Oxidant and antioxidant biomarkers and the risk factor of age on their concentrations in pneumonic Arabian camels *(Camelus dromedarius)*

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Abstract

The research study focused on the relationship between oxidant and antioxidant markers and trace element deficiency, especially hypocuprosis, in addition to the risk factors of significant oxidant and antioxidant markers in pneumonic Arabian camels based on their age**.** This study was conducted on a total of 30 adult camels which were classified into 2 groups. Group 1 included 20 apparently healthy camels free from external and internal parasites and showing no clinical signs of illness as a control group. Group 2 included 10 camels that suffered from bilateral nasal discharge, inappetence, dyspnea, cough with harsh lung sounds, which were divided according to their age into 2 subgroups. The first subgroup included pneumonic camels aged from 2-4 years, and the second subgroup included pneumonic camels aged from 5-8 years. Serum samples were collected from all camel groups during field study for mineral profile analysis and to determine the oxidant/antioxidant status. The statistical results showed a significant increase in malondialdehyde and reduced total antioxidant capacity and catalase in pneumonic camels compared to healthy ones ($P< 0.01$). There was a Pearson negative correlation between copper and malondialdehyde ($r = - 0.6$; $P<0.05$) in pneumonic camels ranging from 2-4 years. Young age pneumonic camels 2-4 years old were more likely to have high malondialdehyde (MDA) (OR=2.4; 95% CI=1.28-6.82; P=0.03), and low TAC concentrations (OR=3.2; 95% CI= 1.17-12.30; P=0.02).

Keywords: antioxidant, camel, copper, oxidant, pneumonia.

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Introduction

Old World camels have served people in cross-continental caravans, transporting people, connecting different cultures and providing milk, meat, wool and draught since their domestication around 3000–6000 years ago (Burger et al., 2019). Acute respiratory infection (acute pneumonia) affects both adults and camel calves and is manifested by sneezing, mucus discharge from the nostrils, fever, depression and reduced feed intake. Mucus discharge eventually becomes pus and blocks the nostrils, leading to difficulties in breathing and death. Respiratory infection appears severe in camels, due to the fact that the primary infection is induced by virus and further complicated by bacteria. Specific signs of chronic respiratory infection (pneumonia) are loud, severe open mouth coughing, weight loss, restlessness, dullness, lacrimation, prolonged recumbency, weakness, and reduced milk yield in lactating (Abbas and Omer, 2005).

Oxidative stress is commonly defined as a cellular or individual level imbalance between oxidants and antioxidants. Oxidative damage is one result of such an imbalance and involves oxidizing cellular macromolecules, cell death by apoptosis or necrosis, as well as damage to the structural tissue. Oxidants are compounds, which can oxidize target molecules and this can be done through one of three actions**:** hydrogen atom abstraction, electron abstraction or oxygen addition (Lykkesfeldt and Svendsen, 2007). The body fights excess free radicals through the antioxidant defenses of antioxidant enzyme (superoxide dismutase, glutathione peroxidase) and antioxidants including ascorbic acid (vitamin C), α-tocopherol (vitamin E), and ceruloplasmin and other natural antioxidants (Dimri et al., 2010). In addition, some endogenous antioxidants may include trace components such as zinc, copper, iron and selenium (Aruoma, 1998). These trace elements share a vital role in the prevention of free-radical-induced deterioration of tissues for the maintaining of health and production (Oteiza et al., 1995). Deficiency of some trace minerals may weaken host protection against oxidative stress. Via its interaction with the Cu-Zn superoxide dismutase (SOD) and ceruloplasmin enzymes, copper is active in the antioxidant mechanism (Halliwell and Gutteridge, 1999). With normal copper body levels, it functions like an indirect antioxidant because of its complementary role in the antioxidation cycle (Saleha et al., 2008).

Malondialdehyde (MDA), which is the last lipid peroxidation product, enzymatic antioxidants such as superoxide dismutase, glutathione peroxidase and catalase, non-enzymatic antioxidants such as decreased glutathione, vitamins C and E, β-carotene, ceruloplasmine and bilirubin, total antioxidant capacity (TAC) and total oxidant status (TOS) are widely used to determine oxidant stress (Celi, 2010). The occurrence of oxidative stress is demonstrated under various conditions, such as sepsis, mastitis, enteritis, respiratory and joint disease, endoparasitis, transport and pregnancy in ruminants, and oxidative stress should be considered for the prognosis and effective treatment of these conditions (Piccione et al., 2013). The aims of the present study were to focus on the relationship between oxidant and antioxidant markers and trace element deficiency (hypocuperosis) in addition to the risk factors of significant oxidant and antioxidant markers in pneumonic camels based on their age. We hypothesized that trace element deficiency will be frequent in pneumonic camels and will be a risk factor for oxidative stress.

Material and methods

Animals' criteria

A total of 30 adult male and female Arabian camels (*Camelus dromedarius*) were examined from separate locations in the Menofia and Behera Governorates, Egypt, and covered an age range of 2–8 years and 200-550 kg body weight (BW) between September 2018 and May 2019. The examined camels received a mixture of silage, hay, roughage and clover. According to general health conditions, body condition scores and physical examination of the camels, they were split into two main groups: Group 1 (n=20) included apparently healthy camels free from external and internal parasites and showing no clinical signs of illness; Group 2 (n=10) included camels that suffered from bilateral nasal discharge, inappetence, dyspnea, cough with harsh lung sounds. Pneumonic camels were further divided according to their age into 2 subgroups. The first subgroup included pneumonic camels aged from 2-4 years (n=4), and the second subgroup included pneumonic camels aged from 5-8 years (n=6).

Clinical information

The initial general clinical examination was performed by techniques outlined by Higgins (1984) and Abd El-Rahman et al. (2003).

Blood samples

Ten ml of blood was collected in dry clean centrifuge tube and kept in a sloping position without agitation until coagulation occurred. The clotted samples were centrifuged at 3000 rpm for 10 minutes for separation of only clear non hemolyzed sera and were stored at -80°C until analyzed.

Biochemical measurements

Commercial kits were used for spectrophotometric determination of iron, phosphorus, calcium, sodium and magnesium (Bio-Diagnostic, Giza, Egypt), in accordance with the method specified. MDA was measured in serum samples as previously described by (Ohkawa et al., 1979) using a kit from the Bio-Diagnostic Company in which MDA reacts directly at optimum pH (3.5) with thiobarbituric acid to produce a spectrophotometrically determined red color. Catalase was measured in serum sample as previously described by Aebi (1984), using a kit from the Bio-Diagnostic Company. In the presence of peroxidase (HRP), the remaining H_2O_2 reacts with 3,5-Dichloro -2-hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample. TAC was determined according to the method described by Koracevic *et al.* (2001), using a kit from the Bio-Diagnostic Company in which a Fe-EDTA complex reacts by a Fenton-type reaction with hydrogen peroxide, resulting in the formation of hydroxyl radicals that degrade benzoate, followed by the release of reactive substances (thiobarbituric acid).

Statistical analysis

Data from healthy and diseased camels were compared by means of a one-way ANOVA by using the statistical package for social science (SPSS) for Windows (IBM SPSS Bootstrapping 24, 2016). Results were expressed as the mean ± standard error (SEM). Univariate analysis was based on the age of diseased camels with reference to 5-95% confidence interval from healthy camels. Receiver operating characteristic (ROC) analysis was carried out using a GraphPad Prism 8. Significance was set at $P<0.05$.

Results and Discussion

Although the mean values of body temperature, respiratory rate and pulse rate were significantly increased in pneumonic camels compared to apparently healthy camels ($P < 0.05$), the mean values of ruminal motility showed a significant decrease in pneumonic camels ($P < 0.05$) than apparently healthy ones (Table 1). There was also a statistical elevation in the values of respiratory and pulse rate in pneumonic younger camels (2-4 years) than in older ones (5-8 years) ($P <0.05$). These results were nearly similar to those obtained by Abubakar and Saad (2011). Cytokines cause a cascade of events that can lead to common clinical symptoms: pyrexia, anorexia and dullness (Gabay and Kushner, 1999). The mean values of rectal temperature, respiratory and pulse rates were more elevated in Group 2 than in the healthy ones in Group 1, which may be correlated with bacteremia (Benkirane and De Alwis, 2002).

Table 1. Clinical examination in apparently healthy and pneumonic camels (mean ± SE).

Variables	**Healthy (n=20)**	**Pneumonic camels (n=10)**	
		2-4 years (n=4)	**5-8 years (n=6)**
Body temperature (°C)	37.5±0.09 [a]	39.2±0.10[b]	38.8±0.28 [b]
Respiratory rate (cycle/min)	12.6±0.303 [a]	22±0.08 [b]	18.2±1.15 [c]
Pulse rate (beat/min)	33.2±0.625 [a]	46.4±0.20 [b]	42.2±0.75 [c]
Ruminal motility (contraction/2 min)	4.3±0.143 [a]	2.1±0.02 [b]	2.0±0.10[b]

n, number. Means within the same row having the different superscripts differ significantly different at (P<0.05).

Table 2 showed that the mean values of phosphorus, magnesium and copper were significantly diminished in pneumonic camels compared to apparently healthy ones (P <0.05), with a significant decrease of these elements in pneumonic camels aged from 5-8 years compared to the young diseased ones (2-4 years), except for the copper level which is statically decreased in young camels compared to elder ones (P <0.05), while the mean values of these elements (iron, calcium and zinc) were not significantly different between apparently healthy camels and diseased camels (P >0.05). Almost identical outcomes were documented in pneumonic calves and diseased camels by Ibrahim *et al*. (1988), Hanzlicek *et al*. (2010), and Hassan *et al*. (2019). Rumen magnesium absorption may be enhanced by lower pH (which increases Mg solubility) and diminished by microbial infection (Kennedy and Bunting, 1991). Serum Mg may decrease during lipolysis associated with stress, cold, or starvation (Rayssiguier, 1984). Serum copper was significantly decreased in pneumonic camels; the reduction of copper mean values may be due to the disturbance of tissue oxidation (Doherty and Mulville, 1992).

Table 2. Major and minor elements profile in apparently healthy and pneumonic camels (mean ± SE).

Variables	**Healthy (n=20)**	**Pneumonic camels (n=10)**	
		2-4 years (n=4)	**5-8 years (n=6)**
Iron (µg/dl)	116.3±1.554 [a]	108.4±0.8 [b]	103.58±0.58 [c]
Phosphorus (mmol/l)	3.532±0.082 [a]	2.6±0.08 [b]	2.5±0.14 [b]
Calcium (mmol/l)	1.67±0.065 a	1.49±0.05 a	1.61±0.02 a
Magnesium(mmol/l)	1.069±0.029 [a]	0.84±0.02 [b]	0.92±0.06 [b]
Copper (µg/dl)	93.7±1.4 [a]	82.66±0.5 [b]	87.4±0.2 [c]
Zinc (µg/dl)	121.9±1.57[a]	120.4±0.3 [a]	119.8±0.8 [a]

n, number. Means within the same row having the different superscripts are significantly different at (P<0.05).

The present study showed a significant increase in the levels of MDA in diseased camels when compared to its levels in the healthy ones, and a significant decrease in the levels of TAC and catalase compared to the healthy group (P<0.05; Table 3). Also, there were static variations in the values of MDA and catalase between young aged pneumonic camels (2-4 years) and pneumonic camels aged from 5-8 years, as shown in Table 3. The same attribution was obtained by El-Deeb (2015). Decreased antioxidants (TAC and catalase), as identified in the current study, can be attributed to the cell protection consumption of those enzymes by preventing the initiation of peroxidization and production of final products, such as TBARS, that are capable of leading to serious cell damage (Halliwell, 1996). The significant elevation of the MDA level may be associated with the risk effect of cellular damage and inflammation, which are linked to bronchopneumonia and broncho-interstitial pneumonia, in addition to the destruction of epithelial cells and fibrinous reaction resulting from vascular damage (Jarikre et al., 2017).

Table 3. Oxidant and antioxidant status in apparently healthy and pneumonic camels (mean ± SE)

Variables	**Healthy (n=20)**	**Pneumonic camels (n=10)**	
		2-4 years (n=4)	**5-8 years (n=6)**
Malondialdehyde (nmol/ml)	2.6±0.09[a]	6.2±0.06 [b]	5.4±0.1 [c]
Total antioxidant capacity (mmol/L)	0.81±0.02[a]	0.28±0.03 [b]	0.22±0.08 [b]
Catalase (U / L)	78.3±0.4[a]	67.8±0. 1 [b]	72.2±0.3 [c]

n, number. Means within the same row having the different superscripts differ significantly different at (P<0.05).

In the present study, there was negative correlation between copper and MDA (r=-0.6; P<0.05) in the pneumonic camels ranging in age from 2-4 years (Fig 1). Our results are in agreement with those obtained by Zhang *et al*. (2012), who indicated that copper deficient goats were associated with increased malondialdehyde (MDA) levels, while supplemental Cu increased the activities of antioxidant enzymes and decreased the serum MDA content in cashmere goats. In our research study, there was a significant decrease in the level of copper. Similar results were obtained by Saleha *et al*. (2008).

Table 4 shows that young age pneumonic camels 2-4 years old were more likely to have high MDA (OR=2.4; 95% CI= 1.28-6.82; P=0.03), and low TAC concentrations (OR=3.2; 95% CI= 1.17-12.30; P=0.02). Our results were similar to the findings obtained by Barja de Quiroga *et al*. (1990), who said that antioxidant status as catalase activity has been shown to be responsible for the detoxification of significant amounts of H_2O_2. The activity of this enzyme is inhibited by aging due to increased production of free radicals. Furthermore, we have documented that young age pneumonic camels of 2-4 years old were more likely to have low catalase (OR=1.4; 95% CI= 1.02-5.38; P=0.03). Additionally, catalase had good specificity and sensitivity as an antioxidant in pneumonic camels.

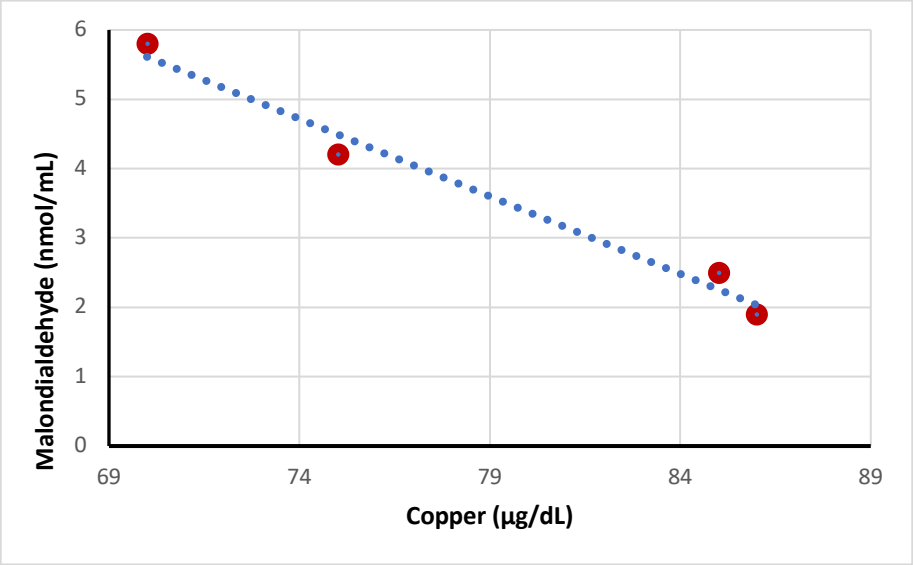

Figure 1. Pearson negative correlation between copper and malondialdehyde (MDA) (r=-0.6; P<0.05) in pneumonic camels ranged from 2-4 years.

Table 4. Risk factors of significant oxidant and antioxidant markers in pneumonic camels based on age.

Malondialdehyde >2.6 (nmol/mL)		**OR**	**95% CI**	**P-Value**
Age	2-4	2.4*	1.28-6.82	0.03
	5-8	1.61	0.53-6.9	0.5
Total antioxidant capacity <0.81 (mmol/L)		**OR**	**95% CI**	**P-Value**
Age	2-4 years	3.2*	1.17-12.3	0.02
	5-8 years	1.82	0.75-3.82	0.5
Catalase <78.3 (U / L)		**OR**	**95% CI**	**P-Value**
Age	2-4 years	1.4*	1.02-5.38	0.03
	5-8 years	0.76	0.51-3.48	0.6

OR, odds ratios; CI, confidence interval. *P<0.05 compared to referent

Conclusions

There was a strong relationship between copper deficiency and the incidence of oxidative stress expressed by an elevated level of MDA in pneumonic camels. Reduced TAC and catalase together with increased MDA concentrations in pneumonic camels, especially in young aged camels (2-4 years) compared to aged ones (5-8 years), could indicate severe oxidative stress in young aged camels.

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Conflict of Interests

No conflict of interest was declared.

References

Abbas, B. and Omer, O. H., 2005. Review of infectious diseases of the camel. Veterinary Bulletin 75, 1N- 16N.

Abd El-Rahman, M.A., Ahmed, M.M. and Derar, I. 2003. Applied study on physiological restraint during ultra-sonography, as stress on health status and some blood parameters of one humped camel (*Camelus dromedarius*) in Upper Egypt. *Ass. Univ. Bull. Everson. Res.* 6 (1): 73-77.

Abubakar, M.S. and Saad, M.Z. 2011. Clinico-pathological changes in buffalo calves following oral exposure to *Pasteurella multocida* B: 2. *Basic and Applied Pathology* 4: 130- 135.

Aebi, H. 1984. Catalase in vitro. *Methods Enzymol*. 105 (2): 121- 126.

Aruoma, O. I. 1998. Free radicals, oxidative stress, and antioxidants in human health and disease. *J Am Chem Soc*. 75:199.

Barja de Quiroga, G., Perez-Campo, R., Lopez-Torres, M. 1990. Antioxidant defenses and peroxidation in liver and brain of aged rats. *Biochem. J.* 272: 247-250.

Benkirane, A., De Alwis, M.C. 2002. Haemorrhagic septicaemia, its significance, prevention and control in Asia. *Vet Med-Czech*. 47: 234- 240.

Burger, P. A., Ciani, E., Faye, B. 2019. Old World camels in a modern world - a balancing act between conservation and genetic improvement. John Wiley & Sons Ltd on behalf of The Stichting International Foundation for Animal Genetics, 50: 598-612.

Celi, P. 2010. The role of oxidative stress in small ruminants' health and production. R Bras Zootec, 39: 348-363.

Dimri, U., Sharm, M.C., Yamdagni, A., Ranjan, R., Zama, M.M.S. 2010. Psoroptic mange infestation increases oxidative stress and decreases antioxidant status in sheep. Vet Parasitol 168: 318-322.

Doherty, T.J, Mulville, J.P. 1992. Diagnosis and treatment of large animal diseases. W. B. Saunders Company. Philadelphia, London, Toronto, Montreal, Sydney, Tokyo.

El-Deeb, W.M. 2015. Acute phase response and oxidative stress parameters in pneumonic camel calves (*Camelus dromedarius*). *Bulg. J. Vet. Med.* 18 (3): 258- 269.

Gabay, C., Kushner, I. 1999. Acute-phase proteins and other systemic responses to inflammation. *New Engl J Med.* 340: 448- 454.

Gutteridge, J. M. C. 1995. Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin. Chem.* 41: 1819-1828.

Halliwell, B. 1996. Antioxidants in human health and disease. *Annu. Rev. Nutr.* 16: 33-50.

Halliwell, B., Gutteridge, J. M. C. 1999. In: Free Radicals in Biology and Medicine, third ed. Oxford University Press, New York, USA.

Hassan, H.Y., Gadallah, S., Kamr, A., Abdelazeim, A. 2019. Serum Iron, Calcium, Phosphorus and Magnesium Concentrations and Their Effects on Hemato-immune Dynamics in Diseased Camels (*Camelus dromedarius*). *EC Veterinary Science* 4 (10): 01-11.

Hanzlicek, G.A., White, B.J., Mosier, D., Renter, D.G., Anderson, D.E. 2010. Serial evaluation of physiologic, pathological, and behavioral changes related to disease progression of experimentally induced Mannheimia haemolytica pneumonia in postweaned calves. *Am J Vet Res*. 71: 359-369

Higgins, A.J. 1984. The camel in health and disease. Introduction. *Br. Vet J.* 140(5): 482-484.

IBM SPSS Bootstrapping 24. 2016. IBM Corporation, North Castle Drive, Armonk, New York, USA.

Ibrahim, I.A., El-Ghannam, M.A., Yousef, S.M., El-Magawry, S.M., Dowider, M.F. 1988. Some biochemical and bacteriological alterations associated with transported pneumonic Friesian calves. *Alex. J. Vet. Sci.* 4 (1): 523-532.

Jain, S.K., Mohandas, N., Clark, M.R., Shohet, S.B. 1983. The effect of malonyldialdehyde, a product of lipid peroxidation, on the deformability, dehydration and 51Cr-survival of erythrocytes. *Br. J. Haematol.* 53: 247-255.

Jain, S.K., Williams, D.M., 1988. Copper deficiency anemia: altered red blood cell lipids and viscosity in rats. *Am. J. Clin. Nutr.* 48: 637-640.

Jarikre, T.A., Ohore, G.O., Oyagbemi, A.A., Emikpe, B.O. 2017. Evaluation of oxidative stress in caprine bronchoalveolar lavage fluid of pneumonic and normal lungs. *Intern. J. Vet Sci. Med.* 5: 143-147.

Kennedy, D.W., Bunting, L.D. 1991. Alterations in ruminal utilization of magnesium and zinc in lambs fed different ratios of concentrate. *J. Vit. Nutr. Res.* 61: 67-71.

Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., Cosic, V. 2001. Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.* 54: 356- 361.

Lykkesfeldt, J. Svendsen, O. 2007. Oxidants and antioxidants in disease: Oxidative stress in farm animals. *Vet. J.* 173: 502-511.

Ohkawa, H., Ohishi, W., Yagi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95 (2): 351-358.

Oteiza, P.I., Olin, K.L., Fraga, C.G., Keen, C.L. 1995. Zinc deficiency causes oxidative damage to proteins, lipids and DNA in rat testes. *J. Nutr.* 125: 823-829.

Piccione, G., Casella, S., Giannetto, C., Bazzano, M., Giudice, E., Fazio, F. 2013. Oxidative stress associated with road transportation in ewes. *Small Ruminant Res.* 112: 235-238.

Rayssiguier, Y. 1984. Magnesium and disorders associated with magnesium deficiency (in goats). Les maladies de la chevre, colloque international, Niort, France 28: 411-414.

Rock, E., Gueux E., Mazur, A., Mott, C., Rayssiguier, Y. 1995. Anemia in copper-deficient rats: role of alterations in erythrocyte membrane fluidity and oxidative damage. *Am. J. Physiol.* 269.5.C: 1245- 1249.

Saleha, M.A., Al-Salahyb, M.B., Sanousic, S.A. 2008. Corpuscular oxidative stress in desert sheep naturally deficient in copper. *Small Ruminant Res.* 80: 33-38.

Suttle, N.F., Jones, D.G., Woolliams, C., Woolliams, J.A. 1987. Heinz body anaemia in lambs with deficiencies of copper or selenium. *Br. J. Nutr.* 58: 539-548.

Uriu-Adams, J.Y., Keen, C.L. 2005. Copper, oxidative stress, and human health. *Mol. Aspects Med.* 26: 268–298.

Zhang, W., Zhang, Y., Zhang, W.S., Song, Z.X., Jia, H.Z., Wang, L.R. 2012. Effect of different levels of copper and molybdenum supplements on serum lipid profiles and antioxidant status in cashmere goats. *Biol Trace Elem Res*. 148: 309-315.