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Effect of Dried Rosemary Supplement as Antioxidant Agent on Blood Biochemical Changes in Relation to Growth Performance of Heat-Stressed Crossbred (Brown Swiss × Baladi) Calves

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ABSTRACT

Heat exposure is a systemic stressor that adversely influences growth and reproductive performances in cattle. This trial aimed to study the effects of Rosemary (RM) supplementation on reducing the side effect of oxidative stress and its relation with growth performance under heat stress condition. Fifteen male calves were divided into three equal groups, the first was offered the basal diet as a control group, whereas the second and the third groups were fed the same basal diet as in control, in addition to a daily supplement of 3g and 6g dried grinded RM/kg concentrate, respectively, for a period of one month. The results showed that supplement of 3 and 6g dry grinded RM/kg concentrate led to a highly significant (P<0.01) decrease in oxidant status and an increase in total antioxidant capacity, as well as significant (P<0.01) declines were noted in the levels of lipids profile, kidney and liver function indicators, and iron concentration. However, RM supplemented groups showed significant (P<0.01) elevations of feed efficiency and daily weight gain copper and triiodothyronine concentrations. In conclusion, RM improved the calves' growth performance through alleviating oxidative stress side effects under hot summer conditions to improve economic returns.

Key words: Antioxidant agent, Blood biochemical, Egyptian desert, Growing calves, Oxidative stress

INTRODUCTION

Rosemary (RM, *Rosmarinus officinalis*) is accepted as one of the medicinal spices with the high antioxidant and anti-carcinogenic activities, as it contains flavonoids, phenols, volatile oil and terpenoids, and natural polyphenols which can lead to a considerable free-radical scavenging activity (Andrade et al., 2018; Petiwala and Johnson, 2015). Heat exposure is a systemic stressor that adversely influences growth and reproductive performances in cattle by disturbing their normal water, minerals and protein metabolism, physiological and blood biochemical processes.

High air temperature, comparative moisture, and radiating energy impair the ability of farm animals, particularly the fattening calves to temperature disposal, resulting in animals subjected to heat stress (Abdalla et al., 2009). A relationship between heat stress and oxidative stress has been established. One of the main reasons for oxidative stress in animals occurs when exposed to heat stress during hot summer conditions in pigs (Montilla et al., 2014), rams (Dehghan et al., 2010), poultry (Abdollah et al., 2016) dairy cows and buffaloes (Kargar et al., 2015 and Maha et al., 2018).

Oxidative stress is a consequence of defect in balance between reactive oxygen species (ROS) generation and efficiency of the antioxidant defense system. While molecular oxygen is needed to continue of normal cellular functions in mammals, excess level of ROS can cause many deleterious effects on organelle, cell and tissue , and disruption of normal metabolism and physiology by oxidative stress (Long et al., 2017). Oxidative stress in animals should be controlled by adding antioxidant nutrients and by minimizing effects of substances that stimulate ROS (Ganaie et al., 2013). The possible role of rosemary as a natural antioxidant was studied on goat's milk (Boutoial et al., 2013), sheep's meat (Vasta et al., 2013), and reproductive performance (Zhong and Zhou, 2013). While the data of the effects of such natural antioxidant especially growing calves under heat stress condition is limited.

Therefore, the objective of the current study was to evaluate the impact of rosemary on antioxidant enzymes, and some biochemical parameters as well as growth performance in heat-stressed calves.

MATERIALS AND METHODS

This study was carried out at a farm belonging to the improving of Cattle production project, in the nuclear research center of the Egyptian atomic energy authority, which was conducted in the desert region of Inshas, Egypt.

Animals and experimental design

The current study involved a two-week adaptation period to the diet followed by four weeks of feeding the experimental diets. The experimental work was carried out during the month of August 2013, where 15 crossbred

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(Brown Swiss × Baladi) male calves aging eight to ten months old with average live body weight of 152 kg were used at the beginning of the experiment. The animals were randomly divided into three equal groups (five calves each). The first group was offered the basal diet which was considered as a control group (G1), whereas the second and third treated groups (G2 and G3, treated groups) were fed the same basal diet as in control, in addition to a daily supplement of 3g and 6g dried grinded RM/kg concentrate, respectively.

Ethical approval

This experiment was performed according to all ethics and animal rights in Ain Shams University, Egypt.

Feed and feeding

Feed allowances were offered once a day in the morning at 10 am. The animals were fed in groups. The concentrate feed mixture (CFM) and rice straw were presented on the basis of the average body weight according to NRC (2001). Fresh drinking water was available to all animals at all times in clean basins full of fresh water.

Fresh plant leaves of RM (*Rosmarinus officinalis*) were collected from the Horticulture. Research Institute, agricultural research center, Giza, Egypt. The leaves were dried in a cool dark place at room temperature (25 °C) for 4 days. The average moisture content for the dry plant material was 11%, and then it was grinded very fine using electric grinder (Moulinex-France). Samples of dried RM and concentrate rations were (biweekly) collected. Samples were ground in an hammer mill provided with a 1-mm pore size screen and analysed in triplicate for their content in dry matter (DM) (forced-air oven at 65°C and dried to a constant weight), ash, crude protein (CP) (N × 6.25), crude fibre (CF), ether extract (EE) according to AOAC (2000). Nitrogen Free Extract (NFE) was calculated by differences. The ingredients of CFM are shown in table 1. The chemical compositions and nutritive values of the experimental feedstuffs on dry matter basis are shown in table 2. The chemical compositions of dried rosemary are presented in table 3. Bioactive compound of rosemary was determined using gas chromatography–mass spectrometry (GC–MS).

Table 1. Ingredients of the concentrate feed mixture

Items	CFM
Ingredients (%)	
Crushed yellow maize	50.00
Wheat bran	20.00
Soybean meal	5.00
Undecorticated cotton seed meal	22.50
Lime stone	1.00
Sodium chloride	1.00
Minerals mixture*	0.10
Vitamin mixture**	0.10
Sodium bicarbonate	0.20
Antitoxin	0.10

* Mineral mixture contains: 5g Cu ,30g Fe, 40g Mn , 45g Zn , 0.3g I, 0.1g Se and 881.6g Caco3/ kg mixture. **vitamin mixture contains: 20 million (I.U) vit A, 2 million (I.U) vit D3 and 2g vit E / kg mixture. *** Concentrate Feed Mixture (CFM)

Table 2. The chemical compositions and nutritive values of the experimental feedstuffs on dry matter basis during August 2013

Items	CFM	Rice straw
Chemical composition (%)		
Moisture	12.40	7.50
Dry matter (DM)	100	100
Organic matter (OM)	94.10	81.82
Crude protein (CP)	16.80	3.20
Crude fiber (CF)	8.00	34.05
Ether extract (EE)	2.60	1.94
Ash	5.90	18.18
Nitrogen – free extract (NFE)	66.70	42.63
Nutritive values		
GE (Mcal/kg DM)*	4.30	3.55
NE (Mcal/kg DM)**	1.60	0.94
TDN (%/kg DM)**	70.20	43.24

% NFE = % DM - (% EE + % CP + % ash + % CF) *, GE (Mcal/kg DM) = 0.057 CP % + 0.094 ether extract (EE) % + 0.0415 carbohydrate % (NRC, 2001), **; NE (Mcal/kg DM) = 0.0245 X TDN % - 0.12 (NRC, 2001),***; TDN (%/kg DM) according to the Central Lab for Food and Feed (CLFF), Agriculture Research Center, Egypt (2001). **** Concentrate Feed Mixture (CFM)

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Table 3. Chemical analysis of rosemary leaves

Components	Amount
Active components of essential oil %	
Camphor	13.61
Alpha-pinene	17.29
Cineole	34.11
Mineral Elements	
Potassium (%)	1.31
Calcium (%)	2.45
Copper (ppm)	3.4
Zinc (ppm)	31.2
Manganese (ppm)	14.6
Proximate analysis %	
Moisture	8.62
Crude protein	5.08
Ether extract	16
Crude fibre	18.94
Ash	7.52
Nitrogen free extract	43.84
Dried Rosemary obtained from Agricultural Research Center	Giza
Hemicellulose.	6.82
Lignin	6.03
Essential oil	1.33

Meteorological data

The animals were housed in a shaded free stall barn and all experimental groups were kept under the same environmental conditions throughout the experimental period. This experiment was carried out for a period of one month, during August 2013, air temperature (Ta) and the relative humidity (RH) during day and night were recorded using thermo-hygrometer, and the average of each item was calculated, where the Ta and the RH during the days times averaged 38.70°C ± 0.33 and 61.55 % ± 0.74, [equivalent to temperature humidity index (THI) 92]. While the average of Ta was 29.10 °C ± 0.24 and in RH was 80.34 % ± 0.71, (equivalent to THI 81) during the night times.

THI was calculated using the equation proposed by amundson et al. (2006), where,

$THI = (0.8\times Ta\ °C) + [(RH\ \%) \times (Ta\ °C - 14.4)/100)] + 46.4.$

Blood sampling and analysis

Blood samples were collected before feeding at 10 am from the jugular vein at the beginning and end of the experiment. Serum was separated from clotted blood by centrifugation (20 min, 3000 ×*g*) and clear serum collected and stored at -20°C until the biochemical and hormonal determinations. All the following parameters were determined using commercial kits manufactured by bio-diagnostic company, Egypt, unless otherwise indicated.

These parameters were glutathione reductase (GR) and catalase (CAT), uric acid, total antioxidant capacity (TAC), serum Malondialdehyde (MDA). The estimated serum lipids were total cholesterol, high density lipoprotein cholesterol (HDL-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol), and triglycerides. Serum concentration of urea-N and creatinine were determined to indicate kidney functions. For liver function evaluation we evaluated serum concentration of aspartate amino transferase (AST) and alanine amino transferase (ALT). Serum concentrations of iron and copper were determined.

Values of serum very low Density Lipoprotein Cholesterol (vLDL-cholesterol) and phospholipids were determined according to Ellefson and Caraway (1976) using the following equations, vLDL cholesterol = Triglycerides/5, and Phospholipids =68 + (0.89×Total cholesterol). Serum concentration of Triiodothyronine (T_3) was determined by using ^{125}I-RIA and antibody-coated tubes kit purchased from Immunotech Beckman Coulter, Inc., Incorporation Prague, Czech Republic, Europe.

Growth performance

Body weight of calves was recorded by digital balance before daily feeding and drinking at the beginning and the end of experimental period. Total and daily body weight gains were calculated for each calf. Dry matter intake (DMI) was determined as kg of calf^{-1}. Feed / gain ratio per day was calculated as kg gain/kg DMI.

The economic gain for rosemary supplementation

The economic gain was calculated as the market values of the total income from body weight gain after subtracting feed cost, and cost of rosemary during the experimental period (one month). Exchange value for Egyptian pounds (LE) to United State Dollar (USD) was equivalent to 8.90 LE at the time of the experiment. The following criteria were calculated in USD: Feed cost per kilogram, total cost of feedstuffs, feed cost per kilogram of gained weight, and net income (total income– expenditure) as presented in table 4.

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Table 4. Economic gain per animal during the experimental period (one month) with rosemary supplementation to crossbred calves under Egyptian summer condition during August 2013

Economic indicator \ Experimental group	Control	3g RM	6g RM
Total gain (kg)	21.04	31.12	33.40
Total income from gain($)	70.92	104.90	112.58
Concentrate feed mixture (kg)	120.00	120.00	120.00
Rice Straw (kg)	60.00	60.00	60.00
Cost of feedstuffs ($)	38.15	38.15	38.15
Cost of rosemary in one month ($)	--	0.51	1.01
Total expenditure	38.15	38.66	39.16
Net income =Total income –Expenditure	32.76	66.23	73.42
Percentage of gain over control	--	102.15	124.07

Price of kg live body weight = $3.37; Price of rosemary = $5.62; Price of feedstuffs (CFM and BH) = $0.30 and $0.04, respectively, quoted in Egypt in 2013. RM means rosemary.

Statistical analysis

Data were statistically analyzed using the general linear model procedure of GLM ANOVA procedure of SAS (2000).

The statistical model used was: $Y_{ij} = \mu + T_i + e_{ij}$

Y = the dependent variable,

μ = the overall mean,

T_i = the fixed effect of treatment (1= control, 2= treatment dose 1, 3 = treatment dose 2), e_{ij} = random error.

Significant differences between the means were verified by Duncan (1955).

RESULTS AND DISCUSSION

Chemical analysis of the experimental rosemary leaves

The results of chemical analysis are shown in table 3. The experimental rosemary leaves contained 8.62% moisture, 5.08% crude protein, 16.0% ether extract, 7.52% ash, 18.94% crude fiber and 43.84% nitrogen free extract. The cell walls of rosemary leaves contained high level of cellulose (16.08%), hemicellulose (6.82%) and lignin (6.03%). Furthermore, there are moderate amounts of some macro (calcium, 2.45% and potassium, 1.31%) and micronutrients (Zinc 31.20 mg/kg, Manganese 14.60 mg/kg, Copper 3.40 mg/kg and Zinc 31.2 mg/kg). The percentage of volatile components in the experimental rosemary leaves was 39.31 % for 1,8 cineole, 14.69 % for camphor, 13.85 % for α-pinene, 9.87 % for β- pinene, 6.17 % for camphene, 3.17 % for limonene, 2.58 % for p-cymene, 2.33 % for borneol, 2.02 % for myrcene, for α-terpineol, 1.46 % for bornyl acetate and was 2.27 % for others.

Oxidant and antioxidant status

Both GR and CAT are indicators for serum antioxidant enzymes activities (Sheweita et al., 2016) as well as TAC value is an as antioxidant biomarker (Rubio et al., 2016). Serum MDA level was determined as lipid peroxidation marker (Sauriasari et al., 2015).

Data of oxidant and antioxidant parameters are shown in figure 1. Heat stressed calves supplemented with G2 exhibited significant (P<0.01) increase in GR activity by 2.21% and in CAT enzyme activity by 12.75%, while non-significant elevation in the activity of these enzymes was found in G3 (1-A, B). This result indicated that 3g RM/kg concentrate enhanced the activities of oxidant related enzymes. The result in figure (1-C) showed that MDA level was significantly (P<0.01) decreased by 29.74 %, in G3, while insignificant difference was in G2 in compare with the control. The difference between treated groups was insignificant. A significant (P<0.01) increase in TAC concentration was found in RM the treated groups, values were increased by 22.89 and 19.23% in G2 and G3 respectively in compare with the control group, while the difference between RM treated groups was insignificant (Figure 1-D).

Different enzymatic and non-enzymatic mechanisms of the functional ROS as antioxidant have been reported. Since oxidation of reduced glutathione (GSH), acts as the primary non-enzymatic endogenous antioxidant in the cells. Antioxidant enzymes as CAT could promote the reduction of peroxides to alcohols, H_20 or both. GR lowers glutathione disulfide (GSSG), produced through the reduction of peroxides, to the sulfhydryl form of GSH (Zhu et al., 2012).

Oxidation of reduced GSH occurs either directly or through enzymatic means, as free radical (FR) and other reactive oxygen species are scavenged, and GSH; GSSG in heifer was less under hot summer condition than mild condition. A lower ratio is commonly used as an indicator of cellular redox, which indicates an increased oxidative load on the cells or decreased antioxidant defenses, through increasing oxidized glutathione GSSG. Thus one of the main reasons for the occurrence of oxidative stress in animals was the exposure to heat stress during hot summer (Ganaie et al., 2013).

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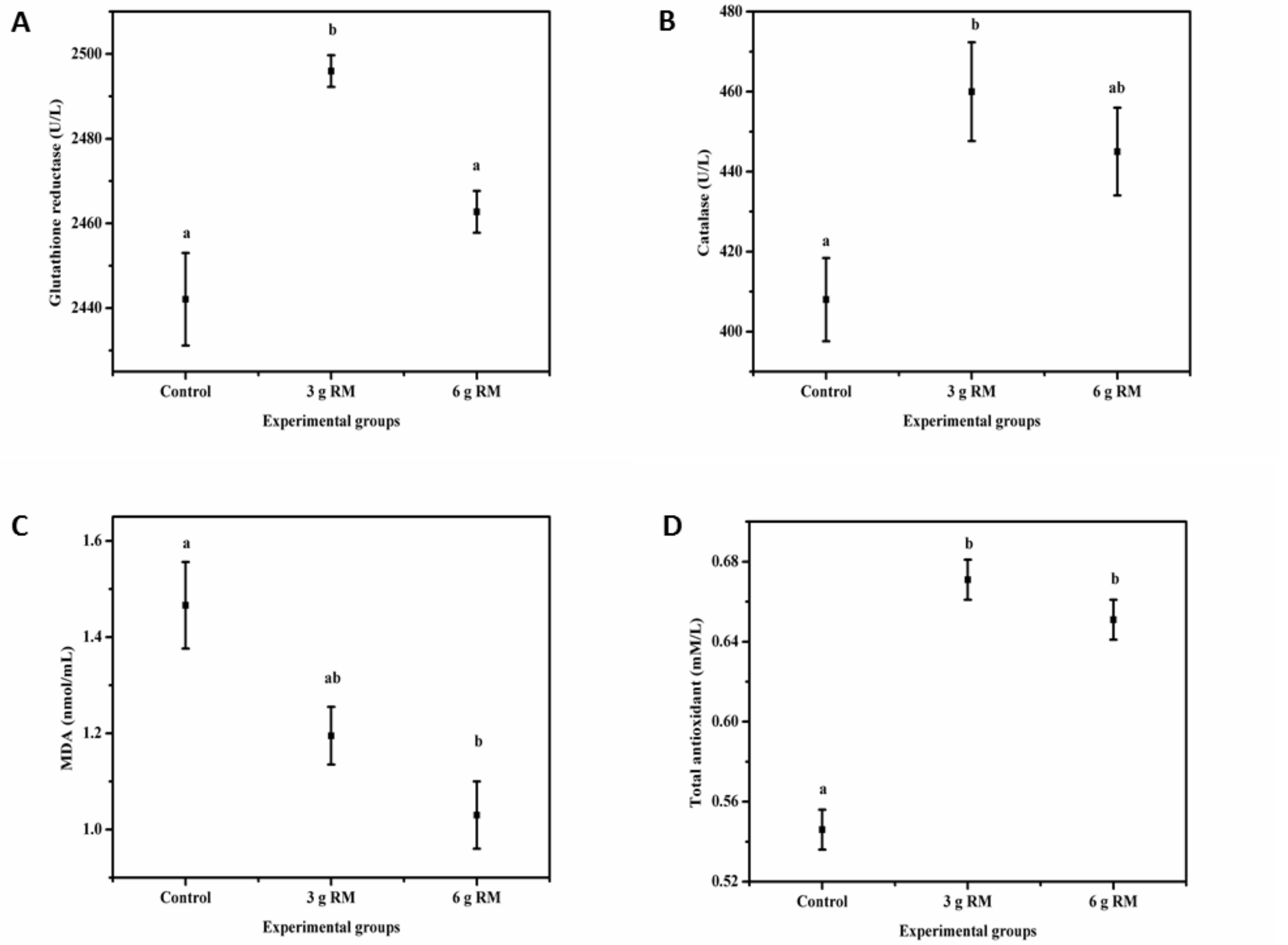

Figure 1. Effect of rosemary supplementation on some oxidant and antioxidant parameters in growing crossbred (Brown Swiss × Baladi) calves under Egyptian summer condition. The means in the same square with a similar superscript are not significantly different (P<0.05)

Trout et al. (1998) found that cattle exposed to heat stress via controlled chambers exhibited no evidence of increased MDA concentration in muscle tissue. While, Bernabucci et al. (2002) noted that the cows exposed to a hot environment display greater MDA than cows exposed to a thermo-neutral environment. Concerning the relationship between hot summer effects and oxidative stress, the use of RM which, has been widely accepted as one of the highest antioxidant agents (Peng et al., 2005), where used as a feed additives because it can alleviate the negative effects of oxidative stress, which occur when the animals were exposed to heat stress during hot summer conditions.

The bioactivities of the RM leaves are comparable with antioxidants components, such as carnosic acid, carnosol, rosemarinic acid, ursolic acid, butylated hydroxyl anisole and butylated hydroxyl toluene (Petiwala and Johnson, 2015). Moreover, RM as an antioxidant has some unique capabilities because most antioxidants need to go through a recycling to maintain antioxidant capability once it is used, while, RM as an antioxidant has a cascade effect. When, the molecule, carnosic acid starts the cascade, after carnosic acid neutralizes aFR it is transformed into carnosol which can neutralize another FR and this cascade continues thus neutralizing several FR without recycling (Masuda et al., 2001). The current findings are comparable with Németh et al. (2004) who found that RM as antioxidant material could lower the oxygen FR formation, increases the antioxidant enzyme activity.

Lipids profile

Data presented in figure (2A and B) shows that G2 and G3 were significantly (P<0.01) lowered the levels of total cholesterol by 7.77%, and phospholipids by 5.28% in compare with control, while the low levels was insignificant in G2. Furthermore, G2 and G3 led to significant (P<0.01) low levels of triglycerides by18.88 and 21.38%, respectively in compare with control (Figure 2C). The same comparison trend was in LDL-cholesterol levels (P<0.01) by 14.52 and 22.8 %, respectively (Figure 2D), and vLDL-cholesterol (P<0.01) by 18.82 and 21.38 %, respectively (Figure 2E). However insignificant increase in HDL-cholesterol was found (Figure 2F). The means in the same square with a similar superscript are not significantly different (P<0.05). Several investigators reported a significant increases in the levels of serum lipid profile under hot summer conditions in cattle (Kalmath et al., 2015), and in Beetal goats (Kumar et al., 2012). The antioxidant activities of RM could reveal their contents, have been found to inhibit lipid peroxidation. Thus, RM treated animals showed a significant decrease in lipid peroxidation concentration and a rise in GSH content (Sancheti and Goyal, 2007). In general, herbs as RM have high antioxidant concentrations that pose the possibility to prevent the oxidation of LDL, so that, the antioxidant properties of RM are of particular interest in alleviating LDL oxidation (Tapsell et al., 2006).

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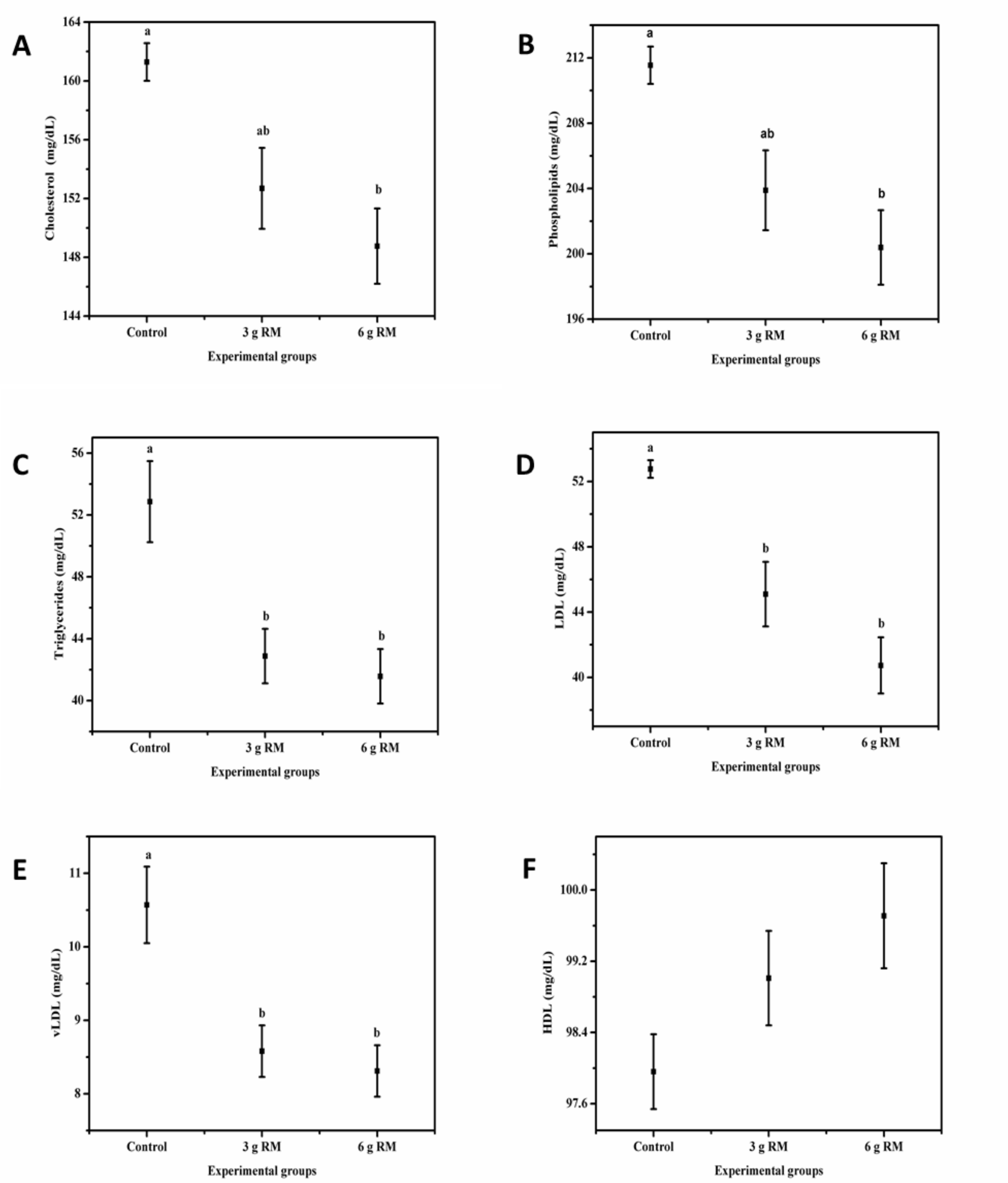

Figure 2. Effect of different rosemary supplementation level g/kg concentrate on lipids profile in growing crossbred (Brown Swiss × Baladi) calves maintained under Egyptian summer condition conditions

Moreover, our present results are consistent with those of Hanafy et al. (2009) who reported that using RM up to 200 mg/kg live body weight as feed additives in rations of Barki growing lambs could decrease serum cholesterol concentration which is positively reflected on lamb's performance. Also, AL-Blooni (2010) found that treating groups of diabetic rats with different levels of dried mixture of chicory and RM induced a significant reduction in serum total cholesterol, triglycerides, LDL and vLDL as compared to control group. On the other hand, Mehmet et al. (2013) reported that total cholesterol, HDL, LDL and triglyceride levels were not significantly different in Rosemary essential oil (REO) groups compared to control groups in Japanese quail under heat stress environmental conditions. This line needs further investigation to identify the possible different actions of RM in both heat stressed birds and animals.

Kidney and liver functions

The influence of RM supplementation on kidney and liver functions are presented in table 5. The results showed G2 and G3 exhibited a significant ($P<0.01$) low levels in concentration of serum creatinine by 33.90 and 32.58%,

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respectively in compare with the control. The same comparison trend in urea –N (P<0.01) lowered by 32.13 and 29.82 %, respectively, in AST (P<0.01), by 20.94 and 27.75 %, respectively, and in ALT (P<0.01) by 16.69 and 14.71 %, respectively.

Uric acid was considered as non-enzymatic antioxidant parameter, because uric acid is a major contributor to total radical trapping capacity (TRAP), which counting approximately 38-47% of the entire TRAP (Kharb, 2000), and uric acid is a better criteria than ascorbate because urate radicals (UH2) unlike ascorbate do not react with oxygen to give another peroxy radical which make it a good antioxidant (Rodionov, 2003).

Data presented in table 5 showed that uric acid as non-enzymtic antioxidant was significantly (P<0.01) increased by 17.29 % in G2, while insignificant difference was noted between G3 and the control group. Generally, the uric acid levels for treated groups were still in the normal range, indicating that used levels of RM improved antioxidant status of animals without the side effect on either the kidney or liver functions.

Ozcan et al. (2004) found lower levels of serum uric acid under different stressors periods, while, it is well known that the stressors is associated with increased FR production and decreased levels of antioxidant defenses. Also, Chaudhari et al. (2010) reported that lower levels of serum uric acid under stress could be related to the high utilization of uric acid for scavenging FR. The higher uric acid concentration found in this study as a result of RM dietary addition could be attributed to the of action of bioactive content of flavonoids, phenols, especially the two phenolic compounds, carnosol and carnosic acid (Almela et al., 2006; Petiwala and Johnson, 2015). These natural phenols could exhibit a considerable FR scavenging activity, and decrease the FR formation (Németh et al., 2004).

Terzano et al. (1997) pointed that urea-N is the main indicator of protein degradation for energetic rumen ammonia level and then blood urea release. In hot condition, several investigations have showed that urea-N values were significantly higher in growing heat stressed calves (Atta et al., 2014) and during summer than in winter in lactating buffaloes (Gudev et al., 2007). Montmurro et al. (1995) suggested that the high level of urea-N was related to the low energy/protein ratio and to gluconeogenesis by protein degradation in conditions of insufficient energy for growth.

Creatinine is a chemical waste molecule that is generated from muscle metabolism, and the decrease in its levels means that no muscular wastage which might have been possibly caused by inadequacy of protein intake to animals under stress. In the present trial, the negative effects of hot condition on kidney functions were lower in RM treated groups than the normal group. The current results are in accordance with lower concentrations of serum urea-N nitrogen and creatinine in RM treated rats (AL-Blooni, 2010), and broiler chickens (Ghazalah and Ali, 2008; AL-Blooni, 2010).

ALT and AST are hepatocellular damage biomarkers. The concentrations of ALT and AST activities in the herein study were significantly lower in G2 and G3 in compare with the control group (table 5). A significant high activities in AST and ALT were reported under hot conditions in cows (El-Masry et al., 2010), while these activities were significantly higher for AST concentration and slightly higher in sheep (Okab et al., 1993). The increase in activities of serum AST and ALT in heat stressed animals may be related to the increase in stimulation of gluconogenesis or gluconeogenesis by corticoids (El-Masry et al., 2010).

In agreement with results reported, Hanafy et al. (2009) showed that AST and ALT were significantly decreased at all RM levels, compared to control groups in Barki growing lambs. Several investigators attributed the decrease in transaminase enzymes in heat stressed calves supplemented with different doses of RM to the physiological effect of phenolic compounds in RM that has a hepatoprotective role (Abdel-Hamid et al., 2011; Rašković et al., 2014). In addition to antilipoper oxidant activity, REO was also found to efficiently reduce the levels of hydrogen peroxide (H_2O_2)- and 2,3-dimethoxy- 1,4-naphthoquinone (DMNQ)-induced oxidative damage of DNA in isolated rat hepatocytes, and testicular cells (Horváthová et al., 2010; Slameňová et al., 2011).

Table 5. Effect of dried rosemary supplementation on kidney and liver functions in growing crossbred (Brown Swiss X Baladi) calves maintained under Egyptian summer conditions during August 2013

Items	Control	3gRM	6gRM	Significance
Uric acid (mg/dL)	$0.399^{a} \pm 0.01$	$0.468^{b} \pm 0.01$	$0.435^{ab} \pm 0.02$	P<0.01
Creatinine (mg/dL)	$1.277^{a} \pm 0.05$	$0.844^{b} \pm 0.03$	$0.861^{b} \pm 0.03$	P<0.01
Urea (mg/dL)	$10.83^{a} \pm 0.01$	$7.35^{b} \pm 0.02$	$7.60^{b} \pm 0.03$	P<0.01
AST (U/mL)	$76.40^{a} \pm 0.93$	$60.40^{b} \pm 0.93$	$55.20^{c} \pm 0.86$	P<0.01
ALT (U/mL)	$29.71^{a} \pm 0.66$	$24.75^{b} \pm 0.58$	$25.34^{b} \pm 0.68$	P<0.01

The means in a row with a similar superscript are not significantly different (P<0.05). RM means rosemary; AST means Aspartate Amino Transferase; ALT means Alanine Amino Transferase.

Serum minerals

Supplementing diets of heat stressed calves with either 3 or 6g RM/kg concentrate significantly (P< 0.01) increased mean values of serum Cu concentrations by 24.55 and 20.76 %, respectively (Table 6). However, serum Fe concentration showed a decrease (P<0.01) by 2.67 and 4.19 %, respectively as compared with non-supplemented heat stressed calves. Many types of macromolecules are affected by oxidative stress especially if accompanied with environmental stresses. Moreover, elevated concentration of iron that acts as pro-oxidant was reported to be correlated with increased protein carbonyl concentrations that results in oxidative damage in animals (Perucchietti and Litjens, 2010). The same authors attributed the depression in Cu and the increase in Fe concentration to RBCs destruction in heat stressed animals and thus one of the main reasons for oxidative stress in animals and the changes in these elements was considered as indicator of oxidative stress.

Also, these macromolecules can act as specific antioxidant protecting macromolecules against the negative effects of oxidative stress (Perucchietti and Litjens, 2010), and the same author attribute the decrease of Cu under oxidative

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stress to relatively high level of Fe, which lead to depressed or lowered absorption of zinc, manganese and Cu. The current result about the increase in Cu concentration and decrease in Fe concentration in heat stressed calves supplemented with two levels of RM can explain the role of RM as antioxidants which decrease the ROS formation therefore, increases the antioxidant enzyme activity (Németh et al., 2004), in alleviation of oxidative stress occurring under heat stress conditions.

Triiodothyronine (T_3) hormone

Data presented in table 6 showed that serum T_3concentrations increased (P<0.01) by 39.43 and 52.87 %, respectively as a function of 3 or 6g RM supplementation per kg concentrate in comparison with the control group. These results confirm that supplementation of heat stressed calves with different doses of RM reduces the negative effect of hot conditions on thyroid activity. Other reports show that, exposure to elevate AT is associated with decrease plasma T_3 and T_4 levels in cattle (El-Masry et al., 2010). Moreover, Sarandöl et al. (2005) showed that this hypothyroidism was accompanied with increased oxidative stress.

The increase in serum T_3 concentration in heat stressed calves fed diets supplemented with RM is related to highest antioxidant activity for RM. The bioactivities of the RM leaves are comparable with known antioxidants constituents, such as carnosic acid, carnosol, rosemarinic acid, ursolic acid, butylated hydroxyl anisole and butylated hydroxyl toluene (Almela et al., 2006; Peng et al., 2005).

Table 6. Effect of dried rosemary supplementation (3 or 6 g/kg concentrate) on some minerals and thyroid hormone in growing crossbred (Brown Swiss X Baladi) calves maintained under Egyptian summer conditions during August 2013

Items	Control	3gRM	6gRM	Significance
Copper (Cu) (µg /dL)	323.42^a± 12.69	402.81^b± 9.93	390.56^b± 11.14	P<0.01
Iron (Fe) (µg/dL)	108.56^a± 0.51	105.66^b± 0.50	104.01^b± 0.46	P<0.01
T_3 (nmol/L)	0.870^a± 0.02	1.213^b ± 0.06	1.330^b± 0.04	P<0.01

The means in a row with a similar superscript are not significantly different (P> 0.05)

Growth performance

The changes in growth performance induced by dried RM additions to the ration of heat stressed calves are presented in table 7. From this table it can be noted before the experimental treatments that the differences in body weight between control and treated groups were insignificant. Addition of 3 or 6g RM/kg concentrate to heat stressed calves showed a significant effect on growth performance, since the mean values of daily gain recorded 47.93 and 58.77 %, respectively, and 47.90 and 58.75 % respectively for total gain as well as 5.00 and 7.12 %, respectively for final body weight, all over the control group.

As a function of supplementation of 3 or 6g RM/kg concentrate to the diet of heat stressed calves, the percentage increase in the mean values of feed efficiency recorded 52.17 and 60.87%, respectively, in comparison with the control group. Moreover, it can be noted that the increases in growth rate of calves were associated with the significant (P<0.01) improvement in feed efficiency as shown in table 7. These results clearly show that supplementation of 3g RM/kg concentrate to heat stressed calves had a significant amelioration in each of daily gain, total gain, final body weight and feed efficiency, while addition 6g RM/kg concentrate to the diet induced higher and better effect on growth performance than those observed by using 3g RM/kg concentrate. Owing the depression in growth performance specially, daily gain in heat stressed animals was observed in growing heat stressed calves. Moreover, the negative changes in protein metabolism, tissues anabolism, most blood constituents, minerals and water metabolism and hormonal levels disturbances, may contribute to such decrease in growth performance in heat stressed cattle (Atta et al., 2014).

Thus, adding RM to the basal diets showed significant alleviation in growth performance of heat stressed calves. These results are in accordance with previous studies which showed good effects of RM plants additive on Seadi lambs performance (Mohamed et al., 2005).

Table 7. Effect of dried rosemary supplementation on growth performance of growing crossbred (Brown Swiss X Baladi) calves maintained under Egyptian summer conditions during August 2013

Items	Control	3g RM*	6gRM	Significance
Initial body weight (kg)	152.60 ± 2.50	151.20 ± 2.33	152.60 ± 1.78	N.S
Daily gain (kg/day)	0.701^a ± 0.01	1.037^b ± 0.01	1.113^c ± 0.01	P<0.01
Total gain (kg)	21.04^a ± 0.22	31.12^b ± 0.22	33.40^c ± 0.22	P<0.01
Final body weight (kg)	173.64^a ± 2.37	182.32^b ± 2.33	186.00^b ± 1.62	P<0.01
Feed / gain ratio (kg/ kg)	0.23^a± 0.01	0.35^b± 0.01	0.37^c ± 0.01	P<0.01

The means in a row with a similar superscript are not significantly different (P<0.05). *: RM means rosemary.

Feed / Gain ratio

Also, RM used as natural additive to animal feed may be a good alternative to artificial antioxidants since they showed beneficial effects also on animal welfare and other physiological functions (Tedesco, 2001). Also, Hanafy et al. (2009) concluded that using RM as feed additives in rations of Barki growing lambs up to 200 mg/kg live body weight could be improve the nutrient digestibility, nutritive value, daily gain, feed conversion, which are positively reflected on lambs performance and economic efficiency. The beneficial effects of REO in reducing the negative effects of heat stress

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in Japanese quail, was reported by Mehmet et al. (2013) that found a significant increase in feed conversion ratio in RM oil groups than control groups under heat stress environmental conditions. The improved performance of REO groups could be related to these positive effects of REO on digestive system. In sheep, Sahraei et al. (2014) found that REO decreased the ruminal total volatile fatty acids, acetate, butyrate and ammonia-N concentration, these changes makes more adaptable to environmental stress, there more efficient digestion.

CONCLUSION

RM contains antioxidant compounds can be used in alleviation of the negative effect of heat stress, especially oxidative enzymes, some blood constituents and growth performance in heat stressed calves. Further studies on the molecular level should be conducted to show up the possible mechanism of the effects of bioactive component of rosemary on the composition and function of rumen microbiota.

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Competing interests

With respect to the research, authorship, and/or publications of this article. The authors declare that they have no competing interests.

Author’s contribution

Dr. Kamel Ahmed El-Masry designed the experiment, article writing and revision, Dr. Essmat Bakry Abdalla designed the experiment, manuscript writing, commenting and approval, Dr. Sana Sayed Emara helped in field study, collected data, laboratory analyses, statistical analysis, tabulation of experimental data and article writing; while, Dr. Abdelhady Farouk Hussein helped in statistical analysis, manuscript writing. All authors have read and approved the final manuscript.

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