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# Effect of Dried *Rosemary*, Vitamin E and Vitamin C on biochemical parameters in Heat Stressed Heifers Calves

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## Abstract

Heat stress (HS) is one of the most stressful factors affecting on animal health, production, and product quality. Ruminants are prone to heat stress due to their rapid metabolic rate rapid growth, high level of production, and species-specific traits such as rumen fermentation, sweating impairment, and skin insulation. HS can lead to oxidative stress that occur when the internal antioxidant mechanisms cannot neutralize an increase in the production of reactive oxygen species (ROS). The present study was established for discussing the effect of heat stress on some physiological and biochemical parameters in calves followed by treatment trials by adding Rosemary and vitamins in their ration. Currently, 90 female calves were used for this study. Animals were divided into 9 groups which exposed to 3 different levels of temperatures at (30-35$^{o}$c, 36-40$^{o}$c and 41-45$^{o}$c) and 3 different types of nutritional supplement (NRC ration, NRC + Vit E& C and NRC + Rosemary). According to the findings, there was a significant increase in the respiratory rate, heart rate and the rectal temperature in heat stressed calves. Also, there was a significant increase in MDA, cortisol, haptoglobin, liver enzymes and renal function tests in blood of these calves. By contrast, the results showed a significant decrease in the level of antioxidants, glucose, and insulin in heat stressed calves when compared with the control. By Adding *Rosemary*, and Vitamin (E&C) in the ration of heat stressed calves, the result showed a significant improvement in the measured parameters of these heat stressed calves.

**Keywords:** Antioxidants, Haptoglobin, MDA & oxidative stress

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**Introduction**

Large domestic animals are exposed to various kinds of stress such as nutritional, psychological, physical, and thermal stress. The changes in factors of the natural environment are main affecting on animal health and production that lead to huge economic losses in the dairy farms production around the world (Behl et al., 2010).

In general, Egypt's summers are marked by high relative humidity and ambient temperature, which causes heat stress and lowers livestock species' productivity. Reactive oxygen species (ROS) production increases under oxidative stress, the capacity of the body's endogenous antioxidant defenses to safely neutralize them. (Hady et al., 2018). Furthermore, heat stress is one of the wide varieties of factors that cause oxidative stress. It results from increased free radicals' production and ROS with a decrease in antioxidant defense mechanisms (Trevisan et al., 2001).

The elevated body temperature and basal metabolic rate that occur during the summer lead to imbalance between oxidant and antioxidants which cause oxidative stress. In order to reduce oxidative stress in animals, antioxidant foods should be added as well as avoiding ROS compounds (Ganaie et al., 2013).

Ration supplemented with plant extracts used as prebiotics such as polyphenols and flavonoids, may offer a relatively healthy and secure way to increase the antioxidant status of animals, reducing the need for antibiotics and increase the disease prevention (Tilahun et al., 2022). Rosemary (*Rosmarinus officinalis L.)* a perennial aromatic plant native to the Mediterranean region, is a member of the Lamiaceae family. Due to the presence of carnosic, carnosol, and rosmarinic acid, it is now frequently utilized in medications, food additives, and cancer prevention. Antibacterial, anti-inflammatory, and antioxidant activities are present in rosemary (Bianchin et al., 2020). These advantages increase its applicability to animals, and numerous studies have demonstrated that dietary supplementation with rosemary extract may enhance the quality of the final product in monogastric animals by reducing heat stress, modulating humoral immunity, regulating gut microbiota, and improving growth, and antioxidant status (Gladine et al., 2007).

Two substances that support the tissue and plasma's ability to act as antioxidants are vitamin E and selenium. The oxidant-antioxidant imbalance is resolved by dietary modification, which includes selenium, and vitamin E (Kalmath et al., 2015). Since heat stress has been documented to be involved in cellular oxidative stress, several authors sought to use antioxidant supplementation to alleviate heat stress's drastic impacts. Natural phytogenic antioxidants have paid great attention as such supplements can be added easily to the daily diet, are available at an affordable price, are effective and are expected to have very few undesirable effects (Tawfeek et al., 2014).

Studies on the impact of rosemary as a natural antioxidant on goat milk, sheep meat, and reproductive performance have all been conducted (Boutoial et al., 2013; Vasta et al., 2013; Zhong and Zhou, 2013). While, there is little information's about these natural antioxidants, particularly on Holstein dairy calves under heat stress.

Due to the high economic importance of calves this study aimed to:

- Estimate the impacts of heat stress on Holstein dairy calves' oxidants, oxidant

malonaldehyde (MDA), antioxidants catalase (CAT), superoxide dismutase (SOD), total antioxidant capacity (TAC), glutathione peroxidase (GPX), haptoglobin, glucose, cortisol, insulin, kidney, and liver function tests.

- Evaluate the effect of adding Vit (E &C), and *Rosemary* to the heat stressed Holstein dairy calves ration on the previous parameters.

## Materials and methods

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

### Animals and experimental design

A total of 90 female Holstein calves imported from Brazil were used for this study, aged about 12 months and weighing 150–160 kg, on (March 2021)

**Table 1: The animals are divided into 9 groups each group containing 10 calves**

| Temp 30-35 ℃ | Temp 36-40 $^0$C | Temp 41-45 $^0$C | Ration |
|---|---|---|---|
| group 1 | **group 2** | **group 3** | **NRC** |
| group 4 | **group 5** | **group 6** | **NRC+ Vit E&C** |
| group 7 | **group 8** | **group 9** | **NRC+ *Rosemery*** |

Group (n=10 per each)

This work was done according to the requirement of the Faculty of Veterinary Medicine, South Valley University research ethics, and the approval number was VM/SVU/23(2)-14. This study involved one-week adaptation period to the diet followed by two weeks of feeding the experimental diets on (March 2021).

### Ration and Water

The ration formed of 50% green glover and 50% berseem deress.

The prepared ration consisted of yellow corn 20%, roughages 17%, vitamins 2%, salt 1%, bran 58% and limestone 2%. Analysis of a sample of the ration, water and *Rosemary* delivered to animals was done in Chemistry and Nutritional Deficiency Department, Animal Health Research Institute, Giza and shown in tables 2, 3 &4.

**Table 2: Ration analysis**

| Items | Examined animal ration |
|---|---|
| **Protein** | **16%** |
| **Fat** | **1.5%** |
| **Humidity** | **10%** |
| **Ash** | **11%** |
| **Carbohydrate** | **41.5%** |
| **Total energy** | **248.7 kcal/100g** |
| **Ca** | **0.77%** |
| **Ph** | **0.4%** |
| **Mg** | **0.59%** |

**Table 3: Water analysis**

| Items | Examined group | Permissible limits by EOS (1589/2005) |
|---|---|---|
| **Appearance color** | **Clear** | **Clear** |
| **PH value** | **7.2** | **6-7.5** |
| **Conductivity (ms/m)** | **580** | **2000(microms/m)** |
| **Calcium (mg/l)** | **60** | **Up to 150** |
| **Calcium carbonate (mg/l)** | **150** | **Up to 400** |
| **Magnesium mg/l** | **8** | **Up to 50** |
| **Magnesium carbonate** | **28** | **Up to 175** |
| **Total hardness as ca $Caco_3$** | **285** | **Up to 500** |

| | | |
|---|---|---|
| **TSS (total suspended solids)** | **30** | **Up to 250** |
| **TDS (total dissolved solids)** | **530** | **Up to 750** |
| **TS (total solids)** | **560** | **Up to 1000** |
| **Chloride (mg/l)** | **100** | **Up to 250 H-500 A** |
| **Ammonia (mg/l)** | **-ve** | **Up to 0.5** |
| **Nitrate (mg/l)** | **-ve** | **Up to 0.02** |
| **Sulphate (mg/l)** | **60** | **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 4: Rosemary analysis**

| Items | % |
|---|---|
| **Protein** | **3.3** |
| **Fat** | **2.8** |
| **Ash** | **11** |
| **Fiber** | **14** |
| **Carbohydrates** | **25** |
| **Sugar** | **22** |
| **Salts (mg/100 mg)** | |
| **Na** | **26** |
| **K** | **570** |
| **Fe** | **7.2** |
| **Ca** | **342** |
| **Ph** | **80** |
| **Mg** | **102** |
| **Zn** | **.87** |
| **Cu** | **.4** |
| **Mn** | **.77** |
| **Amino acids (g/100mg)** | |
| **Tryptophan** | **.1** |
| **Threonine** | **.22** |
| **Lucien** | **.23** |
| **Licin** | **.18** |
| **Methionine** | **.06** |
| **Alfa alanine** | **.22** |
| **Vitamins** | **Iu** |
| **A** | **3100** |
| **D** | **0** |
| **E** | **214** |
| **K** | **0** |
| **C** | **264 mg/dl** |
| **B** | **0.76 mg/dl** |

## Clinical Examination

Rectal temperature (ºC) recorded by using of digital thermometer. Respiratory rate (breaths /minute) was recorded by counting the flank's movements per 1-minute using stopwatch. The heart rate counted by measuring the beat per minute of the heart using a stethoscope (Constable.et al 2017).

## Samples

The Blood samples (10 ml) were collected from the jugular vein of each calves in clean glass vials, for serum preparation. Centrifugation was done at 3000 rpm for 15 min for serum separation

that was stored at -20℃ until testing, according to (Coles, 1986).

The separated sera used for detection of oxidant included MDA (µmol/ml) (Ohkawa et al, 1979) and antioxidants included TAC (Koracevic et al., 2001), SOD (u/ml) (Nishikimi et al., 1972), CAT (u/l) (Aebi, 1984) and GSH (mu/ml) (Paglia and Valentine, 1967) were estimated colorimetrically by means of test kits supplied by Bio-diagnostic company, Egypt by using of spectrophotometer (Spectro UV-VIS Double beam PC scanning spectrophotometer UVD-2950), according to the manufacturer's instructions. Glucose (Young, 2001) by using a (Spectro UV-VIS Double beam PC scanning spectrophotometer UVD-2950). Insulin was measured using a commercial (ELISA kit) supplied by Calbiotech, imported by a beta-diagnostic company, Egypt according to A.O.A.C. (2015). Cortisol level was assayed by the competitive Enzyme-Linked Immunosorbent Assay (ELISA), the kit ADI-900-071 from Enzo Life Sciences (Lausen, Switzerland) according to (Palme and Mostl, 1997). Haptoglobin level determined by using of bovine haptaglobin (HP) ELISA kits with catalogue Nrs. (HAP-11, Life Diagnostics, Inc., West Chester, Pennsylvania), according to the manufacturer's instructions. liver enzymes such as aspartate aminotransferase (AST), alkaline phosphatase (ALP) (u/l) (Breur, 1996 and Young, 1990), and alanine aminotransferase (ALT) (u/l) (Zawata et al., 1994). kidney function tests include urea (mg/dl) (Young, 2001), uric acid (mg/dl) (Young, 1995) and creatinine (mg/dl) (Titez, 1986) were applied by using of spectrophotometer (Spectro UV-VIS Double beam PC scanning spectrophotometer). Test kits supplied by Spectrum, Egyptian Company of biotechnology, according to the manufacturer's instructions.

**Statistical analysis**

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

**Results**

**Effect of heat stress on physiological parameters in animals** (rectal temperature, respiratory and heart rate)

By increasing the temperature levels subjected to calves at age of 12 months (mild and sever heat stress); there was a significant increase in the levels of respiratory rate (46 & 52 breath/minutes), heart rate (128 & 131 beats/minutes) and rectal temperature (40 & 41°c) at the level of P value ≤ 0.05 as showed in table 5.

**Table 5: Effect of heat stress on physiological parameters**

| Parameter Group | Respiratory rate (breath /minutes) | Heart rate (beats/minutes) | Rectal temperature (° c) |
|---|---|---|---|
| **Temperature 30-35⁰ c** | **40 ± 1** | **122± 2** | **39±0.4** |
| **Temperature 36-40° c (mild)** | **46 ± 2[a]** | **128± 1[a]** | **40± 0.3[a]** |
| **Temperature 41-45°c(sever)** | **52 ± 2[a]** | **131± 2[a]** | **41± 0.5[a]** |

Data presented as mean ± SE

a for significant difference of groups according to temperature at the same column

**Effect of heat stress on oxidants and antioxidants parameters**

By increasing the temperature levels subjected to calves at age of 12 months (mild and sever heat stress); there was a significant increase in the levels of MDA (17& 25 µmol/ ml) when compared with the control group (which not exposed to heat stress) (30-35$^0$c) group (11 µmol/ ml). By treatment there was a significant decrease in the level of MDA in treated groups with Rosemary and vit E& C groups (12& 14 and 12& 12 µmol/ ml); respectively during heat stress when compared with the control group (which not exposed to heat stress) (17& 25 µmol/ ml) in the same raw at the level of P value ≤ 0.05 as showed in table 6.

**Table 6: Effect of heat stress on MDA (µmol/ ml)**

| Parameter<br>Group | NRC ration<br>(Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| Temperature 30-35º c | 11± 0.5 | 9.5± 0.2[b] | 9± 0.1[b] |
| Temperature<br>36-40º c (mild) | 17± 0.6[a] | 12± 0.2[b] | 12± 0.3[b] |
| Temperature<br>41-45º c (sever) | 25± 0.7[a] | 14± 0.4[b] | 12± 0.1[b] |

-Data presented as mean ± SE

a for significant difference of groups according to temperature at the same column

-the letter b used to denote the significant difference of groups with NRC group at the same raw

**Effect of heat stress on SOD, CAT, CAT, and TAC**

By increasing the temperature levels subjected to calves at age of 12 months (mild and sever heat stress); there was a significant decrease in the levels of SOD (14& 11.5 u/ml), GPx (52& 41mu/ml), TAC (500& 268 u/ml), and CAT (41& 42.4u/l) when compared to the control group (30-35$^0$c) group (17 u/ml, 68mu/ml, 699u/ml & 60u/l). By treatment there was a significant increase in the level of SOD (17, 17.5& 15, 17 u/ml), GPx (63, 68& 59, 63mu/ml), CAT (61, 61& 60, 57u/l) and TAC (614, 690& 548, 628u/ml) in all supplemented groups when compared to control group (14& 11.5u/ml); (52& 41mu/ml); (41, 42.4u/l), and (500& 268u/ml); respectively in the same raw at the level of P value ≤ 0.05 as showed in tables 7, 8,9& 10.

**Table 7: Effect of heat stress on SOD (u/ml)**

| **Parameter<br>Group** | **NRC ration<br>(Control)** | **NRC ration+ vit E&C** | **NRC ration +<br>*Rosemary*** |
|---|---|---|---|
| **Temperature 30-35º c** | **17± 0.4** | **19± 0.3[b]** | **19± 0.3[b]** |
| **Temperature<br>36-40$^0$ c (mild)** | **14± 0.2[a]** | **17± 0.4[b]** | **17.5± 0.4[b]** |
| **Temperature<br>41-45º c (sever)** | **11.5± 0.2[a]** | **15± 0.1[b]** | **17± 0.3[b]** |

**Table 8: Effect of heat stress on GPx (mu/ml)**

| Parameter Group | NRC ration (Control) | NRC ration+ vit E & C | NRC ration + *Rosemary* |
|---|---|---|---|
| Temperature 30-35° c | 68± 2.5 | 72± 1.5 | 74± 0.9 |
| Temperature 36-40° c (mild) | 52± 3[a] | 63± 1.2[b] | 68± 1.6[b] |
| Temperature 41-45° c (sever) | 41± 1[a] | 59± 2.1[b] | 63± 1[b] |

**Table 9: Effect of heat stress on CAT (u/l)**

| Parameter Group | NRC ration (Control) | NRC ration+ vit E &C | NRC ration + *Rosemary* |
|---|---|---|---|
| Temperature 30-35° c | 60± 1.4 | 63± 1.5 | 64± 0.9 |
| Temperature 36-40° c (mild) | 41± 0.5[a] | 61± 1[b] | 61± 3[b] |
| Temperature 41-45° c (sever) | 42.4± 3[a] | 57± 1[b] | 60± 0.7[b] |

**Table 10: Effect of heat stress on TAC (u/ml)**

| Parameter Group | NRC ration (Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| Temperature 30-35° c | 699± 13 | 814± 3[b] | 852± 5[b] |
| Temperature 36-40° c (mild) | 500± 6[a] | 614± 10[b] | 690± 4.5[b] |
| Temperature 41-45° c (sever) | 268± 15[a] | 548± 16[b] | 628± 9[b] |

-Data presented as mean ± SE
a for significant difference of groups according to temperature at the same column
-the letter b used to denote the significant difference of groups with NRC group at the same raw.

**Effect of heat stress on glucose, cortisol and insulin levels**

By increasing the temperature levels subjected to calves at age of 12 months (mild and sever heat stress); there was a significant decrease in the levels of glucose (59& 50 mg/dl), and insulin (28& 24 iu/ml) when compared to the control group (30-35° c) (72.4mg/dl, and 33 iu/ml) while, there was a significant increase in the level of cortisol in heat stressed groups (12.6& 14.4 nmol/l) when compared to the control group (11.3 nmol/l) at the level of P value ≤ 0.05.

There was a significant increase in the levels of glucose (68, 67 & 64, 62 mg/dl), and insulin (30, 27 & 31, 28 iu/ml) in calves feed on ration according to NRC with addition of Vit E&C, and *Rosemary* when compared to the control group (59& 50mg/dl, and 32& 29 iu/ml) during heat stress at the level of P value ≤ 0.05 as showed in table 11& 12.

On the other hand, there was a significant decrease in the level of cortisol in Vit E&C, and *Rosemary* supplemented groups during heat stress (11.5& 12.2, and 11.7& 11.4nmol/l) when compared to the control group (12.6& 14.3 nmol/l) in the same raw at the level of P value ≤ 0.05 as showed in table 13.

**Table 11: Effect of heat stress on glucose level (mg/dl)**

| Parameter Group | NRC ration (Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| **Temperature 30-35º c** | **72.4± 1.6** | **69± 0.9[b]** | **67± 1.5[b]** |
| **Temperature 36-40º c (mild)** | **59± 0.4[a]** | **68± 0.5[b]** | **67± 1.3[b]** |
| **Temperature 41-45º c (sever)** | **50± 1.7[a]** | **64± 0.8[b]** | **62± 0.8[b]** |

**Table 12: Effect of heat stress on insulin (Iu/ml)**

| parameter Group | NRC ration (Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| **Temperature 30-35º c** | **33± 1** | **32± 0.7** | **29± 0.5[b]** |
| **Temperature 36-40º c (mild)** | **28± 0.6[a]** | **30± 0.9** | **31± 0.9[b]** |
| **Temperature 41-45º c (sever)** | **24± 0.4[a]** | **27± 0.4[b]** | **28± 0.9[b]** |

**Table 13: Effect of heat stress on cortisol (nmol/l)**

| parameter Group | NRC ration (Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| **Temperature 30-35º c** | 11.2± 0.1 | 11.6± 0.1 | 11.8± 0.05 |
| **Temperature 36-40º c (Mild)** | 12.6± 0.1[a] | 11.5± 0.1[b] | 11.7± 0.1[b] |
| **Temperature 41-45º c (sever)** | 14.3± 0.4[a] | 12.2± 0.3[b] | 11.4± 0.1[b] |

-Data presented as mean ± SE
a for significant difference of groups according to temperature at the same column
-the letter b used to denote the significant differences of groups with NRC (control) group at the same raw

**Effect of heat stress on haptoglobin (hgp/l)**

By increasing the temperature levels subjected to calves at age of 12 months (mild and sever heat stress); there was a significant increase in the level of haptoglobin (46& 52 hpg/l) when compared to the control group (30-35º c) (42.4hgp/l).

There was a significant decrease in the level of haptoglobin (42, 44.4& 40, 42 hgp/l) in calves supplemented with Vit E& C, and *Rosemary* supplemented groups during heat stress when compared to the control group (46& 52 hgp/l) in the same raw at the level of P value ≤ 0.05 as showed in table 14.

**Table 14: Effect of heat stress on haptoglobin level (hgp/l)**

| parameter Group | NRC ration (Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| **Temperature 30-35º c** | **42.4± 0.5** | **43± 1** | **41± 0.6** |
| **Temperature 36-40º c (mild)** | **46± 0.7[a]** | **42± 0.5[b]** | **40± 0.5[b]** |
| **Temperature 41-45º c (sever)** | **52± 1.1[a]** | **44.4± 0.5[b]** | **42± 0.8[b]** |

-Data presented as mean ± SE
a for significant difference of groups according to temperature at the same column
-the letter b used to denote the significant difference of groups with NRC group at the same raw.

**Effect of heat stress on kidney function test**

By increasing the temperature levels subjected to calves at age of 12 months (mild and sever heat stress); there was a significant increase in the levels of urea (29, 32 mg/dl), creatinine (1.5& 1.7 mg/dl) and uric acid (4.5& 5.9 mg/dl) when compared to the control animals (30-35° c) (19.2, 1.27 3.9 mg/dl); respectively.

By using of Vit E& C and Rosemary; there was a significant decrease in the urea (22.6, 24.4 & 22.4, 22 mg/dl) creatinine (1.3, 1.04& 1.1, 1.1 mg/dl) and uric acid (4.2, 5.8& 3.7& 4 mg/dl) when compared to the control group (29, 32; 1.5, 1.7, and 4.5, 5.9mg/dl); respectively at the level of P value ≤ 0.05 in the same raw as showed in table 15, 16& 17.

**Table 15: Effect of heat stress on urea level (mg/dl)**

| parameter<br>Group | NRC ration (Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| **Temperature 30-35⁰ c** | **19.2± 0.9** | **17.2± 0.7** | **16 ±0.4[b]** |
| **Temperature 36-40° c (mild)** | **29± 0.7[a]** | **22.6± 0.7[b]** | **22.4± 0.5[b]** |
| **Temperature 41-45°c (sever)** | **32± 0.7[a]** | **24.4± 0.2[b]** | **22± 0.5[b]** |

**Table 16: Effect of heat stress on creatinine level (mg/dl)**

| parameter<br>Group | NRC ration (Control) | NRC ration+ vit E &C | NRC ration + *Rosemary* |
|---|---|---|---|
| **Temperature 30-35° c** | **1.2± 0.02** | **1.09± 0.01[b]** | **1.02± 0.02[b]** |
| **Temperature 36-40° c (mild)** | **1.5±0.03[a]** | **1.3± 0.03[b]** | **1.1± 0.01[b]** |
| **Temperature 41-45° c (sever)** | **1.7± 0.3[a]** | **1.04± 0.02[b]** | **1.1± 0.03[b]** |

**Table 17: Effect of heat stress on uric acid (mg/dl)**

| parameter<br>Group | NRC ration (Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| **Temperature 30-35° c** | **3.9± 0.08** | **4± 0.05[b]** | **3.5± 0.05[b]** |
| **Temperature 36-40° c (mild)** | **4.5± 0.07[a]** | **4.2±0.05[b]** | **3.7± 0.03[b]** |
| **Temperature 41-45° c (sever)** | **5.9± 1[a]** | **5.8± 1** | **4± 0.06[b]** |

-Data presented as mean ± SE
a for significant difference of groups according to temperature
-the letter b used to denote the significant difference of groups with NRC group

**Effect of heat stress on liver function test**

By increasing the temperature levels subjected to calves at age of 12 months (mild and sever heat stress); there was a significant increase in the levels of ALT (53& 72u/l), AST (76& 83 u/l), and ALP (109& 125 u/l) when compared to the control group (30-35° c) (37, 54& 77 u/l); respectively at the level of P value ≤ 0.05.

By using of Vit E& C and Rosemary; there was a significant decrease in the ALT (44, 53& 40, 47 u/l), AST (65, 59& 53, 57 u/l), and ALP (87, 93 & 82, 100 u/l) when compared to the control group (53& 72; 109& 125, and 76& 83 u/l); respectively at the level of P value ≤ 0.05 as the same raw as showed in table 18, 19& 20.

**Table 18: Effect of heat stress on ALT (u/l)**

| parameter<br>Group | NRC ration (Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| **Temperature 30-35º c** | **37± 1.4** | **32± 1.2[b]** | **30± 0.7[b]** |
| **Temperature 36-40º c (mild)** | **53± 0.9[a]** | **44±1.4[b]** | **40± 0.9[b]** |
| **Temperature 41-45º c (sever)** | **72± 2.3[a]** | **53± 0.7[b]** | **47± 1.5[b]** |

**Table 19: Effect of heat stress on AST (u/l)**

| parameter<br>Group | NRC ration (Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| **Temperature 30-35º c** | **54± 1.2** | **49± 0.5[b]** | **44± 0.6[b]** |
| **Temperature 36-40º c (mild)** | **76± 1.8[a]** | **65±1.1[b]** | **53± 0.9[b]** |
| **Temperature 41-45º c (sever)** | **83± 2.3[a]** | **59± 0.8[b]** | **57± 0.6[b]** |

**Table 20: Effect of heat stress on ALP (u/l)**

| parameter<br>Group | NRC ration (Control) | NRC ration+ vit E&C | NRC ration + *Rosemary* |
|---|---|---|---|
| **Temperature 30-35º c** | **77± 3.4** | **74± 2** | **74± 0.7** |
| **Temperature 36-40º c (mild)** | **109± 1.2[a]** | **87±1.1[b]** | **82± 0.9[b]** |
| **Temperature 41-45º c (sever)** | **125± 3.6[a]** | **93± 1.9[b]** | **100± 0.9[b]** |

-Data presented as mean ± SE

a for significant difference of groups according to temperature at the same column

-the letter b used to denote the significant difference of groups with NRC group (control) at the same raw.

## Discussion

**Effect of heat stress on physiological parameters in animals** (rectal temperature, respiratory and heart rate)

The results of the obtained study revealed that there was a significant increase in the level of rectal temperature, respiratory, and heart rate in heat-stressed calves when compared to the control ones.

The sympathetic nervous system and the parasympathetic nervous system make up the body's autonomic nervous system. During stress, the sympathetic nervous system is activated, which triggers a series of hormonal and physiological reactions. Following its activation by the hypothalamus, the adrenal glands release an overdose of catecholamines (epinephrine), which raises the heart rate, and respiration rate (Dalcin et al. 2016).

**Effect of heat stress on oxidative stress parameters**

By increasing the temperature levels subjected to calves at the age of 12 months

(mild and severe heat stress); there was a significant increase in MDA, and a significant decrease in the levels of SOD, GPX, TAC and CAT when compared to those calves which not exposed to heat stress.

The result of the increased MDA level was reported by Li et al. (2020) and Yehia et al. (2021). This increase in MDA levels could be attributed to the increase of free radicals' production and lipid peroxidation accompanied by heat stress.

The decrease of GPX, SOD & CAT in heat stressed groups was in accordance with that reported by Li et al. (2020).

This decline is associated with the mobilization of cellular antioxidants in calves in order to detoxify free radicals generated by heat exposure, and to preserve the steady-state concentrations of ROS (Li et al. 2020).

**Effect of heat stress on glucose, cortisol, haptoglobin, and insulin level**

By increasing the temperature levels subjected to calves at the age of 12 months (mild and severe heat stress); there was a significant decrease in the levels of glucose and insulin when compared to those calves which not exposed to heat stress while, there was a significant increase in the level of cortisol in heat-stressed groups.

A decrease in serum glucose, and insulin levels in the heat-stressed group may be attributed to a variety of variables, such as reduced energy or feed intake, or it may have been a result of the adverse effects of heat on gluconeogenesis as an endocrine adaptation to hot conditions (Abeni et al., 2007).

The animal need for more energy to distribute heat to maintain a normal body temperature or heat stress-induced decreased dry matter intake could be the cause of the decreased glucose concentration (Bhatta et al., 2014).

The increase in cortisol levels was in accordance with that reported by Kim et al. (2018), and Jo et al. (2021). Since cortisol is the typical endocrine response to stress in the animal body, it plays a significant role in heat stress (Kannon et al., 2000). It is used as an indicator of animal welfare, since its level increases during times of distress. Its increase indicated that there was a significant deviation in the levels of blood biochemical that may be due to a metabolic shift in the stressed calves to cope with this imposed stress.

Kumar et al. (2010) reported that thermal stress stimulates the hypothalamus-pituitary-adrenal axis in farm animals leading to increased cortisol production.

By increasing the temperature levels subjected to calves at the age of 12 months (mild and severe heat stress); there was a significant increase in the levels of haptoglobin when compared to the control groups.

The result of haptoglobin was in accordance with Murata et al. (2004), who reported that plasmatic proteins called haptoglobin can change in concentration in response to stressors and in relation to an immunological reaction to the cytokines made by macrophages. Haptoglobin is an α2-globulin synthesized in the animal's liver and is considered a major acute-phase protein in different species of animals. Its concentrations increased with inflammation and stressful situations, so this increase in its level may attribute to heat stress on these calves (Yoshioka et al., 2002).

Situations of poor welfare status are associated with an increase in haptoglobin. In cases of tissue damage, inflammatory processes, infection, and stress,

hepatocytes produce this biomarker in response to macrophage cytokines. Therefore, in both human and animal health, these proteins serve as effective biomarkers for inflammatory processes like illness (Jo et al. 2021).

**Effect of heat stress on kidney function test**

The result of the obtained study revealed that there was a significant increase in the levels of urea, creatinine and uric acid when compared to the control groups. Our findings concurred with those of Rasouli et al. (2004) who noted greater blood urea nitrogen levels in the summer. This rise could be as a result of the use of amino acids as an energy source. The other cause could be because of the release of protein from muscle tissue and an increase in cortisol caused by stress, which speeds up the catabolism of body proteins (Dar et al .2019).

As the desire to eat is decreased due to the heat, an increase in blood creatinine content may be caused by excessive muscle catabolism for energy supply.

**Effect of heat stress on liver function test**

Serum enzymatic activity was measured to assist in the overall evaluation of the animal's health status. The results of the obtained study revealed that by the increasing the temperature levels subjected to calves at the age of 12 months (mild and severe heat stress); there was a significant increase in the levels of ALT, AST, and ALP when compared to the control groups.

This could be explained by the rise in corticosteroid-induced gluconeogenesis stimulation (El-Masry et al., 2010). Another reason may be an impaired liver function or liver damage during heat stress (Bernabucci et al., 2002).

Gaafar et al. (2021) mentioned that in order to compensate for the negative impacts of thermal stress on the physiological and biochemical homeostatic processes, the ALT and AST levels were elevated in heat stress-grouped calves.

**Effect of treatment of heat-stressed calves with Vit E, Vit C, and *Rosemary* on oxidative stress parameters**

There was a significant decrease which returned to normal range in MDA, and increase which returned to normal range in the level of SOD, GPX, CAT and TAC in all supplemented heat-stressed groups when compared to calves feed on ration according to NRC without any addition.

This result of antioxidants in Vit E supplemented group agreed with that reported by Khalifa et al. (2016), Kalmath and Narayana (2020) and Kassab et al. (2020). Vit E can improve the antioxidant in blood during heat stress can improve the antioxidant the animal status (Kassab et al., 2020). Supplementing animal's diets with vitamin E, which is regarded as a key antioxidant of cell membranes, could lower ROS, and stop the lipid peroxidation of biological membranes, which reduces cell oxidative damage (Sordillo 2013).

Rosemary's result was in accordance with that reported by Németh et al. (2004), El-Masry et al. (2018), and Kong et al. (2022).

According to Peng et al. (2005) *Rosemary* is one of the highest antioxidant agents utilized as a feed supplement because it can lessen the harmful effects of oxidative stress during hot summer conditions. The antioxidant compounds carnosol, rosemarinic acid, ursolic acid, carnosic acid, and butylated hydroxyl anisole are responsible for the bioactivities of rosemary leaves. While most antioxidants require recycling to preserve their antioxidant capacity after usage,

rosemary has a cascade effect, which gives it some special advantages over other antioxidants. The cascade is initiated by the molecule carnosic acid. After neutralizing the free radicals, carnosic acid is converted into carnosol, which can then neutralize a second free radical molecule. Thus, neutralizing several free radicals without recycling (Masuda et al., 2001).

**Effect of treatment of heat-stressed calves with Vit E& Vit C and *Rosemary* on glucose, cortisol, haptoglobin, and insulin levels**

There was a significant increase which returned to normal range in the levels of glucose and insulin in Vit E& C and *Rosemary* supplemented groups during heat stress when compared to calves feed on ration according to NRC without any addition.

The result of glucose in Vit E& C groups agreed with that reported by Kassab et al. (2020). This addition improved the animal feed intake as it decreased the stress in calves.

On the other hand, there was a significant decrease which returned to normal range in the level of cortisol in Vit E& C, and *Rosemary*-supplemented groups during heat stress when compared to those calves feed on ration according to NRC without any additions.

The addition of Vit E& C, and *Rosemary* reduced the stress in animals, so the level of cortisol decreased which returned to normal range due to its antioxidative properties (Kim et al. 2012).

There was a significant decrease which returned to normal range in the level of haptoglobin in calves of Vit E& C, and *Rosemary* supplemented groups during heat stress when compared to those feed in ration according to NRC without any addition.

The decrement in haptoglobin level which returned to normal range in all treated groups may attribute to the absence of oxidative stress due to the high anti-oxidative activity of Vit E& C, and *Rosemary* (Meschy, 2000, and Belay, 2002).

**Effect of treatment of heat stressed calves with Vit E, Vit C and *Rosemary* on kidney function tests**

By using of Vit E& C, and *Rosemary* in treatment of heat stressed calves; there was a significant decrease which returned to normal range in the urea, creatinine and uric acid levels when compared to calves feed on NRC ration without any additions.

These results of Vit E& C agreed with that reported by Khalifa et al. (2016), and Kassab et al. (2020). This may be attribute to their antioxidative effect.

The result of *Rosemary* in uric acid was in agreement with that reported by El-Masry et al. (2018). This may be related to that due to the bioactive phenols, flavonoids, and specifically the two phenolic chemicals carnosic acid and carnosol, *Rosemary* increased the antioxidant state of animals (Petiwala and Johnson, 2015). According to Németh et al. (2004), these natural chemicals may have the ability to scavenge free radicals and prevent their production.

**Effect of treatment of heat stressed calves with Vit E& C, and *Rosemary* on liver function tests**

By using of Vit E& C, and *Rosemary*; there was a significant decrease which returned to normal range in the ALT, AST, and ALP levels when compared to calves feed on NRC ration without any additions.

The results of Vit E& C were in agreement of that reported by Razia et al.

(2022) .This result may attribute to the antioxidants properties of Vit E& C in protecting the liver from damage and oxidative stress this reported by (Kim et al. 2012). .

These results of *Rosemary* were in agreement with that reported by El-Masry et al. (2018). The physiological effects of *Rosemary's* phenolic components, which have a strong hepatoprotective impact against oxidative stress, may be responsible for the herb's effects (Abdel-Hamid et al., 2011).

The present work showed the biochemical alterations caused by heat stress in calves and the effect of using Vit E& C, and *Rosemary* powder in the treatment of these calves.

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

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

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

More studies must be conducted on the effect of heat stress on the health of the calves and the use of Vit E& C and *Rosemary* for improving the immunity, and health of the calves.

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