg/dl for the two groups respectively.

Serum enzymes (ALT&AST) are known to be important serum markers to investigate the health of an animal species (Artacho *et al.*, 2007), though (AST) is present in many tissues and is useful in evaluating muscle and liver damage in small and large animals but (ALT) is considered to be liver specific in small animals. This enzyme is present in high concentrations in the cytoplasm of hepatocytes. Plasma concentrations increase with hepatocellular damage, necrosis, hepatocyte proliferation, or hepatocellular degeneration (Boonprong *et al.*, 2007).

In the present study, both (AST) and (ALT) concentrations were higher in cattle from site two as shown in Table (1) and Figures (4 and 5) which may be due to environmental stress. Also the results indicated no significant differences in serum AST and urea between the two groups but there were significant differences in serum creatinine between the two groups as shown in Table (1) and Figures (6 and 7). Clinical picture may be associated with liver or kidney function, so these results agree with previous reports by Kaneko et al., (1997) and Patra et al. (2011). Singh et al. (2002) recorded 52.02 ± 1.93 and 59.38 ± 1.34 (iu/l) for AST and ALT and recorded 15.02±0.67 & $33.14 \pm$ 0.067 and 2.00 ± 0.17 &1.00±0.03mg/dl for urea and creatinine for the two groups of cattle fed different levels of extracted rice bran. It has been reported that the serum ALT is raised only when cells of liver parenchyma were destroyed, while the serum AST may be due to cellular destruction in several extra-hepatic tissues (Amer, 2009). For this reason, serum ALT is more linked with liver diseases than serum AST. Therefore, the observed increases in serum urea and creatinine concentration might be the outcome of the kidney damage and renal dysfunction (Amer, 2009 and Korotkevich et al., 2009) The obtained results of increased levels of serum urea and creatinine confirm the results of El-Bably et al. (2005) who found an increasing of serum urea and creatinine of sheep drinking from polluted water. Under different physiological conditions, variations in haematological values can be due to a variety of factors such as environmental conditions, muscular activity, quality of nutrition and water balance (Abd Ellah et al., 2014).

4. CONCLUSION

In this study after chronic exposure to environmental pollutants the acute phase proteins (Hp & SAA) and biochemical analysis (Alb, ALT, AST, urea and creatinine) were altered in cattle serum. These results indicated that the environmental pollutants as stressors not only alter stress related factors but also affect immune physiological parameters. Also APPs play an important role in stress response which might be helpful for future studies on pollutant stress and immune system attenuation. It is worth noting that chronic exposure to environmental pollutants appears to induce an acute phase response accompanied by increased HP & SAA production. Further investigation should be done to assess the influence of chronic exposure of environmental pollution on the immune response, and it would be interesting to investigate more on causes and effects of pollution to understand if this environmental disturb could be the consequence of stressful situations. This may imply that, even in the absence of clinical symptoms, these cattle were not perfectly healthy. So the study demonstrated that chronic exposure to stress of environmental pollutants were significantly enough to trigger changes in APPs and biochemical analysis in cattle.

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