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Effect of Vitamin E and Selenium and *Spirulina* on oxidative stress and some biochemical parameters in growing calves

Adel El-Sayed Ahmed Mohamed[1*], Mahmoud Mohamed Arafa Mohamed[2], Fatma Ahmed Khalifa[3], Doaa Farag Hassan[4*]

[1]Division of Clinical Laboratory Diagnosis, Department of Animal Medicine, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt, [2]Department of Biochemistry, Animal Health Research Institute, Agriculture Research Center, Dokii, Egypt, [3]Division of Infectious Diseases, Animal Medicine Department, Faculty of Veterinary Medicine, South Valley University, Qena, Egypt, [4*]Department of Biochemistry, Animal Health Research Institute, Agriculture Research Center, Sohag, Egypt.

Abstract

Spirulina platensis is an efficient feed supplement used to improve animal health and growth. Transportation of calves over long distances leads to oxidative stress. The present study was conducted for the determination of biomarkers of transport stress on calves and the evaluation of Vitamin E (Vit E), Selenium (Se), and *Spirulina platensis* powder in the ration of calves on oxidative stress biomarkers, liver and kidney function tests, glucose, insulin, and haptoglobin levels. Currently, a total of 40 female calves imported from Brazil, aged about 8 months, were used for this study. The samples were collected once at the quarantine facility and then three times on the farm. According to the findings, there was a significant increase in ALT, AST, MDA, haptglobin, urea, and glucose, as well as a significant decrease in GSH, SOD, CAT, and TAC levels in the stressed group when compared to the control group; a significant decrease in ALT, AST, MDA, haptglobin, and glucose levels in the *Spirulina platensis* and Vit E and Se groups when compared to the control group There was a significant increment in insulin level in the Vit. E and Se group when compared to the control group.

Keywords: haptoglobin, transportation, MDA, TAC, SOD & CAT.

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*Corresponding Author: Doaa Farag Hassan E-mail: doaafarag570@yahoo.com

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Introduction

Calves play a very important role in the future of animal wealth for replacement in the herd, so it is important for every producer to perform the best calf management practices in order to bring up a healthy replacement stock (Ahmed and Ghada, 2007). Stress is defined as adverse effects in the environment or management system that force changes in the animal's behavior or physiology so that, it can avoid physiological malfunctioning and assist the animal in coping with its environment. Animals can be stressed by various psychological stresses such as restraint, handling, or novelty or physical stresses such as thirst, hunger, fatigue, injury, or thermal extremes (Moberg, 2000). Transport of livestock may expose animals to physical stimuli such as changes in temperature, physiological stimuli such as restrictions of water and feed during transport, and psychological stimuli related to exposure to new environments (NRC, 2006).

Vit E and Se act as micronutrients that share a common biological function in the animal body. Se is a main component of GSH (glutathione peroxidase), which destroys free radicals in the cytoplasm, whereas Vit. E is a non-enzyme scavenger of free radicals, and it reacts with peroxide radicals, leading to the formation of polyunsaturated fatty acids in membrane phospholipids or lipoproteins to yield a stable lipid hydroperoxide. This antioxidant activity of Vit. E in preventing lipid peroxidation is considered one of its main mechanisms by which it stimulates and enhances animal immunity (Shinde et al., 2007).

Spirulina platensis is an edible microalga with a high nutritional value for many agriculturally important animal species (Hoseini et al., 2013). It contains very important vital compounds, such as protein with all essential amino acids (Farag et al., 2016), a source of chlorophyll pigments, carotenoids, and essential fatty acids, alpha-linolenic, gamma-linolenic, and linoleic (Peiretti and Meineri, 2011), photosynthetic pigments (Bermejo et al., 2008), vitamins, including thiamine, folic acid, nicotinamide, riboflavin, vitamins A, D, and E (Hoseini et al., 2013), and minerals like Ca, Mn, Fe, Cr, Cu, P, Mg, Na, Zn, and Se (Babadzhanov et al., 2004). Dietary *Spirulina platensis* can improve animal crude protein digestibility (Peiretti and Meineri 2009).

Supplementation of Vit. E, Se and *Spirulina platensis to* the ration of calves improves their immunity and performance, so, this study aimed for the detection of the effect of oxidative stress on the calves' liver and kidney function tests, oxidant malonaldehyde (MDA), antioxidants superoxide dismutase (SOD), catalase (CAT), total antioxidant capacity (TAC), GSH, haptoglobin, glucose, and insulin, and the evaluation of the effect of adding Vit E, Se, and *Spirulina platensis* to the calves' ration on these parameters.

Materials and method

Work was carried out at the Sakha Animal Production Research Station, the Animal Production Research Institute (APRI), the Agricultural Research Center, and the Ministry of Agriculture during the period from March to July 2021.

A total of 40 female Holstein calves imported from Brazil were used for this study, aged about 8 months and weighing 120–160 kg. The work was divided into two stages. We collected 10 random samples from animals in quarantine under transport stress only once in the first stage. The second stage begins after the rest of the calves on the farm and the samples have been collected three times. The samples collected every two months, in March, May, and July of 2021.

The animals divided into 3 groups

Group 1: 10 calves fed on rations according to the NRC

Group 2: 10 calves fed NRC ration with Vit. E 1% and Se 1% powder (Pfizer Company).

Group 3: 10 calves fed on ration according to the NRC with addition of *Spirulina platensis* powder 0.5 g/kg (from the farm of the National Research Center, Egypt-Alexandria desert road).

This work was done according to the requirement of the Faculty of Veterinary Medicine, South Valley University research ethics under the approval number VM/SVU/23(2)-03.

Samples

We collected samples from animals in the quarantine under stress from transport, and then we collected samples on the farm. Four types of samples were collected for this study: blood, ration, water, and *Spirulina platensis* samples.

The blood samples (7.5 ml) were collected from the jugular vein of each animal in clean glass vials and converted to serum that was aspirated by Pasteur pipettes into Eppendorf tubes at -20℃ until testing, according to Coles (1986).

The separated sera are used for the detection of some liver enzymes such as aspartate aminotransferase (AST), and alanine aminotransferase (ALT) (u/l) (Breur, 1996; Young, 1990); kidney function tests include urea (mg/dl) (Young, 2001), and creatinine (mg/dl) (Titez, 1986).

Also, used for the detection of oxidants MDA (mmol/ml) (Ohkawa et al., 1979), antioxidants included TAC (u/ml) (Koracevic et al., 2001), SOD (u/ml) (Nishikimi et al., 1972), CAT (iu/l) (Aebi, 1984), and GSH (u/ml) (Paglia and Valentine, 1967) by using a (SPECTRO UV-VIS DOUBLE BEAM PC SCANNING SPECTROPHOTOMETER UVD-2950).

The concentration of glucose in the blood was measured colorimetrically at 546 nm using a (SPECTRO UV-VIS DOUBLE BEAM PC SCANNING SPECTROPHOTOMETER UVD-2950). A test kit was supplied by Diamound Diagnostic Company following the method described by Young (2001).

Insulin level was determined by the ELISA method, according to A.O.A.C. (2015).

Haptoglobin was determined by using bovine haptoglobin ELISA kit.

40% green clover and 60% bersem dress made up the ration delivered to animals.

The prepared ration delivered to calves consisted of yellow corn 20%, roughages 28%, vitamins 1%, salt 1%, bran 30%, barely 17%, and limestone 3%, and analysis of samples of the ration, water, and *Spirulina platensis* delivered to animals were done in the Chemistry and Nutritional Deficiency Department, Animal Health Research Institute, Giza, as shown in tables 1, 2, and 3.

Table 1: Ration analysis

Items	Examined animal ration
Protein	16%
Fat	2.6%
Humidity	11%
Ash	12%
Fiber	14%
Carbohydrate	44.4%
Total energy	27.5 kcal/kg
Ca	0.82%
Ph	0.41%
Mg	0.6%

Table 2: water analysis

Items	Examined group	Permissible limits by EOS (1589/2005)
Appearance color	Clear	Clear
PH value	7	6-7.5
Conductivity (ms/m)	620	2000 (microms/m)
Calcium (mg/l)	50	Up to 150
Calcium carbonate (mg/l)	125	Up to 400
Magnesium mg/l	7	Up to 50
Magnesium carbonate	24.5	Up to 175
Total hardness as ca $Caco_3$	285	Up to 500
TSS (total suspended solids)	42	Up to 250
TDS (total dissolved solids)	600	Up to 750
TS (total solids)	642	Up to 1000
Chloride (mg/l)	80	Up to 250 H-500 A
Ammonia (mg/l)	-ve	Up to 0.5
Nitrate & nitrite (mg/l)	-ve	Up to 0.02
Sulphate (mg/l)	64	Up to 25
Phosphorus (mg/l)	-ve	--
Phosphate (mg/l)	-ve	Up to 5
Free chlorine (mg/l)	0.8	0.4-5
Cadmium	-ve	Up to 100
Lead	-ve	

Table 3: *Spirulina platensis* analysis

Items	%
Protein	57
Fat	8
Humidity	62
Ash	11
Fiber	3.6
Carbohydrates	20.7
Sugar	18.2
Salts (mg/100 mg)	
Na	26
K	668
Fe	6.62
Ca	0.4
Ph	0.06
Mg	91
Zn	0.93
Cu	.3
Mn	.96
Amino acids (g/100mg)	
Tryptophan	0.05
Threonine	.14
Lucien	.25
Licin	.14
Methionine	.05
Alfa alanine	.16
Vitamins	Iu
A	2424
D	0
E	31
K	0
C	122mg/dl
B	0.34 mg/dl

Statistical analysis

The obtained data were statistically analyzed after the methods described by (Snedecor and Cochran, 1980) for significance, analysis of variance, least significant difference was also done by statistical package for social science (SPSS) computer program- one-way ANOVA.

Results

Effects of stress on liver and kidney function tests in growing calves

As shown in table 4, there was a significant increase in the levels of ALT (63 u/l), AST (71 u/l), urea (33.6 mg/dl) and creatinine (1.7 mg/dl) in stressed group when compared to control ones.

Table 4: Effect of stress on liver and kidney function tests in growing calves

	ALT (u/l)	AST (u/l)	Urea (mg/dl)	Creatinine (mg/dl)
Control	60±0.7	59±3	30±0.7	1.7±0.3
Stressed	63±0.8[a]	71±2[a]	33.6±0.9[a]	1.7±0.02

Data presented as mean ± SE a for significant difference of stressed group with control at the level of p≤ 0.05

Effects of stress on oxidative stress parameters in growing calves

As shown in table 5, there was a significant increase in the level of MDA (22 mmol/l) while, there was a significant decrease in the levels of GSH (40.2 u/ml), SOD (10.4 u/ml), CAT (31.4 iu/l), and TAC (246 u/ml) in stressed group when compared to control group.

Table 5: Effects of stress on oxidants and antioxidants in growing calves

	MDA (mmol/l)	GSH (u/ml)	SOD (u/ml)	CAT (iu/l)	TAC (u/ml)
Control	17.4±0.4	58.6±1.2	12.6±0.24	39.2±0.8	416±20
Stressed	22±0.7[a]	40.2±1[a]	10.4±0.1[a]	31.4±1[a]	246±7[a]

Data presented as mean ± SE a for significant difference of stressed group with control at the level of p≤ 0.05

Effects of stress on haptoglobin, glucose, insulin, and cortisol in growing calves

As shown in table 6, there was a significant increase in the levels of glucose (79.4 mg/dl), cortisol (14.6 nmol/l), and haptoglobin (50.4 hgp/l) while, there was non- significant decrease in insulin level (24.3 iu/ml) in stressed group in comparison with control group.

Table 6: Effects of stress on haptoglobin, glucose, insulin, and cortisol in growing calves

	Glucose (mg/dl)	Insulin (iu/ml)	Cortisol (nmol/l)	Haptoglobin (hgp/l)
Control	75±0.7	26.4±0.5	13.3±0.1	40.2±0.7
Stressed	79.4±1.4[a]	24.3±0.3	14.6±0.1[a]	50.4±1[a]

Data presented as mean ± SE a for significant difference of stressed group with control at the level of p≤ 0.05

Effects of Vit. E, Se and *Spirulina platensis* on ALT & AST in growing calves

As shown in table 7, there was a significant decrease in the level of ALT in the Vit E& Se treated group (5 4, 40& 34 u/l) at 9, 11& 13 months' age, and in the *Spirulina platensis* group (51, 36& 30 u/l) at 9, 11& 13 months' age in comparison to control group also, the level of AST decreased in Vit. E& Se treated group (54& 49 u/l) at 11& 13 months' age and in the *Spirulina platensis* group there was a significant decrease (53, 51& 44 u/l) at 9, 11& 13 months' age; respectively in comparison to the control group.

Table 7: Effects of Vit E, Se and *Spirulina platensis* on ALT & AST levels in growing calves

Groups / Age		Control	Vit E& Se	*Spirulina platensis*
9 months	ALT (u/l) AST (u/l)	60± 0.7 59± 3	54± 0.8[b] 58± 0.4	51± 1.9[c] 53± 1.5[c]
11 months	ALT (u/l) AST (u/l)	50± 1.07 65± 1.8	40± 0.8[b] 54± 1.3[b]	36± 1.08[c] 51± 1.1[c]
13 months	ALT (u/l) AST (u/l)	46± 1.2 57± 1.5	34± 1.4[b] 49± 1.02[b]	30± 0.8[c] 44± 1.4[c]

Data presented as mean ± SE the letters b and c used to denote the significant difference at the level of $p\leq 0.05$ as follows: b for significant difference of Vit E& Se group with control, c for significant difference of *Spirulina platensis* group with control.

Effects of Vit. E, Se, and *Spirulina platensis* on urea and creatinine in growing calves

As shown in table 8, there was a significant decrease in urea (19 mg/dl) at 13 months' age and in creatinine (12 and 0.96 mg/dl) at 11 and 13 months' age, respectively, in the *Spirulina platensis* treated group when compared to the control group

Table 8: Effects of Vit. E, Se, and *Spirulina platensis* on urea and creatinine in growing calves

Groups / Age		Control	Vit E& Se	*Spirulina platensis*
9 months	Urea (mg/dl) Creatinine (mg/dl)	30± 0.7 1.7±0.3	28.2± 0.6 1.6±0.03	28± 1.3 1.6±0.05
11 months	Urea (mg/dl) Creatinine (mg/dl)	25± 0.8 1.4±0.03	22± 0.9 1.3±0.04	23±1.2 1.2±0.04[c]
13 months	Urea (mg/dl) Creatinine (mg/dl)	24± 0.3 1.2±0.04	21± 0.7 1.1±0.04	19±0.7[c] 0.96± 0.05[c]

Data presented as mean ± SE -the letters b and c used to denote the significant difference at the level of $p\leq 0.05$ as follows: b for significant difference of Vit E& Se group with control, c for significant difference of *Spirulina platensis* group with control

Effects of Vit E, Se, and *Spirulina platensis* on oxidative stress parameters in growing calves

As shown in Table 9, in both treated groups, there was a significant increase in the levels of GSH (58, 61, and 60, 67 u/ml) at 11 and 13 months' age, the level of CAT (43, 55, 56.5, and 49, 60, 65.4 iu/l), the level of SOD (14.3, 16, 17, and 14.4, 17.5, 70 u/ml), and the level of TAC (459, 485, 525, and 482, 533, 586 u/ml) at 9, 11& 13 months' age; respectively. On the other hand, there was a significant decrease in the level of MDA (6.4, 5, 4.3, and 5.2, 4, 3.4 mmol/l) in both groups and all ages, respectively, when compared to the control group.

Table 9: Effects of Vit. E, Se, and *Spirulina platensis* on oxidative stress parameters in growing calves

Group / Age		Control	Vit E& Se	*Spirulina platensis*
9 months	MDA(mmol/l)	17.4± 0.4	6.4± 0.3^{b}	5.2±0.2^{c}
	GSH (u/ml)	58.6± 1.2	62.3± 0.9	60.2± 1.1
	CAT (iu/l)	39.2± 0.8	43± 0.9^{b}	49± 0.6^{c}
	SOD (u/ml)	12.6± 0.24	14.3± 0.3^{b}	14.4± 0.3^{c}
	TAC (u/ml)	416± 20	459± 17.2^{b}	482± 7^{c}
11 months	MDA (mmol/l)	12.7± 0.2	5±0.1^{b}	4± 0.1^{c}
	GSH (u/ml)	53.4± 1.4	58± 1.6^{b}	60± 1.6^{c}
	CAT (iu/l)	41.2± 0.1	55± 0.7^{b}	60± 0.7^{c}
	SOD (u/ml)	13± 0.1	16± 0.4^{b}	17.5± 0.4^{c}
	TAC (u/ml)	432± 5	485± 16^{b}	533±9^{c}
13 months	MDA (mmol/l)	11.3± 0.2	4.3± 0.1^{b}	3.4± 0.1^{c}
	GSH (u/ml)	54.2±1.7	61± 0.7^{b}	67± 2^{c}
	CAT (iu/l)	42± 0.2	56.5± 1^{b}	65.4± 1.2^{c}
	SOD (u/ml)	14.2±0.2	17± 0.3^{b}	20± 0.5^{c}
	TAC (u/ml)	450± 2.5	525±6.5^{b}	586± 5.3^{c}

Data presented as mean ± SE -the letters b and c used to denote the significant difference at the level of $p \leq 0.05$ as follows: b for significant difference of vit E& Se group with control, c for significant difference of *Spirulina platensis* group with control

Effects of Vit. E, Se, and *Spirulina platensis* on haptoglobin, glucose, insulin, and cortisol in growing calves

As shown in table 10, there was a significant decrease in haptoglobin level (39.4, 40.2, 43, and 37, 39, 41.3 hgp/l) and glucose level (70, 68.2, 67.4, and 71, 67.4, 65 mg/l); respectively, in 9, 11& 13 months' age in both treated groups, while, there was a significant increase in the level of insulin in the Vit E and Se group (29.4, 30.6& 30.4 iu/ml); respectively, in all age groups when compared to the control group.

Table 10: Effects of Vit E, Se and *Spirulina platensis* on haptoglobin, glucose, insulin, and cortisol in growing calves

Groups Age		Control	Vit E& Se	*Spirulina platensis*
9 months	Haptoglobin (hgp/l) Glucose (mg/l) Insulin (iu/ml)	40.2± 0.7 75± 0.7 26.4± 0.5	39.4± 0.8[b] 70± 0.8[b] 29.4± 0.5[b]	37± 0.7[c] 71± 0.7[c] 26.2± 0.4
11 months	Haptoglobin (hgp/l) Glucose (mg/l) Insulin (iu/ml)	43.4± 0.7 76.4± 1.2 26.6± 0.5	40.2± 1.7[b] 68.2± 0.8[b] 30.6± 0.5[b]	39± 0.5[c] 67.4± 0.8[c] 27.2± 0.4
13 months	Haptoglobin (hgp/l) Glucose (mg/l) Insulin (iu/ml)	45.2± 0.8 78.2± 3 26± 0.6	43± 0.9 67.4± 2[b] 30.4± 0.5[b]	41.3± 0.6[c] 65± 0.6[c] 25.4± 0.2

Data presented as mean ± SE -the letters b and c used to denote the significant difference at the level of p≤ 0.05 as follows: b for significant difference of vit E& Se group with control, c for significant difference of *Spirulina platensis* group with control

DISCUSSION

Road transportation represents a critical phase in animals' production, and it is often considered one of the main causes of stress, adversely affecting production both in economic and animal welfare terms. It has been shown that road transportation of livestock results in live-weight loss (Ritter et al., 2009). This type of stress could be determined by different types of changes in blood parameters in animals' and can be protected by supplementing animals with different sources of antioxidants such as Vit E, and Se or natural products such as *Spirulina platensis* powder.

The result of the obtained study revealed that there was a significant increase in ALT and AST in the stressed group when compared to the control group.

On one hand, the increase of ALT level in stressed calves agreed with that reported by Brijesh (2012) and disagreed with that reported by Uetake et al. (2009), EL-Deeb and El-Bahr (2014), and Zulkifli et al. (2019).

The increase in AST level in stressed calves agreed with that reported by Uetake et al. (2009), Minka and Ayo (2010), Brijesh (2012), and Zulkifli et al. (2019), but disagreed with that reported by EL-Deeb and El-Bahr (2014).

The elevated ALT and AST were suggested to reflect damage in the internal organs of calves and hepatopathy or hepatocytic destruction due to oxidative stress. Or may be related to the increment in the stimulation of gluconeogenesis by corticoids (El-Masry et al., 2010).

Our results revealed that there was a significant increase in the level of urea in the stressed group when compared to the control group. Earley et al. (2006) and EL-Deeb and El-Bahr (2014) reported similar results.

Urea-N is the main indicator of protein degradation for energetic rumen ammonia levels and, after that, blood urea release. The high level of urea was related to the low energy/protein ratio and to gluconeogenesis by the degradation of proteins in conditions of insufficient energy needed for the growth of animals

(El-Masry et al., 2018). This may also attribute to the increase in muscle breakdown of protein and nucleic acids that resulted from increased cortical concentration and prolonged food deprivation during stressful transport conditions (Guardia et al., 2009).

Our results revealed that there was no significant change in the level of creatinine in the stressed group when compared to the control group. The non-significant change in creatinine level in stressed calves was in accordance with that reported by EL-Deeb and El-Bahr (2014) and disagreed with that reported by Evangelia et al. (2009). This may be due to the fact that transportation stress did not change protein metabolism immediately after animals were unloaded (El-Deeb and El-Bahr, 2014).

Effects of stress on oxidative stress parameters in growing calves

On the one hand, our results revealed that there was a significant increase in MDA levels in the stressed group compared with the control group.

Chirase et al. (2004), Urban-Chmiel (2006), Burke et al. (2009), and EL-Deeb and El-Bahr (2014) concluded that there was an increase in MDA levels in stressed animals. This increase in MDA concentration in stressed calves may be related to lipid peroxidation, which is produced by transportation stress.

On the other hand, the results of the obtained study revealed that there was a significant decrease in GSH, SOD, and CAT levels in stressed group when compared to the control group.

EL-Deeb and El-Bahr (2014) concluded that there was a significant decrease in GSH, SOD, and CAT levels. The decrement in the GSH and CAT levels may be due to the fact that they are considered the first oxidative barrier for free radicals in the body and are used for reactive oxygen species (ROS) scavenging.

SOD catalyzes the dismutation of two molecules of radical oxygen into one molecule of dioxygen and one of H2O2. The decrement in SOD levels shown in transported calves may be attributable to the consumption of this enzyme for the dismutation mechanism (EL-Deeb and El-Bahr, 2014).

The result of the current study revealed that there was a significant decrease in TAC level in the stressed group when compared to the control group. This result in accordance with that reported by Chirase et al. (2004). The decrement of TAC is a reflection of the reductant capability of the antioxidant defense system, with the assumption that all of the antioxidant mechanisms are synergistic and that this occurs in cases of stress.

Effects of stress on haptoglobin, glucose, and insulin in growing calves

On the one hand, our results revealed that there was a significant increase in the level of haptglobin in stressed group when compared to the control group.

Earley et al. (2006), Arthington et al. (2008), EL-Deeb and El-Bahr (2014), and Marcato et al. (2021) concluded that there was an increase in the level of haptglobin in stressed animals, while Sporer et al. (2008) reported that there was a decrease in the level of haptaglobin in stressed bulls. Haptoglobin is an α2-globulin synthesized in the liver and is considered a major acute-phase protein in different species of domesticated animals. Its concentrations increased with inflammation and stressful situations, so its increment may attribute to road transportation stress on these calves (EL-Deeb and El-Bahr, 2014).

On the other hand, there was a significant increase in the level of glucose in the stressed group in comparison with the control group.

The increase in glucose level in the stressed group agreed with that reported by Earley et al. (2006, 2010), and Jung et al. (2022). The reasons for the increment in plasma glucose level following transport may reflect glycogenolysis associated with the increase in circulating catecholamines and glucocorticoids that were released during the stress of transport (Earley et al., 2006). The hyperglycemia may be due to the secretion of epinephrine in response to stressful stimuli that lead to glycogenolysis in muscle and liver, causing a higher blood glucose level (Sharma et al., 2005).

Effects of addition of Vit. E, Se, and *Spirulina platensis* on liver enzymes in growing calves

The result of the obtained study revealed that there was a significant decrease in ALT and AST levels in the Vit E, Se and *Spirulina platensis* groups when compared to the control group at 9, 11, and 13 months' age.

The decrement of ALT level in Vit. E and Se supplemented groups agreed with that reported by Mudgal et al. (2008), Farghaly et al. (2017), and Shams et al. (2020) and disagreed with that reported by Samanta and Dass (2007), Mudgal et al. (2012), and Żarczyńska et al. (2021).

The decrement of AST level in supplemented calves with Vit. E and Se agreed with that reported by Shams et al. (2020) but disagreed with that reported by Singh et al. (2002), Samanta and Dass (2007), Kumar et al. (2008), Shinde et al. (2009), Mudgal et al. (2012), and Farghaly et al. (2017).

Decreased levels of liver enzymes in the Vit E and Se group may be attributable to the antioxidant effect of Vit E& Se in protecting the liver from damage and oxidative stress. The decrease in the ALT and AST levels in the *Spirulina platensis* group disagreed with that reported by Riad et al. (2019). AST and ALT activities are indicators of hepatotoxicity. Treatment with *Spirulina platensis* showed a significant decrement in AST and ALT levels that may attribute to *Spirulina platensis* protective role against liver dysfunctions due to its high contents of phenolic compounds (Bhattacharyya & Mehta, 2012).

Our data revealed that there was no significant change in urea level in the Vit E and Se group, while there was a significant decrease in urea level at 13 months' age and in creatinine level at 11 and 13 months' age in the *Spirulina platensis* treated group when compared to the control group.

Effects of addition of Vit. E, Se and *Spirulina platensis* on kidney function tests in growing calves

The non-significant change in urea level in the Vit E and Se group agreed with that reported by Mudgal et al. (2008), Slavik et al. (2008), Ganie et al. (2012), Wie et al. (2016), and Żarczyńska et al. (2020 and 2021) and disagreed with that reported by Farghaly et al. (2017) and Shams et al. (2020). This may be related to the protective effect of Vit E and Se against stress or to the fact that Vit E and Se do not affect the metabolism of nitrogen (Shinde et al., 2009).

Heidarpour et al. (2011), Ragab et al. (2014), and Riad et al. (2019) proved the decrement in the level of urea in *Spirulina platensis* treated animals.

The result of the obtained study revealed that there was a non-significant change in creatinine level in the Vit. E and Se group when compared to the control group at ages 9, 11, and 13 months, and there was only a significant decrease in its level in the *Spirulina platensis* group when compared to the control group at ages 11 and 13 months.

The result of a non-significant change in creatinine level in calves supplemented with Vit E and Se agreed with that reported by Shinde et al. (2009), Mudgal et al. (2008), and Żarczyńska et al. (2020 and 2021), but disagreed with that reported by Kumar et al. (2008) and Shams et al. (2020). This may indicate that the Vit. E and Se had no effect on nitrogen metabolism. Or, do not compromise kidney function (Żarczyńska et al., 2020).

The decrement of creatinine in the *Spirulina platensis* group when compared to the control group disagreed with that reported by Riad et al. (2019).

Effects of addition of Vit E& Se and *Spirulina platensis* on oxidative stress parameters in growing calves

On the one hand, the result of the obtained study revealed that there was a significant decrease in MDA in Vit. E, Se, and *Spirulina platensis* at all age groups when compared to the control group.

The decrease in MDA level in the Vit E and Se supplemented group disagreed with that reported by Wie et al. (2016). This may be attribute to the antioxidant effect of the Vit E and Se, which prevent the initiation of free radical chain reactions and decrease the lipid peroxidation products.

The decrease in MDA in the *Spirulina platensis* group agreed with that reported by Celli (2010), and this result may be due to *Spirulina platensis* antioxidant properties, which have the ability to decrease oxidative stress with a subsequent decrease in lipid peroxidation levels (EL-Sabagh et al., 2014).

On the other hand, the result of the obtained study revealed that there was a significant increase in GSH in the Vit. E, Se, and *Spirulina platensis* groups when compared to the control group at 11 and 13 months.

The increment in GSH level in the Vit E and Se group compared with the control group agreed with that reported by Beck et al. (2005), Guyot et al. (2007), Lum et al. (2009), Chorfi (2011), Gunter et al. (2013), Alberto et al. (2020), and Żarczyńska et al. (2021), but disagreed with that reported by Wie et al. (2016). The increase in CAT in the Vit E and Se groups disagreed with that reported by Wie et al. (2016). There is a synergistic action between Vit. E and Se to stimulate animals' immune responses, so supplementation with Vit. E and Se increases the concentrations of natural antioxidants in the blood of animals. The antioxidant effect of Vit. E and Se supplementation was reflected by a significant increment in the levels of GSH, CAT, and SOD (Soliman 2015).

The increment of SOD and GSH in the *Spirulina platensis* group agreed with that reported by Celli (2010) and Fasolo et al. (2020). The increase in CAT in the *Spirulina platensis* group agreed with that reported by Reddy et al. (2004) and disagreed with that reported by Fasolo et al. (2020). This may attribute to the *Spirulina platensis* anti-oxidative effect that is related to multiple active ingredients, including polysaccharides, -tocopherol, and ß-carotene, which have potent antioxidant activities working individually or in synergy on free radicals (Al-Sapagh et al., 2014). Also, the antioxidant activity of the phycocyanin component of *Spirulina platensis* is about 20 times higher than that of vitamin C, and it contains SOD, which acts indirectly by decreasing the rate of oxygen radical-generating reactions (Belay, 2002).

Our result also, revealed that there was a significant increase in TAC in the Vit. E, Se, and *Spirulina platensis* groups when compared to the control group at 9, 11, and 13 months.

The result of increased TAC in the Vit E and Se groups disagreed with that reported by Wie et al. (2016). This could be attribute to Vit. E and Se supplementation improving antioxidant status in the animals.

An increase in the level of TAC in the *Spirulina platensis* group agreed with that reported by Ghattas et al. (2019) and disagreed with that reported by Keller et al. (2021). This may be due to the high contents of carotenoids, C-phycocyanin, and some potent antioxidant agents in Spirulina platensis (Abd El-Baky et al., 2007) or the presence of high contents of polyunsaturated fatty acids in *Spirulina platensis* (Ghattas et al., 2019).

Effects of addition of Vit E, Se and *Spirulina platensis* on haptoglobin, glucose and insulin in growing calves

On the one hand, the result of the current study revealed that there was a significant decrease in the level of haptglobin in *Spirulina platensis* and Vit E and Se groups in comparison with the control groups at ages 9, 11, and 13 months.

The decrement in haptoglobin level in the Vit E, Se, and *Spirulina platensis* groups may be due to the absence of oxidative stress due to the high anti-oxidative activity of Vit E, Se, and *Spirulina platensis*.

There was a significant decrease in glucose level in the Vit E, Se, and *Spirulina platensis* groups in comparison with the control group at 9, 11, and 13 months.

The decrease in glucose in the Vit. E and Se group agreed with that reported by Ebrahimi et al. (2009), and disagreed with that reported by Mudgal et al. (2012), and Kanana et al. (2019). This hypoglycemia may be due to the impaired gluconeogenic capability of cells in the animal liver (Sharma et al., 2005). Also, the decrease in glucose level in the *Spirulina platensis* group may be related to the decreased gluconeogenic capability of animals' liver cells.

At 9, 11, and 13 months, there was a significant increase in insulin levels in the Vit. E, and Se groups when compared to the control group. Insulin is considered an important hormone that is responsible for the glucose regulation in the animals' bodies, and Vit. E and Se play a very important role in the absorption of glucose and energy balance so that, it needs more secretion of insulin from the pancreas (Hefnawy & Tortora-Perez, 2010). Also, Se has a role in the activation of the attachment between insulin hormone and its receptors on the target cell and is able to improve post-receptor signals (Spears, 2011).

The present work showed the biochemical alterations caused by oxidative stress in transported calves and the effect of using Vit. E, Se, and *Spirulina platensis* powder in the treatment of these calves.

So, from the above conclusions, we can suggest the following recommendations:

We recommend using Vit. E and Se in the diet of the calves to improve their health condition and protect them from oxidative stress.

We recommend using *Spirulina platensis* powder as feed additives to the ration of calves.

More studies must be conducted on the effect of transport stress on the health of the animal and the use of Vit E and Se and *Spirulina platensis* for improving the immunity and health of the animals.

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