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Effect of adding micronutrient on biochemical parameters during transition period of cows

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ABSTRACT

Dairy cows play an important role in production in our country Egypt. Transition period is the most critical time for those cows that undergo different hormonal and biochemical changes. We take care of animals during this period by addition of mixture of vitamins and trace minerals to the ration in order to improve their general health. The present study was established for evaluation the effect of addition of some trace minerals and vitamin E to ration on some hormonal and biochemical parameters in dairy cows. We used 50 pregnant dairy cows for this study. We divided them into 5 groups. Group 1 (control), supplemented group 2 (vit E), group 3 (Cu sulphate), group 4 (Mg sulphate) and group 5 (Zn oxide). Samples were collected from animals at 2nd, 4th&6th week before parturition and at 2nd, 4th& 6th week after parturition. The result of the present study revealed an increase in the levels of glucose, GSH, SOD, Ca, P, Mg and decrease level of cortisol, MDA& haptoglobin in all supplemented groups when compared to control at different stage of pregnancy.

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INTRODUCTION

Dairy cows are considered as the main important source of animal protein and they are an important source for leather production in our country **(Sarwer et al.2009).** Dairy farm animals are considered as a vital part of agriculture all over the world as they supply many products and milk for the human populace. The earnings which a dairy cow generates come from sale of her offspring and her milk production **(Mondal et al. 2018).**

Transition period is considered as one of the most stressful time in the life of the dairy cows during which they undergo physiological changes in transitioning from pregnant, non-lactating status to a non-pregnant lactating status following calving and moves from demands of foetal growth to calving ،colostrum production and milk production **(Andrieu 2008).**

The important roles of Zn, Cu, and vitamin E on productive and reproductive performance are well established. Cu is important to the antioxidant system as it is part of the copper-zinc superoxide dismutase enzyme which helps in conversion of superoxide radicals to hydrogen peroxide. It is involved in Cu-Zn superoxide dismutase (SOD) enzymes in the antioxidant system. Cu-Zn SOD is essential for dismutation of superoxide radicals to hydrogen peroxide in the cell **(Siciliano-Jones et al. 2008).**

Vit E stimulates the immune status of animal and has an important role in the maintenance of membrane integrity in most cell body against toxic oxygen free radical against its peroxidation. Cu has many antioxidant functions. Zn is implicated in maintaining the epithelial barriers to infection, and has role in udder health **(Qureshi et al. 2010).** Due to the high economic importance of dairy cows this study aimed to Determination the effect of addition vit E and some trace minerals on ration of dairy cows on the level of oxidant malonaldehyde (MDA), superoxide dismutase (SOD), glutathione perioxidase (GPX), haptoglobin, glucose, cortisol, and some minerals (Ca, P & Mg).

MATERIALS and METHODS

Work was carried out at the farm of the Faculty of Agriculture- Sakha- Agriculture of Research Institute.

1. Animals, ration and experimental design

A total of 50 pregnant dairy cows Holstein Frisian breed primiparous aged about 42 months imported from Italy, America and Germany feed on balanced ration apparent healthy and free from diseases and external parasites used for this study in the farm of Faculty of Agriculture- Sakha- Agriculture of Research Institute. The pregnant cows divided into 5 groups each group consisted of 50 cows

Group 1: cows fed on ration according to NRC.

Group 2: cows fed on ration according to NRC + 35 mg/kg body weight zinc oxide.

Group 3: cows fed on ration according to NRC+ 45 mg/kg body weight magnesium sulphate.

Group 4: cows fed on ration according to NRC+ 6 mg/kg body weight Cupper sulphate.

Group 5: cows fed on ration according to NRC+ 6000 Iu of α tocopherol.

2. Samples

Blood samples (7.5 ml) were collected from the jugular vein of each animal in clean glass vials, and converted to serum which was stored at -20℃ until testing, according to **(Coles, 1986).**

The separated sera are used for the detection of some minerals include Mg, Ca, P (mg/dl) **(A.O.A.C, 2015),** oxidants MDA (µmol/ml) **(Ohkawa et al. 1979),** antioxidants included SOD (u/ml) (**Nishikimi et al. 1972),** and GSH level (u/ml) **(Paglia and Valentine 1967),** these were estimated calorimetrically by means of test kits supplied by Biodiagnostic company, Egypt by using of spectrophotometer (Spectro UV-VIS Double beam PC scanning spectrophotometer UVD-2950), according to the manufacturer's instructions. Cortisol concentration was measured by using of the competitive (ELISA), the kit ADI-900-071 from Enzo Life Sciences (Lausen, Switzerland) according to **Palme and Mostl**

(1997). Levels of glucose **(Young, 2001)** determined by using a (Spectro UV-VIS Double beam PC scanning spectrophotometer UVD-2950).

Detection of some serum minerals included calcium (mg/dl), magnesium (mg/dl) and phosphorus (mg/dl) according to **(A.O.A.C, 2015).**

2.5. Statistical analysis

The obtained data were statistically analyzed for significance, analysis of variance by statistical package for social science (SPSS) computer program- one-way ANOVA according to **Snedecor and Cochran (1980).**

3-RESULTS

1- Effect of some addition of Zn oxide, Mg sulphate, Cu sulphate and vit. E on glucose and cortisol levels

-Glucose level

The result of our study revealed that there was a significant increase in glucose level in 4th before and after calving in all groups when compared to control and in Zn, Mg and vit E groups at 6th week before calving. Increase Zn, Cu and vit E at 2nd week before calving, in Mg, Cu and vit E at 2nd week after calving and in vit E at 6th week after calving when compared to control one and when compared to vit E at the level of P value ≤ 0.05 as showed in table 1.

-Cortisol level

There was a significant decrease in cortisol level at 6th week before calving in Cu and vit E groups, in Zn, Mg and vit E at 2nd week before calving and decrease in all supplemented groups after calving and 4th week before calving when compared to control ones while, glucose level increased before and after calving when compared to control at the level of P value≤0.05 as showed in table 1

Table 1. Effect of addition of Zn oxide, Mg sulphate, Cu sulphate and vit. E on glucose, insulin and cortisol levels during different periods of calving

Groups	Calving time / Parameters	Before calving			After calving		
		6th week	4th week	2nd week	2nd week	4thweek	6thweek
Control	Glucose mg/dl	66±3	62±3	60±1.7	59±2	55±2	52±2
	Cortisol nmol/ml	14±1.1	14.4±1.1	16±1.5	14±2	13±2	11.6±1
Zn oxide	Glucose mg/dl	70±0.7*	72±1.6*	74±2.8*	73±3*	72±2*	70±3*
	Cortisol nmol/ml	14±1.6	10±1.1*	13±1.1*	9±2*	8±2*	9±1.5*
Mg sulphate	Glucose mg/dl	71±0.6*	69±2.3*	69±1.7*	68±1.6*	71±2*	73±1*
	Cortisol nmol/ml	13±2	11±1.3*	12±1.5*	9±1.6*	9±1.6*	8±1.6*
Cu sulphate	Glucose mg/dl	69±2.4	69±2.2*	67±3*	69±1.7*	71±1.3*	71±0.7*
	Cortisol nmol/ml	11±2*	9±1.8*	15±1.8	10±1.6*	9±1*	8±1*
Vit E	Glucose mg/dl	73±1.3*	76±1.6*	78±1.5*	70±1.5*	78±3	80±1*
	Cortisol nmol/ml	11±1.5*	10.4±2.4*	12±1.6*	10±2*	8±2*	9±1.6*
P value		0.001	0.001	0.001	0.001	0.001	0.001

Data presented as mean ± SD

-* used for significant difference of group with control at the level of P≤0.05

2-Effect of some additives on some oxidant and antioxidants levels during different periods of pregnancy.

-MDA level

There was a significant decrease in MDA level in all stages of calving in Zn and vit E supplemented groups when compared to control one, decrease in Mg supplemented group at 2nd week before and after calving when compared to control and decrease in Cu supplemented group at 2nd week and all stages after calving when compared to control at the level of P value≤ 0.05 as showed in table 2.

-GSH& SOD levels

There was a significant increase in SOD and GSH levels in all supplemented groups when compared to control one at the level of P value≤ 0.05 as showed in table 2.

Table 2. Effect of addition of Zn oxide, Mg sulphate, Cu sulphate and vit. E on some oxidant and antioxidants levels during different periods of pregnancy

Groups	Period	Before calving			After calving		
	Parameters	6 weeks	4 weeks	2 weeks	2 weeks	4 weeks	6 weeks
Control	MDA µmol/l	8.4±0.8	9±1.5	11±0.5	10±0.2	9±0.9	8±0.2
	GSH mu/ml	41±1.6	37±3	32±2.2	39±1	42±1.5	45±1.3
	SOD u/ml	7±0.3	6±0.4	5±0.2	5.9±0.4	6±0.2	6.4±0.3
Zn oxide	MDA µmol/l	7.4±0.3*	7.5±0.1*	7.7±0.3*	6±0.1*	5±0.2*	5±0.2*
	GSH mu/ml	52±1*	50±1*	49±1.6*	50±1*	58±1*	61±2*
	SOD u/ml	7±0.4	7±0.4*	7±0.4*	6.7±0.2*	7±0.2*	7.5±0.3*
Mg sulphate	MDA µmol/l	8.4±0.1	9±0.1	9±0.1*	9±0.2*	9±0.2	8.2±0.1
	GSH mu/ml	47±1.7*	46±1.6*	45±2*	50±1.6*	51±1.6*	53±2*
	SOD u/ml	7.5±0.3	7±0.2*	7±0.2*	6.8±0.2*	7.3±0.2*	7.4±0.3*
Cu sulphate	MDA µmol/l	8.3±0.2	9±0.2	9±0.2*	9±0.1*	8±0.1*	7.6±0.1*
	GSH mu/ml	48±1.5*	45±1*	40±1.7*	49±1.5*	51±3*	53±2*
	SOD u/ml	7±0.4	6.5±0.1	6±0.2*	7±0.15*	6.7±0.2*	7±0.1*
Vit E	MDA µmol/l	8±0.3*	7±0.1*	7±0.2*	6±0.3*	5±0.1*	4±0.1*
	GSH mu/ml	56±1*	58±3*	60±3*	63±5.5*	64±5*	68±3.5*
	SOD u/ml	7±0.2	7±0.1*	6.7±0.2*	7.2±0.2*	7±0.1*	7.5±0.1*
P value		0.001	0.001	0.001	0.001	0.001	0.001

Data presented as mean ± SD

-* used for significant difference of group with control at the level of P≤0.05

3-Effect of some additives on some mineral levels during different periods of calving

-Ca level

There was a significant increase in Ca level in all supplemented groups when compared to control at all stages of pregnancy at the level of P value ≤ 0.05 as showed in table 3.

-P level

There was a significant increase in P level in all supplemented groups at 2nd week before and 2nd, 4th& 6th after calving when compared to control group at the level of P value ≤ 0.05 as showed in table 3.

-Mg level

There was a significant increase in Mg level in Mg supplemented group at all stages of calving before and after when compared to control and increase in all supplemented groups at 4th week when compared to control at the level of P value ≤ 0.05.

There was non-significant change in Mg level at Cu, Zn& vit E supplemented groups when compared to control one at all stages of calving when compared to control at the level of P value ≤0.05 as showed in table 3.

Table 3. Effect of addition of Zn oxide, Mg sulphat, Cu sulphate and vit. E on some mineral levels during different periods of calving

Groups	Period	Before calving			After calving		
	Parameters	6th week	4th week	2nd week	2nd week	4thweek	6th week
Control	Ca mg/dl	9.3±0.2	9±0.1	8.5±0.3	8.4±0.1	8.5±0.2	8.3±0.3
	P mg/dl	5.2±0.2	4.9±0.2	4.3±0.3	4.2±0.1	4.4±0.2	4.4±0.2
	Mg mg/dl	3.1±0.1	2.9±0.2	2.8±0.2	2.7±0.2	3±0.3	3±0.2
Zn oxide	Ca mg/dl	9.8±0.1*	9.7±0.2*	9.7±0.2*	9.3±0.1	9.8±0.1*	10±0.2*
	P mg/dl	5.4±0.1	5.2±0.1	5.3±0.1*	5.3±0.1*	5.3±0.2*	5.3±0.2*
	Mg mg/dl	3.3±0.1	3.2±0.2	2.9±0.2	3.1±0.1*	3.2±0.1	3.1±0.04
Mg sulphate	Ca mg/dl	9.8±0.2*	9.8±0.2*	9.5±0.1*	9.5±0.2	9.9±0.3*	9.9±0.2*
	P mg/dl	5.3±0.2	5±0.2	4.9±0.2*	5.2±0.1*	5.3±0.1*	5.3±0.1*
	Mg mg/dl	3.6±0.2	3.6±0.2*	3.3±0.2*	3.3±0.1*	3.5±0.1*	3.5±0.2*
Cu sulphate	Ca mg/dl	9.5±0.1	9.7±0.1*	9.7±0.1*	9.4±0.1*	9.8±0.2*	10±0.1*
	P mg/dl	5.2±0.2	4.9±0.2	4.9±0.3*	5±0.2*	5.1±0.2*	5.1±0.2*
	Mg mg/dl	3.2±0.5	2.8±0.2	2.8±0.2	3.1±0.1*	3±0.04	3.1±0.01
Vit E	Ca mg/dl	10±0.2*	9.9±0.3*	9.6±0.2*	9.8±0.1*	9.8±0.2*	9.9±0.1*
	P mg/dl	5.3±0.1	5.2±0.2	5±0.2*	5.2±0.1*	5.3±0.4*	5.3±0.4*
	Mg mg/dl	3.1±0.2	3.1±0.1	3±0.2	3.2±0.1	3.2±0.2	3.3±0.1
P value		0.001	0.001	0.001	0.001	0.001	0.001

Data presented as mean ± SD

-* used for significant difference of group with control at the level of P≤0.05

DISCUSSION

1-Effect of some additives on glucose and cortisol levels during different periods of calving

-Glucose level

The result of our study revealed that there was a significant increase in glucose level in all supplemented groups before and after calving when compared to control. This result agreed with that reported by **Omur et al. (2016)** and disagreed with that reported **by Amanda et al. (2018).**

Plasma glucose level is very important energy metabolites and higher levels in treated groups could be due to more feed intake attributed to vit E and trace element addition to animal ration and by the way increase feed intake and higher levels of glucose recorded (**LeBlanc et al. 2004).**

Cu has an important role in the energy metabolism as it acts as a cofactor of cytochrome c oxidase of electron transport chain that generates an electrical gradient used by the mitochondria in order to produce ATP (**Keshri et al. 2019).**

The adequate level of Mg in dairy animals leads to increase insulin synthesis that increase glucose uptake into blood **(Veronese et al. 2016).**

-Cortisol level

There was a significant decrease in cortisol level in all supplemented groups when compared to control one at different stages of pregnancy. This result disagreed with that reported by **(Collet et al. 2019).**

This may attribute to removal of stress in all supplemented groups as vit E, Cu and Zn are very important for increasing the immunity and anti-oxidative parameters in dairy animals (**Qureshi et al. 2010**) and cortisol is the first indicator of stress so, it decreases.

Zn and Cu are the main component of SOD that scavenges superoxide which considered as one of the components of ROS in the immune cells and by the way improve health status of dairy cows and remove stress factors in the animal (**Siciliano-Jones et al. 2008).**

2-Effect of some additives on some oxidant and antioxidants levels during different periods of calving

-MDA level

The result of the obtained study revealed that there was a significant decrease in MDA level in all stages of calving in Zn and vit E supplemented groups when compared to control one, decrease in Mg supplemented group at 2nd week before and after calving when compared to control and decrease in Cu supplemented group at 2nd week and all stages after calving when compared to control. This result disagreed with that reported by **(Balmurugan et al. 2019).**

Metabolic demands associated with late pregnancy, calving and initiation of lactation in dairy cows would be expected to increase the production of ROS (**Sordillo, 2005**). Supplementation of dairy animals with vitamins and trace elements able to minimize the harmful effects of excessive free radicals' production and by the way decrease the level of MDA **(Politis 2012).**

Addition of trace minerals to the ration of dairy animals reduces the oxidative load in dairy animals by reduction the lipolysis and other forms of gluconeogenesis **(Balamorgan 2011)** so, the level of MDA decrease as it is the main important indicator of oxidative stress.

-GSH and SOD levels

There was a significant increase in SOD and GSH levels in all supplemented groups when compared to control one. This result comes in accordance with that reported by **(Sordillo and Aitken 2009, Khatti et al. 2017, Solda et al. 2017 and Amanda et al. 2018)** and disagreed with that reported by **(Singh et al. 2017).**

Zn and Cu are main components of SOD so, supplemented groups has increase in their levels (**Prasad et al .2004 and Reece 2004**).

The micronutrients act as antioxidants,

which protect the cells from the effects of free radicals (**Kesheri et al. 2019).** Zn and Cu are the main component SOD that scavenges superoxide, the components of ROS in the immune cells in dairy animals. Further, a continuous decrease of endogenous antioxidant molecules result in decreased availability of the endogenous antioxidant defenses to counter the increased oxidant produced leading to oxidative stress in transition period. Trace minerals such as Fe, Cu and Zn are involved in the antioxidant defense system and dairy animals require them to maintain antioxidant activity of their immune system (**Weiss and Spears 2006).** Cu is a cofactor of SOD, and it participates in redox reactions **(Spears and Weiss, 2008).**
Vit E has essential antioxidant functions and is considered as a very important nutrient for cows (**Haga et al. 2018).**

3-Effect of some additives on some mineral levels during different periods of calving

Calcium and Phosphorus levels

The result of the obtained study revealed that there was a significant increase in Ca& P level in all supplemented groups when compared to control at all stages of pregnancy. This result agreed with that reported by **(Bicalho et al. 2014 and Omur et al. 2016)** and disagreed with that reported by **(Khan et al. 2015).**

Treatment with Mg salt cause the decrease in target organs to PTH which result in increment of bone resorption and Ca and P absorption from the digestive tract so, increase their levels happens when we add Mg salt to the ration of dairy animals **(Martens and Shewel 2000 and lean et al. 2006).**

-Magnesium level

The result of the obtained study revealed that there was a significant increase in Mg level in Mg supplemented group at all stages of calving before and after calving when compared to control and increase in all supplemented groups at 4th week when compared to control.

Its level increases due to high Mg intake and ruminal absorption by the animal ruminal papillae (**Schonewille 2008).**

There was non-significant change in Mg level at Cu, Zn& vit E supplemented groups when compared to control one at all stages of calving when compared to control.

4-Effect of some additions on haptoglobin level during different periods of calving
-Haptoglobin

There was a significant decrease in haptoglobin level in all supplemented groups when compared to control one. This result disagreed with that reported by (**Bicalho et al. 2014).**

The decrease in haptoglobin level may attribute to high immunity of animals due to the anti-oxidative effect of vit E or food supplementation to animal by trace minerals **(Haga et al. 2018 and Kesheri et al. 2019).**

CONCLUSION

In this study we pointed to the effect of addition of some minerals and vitamins on the ration of dairy cows on some hormonal and biochemical parameters. We recommend managing the dairy cows carefully during transition period by addition mixture of trace minerals and vitamins in order to improve the health status and immunity of dairy cows.

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