Research Article

Monitoring of Antioxidant Vitamins Concentrations in Some Ovine Diseases

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Abstract | Recently, antioxidant vitamins were prescribed in different treatment and prophylactic programs for human and animal. Unfortunately, few studies investigated their concentrations in different sheep diseases. So, this paper aimed to assess the antioxidant vitamins (E, A, D_3 and C) levels in some sheep physiological and pathological conditions. For this purpose, 200 barki ewes were divided into control group, inflammatory diseases groups (pneumonic group, arthritic group, enteric group), late pregnant groups (normal late pregnant group, pregnancy toxemia group), postpartum groups (normal postpartum group, endometric group, mastitic group and non-inflammatory postpartum disorders group). Blood samples were collected from all ewes and vitamins concentrations were estimated in all groups. The results of this work demonstrated a significant ($P< 0.05$) decrease in vitamin E, A, D_3 and C concentrations in inflammatory diseases groups, late pregnant groups and postpartum groups. Conclusion: antioxidant hypovitaminosis partially participate in different ovine diseases pathogenesis and complications and their supplement for healthy and diseased animals is strongly recommended.

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Introduction

Antioxidant vitamins are organic compounds, necessary for optimum animal and human health. They include vitamin E (α-tocopherol), A (β-carotene), D_3 (cholicalciferol) and C (ascorbic acid) (Nayyar and Jindal, 2010; Das et al., 2013). In human medicine, different researchers referred to their protective effect against wide range of lung, joint, gastrointestinal, periparturient and postpartum diseases (Gangwar et al., 2008; Tahan et al., 2011; Liu et al., 2014; Jalili et al., 2014; Hanson et al., 2016; Yan et al., 2015; Yang and Li, 2015; Pontes et al., 2015; Kheirkhah et al., 2016; Merriman et al., 2018; Myint et al., 2019). They also can reduce pain and minimize inflammatory diseases complications, in addition to their significant role in patient's therapeutic responses enhancement (Kostoglou-Athanassiou et al., 2012; Hansen et al., 2014; Mahomed, 2017; Sharifi et al., 2016).

Unfortunately, most of the available studies in veterinary medicine investigated their effect as feed supplement either for health or diseased animals, rather than monitoring their concentration during ovine diseased conditions. Hence, this study aimed to assess the antioxidant vitamins concentrations (E, A, D_3 and C) in three ovine inflammatory diseases (pneumonia, arthritis, enteritis), sheep normal late pregnancy and pregnancy toxemia, ovine normal postpartum and postpartum endometritis, mastitis and non-inflammatory postpartum disorders.

Materials and Methods

Following the ethical guidelines of animal handling and treatment, this study was carried on 200 barki ewes (3-4 years) from veterinary units at different Matrouh governorate cities, they were divided into:

Control group (CG): 20 apparently healthy ewes neither pregnant nor postpartum (normal ranges of body temperature, pulse rate, respiration rate, good body scores, normal reflexes and appetite).

Inflammatory diseases groups: 60 ewes neither pregnant nor postpartum, subclassified in: Pneumonic group (PG): 20 ewes with respiratory signs (off food, low body weight, nasal and ocular discharges, crusts around nose, elevation in body temperature (40-41°C) and pulse rate, rapid deep abdominal respiration, crackles and wheezes in auscultation). Arthritic group (AG): 20 ewes with swollen joints, lameness, some unable to stand, high body temperature and pulse rate, no appetite, severe pain in examination and low body weight. Enteric group (EG): 20 ewes with sever diarrhea, dehydration, emaciation, no appetite, fever.

Late pregnant groups: 40 late pregnant ewes (15-30 days before lambing) and subclassified into: Normal late pregnant group (NLPG): 20 late pregnant ewes have physiological ranges of body temperature (38-39ºC), body weight, pulse rate (70-80 beat/min), respiration rate, appetite, eye and ear reflexes, BHB < 3 Umo/L, they had normal parturition as well as postpartum period. Pregnancy toxemia group (PTG): 20 late pregnant ewes, suffered from sudden loss of appetite and reflexes, isolated themselves from the rest of the flock, BHB ≥ 3 Umo/L. Later, dullness, emaciation, recumbancy, head dropping, acetone breath, rapid respiration (35-45 breaths/min), low body temp (35-36ºC) and pulse rates (60-65 beat/min) were observed on the animal. Finally, Advanced hypoglycemic encephalopathy, defenses, blindness, tremors, muscle convulsions, smashing mouth, frothy salivation, coma and death. Postmortem examination, revealed dead multiple fetuses, pale large liver (hepatic fatty degeneration).

Postpartum groups: 80 postpartum ewes (15-30 days after parturition), subclassified into: Normal postpartum group (NPG): 20 ewes with normal lambing and postpartum stage. Endometric group (EnG): 20 postpartum ewes with high temperature, offensive colored uterine discharge, loss of appetite, depressed). Mastitic group (MC): 20 postpartum ewes feverish, off food, swollen udder, painful in examination, abnormal milk color and odor, teat cracks). Non-inflammatory postpartum disorders group (NIPG): 20 postpartum ewes suffered from dystocia (5 ewes, usually primiparous females with large fetus), hypocalcaemia (5 ewes suffered from tremors, recumbancy, cold extremities, rapidly respond after calcium supplement), uterine prolapse (5 ewes) and retained placenta (5 ewes).

Blood samples were collected from all ewes by jugular vein puncture in a tube containing heparin calcium 5000 I.U., then blood was centrifugated at 3000 r.p.m for 20 minute and hepranized plasma was collected in clean tightly closed eppendorf tubes. The storage of collected plasma was at -80°C. Later, this plasma was used for vitamins (E, A, D_3) determination by ELISA kits of MyBioSource® Company. While, ascorbic acid (vit C) plasma concentrations were estimated spectrophotomically by kits supplied by Biodiagnostic® Company.

Statistical analysis

All vitamins concentrations were displayed as mean ± standard deviation (SD). SPSS® program version 23 was used to assess the differences between the estimated vitamins means (one-way ANOVA test) and the post hoc differences between means (a multiple comparison Tukey`s HSD test. A difference was considerable significant at $P < 0.05$.

Results and Discussion

Concerning the antioxidant vitamins levels in the inflammatory diseases groups: Table 1 illustrated a significant decline ($P < 0.05$) in vitamin E, A, C, D_3 concentrations in PG, AG, EG in relation to CG. While, these vitamins levels non-significantly changed between PG, AG and EG. Similarly, the Table 2 cleared a significant ($P < 0.05$) decrease in vitamin E, A, C, D_3 plasma concentrations between NLPG, PTG and CG, and between NLPG and PTG. Table 3 explained a significant ($P < 0.05$) reduction in the estimated vitamins levels between NPG, EnG, MG, NIPG and CG, between EnG, MG and NPG, and between NIPG and NPG, EnG, MG.

Table 1: *Comparison between the antioxidants vitamins concentrations in PG, AG, EG and CG. Values are means±SD.*

Parameter	CG	PG	AG	EG
Vit E (µmol/L)	49.07±6.78[d]	14.09±1.48[a]	13.94±1.52[a]	15.54±1.70[a]
Vit A (ng/ml)	5.20±0.82[d]	1.46±0.06[a]	1.46±0.07[a]	1.45±0.07[a]
Vit D3 (ng/ml)	49.68±5.20[d]	13.72±1.08[a]	13.73±1.08[a]	13.69±1.04[a]
Vit C (mg/L)	18.20±1.44[d]	5.07±0.27[a]	5.17±0.48[a]	5.02±0.37[a]

[a]: significant with CG; [b]: significant with PG; [c]: significant with AG; [d]: significant between the four groups; considered significant when P<0.05.

Table 2: *Comparison between the antioxidants vitamins concentrations in NLPG, PTG and CG. Values are means±SD.*

Parameter	CG	NLPG	PTG
VitE (µmol/L)	49.07±6.78[c]	36.70±1.44[a]	26.70±1.44[a,b]
VitA (ng/ml)	5.20±0.82[c]	3.65±0.22[a]	2.36±0.13[a,b]
VitD3 (ng/ml)	49.68±5.20[c]	33.06±1.60[a]	24.67±2.27[a,b]
VitC (mg/L)	18.20±1.44[c]	13.37±1.41[a]	9.03±1.02[a,b]

[a]: significant with CG; [b]: significant with LPG; [c]: significant between the three groups; considered significant when P<0.05.

Table 3: *Comparison between the antioxidants vitamins concentrations in NPG, EG, MG, NIPG and CG. Values are means±SD.*

Parameter	CG	NPG	EnG	MG	NIPG
VitE (µmol/L)	49.07±6.78[e]	36.31±2.38[a]	14.48±1.15[a,b]	14.34±1.14[a,b]	26.70±1.44[a,b,c,d]
VitA (ng/ml)	5.20±0.82[e]	3.64±0.19[a]	1.45±0.07[a,b]	1.47±0.06[a,b]	2.37±0.13[a,b,c,d]
VitD3 (ng/ml)	49.68±5.20[e]	33.19±1.64[a]	13.74±1.09[a,b]	13.83±1.05[a,b]	22.95±1.83[a,b,c,d]
VitC (mg/L)	18.20±1.44[e]	13.80±1.62[a]	5.17±0.59[a,b]	5.22±0.54[a,b]	8.85±1.09[a,b,c,d]

[a]: significant with CG; [b]: significant with NPG; [c]: significant with EnG; [d]: significant with MG; [e]: significant between all the groups; considered significant when P<0.05.

Antioxidant vitamins or non-enzymatic antioxidants are these vitamins which work on keeping oxidant/antioxidant balance in order to prevent the appearance of oxidative stress which a major predisposing factor for different animal and human diseases (Nayyar and Jindal, 2010; Das et al., 2013). Beside their action as antioxidants they have a role in immune response regulation, cellular proliferation and differentiation and reducing malignancy and aging. Furthermore, they are important for the anti-inflammatory interleukin (IL-10) production and their deficiency is tightly connected with multiplication of the incidence, complications and severity of respiratory, joints, gastrointestinal, peripartum and postpartum diseases (Raman et al., 2011; Caram et al., 2015; Mahomed, 2017; Chin and Ima-Nirwana, 2018).

In accordance to this assumption, a pronounced decrease in vitamins E, A, C and D_3 concentrations was obtained in all studied groups in the current work. Generally, this hypovitaminosis in all investigated groups was attributed to the oxidative stress which is an expected outcome to the pro-inflammatory cytokines stimulation in the inflammatory diseases groups. As this pro-inflammatory cytokines promote extensively the free radical generation which consume the enzymatic anti-oxidants and disrupt the oxidant/antioxidant balance leading to the appearance of the oxidative stress and the subsequent outstanding hypovitaminosis. Similar findings were mentioned before in animal or human pneumonia (Elnisr et al., 2012), arthritis (Rodríguez-Carrio et al., 2004), enteritis (Sahinduran and Albay, 2004).

On the other hand, the oxidative stress was assigned in the normal late pregnancy group to its physiological role in facilitating placental blood movement from mother to her fetus and vice versa through increasing endothelial cell proliferation and cell death inhibition during the late pregnancy. The high fetal oxygen needs in the mother circulation and the augmented lipids peroxidation process take apart in this oxidative stress occurrence. Furthermore, the increased NEFAs and BHB concentrations due to catabolic energy shift in late pregnancy and pregnancy toxemia is a reasonable cause for elevated pro-inflammatory

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cytokines production and depended raised free radicals liberation and oxidative stress. obviously, the remarkable decreases in the antioxidant vitamins concentrations observed between PTG compared to NLPG, is connected to the higher levels of NEFAs and BHB and related higher pro-inflammatory cytokines activation in PTG in relation to NLPG (Darwish, 2019).

Similarly, the postpartum stage normally associated with NEFAs and BHB accumulation and raised pro-inflammatory cytokines activity. No doubt, the pro-inflammatory action and the oxidative stress will be more observable in postpartum pathological conditions (inflammatory or non-inflammatory) than the physiological postpartum stage due to stress and pain. Meanwhile, the distinguishable hypovitaminosis in EnG and MG related to NIPG indicated a higher degree of oxidative stress because of possibility of infection and presence of microorganism and more tissue damages. Thereby, the suspected inflammatory immune response will be more potent and oxidative stress will be exaggerated. This data agreed with previous data reported in normal postpartum period (Milad et al., 2001), endometeritis (Kaya et al., 2017), mastitis (Dimri et al., 2013; El-Deeb, 2013) and NIPG (LeBlanc et al., 2002; LeBlanc et al., 2004).

Additionally, all above-illustrated physiological and diseased conditions, anorexia and decreased available vitamins in the animal rations played a fundamental role in the noticed hypovitaminosis and in maximizing the aforementioned oxidative stress through lowering enzymatic antioxidants synthesis due to trace element deficiency (Nayyar and Jindal, 2010; Das et al., 2013). Logically, destruction of intestinal mucosa and villi and decrease intestinal absorptive surface is a more specific reason for vitamins deficiency in EG (Sahinduran and Albay, 2004). While increased fetal growth requirements is another possible explanation for this hypovitaminosis in NLPG (Darwish, 2019).

Finally, it can be concluded that vitamins supplements will be a non-expensive addition to our therapeutic as well as prophylactic measures in sheep pneumonia, arthritis and enteritis and they are strongly recommended to reduce physiological stressors during the late pregnancy and postpartum stages in sheep to decrease the incidence rate of pregnancy toxemia and postpartum endometritis, mastitis and non-inflammatory postpartum disorders respectively.

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Author's Contribution

All authors equally contributed to the work.

Statement of conflict of interest

The authors have declared no conflict of interest.

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