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Some haematobiochemical alterations in buffaloes suffering from milk fever with trail of treatment

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ABSTRACT

This study was planned to investigate the effect of milk fever and treatment on haemato-biochemical parameters alterations in buffaloes. About 15, 4-5 years old buffaloes (5 healthy -10 suffering from milk fever) were divided into 3 groups (5/each). 1st group healthy buffaloes served as control, 2nd group buffaloes suffering from slight milk fever or the first stage of milk fever (tremors in head and legs) and treated by 500 ml Calcium borogluconate (I/V) as one dose, along with 25 ml phosphonic acid/ buffalo (I/V), while the 3rd group buffaloes were suffering from severe milk fever (depression, lateral recumbancy beside unconsciousness) and treated by 500 ml Calcium borogluconate (I/V) as one dose and 25 ml phosphonic acid (I/V)/buffaloe along with 1.5ml/50 kg bwt vitamin D (I/M) daily for 5 days. Blood samples were taken from each buffalo one pre and at 5th days post treatment for determination of hematobiochemical parameters changes.

Buffaloes suffering from milk fever revealed a significant reduction in calcium, phosphorus, α, β globulins, superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GSH-px) beside an insignificant reduction in magnesium, potassium, red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), lymphocyte, basophil, monocyte, total protein, albumin , total globulin coupled with a significant increase in glucose, white blood cells (WBCs), neutrophil, eosinophil, cortisol, parathyroid, aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), creatinin kinase (CK), creatinin phosphkinase (CPK), lactate dehydrogenase (LDH), and malondialdehyde (MDA) associated with significant increase in γ globulin.

Treatment of diseased buffaloes using Calcium borogluconate, phosphonic acid and vitamin D improved its healthy status as well the hematobiochemical parameters and returned to nearly normal levels at 5th day post treatment when compared with healthy control buffaloes.

Summaring up our observations, it could be concluded that milk fever in buffaloes induced some adverse effects in hematobiomcheical parameters

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and oxidation. Treatment of diseased buffaloes by Calcium borogluconate, phosphonic acid and vitamin D induced cure of diseased buffaloes and improved the adverse effects of milk fever.

INTRODUCTION

Buffaloes are considered the main important animals in milk production in Egypt (**Abou-Bakr 2009**). Egyptian consumer prefers buffaloes milk due to its white color, acceptable flavor (**El-Salam and El-Shibiny 2011**). Buffalo milk is higher in protein content than cattle milk. (**Hernández et al. 2019**).

Metabolic diseases are the most important diseases in farm animals which mainly concerned with period commencing of parturition and extending until the peak of lactation (**Radostitis et al. 2000**). Milk fever is a complex mineral-related metabolic diseases affects high-productive cows from three years and older dairy animals known as parturient paresis or post parturient hypocalcaemia, causing some economic losses due to decrease of milk production **(Laurent and Alexander 2007).** It is one of the metabolic disorders of dairy animals related to parturition which occurred during final months of pregnancy or postparturation and develops within 48 hours after parturition, especially in high milk producing dairy animals **(Seifi et al. 2004).** It induces a drop of serum calcium, phosphorus levels and milk production **(Jesse 2018).** Milk fever is related to high milk production due to drain of calcium and phosphorus in milk at the onset of lactation **(Braun et al. 2012)** or due to decreased feed intake during last few days preparturition as well as gastrointestinal tract stasis at parturition leading to a decrease of calcium available for absorption and subsequent hypocalcaemia **(Peter and Ian 2008)** beside at first days after calving colostrum synthesis and secretion need a large amount of calcium leading to reduction of calcium in serum **(Martinez et al. 2012).** Milk fever is divided into three stages according to the severity of reduction of calcium level. The 1st stage or subclinical milk fever is characterized by slight clinical signs as slight muscle tremors in head and limbs **(Houe et al. 2001),** The 2nd stage occurs when there is a sever reduction in serum calcium level and the most prominent signs are subnormal body temperature, dry muzzle, cold extremities, reduction in ruminal movement, constipation and laying down with head turned into flank **(Goff and Horst 1997).** The 3rd stage characterized by lateral recumbency, comatosed animal and complete flaccidity and passive movement of limbs **(Ramos et al. 2009).** Administration of calcium solutions around calving either as oral drench or by intravenous injection play an important role in treatment and prevention of milk fever **(Thilsing et al. 2002).**

The present study focused on evaluating the effect of milk fever on some haematobiochemical, hormone, enzymes and antioxidant enzymes with trial of treatment

MATERIALS AND METHODS

1) Drug

a) Calcium borogluconate: produced by Pfizer for chemical pharmaceuticals company Egypt.
b) Phosphonic acid: produced from Intervet company for pharmaceuticals under the trade name of Tonophosphan and each 100ml contain sodium salt of 4-dimethylamine,2-methylphenyl-phosphonic acid 0.2 g.
c) Vitamin D: produced by Interchemie company for veterinary medicines under the trade name of Vitol -140.

2) **Animals**:-

A total number of 15 buffaloes aged from 4 -5 years old (5 healthy -10 suffering from milk fever in a private farm at Abu Hamad city (El-Sharkia Province) were used in this study. Buffaloes were divided into 3 groups (5/each).1st group is clinically healthy buffaloes (control), 2nd buffaloes group are suffering from slight milk fever (tremors in head and legs) and treated by I/V injection of 500 ml of calcium borogluconate, along with I/V injection of 25 ml phosphonic acid /buffalo. The 3rd buffaloes group was suffering from a severe milk fever (depression, lateral recumbancy and unconsciousness) and treated by I/V injection of 500 ml calcium borogluconate and I/V injection of 25 ml phosphonic acid/buffalo beside I/M in-

jection of 1.5ml/50 kg bwt vitamin D daily for 5 days. Three blood samples were taken from each buffalo at pretreatment and 5th days post treatment. The 1st blood sample was taken on a tube contained EDTA for estimation of blood picture (**Jain 1986).**

The 2nd blood sample was taken in a tube contained heparin for measuring of phagocytic cells% and killing cells % **(Wilkinson 1977 and Lucy and Larry 1982**).
The 3rd blood sample was taken to obtain serum for estimation of total protein (**Doumas et al 1981**) albumin (**Bauer 1982)**, protein fractions (**Kaneko 1989**), transaminases enzymes (AST-ALT) (**Reitman and Frankel 1957**), ALP **(Kind and King 1954**), calcium (Ca) (**Gindler 1972),** phosphorus (Ph) (**Goldenberg 1966**), magnesium (Mg) (**Gind1er and King 1971**), potassium (K) (**Oser 1979**), glucose (**Siet, et al 1981**), Creatine kinase (CK) (**Horder et al. 1989**), Creatine phosphokinase (CPK) (**Forster et al. 1974**), lactate dehydrogenase (LDH) (**Buhl and Jackson 1978),** cortisol by radioimmunoassay method **(Abraham et.al. 1972),** parathyroid hormone (PTH) by radioimmunoassay **Mayer et al. (1979),** Superoxide dismutase (SOD) (**Nishikimi et al. 1972**), catalase (CAT) (**Sinha 1972**), Malanodialdhyde (MDA) (**Nielsen et al 1997**), glutathione (GSH) (**Ellman 1959**) and glutathione peroxidase (GSH-px) (**Palgia and Valentine 1967**).
3**) Statistical analysis**: - The obtained data were analyzed by using computerized SPSS program version 16 according (**Tambane and Dunlop 2000**).

RESULTS

Buffaloes suffering from milk fever revealed significant reduction in calcium, phosphorus , α, β, globulins, SOD, CAT, GSH, GSH-px beside insignificant reduction in Mg, K, RBCs, Hb, PCV, lymphocytes, basophils, monocytes , total protein, albumin , total globulin coupled with significant increase in glucose, WBCs, neutrophils, eosinophils, cortisol, parathyroid, AST, ALT, ALP, CK, CPK, LDH, MDA associated with significant increase in γ globulins. Serum parameters were returned to nearly normal levels at 5th day post treatment (table 1- 5).

Table 1. Effect of milk fever and treatment on some serum mineral and glucose in buffaloes (n= 5)

Groups	GP 1	GP 2		GP 3	
Parameters		Pre Treatment	5th day post Treatment	Pre treatment	5th day post treatment
Ca (gm/%)	9.25±0.87a	4.21±0.64c	8.77±0.64b	4.38±0.75c	9.21±0.44b
Ph (gm/%)	5.56±0.63a	3.12±0.58b	4.89±0.74a	3.32±0.64b	4.97±0.89a
Mg (gm/%)	3.87±0.18a	3.70±0.71a	3.79±0.55a	3.85±0.44a	3.81±0.37a
K (Meq/l)	5.21±0.68a	4.32±0.42a	4.89±0.36a	4.29±0.41a	4.90±0.59a
Glucose (mg/dl)	72.41±0.87b	78.07±0.79a	71.89±0.55b	79.13±0.99a	71.06±0.93b

Different superscripts (a, b and c) within the same row indicate significant differences at $p < 0.05$

Table 2. Effect of milk fever and treatment on blood picture in buffaloes (n= 5)

Groups	GP 1	GP 2		GP 3	
Parameters		Pre treatment	5th day post treatment	Pre treatment	5th day post treatment
RBCs(10^6/c.mm)	6.84±0.38a	6.69±0.87a	6.77±0.55a	6.70±0.58a	6.79±0.89a
HB (gm)	13.63±0.71a	12.68±0.23a	12.94±0.68a	12.81±0.88a	12.97±0.95a
PCV (%)	35.09±0.86a	34.87±0.91a	34.98±0.89a	34.89±0.78a	34.99±0.59a
Total WBCs (10^3/c.mm)	8.30±0.83b	10.14±0.87a	8.67±0.89b	10.18±0.85a	8.23±0.92b
neutrophil	3.23±0.41b	5.26±0.45a	3.74±0.55b	5.10±0.45a	3.30±0.61b
lymphocyte	2.11±0.26a	2.02±0.41a	2.07±0.32a	2.10±0.35a	2.01±0.48a
Differentia eosinophil	0.98±0.21b	1.29±0.15a	0.99±0.12b	1.21±0.12a	1.07±0.16a
(10^3/c.mm) basophil	0.97±0.19a	0.79±0.11a	0.91±0.14a	0.92±0.13a	0.90±0.12a
monocyte	1.01±0.08a	0.78±0.16a	0.96±0.14a	0.85±0.17a	0.95±0.16a
Phagocytic %	39.31±0.78a	40.98±0.65a	39.51±0.69a	40.98±0.54a	39.55±0.87a
index	5.46±0.66 a	5.80±0.49a	5.68±0.89a	5.81±0.46a	5.66±0.83a

Different superscripts (a, b and c) within the same row indicate significant differences at $p < 0.05$

Table 3. Effect of milk fever and treatment on liver functions in buffaloes (n= 5)

Groups Parameters		GP 1	GP 2		GP 3	
			Pre treatment	5th day post Treatment	Pre treatment	5th day post treatment
Protein Profile (mg/dl)	T. protein	6.55±0.97a	5.72±0.85a	6.45±0.89a	5.52±0.77a	6.30±0.69a
	albumin	3.32±0.38a	2.88±0.16a	3.26±0.87a	2.81±0.51a	3.19±0.55a
	globulin α	1.08±0.21a	0.75±0.08b	1.07±0.07a	0.72±0.11b	1.02±0.10a
	β	1.02±0.19a	0.79±0.08b	0.99±0.09ab	0.80±0.12b	0.95±0.13b
	γ	1.13±0.13a	1.20±0.15a	1.13±0.17a	1.19±0.13a	1.14±0.17a
	total	3.23±0.51a	2.84±0.43a	3.19±0.69a	2.71±0.28a	3.11±0.46a
	A G ratio	1.03±0.15a	1.02±0.12a	1.03±0.13a	1.04±0.17a	1.03±0.17a
Liver enzymes (IU/L)	AST	22.58±0.33b	29.61±0.95a	23.18±0.61b	30.04±0.89a	25.04±0.97b
	ALT	13.89±0.94b	18.77±0.87a	14.05±0.79b	18.93±0.99a	13.97±0.99b
	ALP	43.62±2.43c	59.05±2.89a	48.08±2.87b	57.32±2.93a	48.62±2.99b

Different superscripts (a, b and c) within the same row indicate significant differences at $p < 0.05$

Table 4. Effect of milk fever and treatment on cortisol and parathyroid hormone in buffaloes (n= 5)

Groups	GP 1	GP 2		GP 3	
Parameters		Pre treatment	5th day post Treatment	Pre treatment	5th day post treatment
Cortisol (ng/ml)	3.94±0.21c	12.44±0.54a	5.37±0.66b	12.34±0.33a	5.88±0.48b
Parathyroid (ng/dl)	3.97±0.89b	9.46±0.68a	4.67±0.94b	8.99±0.93a	4.76±0.95b

Different superscripts (a, b and c) within the same row indicate significant differences at $p < 0.05$

Table 5. Effect of milk fever and treatment on some enzymes and oxidation in buffaloes (n= 5)

Groups	GP 1	GP 2		GP 3	
Parameters		Pre treatment	5th day post Treatment	Pre treatment	5th day post treatment
CK (IU/L)	84.36±0.69c	97.32±0.94a	86.07±0.83b	96.58±0.84a	87.04±0.79b
CPK (IU/L)	248.08±4.21c	264.21±3.83a	253.21±3.78b	265.72±3.93a	250.84±3.89ab
LDH (IU/L)	412.08±2.13c	465.12±2.32a	421.44±2.42ab	459.12±20.54b	420.65±2.27ab
MDA (ml/nmol)	8.21±0.94ab	12.41±0.99a	9.64±0.89b	12.08±0.79a	9.95±0.92b
SOD (U/ml)	5.18±0.89a	3.09±0.46b	4.92± 0.81a	3.15±0.73b	4.98±0.77a
CAT(U/mL)	85.21 ± 1.23a	78.32 ± 0.85b	83.96 ± 0.a	79.09 ± 0.69b	84.79 ± 0.87a
GSH	24.35 ± 0.52a	17.87 ± 0.62c	22.17 ± 0.87b	18.10 ± 0.33c	22.76 ± 0.46b
GSH-px	5.36± 0.56a	3.65± 0.36b	5.08± 0.78a	3.97± 0.63b	4.99± 0.55a

Different superscripts (a, b and c) within the same row indicate significant differences at $p < 0.05$

DISCUSSION

The main clinical signs observed in animals suffering from milk fever were, in a slight stage, subnormal body temperature, deprived appetite, tremors in the head and legs, protrusion of the tongue, and grinding of teeth, but in a severe case, depression, lateral recumbency, and unconsciousness. The above-mentioned observed clinical signs were previously supported by **Abd El-Raof and Mobarak (2006),** who found that cattle suffering from milk fever showed a subnormal rectal temperature beside depression, lateral recumbency, and unconsciousness. Same clinical signs were observed by **Hassan et al. (2020)** in cows suffering from milk fever.

Our findings revealed that buffaloes suffering from milk fever revealed a significant decrease in calcium and phosphorus levels along with a significant increase in glucose levels when compared with healthy control buffaloes. Reduction of calcium in diseased buffaloes may be due to the drain of calcium to the mammary gland at a rate greater than intestinal calcium absorption and high calcium demand following the onset of lactation (**Goff and Horst 1997).** Elevation in glucose level may be related to the fact that hypocalcemia prevents the secretion of insulin (**Radostitis et al., 2000).** Milk fever is characterized by a significant decrease in calcium and phosphorus (**Beede et al.., 2001**). A close similarity was seen between these findings and those obtained by **Lean et al. (2006),** who indicated that milk fever in cattle induced a significant reduction in calcium and phosphorus and an elevation in glucose levels. Milk fever induces a significant decrease in calcium, inorganic phosphorus, and magnesium (**Hassan, et al. 2020).** These reductions in calcium, phosphorus, and magnesium levels and elevations in glucose levels were observed previously by **Sweety and Pradeep (2021)** in buffaloes suffering from milk fever. Our findings were in agreement with those obtained by **Kulajit and Chayanika (2023),** who **s**tated that milk fever in buffaloes is associated with a reduction in calcium and phosphorus and an elevation in glucose levels.

In the present investigation, it has been shown that milk fever in buffaloes revealed a non-significant decrease in RBCs, Hb, PCV, lymphocytes, basophils, and monocytes, along with a significant increase in WBCs, neutrophils, and eosinophils, and an insignificant increase in phagocytosis% index when compared with control healthy buffaloes. Elevation in leukocytic count, eosinopenia, neutrophilia, and lymphopenia were observed in buffaloes suffering from milk fever due to increased adrenocortical hormone activities in response to the stress of hypocalcemia and parturition **(Berger and Gerber 199**7). Cattle affected by milk fever showed leukocytosis, eosinopenia, neutrophilia, and lymphopenia **(Coles 1997).** Our observed data fit with those reported by **Abd El-Raof and Mobarak (2006)**, who reported that cows suffering from milk fever showed a reduction in erythrocytic count, hemoglobin, packed cell volume levels, and an

elevation in the counts of leukocytes. Our results are supported by **Hassan et al. (2020),** who recorded that milk fever induced a non-significant decrease in RBCs, Hb, PCV, lymphocytes basophils, and monocytes, coupled with an increase in WBCs and neutrophils.

The present work declared that diseased buffaloes showed an insignificant decrease in total protein, albumin, and total globulin, along with a significant decrease in α and β globulin associated with an insignificant increase in γ globulin compared with control healthy buffaloes. These results are comparable with those obtained previously by **Feitosa and Brigle (2000),** who observed a significant reduction in serum total proteins and protein fractions (albumin, α, β, and total globulin) along with a non-significant increase in γ globulin in cattle suffering from milk fever. Our results go hand in hand with those reported by **Abd El-Raof and Mobarak (2006),** who reported that the serum protein profile is significantly decreased in cattle suffering from milk fever. This finding is closely aligned with **Hassan et al. (2020),** who found that local cows suffering from milk fever showed a significant decrease in total proteins, albumin, α, and β globulins.

Biochemical analysis of buffaloes suffering from milk fever indicated a significant increase in cortisol and parathyroid hormones as compared with control buffaloes, and this agreed with **Horst et al. (1997) and Radostitis et al. (2000)**, who stated an elevation in parathyroid and cortisol hormones in cattle affected by milk fever.

In the present study, it has been shown that milk fever resulted in a significant increase in AST, ALT, ALP, CK, CPK, and LDH when compared with control healthy buffaloes, which may be explained by **Hanif et al. (1990),** who stated that hypocalcemia is associated with increased liver and muscle enzyme activity due to degenerative change and necrosis of the liver and muscles of cattle suffering from milk fever. Similar findings were recorded by **Yamagishi et al. (1999),** who stated that milk fever cows show a significant increase in muscle enzymes (CK, CPK, and LDH). In keeping with these lines**, (Lopes 1999)** observed that serum levels of muscle enzymes were increased in hypocalcaemia. These results were in harmony with **Bogumiła et al. (2012)**, who stated that liver and muscle enzymes were decreased in the sera of cows suffering from hypocalcaemia. Elevation in activity of AST, ALT, ALP, CK, and CPK in hypocalcaemic buffaloes coincided with **De Garis and Lean (2018**) and **Hassan et al. (2020)**, who stated that milk fever induced a significant increase in serum AST, ALT, ALP, CK, CPK, and LDH .

In the present study, the analytical findings of the serum constituents of buffaloes suffering from milk fever revealed a significant reduction in SOD, catalase, GSH, and GSH-px, along with an elevation in MDA, compared with control healthy buffaloes. Our results were in agreement with **Hanif et al. (1990)**, who stated that hypocalcemia is associated with an increase in MDA and a reduction in anti-oxidant enzymes. In the same direction, **Thilsing et al. (2002)** reported that milk fever induced a decrease in antioxidant enzymes alongside an increase in MDA. The same changes in GSH and GSH-px were observed by **Bogumiła et al. (2012)** in sera of cows suffering from hypocalcaemia. Also, **Puppel and Kuczynska (2016)** stated that milk fever induced a reduction in CAT, SOD, and GSH.

It has been shown that treatment of diseased buffaloes with calcium borogluconate and phosphonic acid resulted in improved health status, and the hematobiochemical parameters returned to nearly normal levels at the 5th day post-treatment when compared with control healthy buffaloes. These improvements in the health status and hematobiochemical parameters may be due to improved blood calcium and phosphorus in diseased buffaloes **(Radostitis et al. 2000**). These results were supported by the studies of **Abd El-Raof and Mobarak (2006) and Hassan et al. (2020**), which showed that cows suffering from milk fever and treated with calcium and phosphorus revealed improved health status and hematobiochemical parameters.

Summarizing our observations, it could be concluded that milk fever in buffaloes induced

some adverse effects in hematobiochemical parameters and the oxidation reduction potential profile of these animals, and treatment of the diseased buffaloes with calcium borogluconate, phosphonic acid, and vitamin D induced a great reduction of the adverse effects of milk fever.

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