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RESEARCH ARTICLE

Effect of β-carotene as Antioxidant on Immune Response and Blood Biochemical Changes in Relation to Growth Performance of Heat-Stressed Calves

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ABSTRACT

Twenty-four male crossbred calves of 6 –7 months old and 144–150 kg body weight were used to investigate the effect of β-carotene on immunoglobulins (Igs) and some blood constituents, and its relation to growth performance in heat-stressed calves. The animals were maintained under hot summer environmental conditions and randomly divided into two equal groups (12 calves each). The first (control) was offered the basal diet, whereas the second group (treatment) was fed the basal diet plus 50,000 IU β-carotene/calf/day, for 1 month as an experimental period. Total and daily body weight gains were calculated for each calf. Blood samples were collected at the end of the experiment to determine some blood constituents and immunological indices. β-carotene addition to calves' diet reduced respiration rate, rectal temperature (RT), malondialdehyde, lipid fractions, and a decline in aspartate transaminase and alanine aminotransferase activities. β-carotene significantly increased glutathione reductase, catalase enzyme activities, total antioxidant capacity, and concentrations of total protein, albumin, globulin, calcium, and phosphorus, also improved immunological indices, that is, β-globulin, γ-globulin, and IgG. Moreover, β-carotene enhanced T3 and T4 levels and improved feed efficiency and daily gain. In conclusion, supplementation of growing calves with β-carotene under Egyptian hot summer conditions reduced heat stress effect by RT decrement and modifying most blood constituents and thyroid function, antioxidant, and immunological indices which lead to an improvement in growing calves.

Key words: Blood constituents, growing calves, heat stress, immunoglobulin G, β-carotene

INTRODUCTION

One of the greatest challenges to animal production is heat stress because it can disrupt the physiology, productive, and reproductive performance of livestock. High ambient temperature, relative humidity (RH), and radiant energy impair the ability of farm animals, particularly the feedlot calves to dissipate heat, resulting in heat stress.[1-3]

β-carotene like other carotenoids is mainly converted into Vitamin A in the intestinal mucosa as well as in the liver and other body tissues.[4] Vitamin A is an important micronutrient that supports all the critical biological processes in the body. However, the body cannot synthesize Vitamin-A, so it should be added to the diet as a preformed provitamin-A or as carotenoids. Carotenoids contain natural plant fat-soluble pigments, about 50 of these, including β-carotene, exhibit provitamin-A activity. β-carotene is the most bountiful source of provitamin-A in the feedstuff. Besides, it also functions independently as a strong antioxidant which can enhance immunity with possible productive traits.[5] Vitamin A and β-carotene have many diverse functions such as reproduction, immune functions, and health, nevertheless, further studies needed to better

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understand the precise physiological actions of β-carotene on different reproductive outcomes and physiological scenarios.[6]

Therefore, to reduce the side effect of heat stress in heat-stressed calves, β-carotene has been used in our experiment. When the studies about growth performance responses to β-carotene in heat-stressed calves are limited and controversial, therefore, the present research objective is to estimate β-carotene effects on the antioxidant status, blood biochemical levels, thyroid function, and some immunological indices in relation to growth performance in crossbred calves under Egyptian hot summer conditions.

MATERIALS AND METHODS

Experimental location and ethics

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 (latitude 310 12' N to 220 2' N, longitude 250 53' E to 350 53' E). This work was reviewed and approved by the Animal Care and Welfare committee of Egyptian Atomic Energy Authority standard operating procedures. These ethics contain relevant information on the endeavor to reduce animal suffering and adherence to best practices in veterinary care according to the International Council for Laboratory Animal Science guidelines. The experimental procedures were carried out according to the Local Experimental Animals Care committee and approved by the Institutional Ethics Committee.

Animals and experimental design

The current study involved a 2-week adaptation period to the diet using the same ration components which used over the experiment, followed by 4 weeks of feeding the experimental diets, where 24 crossbred (Brown Swiss × Baladi) male calves aging 6–7 months old with an average live body weight of 144–150 kg were used at the beginning of the experiment. The animals were randomly divided into two equal groups (12 calves each). The first group was offered the basal diet which was considered as a control group, whereas the second group was fed the same basal diet as in control, in addition to a daily oral individual supplement by a two bolus of β-carotene (soft gelatin capsules; 25,000 IU; Pharco Pharmaceuticals, Egypt), with total dose 50,000 IU β-carotene/calf/day and considered as a treated group, while the dose chosen according to the pervious literature in this field.

Feed and feeding

Feed allowances were offered once a day in the morning at 10 a.m. The animals were fed in groups. The concentrate feed mixture (CFM) and rice straw were presented separately based on the average body weight according to NRC, [7]. In quantities ranging from 3.75 to 4.5 kg/calf/day for CFM and 2.5 to 3 kg/calf/day for rice straw over the course of the experiment as the amounts of feed are raised with the increase in the weights of the animals, with the percentage of concentrate: roughage 60:40 %. Fresh drinking water was always available to all animals in clean basins full of freshwater.

Samples of rations were (biweekly) collected. Samples were ground in a hammer mill provided with a 1 mm pore size screen and analyzed in triplicate for their content in dry matter (DM) (forced air oven at 65°C and dried to constant weight), ash, crude protein (CP) (N × 6.25), crude fiber (CF), and ether extract (EE) according to the Association of Official Analytical Chemists International.[8] 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 DM basis are shown in Table 2.

Meteorological data

The animals were housed in a shaded freestall 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 6 weeks, from mid of July to last of August 2018. Air temperature (AT) and the RH during day and night were recorded daily from the meteorological station of Atomic Energy Authority during the whole experimental period, and the

Table 1: Ingredients of the CFM

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 5 g Cu, 30 g Fe, 40 g Mn, 45 g Zn, 0.3 g I, 0.1 g Se, and 881.6 g $Caco_3$/ kg mixture. **Vitamin mixture contains 2 million (I.U) Vitamin A, 2 million (I.U) Vitamin D3, and 2 g Vitamin E/kg mixture. CFM: Concentrate feed mixture

Table 2: The chemical compositions and nutritive values of the experimental feedstuffs on dry matter basis

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

*On dry matter basis, while, the chemical composition presents as a percentage of dry matter, so we consider dry matter as 100%, ** % NFE = % DM – (% EE + % CP + % ash + % CF). ***% OM = % DM – % Ash or = % EE + % CP + % NFE + % CF. ****GE (Mcal/kg DM) = 0.057 CP% + 0.094 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), Agric. Res. Center, Egypt (2001), CP: Crude protein, CF: Crude fiber, EE: Ether extract, DM: Dry matter, OM: Organic matter, NFE: Nitrogen-free extract, CFM: Concentrate feed mixture, GE: Growth energy, NE: Net energy, TDN: Total digestible nutrient

average of each item was calculated, where the AT and the RH during the day's times averaged 38.70°C ± 0.33 and 61.55% ± 0.74 (equivalent to temperature–humidity index [THI] 92), while, the average of AT 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*.[9] where, THI = (0.8 × AT °C) + [(RH %) × (AT °C − 14.4)/100)] + 46.4.

Blood sampling and analysis

Blood samples were collected before feeding at 10 a.m. from the jugular vein at the beginning and end of the experiment, at clean tube with 10 ml volume. Serum was separated from clotted blood by centrifugation (20 min, 3000× *g*) and clear serum collected and stored at –70°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.

Serum samples were used to determine antioxidant enzymes activities (glutathione reductase [GR] and catalase), total antioxidant capacity as antioxidant biomarkers, serum malondialdehyde (MDA) as a lipid peroxidation marker, serum total protein, and albumin. The estimated serum lipids were total cholesterol, high-density lipoprotein cholesterol (HDL cholesterol), low-density lipoprotein cholesterol (LDL cholesterol), and triglycerides. For liver function evaluation, we evaluated serum concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Serum concentrations of calcium (Ca) and phosphorus (P) were determined.

Values of serum very LDL cholesterol (VLDL cholesterol) and phospholipids were determined according to Ellefson and Caraway,[10] using the following equations: VLDL cholesterol = Triglycerides/5 and Phospholipids = 68 + (0.89 × Total cholesterol).

Serum proteins were fractionated according to the method of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of protein as described by Johnstone and Thorpe.[11] Serum immunoglobulin G (IgG) was measured using a kit for IgG of the Binding Site Limited Company, Birmingham. Serum concentrations of triiodothyronine (T_3) and thyroxine (T4) were determined using ^{125} I-RIA and antibody coated tubes kit purchased from Immunotech Beckman Coulter, Inc., Prague, Czech Republic, Europe.

Physiological parameters

Rectal temperature (RT) was measured daily using a clinical thermometer inserted into the rectum to 3 inches and left for 2 min. Respiration rate

(RR) was determined daily by counting the flank movement for a period of 1 min using stop watch. The count was expressed as breaths per minute (breath/minute). RR necessary carried out before measuring the RT to avoid animal excitation.

Growth performance

Bodyweight of calves was recorded before feeding and drinking weekly until the end of the experimental period. Total and daily body weight gains were calculated for each calf. Dry matter intake (DMI) was determined by calculating the average of intake kg/calf/day over the course of experiment by recording the residual for each group 3 times a week. Gain/feed was calculated as kg gain/kg DMI and showed as the average in the results.

Statistical analysis

The differences between the mean values of treatment and control groups were tested using the unpaired varieties of "*t*" test of significance, according to the statistical analysis system software program.[12] The model used is: $Y_{ij} = \mu + T_i + e_{ij}$. Where, Y = the dependent variable, μ = the overall mean, T_i = the fixed effect of treatment (1= control, 2= treatment), and eij = random error.

RESULTS

As shown in Table 3, RT and RR decreased ($P < 0.001$) by –1.73 and –25.49%, respectively, as a function of supplementation of β-carotene for heat-stressed crossbred (Brown Swiss × Baladi) calves.

Data presented in Table 4 showed that when the heat-stressed calves were supplemented with β-carotene MDA level as an indicator of the damage of cell membrane was significantly ($P < 0.001$) decreased by –32.87%. However, a significant ($P < 0.001$) increase in GR and catalase activities as antioxidant enzymes and total antioxidant concentration was recorded by 16.58, 22.15, and 46.78%, respectively.

Supplementation of β-carotene to heat-stressed calves induced a significant ($P < 0.001$) increase in serum total protein, albumin, and globulin concentrations by 15.99, 12.47, and 20.98%, respectively, while the albumin/globulin ratio decreases by 6.99%; $P < 0.001$, as shown in Table 5.

Alpha-globulin, β-globulin, gamma-globulin, and IgG concentrations results showed a significant ($P < 0.001$) improvement in supplemented calves by 7.93, 21.82, 26.55, and 35.90%, respectively, as compared with non-supplemented calves in Table 5.

Data presented in Table 6 showed that supplementation of β-carotene to heat-stressed calves induced a significant ($P < 0.001$) decrease in the concentrations of each of total cholesterol by 7.27%, triglycerides by 18.71, phospholipids by 5.13, LDL cholesterol by 17.32, and VLDL cholesterol by 18.72%, while, a significant ($P < 0.001$) increase by 15.45% in HDL cholesterol concentration was observed.

Supplementation of heat-stressed calves with β-carotene had a great effect on Ca and P concentrations represented in a significant rise

Table 3: Effect of β-carotene supplementation (50,000 IU/calf/day) to the diet on RT and RR in growing calves maintained under hot environmental conditions

Item	Control mean±SE	Treatment mean±SE	Change %	*P*-value
RT (°C)*	39.93±0.04	39.24±0.06	–1.73	0.001
RR (breaths/min)**	93.50±0.66	69.67±0.69	–25.49	0.001

*RT: Rectal temperature, **RR: Respiration rate

Table 4: Effect of β-carotene supplementation (50,000 IU/calf/day) to the diet on oxidant and maintained under hot environmental conditions

Items	Control mean±SE	Treatment mean±SE	Change %	*P*-value
MDA (nmol/ml)*	4.35±0.08	2.92±0.08	–32.87	0.001
GR (U/L)	1909.17±10.76	2225.74±20.35	16.58	0.001
Catalase (U/L)	247.92±6.30	302.83±4.37	22.15	0.001
Total antioxidant (mM/L)	0.466±0.01	0.684±0.01	46.78	0.001

*MDA: Malondialdehyde, GR: Glutathione reductase

Table 5: Effect of β-carotene supplementation (50,000 IU/calf/day) to the diet on serum total protein, albumin, globulin fractions, and IgG in growing calves maintained under hot environmental conditions

Item	Control mean±SE	Treatment mean±SE	Change (%)	*P*-value
Total protein (g/dl)	6.49±0.04	8.05±0.04	15.99	0.001
Albumin (g/dl)	4.09±0.01	4.60±0.02	12.47	0.001
Globulin (g/dl)	2.86±0.03	3.46±0.02	20.98	0.001
Albumin/globulin	1.43±0.01	1.33±0.01	–6.99	0.001
Alpha-globulin (g/dl)	0.63±0.01	0.68±0.01	7.93	0.001
Beta-globulin (g/dl)	1.10±0.01	1.34±0.01	21.82	0.001
Gamma-globulin (g/dl)	1.13±0.01	1.43±0.01	26.55	0.001
IgG (g/dl)	0.78±0.01	1.06±0.01	35.90	0.001

IgG: Immunoglobulin G

Table 6: Effect of β-carotene supplementation (50,000 IU/calf/day) to the diet on serum lipids profile concentrations in growing calves maintained under hot environmental conditions

Item	Control mean±SE	Treatment mean±SE	Change (%)	*P*-value
Total cholesterol (mg/dl)	182.81±0.97	169.51±1.15	–7.27	0.001
HDL cholesterol (mg/dl)*	57.01±0.63	65.82±0.65	15.45	0.001
LDL cholesterol (mg/dl)**	102.45±0.47	84.71±0.82	–17.32	0.001
VLDL cholesterol (mg/dl)***	23.35±0.23	18.98±0.16	–18.72	0.001
Triglycerides (mg/dl)	116.74±1.15	94.90±0.88	–18.71	0.001
Phospholipids (mg/dl)	230.70±0.86	218.86±1.02	–5.13	0.001

*HDL: High-density lipoprotein, **LDL: Low-density lipoprotein, ***VLDL: Very low-density lipoprotein

Table 7: Effect of β-carotene supplementation (50,000 IU/calf/day) to the diet on serum liver enzyme activity, and Ca, P, T3, and T4 concentrations in growing calves maintained under hot environmental conditions

Item	Control period mean±SE	Treatment period mean±SE	Change (%)	*P*-value
Ca (mg/dl)	11.19±0.15	13.38±0.26	19.57	0.0001
P (mg/dl)	9.49±0.13	12.57±0.19	32.46	0.0001
AST (μ/l)*	40.67±0.21	34.83±0.22	–14.36	0.000
ALT (μ/l)**	8.08±0.19	6.58±0.15	–18.56	0.0001
T_3 (ng/ml)***	2.53±0.09	3.24±0.08	28.06	0.001
T_4 (ng/ml)****	42.14 ±0.43	66.72±0.44	58.33	0.001

*AST: Aspartate aminotransferase, **ALT: Alanine transaminase, ***triiodothyronine, ****tetraiodothyronine (thyroxine), Ca: Calcium, P: Phosphorus

($P < 0.0001$) in serum Ca by 19.57% and P by 32.46%, as shown in Table 7. Current results indicated AST and ALT levels decrement ($P < 0.0001$) by 14.36 and 18.56%, respectively, when the β-carotene was added to the ration of heat-stressed calves [Table 7]. Supplementation of heat-stressed calves with β-carotene had a positive effect on thyroid hormone concentrations, where serum T_3 and T_4 were significantly ($P < 0.001$) increased by 28.06 and 58.33%, respectively, but around its normal range, Table 7.

Heat-stressed calves supplemented with β-carotene showed a significant ($P < 0.0001$) improvement in total gain and daily gain by 42.68 and 43.28%, respectively, as compared to non-supplemented calves. Data revealed positive insignificant change in DMI according to the change in total gain over the experiment by 2.85% and an improvement ($P < 0.0001$) in gain/feed ratio by 41.18%, with 0.17 and 0.24 for control and supplemented calves, respectively, due to β-carotene supplementation [Table 8].

DISCUSSION

In the current study, β-carotene treatment exerted significant decrease in RT and RR of heat-stressed calves when compared to control. A significant effects due to seasonal variations especially hot summer conditions on some physiological

Table 8: Effect of β-carotene supplementation (50,000 IU/calf/day) to the diet on growth performance of growing calves maintained under hot environmental conditions

Item	Control period Mean±SE	Treatment period Mean±SE	Change (%)	*P*-value
Initial body weight (kg)	147.20±0.49	148.02±0.41	0.55	0.21
Final body weight (kg)	167.42±0.44	176.87±0.59	5.64	0.0001
Total gain (kg)	20.22±0.27	28.85±0.50	42.68	0.0001
Daily gain (kg/day)	0.67±0.01	0.96±0.02	43.28	0.0001
DMI (kg/day)*	3.86±0.07	3.97±0.11	2.85	0.46
Gain/feed (kg/kg)**	0.17±0.003	0.24±0.003	41.18	0.0001

*DM: Dry matter intake, **(kg gain/kg DMI)

parameters (RT and RR) changes in Baladi cattle in the Middle of Egypt recorded by El-Gaafarawy *et al.*,[13] in accordant to our results. Concerning the relationship between hot summer conditions and oxidative stress,[14] antioxidants additives to feedstuff help to counter the deleterious effects of oxidative stress, which occur when the animals exposed to heat stress during hot summer conditions.

In agreement of our results, β-carotene showed an increase in antioxidant enzymes such as glutathione (GSH), glutathione peroxidase, catalase, and superoxide dismutase, while decreased MDA.[15,16] Furthermore, the activity of GR was less under hot summer compared to mild conditions, and as the predominant low-molecular-weight thiol in animal cells reduced GSH also, serves as a crucial antioxidant to offset environmentally derived oxidative stress, and under heat stress which is considered one of the main reasons for oxidative stress in animals.[17,18] The antioxidant activities of carotenoids and biochemical properties influencing signaling pathways have been discussed as basic mechanisms of prevention. Carotenoids are unique constituents of a healthy diet and play an important role in the network of antioxidant vitamins and phytochemicals. The lipophilicity of carotenoids determines their subcellular distribution; they are enriched in membranes and other lipophilic compartments.[19] Our results are in agreement with β-carotene which plays an important role as an antioxidant, efficiently contributes to the defense against lipid peroxidation, and scavenges peroxyl radicals.[20] β-carotene and other carotenoids are widely regarded as biological antioxidants. Carotenoids can inhibit the propagation of radical initiated lipid peroxidation. Modulation of lipid peroxidation by β-carotene may be an important mechanism for reducing oxidative stress. β-carotene is a potent antioxidant because it is reactive toward peroxyl radicals.[21] And also, β-carotene provides protection against singlet oxygen-mediated lipid peroxidation and can function as an effective antioxidant not only against $^1O2$ but also against lipid peroxidation and the highly destructive, hydroxyl radical HO.[22] Moreover, β-carotene supplementation increases antioxidant enzymes in tissues and prevent their decrease.[23] Furthermore, the supplementation of β-carotene in rats resulted in a significant reduction of liver MDA and a significant elevation of liver and blood GSH.[24]

In concomitant with the current serum proteins results,[25] established β-carotene supplementation generated serum increases of total protein. Besides, β-carotene nutritional benefits as provitamin-A, it also functions independently as a strong antioxidant which can enhance immunity with possible productive traits. About the studies on the role of carotenoids on immune response, Igs were used to assays immune function. While, Ig serves to neutralize toxins, immobilize certain microorganisms, neutralize viral activity, agglutinate microorganisms or antigen particles, and precipitate soluble antigens. Blood neutrophils isolated from cattle fed carotene had higher killing ability during the stressful period. The increased bacterial killing could be accounted for partly by increased myeloperoxidase activity in the neutrophils.[26,27]

Orally administered β-carotene increased the uptake of β-carotene by lymphocytes. The high concentration of β-carotene in lymphocytes may, therefore, protect these immune cells against oxidant stressors and thereby maintain optimum immune function. Furthermore, β-carotene may directly regulate cellular events related to lymphoblastogenesis.[28] The mechanism by which β-carotene modulates immunity is not understood

still now, while, β-carotene may serve as an antioxidant against lipid peroxidation.[29] Moreover, the high concentration of β-carotene in lymphocytes may protect these immune cells against oxidant stressors and thereby maintain optimum immune function. Besides, β-carotene may directly regulate cellular events related to lymphoblastogenesis.[30]

Several investigators reported significant increases in cholesterol, phospholipids, total lipids, and triglycerides under hot summer conditions in white rabbit males,[31] and in LDL, VLDL, and triglycerides in heat-stressed calves.[3,32] And in agreement of our results, β-carotene showed a decrease in plasma cholesterol, LDL, and VLDL when add to the ration.[15,33] However, on contrary, β-carotene supplementation generated serum increases of total cholesterol reported by Meza-Herrera *et al*.[25]

The highest percentage of total β-carotene in the blood was associated with HDL, where, in cattle, carotene is mainly transported in plasma bounded to HDL.[34] Their absorption involves several steps from the breakdown of the food matrix and release of carotenoids into the lumen of the gastrointestinal tract through their incorporation into lymphatic lipoproteins,[35] whereas LDL and VLDL accounted for 35 and 7% of total β-carotene, respectively.

This distribution was in general agreement with another study in calves and is expected because HDL cholesterol accounts for the majority of total blood cholesterol in cattle. β-carotene supplementation increased the concentration of β-carotene in all lipoprotein fractions.[36] In contrast, β-carotene associated with the lipoprotein fractions in untreated calves remained relatively constant across all periods and average 62%, 29%, and 9% for HDL, LDL, and VLDL, respectively.[28]

Ca is needed for the formation of skeletal tissues, normal nervous impulses transmission, blood clotting, and for milk production. Intracellular Ca is also important for enzymatic function and intracellular communication. P is a very important mineral for animals. About 80% is incorporated in bones and teeth together with Ca. P is important for almost all energy transactions that involve formation or breaking of high-energy bonds that link oxides of phosphate to carbon (nitrogen) such as adenosine triphosphate. Another important role is participating in the acid-base buffer systems of the blood and other body fluids. Because of Ca loss by sweating under hot condition as well as in phosphorous, it documented that during periods of heat stress, the supplementation of addition P might be needed; this makes the animal more tolerant to high ambient temperatures than those fed ratios with normal P levels.[37] Moreover Agrawall,[38] noticed that seasonal variation in plasma P concentration may be due to the varying availability of green fodder during a different season. During summer, the rise in ambient temperature was associated with a decrease in plasma sodium, potassium, Ca, and P in Baladi cows. In this study, β-carotene administration resulted in higher serum Ca and P levels than control showed more ameliorative effect against heat stress.

Exposure to environmental heat stress significantly elevated serum liver ALT and AST activities[39] in heat-stressed animals which may attributed to the increase in stimulation of gluconeogenesis by corticoids,[40] but it was reversed on β-carotene supplementation in our results which agreeing with Okechukwu *et al*.[41] β-carotene links to its provitamin-A effects which means that β-carotene exerts its effects by functioning as a precursor of Vitamin A. Thus, β-carotene functions as a lipid-lowering agent and a lipid-soluble antioxidant, and helps to protect the liver against damage by maintaining the levels of the serum total proteins, liver enzymes, and lipid profile.[42]

In agreement with our results Draeger *et al*.,[43] stated that Vitamin A supplementation was significantly decreased serum levels of the liver function biomarkers, AST and ALT. This may be explained by the antioxidant activity of Vitamin A which can protect the liver from free radicals generated by metabolic reactions. Moreover, when the β-carotene considered the most abundant source and nutritional benefits in the diet as provitamin-A,[26] so it may achieve the same results of Vitamin A.

The present data revealed improvement ($P < 0.01$) in thyroid hormones (T_3 and T_4) concentration due to β-carotene supplementation. It has been shown that exposure to high environmental temperatures depresses thyroid activity, whereas exposure to cool environments increases thyroid activity.[44] It is reported that heat stress decreased plasma T_3 and T_3 levels during the summer season helps the animals to decrease the endogenous heat production to tolerate the heat.[45] Moreover El-Masry *et al*.,[39]

recorded a significant decrease in T_3 hormone level under hot conditions in cows.

In agreement with our results, serum T_3 concentration increased in Vitamin A-treated group, while, Vitamin A and its retinoid derivate play an important role in regulation of normal growth and development. Vitamin A has been shown to regulate thyroid hormone metabolism and inhibit thyroid-stimulating hormone (TSH) secretion through downregulation of TSH-β gene expression.[46] β-carotene supplementation of heat-stressed calves showed an enhancement in daily and total gain, DMI, and gain/feed in comparison to control. The results about the depression in growth performance, especially daily gain in growing heat-stressed calves,[47] as well as, the negative changes in protein metabolism, most blood constituents, and hormonal levels,[2] may contribute in such decrease in growth performance for untreated group. Earlier studies of Kohlmeier and Burroughs,[48] Perry *et al*.[49] demonstrated that supplementing feedlot cattle diets that contained 1.2–2.4 mg/kg of β-carotene with 1433–3087 IU/kg VA increased body weight and average daily gain compared to unsupplemented cattle.

The enhancement of growth performance with β-carotene supplementation may be due to that calves have been shown that the immunomodulating effects of beta-carotene include an increased IgG concentration. This can lower the diseases infection cases (such as diarrhea and upper respiratory diseases), together with an increase in growth rate.[50] Carotenoids degradation occurs rapidly by oxidation. This happens mainly by light exposure and solar radiation. Subsequently, when animals ingest carotenoids, these are released from the food matrix during the physical and chemical processes that occur during the chewing and rumen fermentation.[51] Carotenoids flow from the rumen to the small intestine with the presence of double bonds. After it, they are solubilized by the action of bile components and follow the same absorptive pathways as other dietary lipids and become a part of the ruminant micelles absorbed in the intestinal villi.[52] And also, the digestion in ruminants of the total carotenoids in forages should correspond with the digestion of the DM in the forages.[53]

On contrary, supplementation feedlot cattle with increased concentrations of dietary β-carotene did not affect growth performance in terms of daily DMI and body weight gain.[54]

CONCLUSION

From the results cited herein, it can be concluded that supplementation of β-carotene to the diet at the rate of 50,000 IU/calf/day may modify most blood metabolites and improve some immunological and antioxidant indices and thyroid activity. Hence, this level of β-carotene has been able to improve significantly feed efficiency and growth performance of heat-stressed calves. In a way that supports the increase in economic income and improvement of the national revenue in tropical and subtropical regions of different countries, while, high concentration of carotenoids imparts an undesirable yellow color to cattle fat and this presentation positively affects the price and consumption of meat.[53]

DISCLOSURE STATEMENT

The authors report no conflicts of interest.

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