Ameliorating Effect of β-Carotene on Haemato-Biochemical Parameters and Oxidative Stress Status during Gestational Stages in Goats

Anhar I. El-Hanafy1, Abdellkarim I.M. El-Sayed2, Mahmoud R.El-Mahdi2, Hassan A.M. Farghaly1 and Akram A. El-Tarabany1

1. Department of Biological Applications, Radioisotopes Applications Division, Nuclear Research Center, Atomic Energy Authority, Inshas, Cairo, Egypt, P.O.13759
2. Department of Animal Production, Faculty of Agriculture, Benha University, Benha, Egypt, P.O.13736.

Corresponding author: anhar_elhanafy@yahoo.com

Abstract

The possible effects of β-carotene orally supplementation upon gestational stages changes in serum concentrations across time for total protein (TP), albumin (ALB), globulin (GLB), total cholesterol (TC), triglyceride (TG) and alanine transaminase (ALT) and aspartate transaminase (AST), Haemoglobin (Hb), Red blood cells count (RBCs), packed cell volume (PCV), malondialdehyde (MDA), reduced glutathione (GSH), total antioxidant capacity (TAC) and progesterone (P4) were evaluated. Experiments were carried out from May to October and were constructed into two groups of gestational goats (20 does each) with 2–3 years (body weight 26±2kg) crossbred does 50% Zaraibi x 50% Baladi. The first group was considered as control (C) while the other one was β-carotene (BC) group in which 50 mg BC was oral supplemented/day/goat. Serum blood samples were collected in order to quantify progesterone concentrations (P4) through radioimmunoassay. TP, ALB, GLB, TC, TG and PCV were analyzed spectrophotometrically. Oral supplementation with β-carotene significantly increased (p ≤ 0.05) the concentrations of TP, ALB, GLB, GSH, TAC and P4 with the associated significant decrease in TC, TG and MDA concentrations, compared with the control. Gestation stages showed significant differences (p ≤ 0.002 and p ≤ 0.05) in all biochemical parameters, haematological, oxidative stress parameters and P4 level. However, a treatment × gestation stages interaction occurred between treatments for TP, ALT, AST, Hb, RBCs, PCV, MDA, GSH, TAC and P4 (P < 0.002 and p ≤ 0.05) favoring the β-carotene group.

Key words: Goats, gestation, β-carotene, haemato-biochemical parameters, oxidative stress parameters, Hormone}

Introduction

Gestation is one of the important physiological stages in which nutritional needs are enhanced for supporting the growth and development of fetus. Nutrients including β-carotene are transmitted via maternal circulation and placental transfer to the fetus during pregnancy. During pregnancy, females are also more prone to oxidative stress caused by the imbalance between the prooxidant-antioxidant levels (Toescu et al., 2002). Thus, it is most likely that maternal β-carotene deficiency during pregnancy can lead to abnormal physiological function and poor health condition. Supplementation of either vitamin A or its precursor β-carotene promotes an ample range of biological processes such as cellular development, differentiation and morphogenesis through the action of retinoic acid (RA) (Amann et al., 2011). β-carotene is a potent scavenger of free radicals, especially singlet oxygen (Schweigert et al., 2003). Since RA interacts with nuclear receptors, it has the ability to modulate many gene products linked to reproductive performance (Schweigert et al., 2003; Amann et al., 2011).

In herbivorous ungulates, the largest β-carotene accumulation occurs in the liver with cattle and horses reflecting the highest β-carotene liver content, followed by goats, buffalo and sheep. Goats’ β-carotene liver concentration is around 3.4 μg/g tissue (Darwish et al., 2016). Even though an optimal intake of β-carotene is hypothesized to affect ruminant reproduction, both negative (Folman et al., 1987) and positive effects have been reported (Kawashima et al., 2009).

Blood is an important and reliable medium for indicating the health status of individual animals. The blood parameters of animals can be greatly altered by numerous factors such as nutrition, disease, stress, parturition and climate. Therefore the hematological investigations served as basic information for animal health assistance. Previous studies have suggested that β-carotene supplementation can improve the immune function, reproductive and growth performance in goats (Dominic, 2016). Blood antioxidant status, hematological and biochemical parameters of goats can also be influenced by the β-carotene supplemented in diet (El-Demerdash et al., 2004). However, research work about the influence of dietary β-carotene on the antioxidant status, hormone profile and haemato-biochemical parameters of pregnant animals is rather limited. The objective of the present study was to gain insights regarding a possible relationship between β-carotene supplementation of crossbred goat does (50% Zaraibi x 50% Baladi) and some of their blood biochemical, haematological and oxidative stress parameters as well as their hormonal levels, during gestation stages.
Material and methods

Animals and Feeding

The experiment was carried out in the Experimental Farms Project (Goats Farm), Nuclear Research Center, Atomic Energy Authority, Inshas. The experimental goats were fed basal ration of concentrate feed mixture (CFM) according to the allowances of NRC (2007) of goats. The CFM composed of 37.4% wheat bran, 27% yellow corn, 12.5% soybean meal, 10.0% undecorticated cottonseed cake, 5% rice bran, 4% sugarcane molasses, 3% limestone, 1% sodium chloride and 0.1 vitamin and minerals premix. Concentrate feed mixture (3.5% of body weight) was offered once daily at 10 am. Barseem hay was offered ad libitum. Fresh drinking water was available at all time.

Experimental Design

Twenty pregnant female goats aged 2-3 years with average initial body weight 26±2 kg were randomly divided into two equal groups. Animals in the 1st group were fed on basal ration without any additives (control), while the 2nd group was fed basal ration and orally supplemented with 50 mg β-carotene /head /daily.

Ambient temperature, relative humidity and temperature humidity index

The ambient temperature and relative humidity were obtained daily from meteorological station of Atomic Energy Authority during the whole experimental period. The temperature humidity index (THI) was calculated during the whole pregnancy period from (May, 2014 to October, 2014) according to Maraet et al. (2000) as: THI = db°C - [(0.31-0.31 RH) × (db°C-14.4)], where, THI= temperature humidity index, db C= dry bulb temperature in Celsius and RH = relative humidity%. Since the averages of ambient temperature (AT), relative humidity (RH %) and temperature humidity index (THI) were 32.79°C, 76.16% and 31.43, respectively.

Haemato-biochemical parameter analysis

Before the morning feeding two blood samples were collected biweekly from each animal via the jugular vein puncture. The first blood sample (3 ml) was collected into an EDTA tube. In order to determine the hematological parameters, an automated analyzer (Autolysar AL 820, Swiss) was utilized to measure red blood cell (RBC) counts, hemoglobin (Hb) concentration and the total leucocytes count (WBC). The second blood sample was handled to harvest serum samples and persevered at ~ 20°C. Serum total protein was determined via Biuret method (Armstrong and Carr, 1964). Serum cholesterol concentration was calibrated colorimetrically as described by Watson (1960).

Assessment of oxidative stress and hormonal assay

Reduced Glutathione (GSH) expressed as (umol/dl) were detected by High Performance Liquid Chromatography (HPLC) using the method of Jayatilleke and Shaw (1993). Total MDA (umol/dl) (Karatepe, 2004). Total antioxidant capacity (TAC), and was expressed as (nmol/dl) (El-Deeb and Younis, 2009). Hormonal analysis of progesterone (P4), were analyzed by direct radioimmunoassay technique (RIA).

Statistical analysis

Data were expressed as mean ± SE. The data were analyzed statistically by GLM procedure of the SAS program (SAS, 1998). Duncan’s Multiple Range test was used to detect the significant differences among means of the experimental groups (Duncan, 1955).

Using the following model: Yijk = μ + Ti + Sj + (TS)ij + eijk Where: Yijk = the dependent variables estimated, μ = Overall mean, Ti = the effect of treatment (1 = control, 2 = beta carotene) Sj = the effect of gestation stages (1 = Early, 2 = Mid and 3 = Late), TSij = the effect of interaction between treatment and gestation stages, eijk = random error.

Results

Biochemical parameters

Biochemical parameters of pregnant does are presented in Tables 1and2. Total protein (8.730±0.075g/dL), albumin (5.237±0.065g/dL) and Globulin (3.500±0.052g/dL), were significantly (p≤0.05) increased by the β-carotene orally supplementation. While Total cholesterol (139.59±0.351mg/dL), Triglyceride (122.67±0.301 mg/dL) and the activities of ALT(39.50±0.114U/L) and AST (53.04±0.095) were significantly (P<0.001) decreased by the β-carotene orally supplementation when compared with the control. Gestation stages showed a significant (p≤0.05) effect and late gestation showed the highest mean concentration for TP, ALB, GLB, TC, TG concentrations and ALT and AST activities. Interestingly the interaction between β-carotene and gestation stages showed significant differences, except for ALB, GLB and TC the interaction showed no significant differences, such differences favored the β-carotene group.
Table 1. Serum biochemical parameters of does during gestation, as affected by treatment, gestation stages and their interactions.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment (T)</th>
<th>Gestation stages (S)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP (g/dL)</td>
<td>ALB (g/dL)</td>
<td>GLB (g/dL)</td>
</tr>
<tr>
<td>Control</td>
<td>6.16^B±0.074</td>
<td>3.70^B±0.064</td>
<td>2.46^B±0.051</td>
</tr>
<tr>
<td>Beta carotene</td>
<td>8.73^A±0.075</td>
<td>5.24^A±0.065</td>
<td>3.50^A±0.052</td>
</tr>
</tbody>
</table>

Means with different letters (A, B and C or a, b, c,...) in the same column within the same factor are significantly different at (P<0.05).

TP= Total protein; ALB=Albumin; GLB=Globulin.

Table 2. Serum biochemical parameters of does during gestation as affected by treatment, gestation stages and their interactions.

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment (T)</th>
<th>Gestation stages (S)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ALT (U/L)</td>
<td>AST (U/L)</td>
<td>TC (mg/dl)</td>
</tr>
<tr>
<td>Control</td>
<td>40.20^B±0.115</td>
<td>57.99^B±0.096</td>
<td>142.00^B±0.352</td>
</tr>
<tr>
<td>Beta carotene</td>
<td>39.50^B±0.114</td>
<td>53.04^B±0.095</td>
<td>139.59^B±0.351</td>
</tr>
</tbody>
</table>

Means with different letters (A, B and C or a, b, c,...) in the same column within the same factor are significantly different at (P<0.05).

ALT= Alanine aminotransferase; AST= Aspartate aminotransferase; TC= Total Cholesterol; TG=Tri glyceride.

Haematological parameters

Haematological parameters of pregnant does are presented in Table3. Hb (9.36±0.063 g/dl), RBCs (11.30±0.064 10^6/µl), PCV% (27.10±0.068 %), and WBCs (10.70±0.071 10^3/µl) were significantly (p<0.05) increased by the β-carotene orally supplementation, when compared with the control. Gestation stages showed a significant (p<0.05) effect and earlygestation showed the highest mean for Hb concentration, RBCs and WBCs counts. Interestingly the interaction between β-carotene and gestation stages showed significant differences, such differences favored the β-carotene group for Hb concentration, RBCs count and PCV%. Except for WBCs count the interaction showed no significant differences.
Oxidative stress status parameters and hormonal level

Oxidative stress status parameters of pregnant does are presented in Table 4. MDA (1.09±0.022 umol/dL), GSH (7.09±0.069 umol/dL), TAC (7.70±0.074 mmol/dL), and P4 (24.89±0.074 ng/mL) were significantly (p<0.05) increased by the β-carotene orally supplementation, when compared with the control. Gestation stages showed a significant (p<0.05) effect and mid gestation showed the highest mean for MDA, GSH, TAC and P4 concentrations while the lowest ones were in early gestation for MDA, GSH and P4 and late gestation for TAC. Interestingly the interaction between β-carotene and gestation stages showed significant differences, such differences favored the β-carotene group for MDA, GSH, TAC and P4 concentration.

Discussion

Current results support our working hypothesis in that β-carotene supplementation improved haematobiochemical, oxidative stress status and hormonal levels during gestation stages. β-carotene supplementation promotes the increasing, across gestation period, in blood metabolites specifically TP, ALB, GLB. The main increases were observed towards the late pregnancy. Despite our fragmentary knowledge regarding the mechanisms modulating the intermediate metabolism (Meza-Herrera and Tena-Sempere, 2012), results of our study suggest that such neurophysiologic scenario observed in the β-carotene supplemented gestational goats may potentially involve BC as an acting molecule involved in the intermediate metabolism, specially upon protein, carbohydrate, and lipid metabolism. However, TC and TG were decreased by supplementation of β-carotene while the interaction between β-carotene and its precursor lycopene, suppress cellular cholesterol synthesis from acetate, but not from mevalonate in a concentration-dependent manner. This inhibition was concomitant with a stimulation of the LDL receptor in macrophages which could lead to enhanced of LDL from the plasma. Levy et al (1993) and Levy et al (1995) previously have shown that carotenoids can bind to lipoproteins and to macrophages (Levy et al, 1996) and to affect their oxidative state.

Table 3. Haematological parameters of does during gestation as affected by treatment, gestation stages and their interactions.

<table>
<thead>
<tr>
<th>Item</th>
<th>Hb (g/dl)</th>
<th>RBCs (10^6/µl)</th>
<th>PCV (%)</th>
<th>WBCs (10^3/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8.72±0.064</td>
<td>10.91±0.053</td>
<td>26.47±0.069</td>
<td>9.70±0.074</td>
</tr>
<tr>
<td>Beta carotene</td>
<td>9.36±0.063</td>
<td>11.30±0.052</td>
<td>27.10±0.068</td>
<td>10.10±0.071</td>
</tr>
<tr>
<td><strong>P values</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>Gestation stages (S)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>9.35±0.055</td>
<td>11.68±0.059</td>
<td>26.75±0.060</td>
<td>10.23±0.064</td>
</tr>
<tr>
<td>Mid</td>
<td>8.90±0.054</td>
<td>10.48±0.058</td>
<td>25.62±0.061</td>
<td>9.55±0.063</td>
</tr>
<tr>
<td>Late</td>
<td>8.89±0.053</td>
<td>11.15±0.057</td>
<td>27.99±0.062</td>
<td>9.96±0.062</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td><strong>0.002</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.05</strong></td>
</tr>
<tr>
<td>(T * S)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>8.90±0.106</td>
<td>10.56±0.114</td>
<td>26.50±0.119</td>
<td>9.90±0.129</td>
</tr>
<tr>
<td>Mid</td>
<td>8.41±0.107</td>
<td>10.16±0.113</td>
<td>25.03±0.118</td>
<td>9.30±0.128</td>
</tr>
<tr>
<td>Late</td>
<td>8.88±0.108</td>
<td>11.00±0.112</td>
<td>27.89±0.117</td>
<td>9.90±0.127</td>
</tr>
<tr>
<td>Beta carotene</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early</td>
<td>9.80±0.101</td>
<td>11.80±0.117</td>
<td>27.00±0.116</td>
<td>10.50±0.126</td>
</tr>
<tr>
<td>Mid</td>
<td>9.40±0.102</td>
<td>10.80±0.116</td>
<td>26.20±0.115</td>
<td>9.80±0.125</td>
</tr>
<tr>
<td>Late</td>
<td>8.90±0.103</td>
<td>11.30±0.115</td>
<td>28.10±0.114</td>
<td>10.01±0.124</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.05</strong></td>
<td><strong>0.02</strong></td>
</tr>
</tbody>
</table>

Means with different letters (A, B and C or a, b, c, …) in the same column within the same factor are significantly different at (P<0.05).

Hb= Haemoglobin; RBCs= Red blood cells count; PCV= Packed cell volume; WBCs= White blood cells count.
These results may partly were discussed. Concerning P4 picant - which can generally be used as a al antioxidants to remove harmful and from environmental stressors, thereby function as natur environmental sources of free radicals. Vitamin A can protected its immune responses to certain suppletion rat with the vitamin A or β Bendich and Shapiro (1986) immune function of animals regarded that improving antioxidant status enha its supplementation in diet.

on increasing the levels of RBCs, Hb, and PCV upon alterations in membrane fluidity, which account to those of Bendich (1991), AMAR et al. 2000 and El-Demerdashet al. (2004) whom illustrated that carotenoids have been implicated in enhancing the immune response such as proliferation, induction of specific effector cells as well as the secretion of cytokines. β-carotene levels up to 200 mg kg⁻¹ diet enhanced some immune parameters like serum complement and total plasma immunoglobulin in the experimental rainbow trout. Moreover, immuno-enhancement of β-carotene involves the quenching of free radicals or the lowering of lipid peroxide levels and alterations in membrane fluidity, which account on increasing the levels of RBCs, Hb, and PCV upon its supplementation in diet. These results may partly due to theantioxidant activity of vitamin A. It has been regarded that improving antioxidant status enhanced immune function of animals (Grimble, 2001).

Bendich and Shapiro (1986) reported that supplementation rat with the vitamin A or β-carotene protected its immune responses to certain environmental sources of free radicals. Vitamin A can function as natural antioxidants to remove harmful free radicals produced through normal cellular activity and from environmental stressors, thereby maintaining the structural integrity of immune cells (Chew, 1996).

Malondialdehyde is a good indicator of lipid peroxidation which can generally be used as a biomarker for radical induced damage and endogenous lipid peroxidation (Wang et al., 2008). In the present study, MDA concentration in the serum was reduced by inclusion of β-carotene supplementation. This may be due to what stated by Tsuchihashi et al. (1995) that beta-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 alpha-tocopherol or betacarotene may be an important mechanism for reducing oxidative stress. And what had demonstrated by Salem (2015) that the supplementation offlycopene, β-carotene or their mixture in rats resulted in a significant reduction of liver MDA, and asficient elevation of liver and blood glutathione (GSH), in comparison with the levels of high fat dietgroup. Thesupplementation offlycopene, β-carotene or their mixture resulted in a significant reduction of liver MDA, and asficient elevation of liver and blood glutathione (GSH), in comparison with the levels of high fat dietgroup.

The present results elucidated that β-carotene elivated progesterone(P4) concentration during gestation periods. A number of studies have reported either positive effect or no effect of supplemental β-carotene on concentration of reproductive hormones in various animal species. However, limited studies have been conducted in goats, therefore studies from other species were discussed. Concerning P4
concentrations, contradicting results have been reported. It was found that the P4 concentration was increased through supplemental β-carotene in cattle (Greenberg et al., 1986) and goats (Arellano-Rodriguez et al., 2009).

Conclusion
The current study reported that β-carotene supplementation (50mg/day/doe) generates serum biochemical, haematological, oxidative stress parameters as well as progesterone concentrations across gestation stages in does goats.

References


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