Dept. of Animal Prod.,
Fac. of Agric., Minia Univ., Minia, Egypt.

PHYSIOLOGICAL REACTIONS AND GROWTH PERFORMANCE OF LAMBS SUPPLEMENTED BY VITAMIN A WITH ZINC UNDER SUMMER CONDITIONS

(With 5 Tables)

By

E.B. SOLIMAN

(Received at 23/5/2005)

الإستجابات الفسيولوجية وأداء النمو للحملان نتيجة التأثير المشترك للإمداد بفيتامين أ والزنك تحت ظروف الصيف

عصام بسيونى سليمان

أجريت هذه الدراسة على عدد ٢٤ من الحملان الاوسيمى (عمر ٣ شهور) بمتوسط وزن ١٧,٥٤ ± ٠,٩٥ كجم وذلك لتقييم تأثير إمداد هذه الحملان بفيتامين أ والزنك علي أداء النمو والأستجابات الفسيولوجية تحت ظروف الصيف. قسمت الحملان عشوائيا إلى أربعة مجموعات متساوية الأولى للمقارنه (الكنترول) بينما أعطيت حملان المجموعة الثانية فيتامين أ عن طريق الفم بمعدل ١٠,٠٠٠ وحدة دولية / رأس / إسبوعيا ، واعطيت حملان المجموعة الثالثة الزنك بمعدل ٣٠ ملجم/ كجم مادة جافة بينما أعطيت حملان المجموعة الرابعة فيتامين أ بمعدل ١٠,٠٠٠ وحدة دولية / رأس / إسبوعيا+الزنك بمعدل ٣٠ ملجم/كجم مادة جافة. وقد أظهرت النتائج :- أن حملان المجموعة الثانية والثالثة والرابعة سجلت قيم أعلى معنويا فى متوسطات معدل الزيادة اليومية فى الوزن وزيادة معنوية فى كفاءة التحويل الغذائى مقارنة بالكنترول. سجلت المجموعة الرابعة قيم أعلى معنويا فى متوسطات وزن الجسم النهائى مقارنة بالكنترول. سجلت المجموعة الرابعة قيم أعلى معنويا فى متوسطات معدل الزيادة اليومية فى الوزن ، وزن الجسم النهائى وكفاءة التحويل الغذائى مقارنة بالمجموعة الثانية والثالثة. أظهرت حملان المجموعة الثانية والرابعة زيادة معنوية فى تركيز هيموجلوبين الدم، متوسط هيموجلوبين الخلايا، متوسط تركيز هيموجلوبين الخلايا مقارنة بالكنترول. لوحظت زيادة معنوية فى العدد الكلى لكرات الدم البيضاء فى المجموعة الرابعة مقارنة بالكنترول. أظهرت حملان المجموعة الثانية والرابعة انخفاض معنوى فى نسبة الكرات المتعادلة مقارنة بالكنترول بينما زادت نسبة الكرات الليمفاوية فى المجموعات الثانية والثالثة والرابعة مقارنة بالكنترول. لوحظ زيادة معنوية فى محتوى البلازما من البروتينات الكلية والألبيومين فى المجموعات الثانية والثالثة والرابعة مقارنة بالكنترول مع زيادتها معنويا فى المجموعة الرابعة مقارنة بالمجموعة الثانية والثالثة. كما لوحظ زيادة معنوية فى مستوى الجلوبيولين فى البلازما للمجموعة الثالثة والرابعة مقارنة بالكنترول.

أظهـــرت حمـــلان المجموعة الثانية والرابعة زيادة معنوية فى محتوى البلازما من فيتامين أ مقارنة بالكنترول بينما زاد محتوى البلازما من الزنك فى المجموعات الثالثة والرابعة مقارنة بالكنـــترول. زاد محـــتوى الـــبلازما معنويا من هرمون تراى ايودو ثيرونين (T_3) لحملان المجموعـــة الثانية والثالثة والرابعة مقارنة بالكنترول مع زيادة معنوية فى تركيز هرمون T_3 لحمـــلان المجموعـــة الرابعة مقارنة بالمجموعة الثانية والثالثة. أظهرت النتائج أن معاملات التجربة لم يكن لها تأثير معنوى على بعض القياسات مثل معدل استهلاك الغذاء يوميا، درجة حـــرارة المستقيم، معدل التنفس، معدل النبض، كرات الدم الحمراء، النسبة المئوية لمكونات الـــدم الخلويـــة، حجـــم مكونـــات الدم الخلوية، كرات الدم البيضاء حمضية الصبغ، الكرات الأحاديـــة والكـــرات قاعدية الصبغ وبعض مكونات البلازما مثل الجلوكوز واللبيدات الكلية. نستنتج من هذه الدراسة أن التأثير المشترك لإمداد الحملان الذكور النامية بفيتامين أ + الزنك تحت ظروف الصيف أدى إلى تحسين اضافي معنوى في أداء النمو نتيجة لزيادة الاستجابات الفســـيولوجية وكفـــاءة الانشطة الحيوية لهذه الحملان مقارنة بتلك التى عوملت بفيتامين أ او الزنك فقط.

SUMMARY

Twenty four males of Ossimi lambs averaged 3 months of age and 17.54 ± 0.95 kg body weight were used during summer to evaluate their growth performance and physiological reactions to supplemental vitamin A, zinc (Zn) and vitamin A with Zn. Animals were randomly divided into 4 equal groups (6 lambs in each). First group served as control while the second group (T1) received oral administration of vitamin A (retinol) at 10.000 IU/head/weekly, and the third group (T2) received 30 mg Zn/kg DM (132.2 mg Zn sulfate/kg DM), and the fourth group (T3) received vitamin A at 10.000 IU/head/weekly with 30 mg Zn /kg DM. Results showed that averages of daily weight gain (DWG) increased ($P<0.01$) for lambs of T1, T2 and T3 compared with control. Lambs of T3 had higher ($P<0.05$) averages of final body weight (FBW) than control. Feed conversion (FC) rates were improved ($P<0.05$) for T1, T2 and T3 compared to control. Averages of DWG, FBW and FC were improved ($P<0.05$ and $P<0.01$) for T3 than those of T1 and T2. Blood Hb, MCH and MCHC increased ($P<0.05$ and $P<0.01$) for lambs received T1 and T3 compared to control. Total count of leucocytes increased for T3 lambs compared to control. T1 and T3 lambs showed marked decrease ($P<0.05$) in neutrophils compared with control. T1, T2 and T3 exhibited significant ($P<0.05$) increases in lymphocytes compared with control. Lambs of T1, T2 and T3 exhibited higher ($P<0.05$) levels of plasma total protein than control, and its concentration in T3 was greater ($P<0.05$) than T1 and T2. Plasma albumin levels were higher ($P<0.05$) in all treatments than control while plasma globulin increased ($P<0.01$) for T2

and T3 lambs compared to control. Plasma vitamin A concentrations were greater (P<0.05) for T1 and T3 than control. Plasma Zn increased (P<0.01) for T2 and T3 lambs comparing to control. Plasma T_3 hormone concentrations were greater (P<0.001) for T3 lambs than those of T1 and T2. No significant changes observed in feed intake, rectal temperature, respiration rate, pulse rate, blood RBC, PCV, MCV, eosinophils, basophils, monocytes %, plasma concentrations of glucose and total lipids for lambs received T1, T2 and T3 compared with control. The results indicated that lambs received supplemental vitamin A with Zn exhibited more favorable signs in their physiolgical reactions than those received each of vitamin A or Zn alone, indicating a synergistic relationship between both nutrients and reflecting superiority of their efficient metabolic activities and growth response under summer conditions.

***Key words**: Vitamin A, Zinc, Growing Lambs, Physiological Reactions.*

INTRODUCTION

Deficiencies of trace mineral and vitamin for livestock are common, affecting their productivity, and are associated with a wide variety of clinical, physiological and pathological disorders. Deficiency can be caused by inadequate intake or by the presence of antagonists in the diet interfere with absorption and/or metabolism. Vitamin A plays an essencial role in some animal physiological functions including stimulation of growth, proper development of skeletal tissue, cell division and differentiation. So, its deficiency could be resulted in clinical signs such as metabolic disorders and growth retardation (Pond *et al.*, 1995). Animal immune function and health could be impaired by inadequacies in vitamin A and ß-carotene as antioxidant defence (Chew, 1987). Shortage of green fodder resources during summer led to the lack of vitamin A. Also, farm animal's requirements for vitamin A increased during hot summer months (Swells, 1993).

On the other hand, importance of Zn as an essential nutrient has been recognized for many years and over 200 Zn-dependent enzymes have identified in all major biochemical pathways in animal body. Zn has been associated with appetite growth, male sexual development and wound healing. There are no significant stores of body Zn, so the animal must rely on a daily supply to meet requirements (Mayland *et al.*, 1987). Zn plays a key role in the immune system, thereby; Zn deficiency causes decreased immunity and loss of T-cell function (Shankar and Prasad,

1998). Thus, the amounts of vitamin A and Zn needed for immuno-enhancement in ruminants are higher than the suggested required amounts by NRC (Nockles and Blair, 1996).

In growing calves, El-Masry *et al.* (1998) showed an interactive effect for Zn with vitamin A to improve growth performance and immune response. In addition, supplemental vitamin A to ewes at late pregnancy and during suckling period improved growth and physiological reactions of their male lambs (Soliman, 2002). Zn status influences vitamin A metabolism, including its absorption, transport, and utilization (Parul and Keith, 1998). Vitamin A has stimulatory effect on Zn absorption (Bersin, 1988). So, deficiency of either vitamin A or Zn can trigger chronic problems in the metabolism of both nutrients (Bondi and Sklan, 1984). In sheep, however, this synergistic effect of vitamin A and Zn has not been fully documented.

This study, therefore, focused some mechanistic aspects through which vitamin A, Zn and vitamin A with Zn supplementation may influence growing lambs' performance and related physiological reactions under summer conditions.

MATERIALS and METHODS

Twenty four males of Ossimi lambs averaged three months of age and 17.54 ± 0.95 kg body weights were used in this experiment for 10 weeks during the months of July, August and September at the farm of Animal Production Department, Faculty of Agriculture, El-Minia University. Animals were randomly divided into 4 equal groups (6 lambs in each). First group served as control while the second group received oral administration of vitamin A (retinol) at 10.000 IU/head/week, and the third group received 30 mg Zn (as Zn sulfate, $ZnSO_4.7H_2O$)/kg DM (132.2 mg Zn sulfate/kg DM), and the fourth group received vitamin A at 10.000 IU/head/weekly with 30 mg Zn/kg DM. Animals were fed on concentrate feed mixture and bean straw to cover their nutrient requierments according to their live body weight. They were fed on bean straw in amounts that represent 1% of their body weights. The constituents of concentrate feed mixture and its feeding values calculated according to NRC (1985) are presented in Table 1. The calculated concentrations of Zn and ß-carotene in concentrate mixture were 51.11 mg/kg DM and 1.50 mg/kg DM, respectively. The NRC requirements for growing lambs are 69 µg of ß-carotene/kg live weight/day (47 IU of vitamin A/kg live weight/day) and 33 mg Zn/kg DM. The treated

animals received 1.5 mg/kg DM of dietary ß-carotene with 10.000 IU/head/week of vitamin A as oral administration. In case of Zn, they were fed on concentrate mixture contained 51.11 mg Zn/kg DM and supplemented by 30 mg Zn/kg DM as Zn sulfate. Feed was offered twice a day at 8 a.m and 2 p.m and mineral blocks and drinking water were available to the animals all times. The animals were apparently healthy and proved to be free from internal and external parasites.

Body weights of lambs were recorded at starting the experiment and at biweekly thereafter. Feed intakes were recorded daily. Averages of daily gain and feed conversion rates of lambs were calculated. The absolute weight of lambs gives an idea about the weight development during experimental period; the growth was measured and expressed in percentage relative to the body weight in order to compare the different groups in relation to its relative growth rate.

$$\text{Relative growth rate} = \frac{(W_2 - W_1) \times 100}{\frac{1}{2}(W_2 + W_1)}$$

Measurements of rectal temperature (RT), respiration rate (RR) and pulse rate (PR) were recorded biweekly for lambs at 8-9 a.m. Averages of ambient temperature and relative humidity during the experimental period were 27.50 °C and 63.0 % respectively at 8-9 a.m. Heparinized blood samples (5 ml) were collected biweekly at 8 a.m. from the jugular vein of each animal before feeding and drinking. Whole blood samples were analyzed shortly for blood hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) and leucocyte counts using conventional methods. Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated mathematically. Stained blood smears with Lieshman's stain were prepared for the differential leucocytes count (Dacie and Lewis, 1991). Plasma samples obtained and stored at -20°C utill assayed for biochemical analysis. Plasma Zn concentrations determined using atomic absorption spectrophotometry. Plasma vitamin A concentrations determined using the method described by Neeld and Pearson (1963). Plasma total protein, albumin, total lipids and glucose measured spectrophotometrically using standard test kits supplied from Bio-Merieux (Marcy-1, Etolie Charbonnieres- Les Bains, France) and Bio-Analytics kits (USA). Globulin calculated mathematically by substracting the difference between total protein and albumin. Plasma triiodothyronine (T_3) concentrations was determined by a direct solid-phase I^{125} radioimmunoassay techniques using (coat-A-

count TKT_3) RIA kits purchased from diagnostic products corporation (DPC, LA, CA, 90045-559, USA).

The data were analyzed by least square means analysis of variance using General Linear Models (GLM) procedure of the statistical analysis system (SAS, 1992). The model used to analyze the different traits studied for lambs was as follows:

$Y_{ij} = \mu + T_i + e_{ij}$. Where: Y_{ij}= ij^{th} Observation, μ = Population mean; T_i = Effect of i^{th} treatments and eij= Random error. Duncan's Multiple Range test was used to detect differences between means of the experimental groups (Duncan, 1955).

RESULTS

Averages of daily weight gain (DWG) increased ($P<0.01$) by 16, 20 and 44 % for lambs received vitamin A, Zn and vitamin A with Zn, respectively compared with control (Table 2). Lambs received vitamin A with Zn had higher ($P<0.05$) averages of final body weight (FBW) by 17 % than control. Feed conversion (FC) rates were improved ($P<0.05$) by 12.6, 14.2 and 25.4 % for vitamin A, Zn and vitamin A with Zn-supplemented lambs compared to control. Differences in feed intake (FI) for lambs due to treatments were not significant but tended to increase by 6.6 % with vitamin A with Zn than control. Averages of DWG, FBW and FC were improved ($P<0.05$ and $P<0.01$) for vitamin A with Zn-supplemented lambs than those each of vitamin A or Zn alone.

As shown in Table 3, supplemental vitamin A, Zn and vitamin A with Zn had no significant effect on thermal and cardio-respiratory responses (RT, RR and PR) of lambs. There were no significant change in blood RBC, PCV and MCV for lambs received vitamin A, Zn or vitamin A with Zn compared with control. Significant increases amounted by 15.5 and 17.5 % in concentrations of blood Hb ($P<0.01$); by 8.9 and 9.7 % in MCH ($P<0.05$); and by 13.6 and 12.3 % in MCHC ($P<0.05$) observed for lambs received vitamin A and vitamin A with Zn, respectively compared with control.

Table 4 shows that total count of leucocytes increased ($P<0.05$) by 15 % for vitamin A with Zn-supplemented lambs. Vitamin A and vitamin A with Zn-supplemented lambs showed marked decrease in neutrophils % by 20.0 and 24.4 % repectively compared with control. Supplemental vitamin A, Zn and vitamin A with Zn induced significant ($P<0.05$) increases in lymphocytes % by 13.5, 7.3 and 20.3 % respectively compaared with control. Results indicated that lymphocyte

% was greater (P<0.05) for vitamin A with Zn-supplemented lambs than those of Zn alone. Insignificant changes were observed in eosinophils, basophils and monocytes percentages due to experimental treatments.

Table 5 shows that vitamin A, Zn and vitamin A with Zn-supplemented lambs exhibited higher (P<0.05) plasma total protein by 9.4, 15 and 27.8 % than control, and its concentration due to vitamin A with Zn was greater (P<0.05) than each of vitamin A or Zn alone. Plasma albumin increased (P<0.05) by 18.2, 15.2 and 21.2 % respectively for vitamin A, Zn and vitamin A with Zn-supplemented lambs compared to control. Plasma globulin increased (P<0.01) by 14.8 and 32.7 % respectively for Zn and vitamin A with Zn-supplemented lambs than control. Vitamin A or vitamin A with Zn induced greater (P<0.05) increase in plasma vitamin A by 22.0 and 31.8 % respectively than control. Plasma Zn increased (P<0.01) by 21.0 and 28.9 % respectively for Zn and vitamin A with Zn-supplemented lambs than control. Plasma T_3 concentrations increased (P<0.001) by 16.5, 27.1 and 46.6 % respectively for vitamin A, Zn and vitamin A with Zn respectively compared to control. No significant changes observed in plasma glucose and total lipids concentrations due to treatments.

DISCUSSION

This study showed that supplementation of each vitamin A or Zn alone to growing lambs enhanced their growth performane with no significant changes in their FI. Lambs given vitamin A or Zn alone recorded higher averages of DWG and better FC rates comparing with non-supplemented lambs. This improvement may signify a higher efficiency of feed utilization for those lambs. These findings on lambs are consistent with some studies working on other ruminants. El-Masry *et al.* (1998) noticed an improvement in growth performance of growing calves to vitamin A or Zn, but they failed to show a significant increase in DWG with vitamin A. Vitamin A has a major function in metabolism to preserve stability, structural intergrity and normal permeability of cell and subcellular membranes, by which it has positive effects on tissue biosynthesis and growth promotion (Chew, 1993). Vitamin A has a role in regulating energy homeostasis by enhancing uncoupling protein 1 (UCP1) mRNA gene expression and decreasing serum leptin levels (Kumar *et al.*, 1999). Thus, clinical signs such as metabolic disorder, reduced feed efficiency, slowed gains and growth retardation could be occurred with lack of vitamin A (Pond *et al.*, 1995). Zn may exert its

effect via affecting the activity of thymidin kinase, requiered for DNA synthesis and cell division (Harper *et al.*, 1979). Zn enhanced metabolic processes and many enzymes systems, which are concern with utilization and metabolism of feed nutrients, require Zn for proper functioning both as a part of the molecule and as an activator (McDowell *et al.*, 1993). As a result of early Zn deficiency in ruminants, feed intake, feed efficiency and growth rate will be reduced.

It is important to notice that supplemental vitamin A with Zn had a significant additive effect to improve growth performance since lambs received vitamin A with Zn recorded higher averages of DWG and FBW as well as better FC rates than each of vitamin A or Zn alone. This finding may indicate a synergistic role for vitamin A and Zn to improve nutrient absorption and utilization for enhancing growth. Zn may play a beneficial role in this respect. Supplemental Zn (170 mg/kg diet) increased DWG, FC, and digestibility of nutrients with no changes in their feed intake (Abd El-Rahim *et al.*, 1995). Nitrogen retention was increased in ram lambs fed Zn-supplemented diet at 30 ppm compared with Zn-deficient animals, suggesting that protein utilization is impaired in sheep with Zn deficiency (Somers and Underwood, 2003).

Supplementation each of vitamin A and Zn to lambs had no significant effect on their thermal and cardio-respirstory reactions, maintaining their RT, RR and PR under hot summer conditions. This indicated that each of supplemental vitamin A and Zn was adequate to maintain normal thermoregulation and adaptive responses of growing lambs. In this regard, vitamin A supplementation to ewes had no significant effect on RT, RR and PR either for ewes or their male lambs (Soliman, 2002). Hot conditions are believed to interfere with the animal's ability to convert carotene to vitamin A and to depress the efficiency with which vitamin A can be used to meet needs, and this may increase the animal's requirements for vitamin A (Swells, 1993). Also, thermoregulation could be negatively altered in Zn-deficient animals (Topping *et al.*, 1981).

Blood Hb, MCH and MCHC were changed up for vitamin A and vitamin A with Zn-supplemented lambs. An increase in Hb values amounted by 8.7 % were observed for lambs born to vitamin A-supplemented ewes (Soliman, 2002). With this respect, vitamin A deficiency may associated with altered Fe metabolism, including reduced plasma Fe and sometimes anemia; and this effect does not appear to be caused by increased RBC destruction (Pond *et al.*, 1995). With supplemental Zn, those parameters were not significantly different from

control but they were lowered than with vitamin A or vitamin A with Zn. There is a wide margin of safety between Zn requierments and amount that are toxic in sheep. The excessive and toxic levels of Zn has been described for growing lambs to be between 750 and 1000 mg/kg diet, reducing their FI, DWG and FC rates (NRC, 1985). When Zn supplied at those excessive levels, it may interfere with Fe absorption and utilization by impairment of iron incorporation into ferritin (Underwood, 1977); and induce severe Cu deficiency in sheep (NRC, 1985). However, plasma Cu and Fe concentrations were not altered by elevated dietary Zn up to 0.65 % in cattle (Gaynor *et al.,* 1988); and feeding Zn (100 mg/kg DM) did not negatively impact liver Cu status in sheep (Hatfield *et al.,* 2001). Supplemental Zn at 30 mg/kg DM, in the present study, did not change blood hematology indicating that such level of Zn was adequate for normal hematopoieses. It could be noticed that changes in blood hematological parameters studied were within the normal values of sheep (Blunt, 1975).

Vitamin A-supplemented lambs exhibited an increase in lymphocyte % accompanied with a decrease in their neutrophils. Supplemental Zn also increased lymphocytes %, but it had no significant effect on neutrophils. These findings indicated that each of vitamin A or Zn may benefit immune function in sheep. Some reports focused the essencial role of both micronutrients in animal immune system. *In vitro* study on cattle, vitamin A stimulated lymphocytes function and proliferation (Tjoelker *et al.,* 1988b), while its effect on neutrophils function was null or negative, since its phagocytosis was suppressed (Tjoelker *et al.,* 1988a). Enhanced proliferation of lymphocytes and immune function was noticed in ß-carotene- or vitamin A-supplemented cows (Michal *et al.,* 1994). This protective role of vitamin A may be mediated stimulating immunoglobulin synthesis, stimulating naturall killer cells and cytotoxic T-lymphocytes (Dennert, 1984); and enahncing resistance to intracellular pathogens (Chew, 1993). Vitamin A influences broad aspects of leucocyte function in terms of DNA synthesis and increased mitogen-stimulated mononuclear leucocytes, suggesting that the bioavailability of this vitamin may alter immune competency and disease susceptability of newborn calves (Nonnecke *et al.* 2000). According to Shankar and Prasad (1998), Zn plays a role in T-lymphocyte activation and signal transduction since it implicated in early steps of T- lymphocyte activation via stimulating autophosphorylation of tyrosine residues, and subsequent phosphorylation of the T-lymphocyte receptor complex by which the subsequent changes, through protein

phosphorylation, regulate activation and lymphocyte cell proliferation. This immunologic role of Zn could be mediated basic cellular functions such as DNA replication, RNA transcription, cell division, and cell activation. Zn also is crucial for normal development and function of neutrophils and natural killer cells. Thus, maintaining of neutrophils and monocytes and activating of lymphocytes of growing lambs to supplemental Zn may considered a useful response to improve their immune function, disease resistance, and general health enhancing their adaptability against adverse environmental conditions. In case of vitamin A with Zn, there was a higher total count of leucocytes than each of vitamin A or Zn alone; and higher increase in lymphocytes % than with Zn alone. This result might indicate a synergistic role of both micronutreints to enhance immune status in sheep. In cattle, an increase in leucocytes count was noticed in response to supplemental vitamin A with Zn accompanied with a rise in some immune indices such as α1, β and γ-globulin levels (El-Masry *et al.,* 1998). In addition, weaned calves fed Zn-supplemented milk exhibited higher IgG and IgM responses, showing a stronger humoral immune response, probably as a result of the beneficial effect of Zn on the interaction between T helper cells and B cells (Prasad and Kundu, 1995).

In the present study, some plasma factors were positively changed up due to vitamin A, Zn or vitamin A with Zn. There was an additive effect for vitamin A with Zn to increase plasma total protein and globulin levels than each vitamin A or Zn alone. This increase could be ascribed to the role of Zn which is required for normal protein synthesis and metabolism. Research evidence the important of Zn on the efficiency of utilizing of absorbed amino acids in protein synthesis for growing lambs and calves (McDowell, 1995). Also, supplemental Zn increased digestibility of nutrients and apperent absorption and retention of nitrogen (Abd El-Rahim *et al.,* 1995). These findings indicate that protein metabolism might be altered for the trend towards higher growth performance which attained, in the present study, for vitamin A with Zn-supplemented lambs. In additon, complementary role of vitamin A with Zn was apparent to elevate plasma globulin concentrations. This finding may related to the improved globulin fraction levels attributed to activity of supplemental Zn in lambs (Kegley and Spears, 1995); and Zn or Zn with vitamin A in growing calves, reflecting their improved immune function and growth responses (El-Masry *et al.,* 1998).

The increase in plasma vitamin A levels for vitamin A-supplemented lambs agree with similar trend reported in ruminants as

dairy cows (Mehrez, 1989); buffaloes (El-Barody *et al.*, 1993) and suckling ewes (Soliman, 2002). In Japanese Black beef calves, the increase in plasma vitamin A was associated with an elevation in plasma insulin like-growth factor-1 (IGF-1) in clinically healthy calves compared to retarded growth calves (Ishibashi *et al.*, 1999). Plasma vitamin A levels tended to be insignificantly greater by 7.9 % due to vitamin A with Zn than vitamin A alone. This may indicated that adding Zn to vitamin A supplementation improves vitamin A status. Zn is necessary to maintain normal concentrations of plasma vitamin A (Harper *et al.*, 1979), via affecing aspects of vitamin A metabolism, including its absorption, normal mobilaztion of vitamin A from liver, and utilization (Parul and Keith, 1998). They explained this dependence via regulatory role of Zn in vitamin A transport mediated through protein synthesis, and the oxidative conversion of retinol to retinal that requires the action of a Zn-dependent retinol dehydrogenase enzyme. In accordance to Rahman *et al.* (2002), Zn deficiency is accompanied by reduced circulating retinol concentrations in which supplementation with vitamin A alone fails to correct; and when the animals are given either Zn supplements or Zn-containing diets, their serum retinol concentrations do improve, while Zn-deficient rats showed a simultaneous reduction in retinol and retinol binding protein (RBP). The increases in plasma vitamin A may related to increased plasma total protein, in this study, since vitamin A is transported from the liver to peripheral tissues as free retinol bound to RBP. So, it is indicated that when there may be a reduction in serum total protein, it may be observed that vitamin A levels are low (Harper *et al.*, 1979). In the same respect, protein deficiency causes reduced plasma vitamin A concentrations which will be reduced as a result from reduced transport of vitamin A from liver because of reduced serum albumin, the carrier protein for vitamin A in blood (Pond *et al.*, 1995). This may explain the increase in plasma albumin and total protein due to vitamin A or vitamin A with Zn.

Supplemental Zn or vitamin A with Zn appear to provide for additional increases in plasma Zn for growing lambs revealing an increase in their Zn absorption. These results are compatible with those reported on plasma Zn concentrations in response to supplemental Zn (Gaynor *et al.*, 1988); and Zn or vitamin A with Zn (El-Masry *et al.*, 1998) in other ruminants. Plasma Zn levels have been reported to be in ranges 20-40, 50-80 and 80-140 µg/dl respectively for Zn-deficient, Zn-marginal and Zn-adequate diets in ruminants (Kincaid, 1999). In the present study, averages of plasma Zn levels were 70.5, 73.5, 85.3 and

90.9 µg/dl respectively for control, vitamin A, Zn and vitamin A with Zn-supplemented lambs. This may indicate that lambs supplemented with Zn or Zn with vitamin A were Zn-adequated. Serum Zn values increased from 44 µg/dl in Zn-deficient sheep to 78 µg/dl when they were given a supplemental Zn (Soliman *et al.*, 1988).

This study showed an additive effect for vitamin A with Zn to increase plasma T_3 than each of vitamin A or Zn alone. Thyroxin stimulates conversion of carotenoids to vitamin A therby vitamin A deficiency reduces thyroxin secretion (Pond *et al.*, 1995). However, such synergistic effect of vitamin A with Zn on thyroid activity seems to be attributed more to the role of Zn. Thyroid T_3 and T_4 levels and hypothalamic TRH content were declined in Zn-deficient compared with Zn-adequate rats (Morley *et al.*, 1980). They noticed that iodine uptake was similar between the two groups, suggesting that Zn-deficiency interferes with the deiodinase enzyme conversion of T_4 to T_3. Zn deficiency decreased serum T_3 and free T_4 by approximately 30% with a decrease in hepatic type I 5'deiodinase activity by 67% with Zn deficiency compared to Zn-adequate rats, showing that Zn deficiency affects the metabolism of thyroid hormones (Kralik *et al.*, 1996). This decline in thyroid function exhibited histopathological changes such as atrophy and degeneration in the follicles, concluding that decreasing serum T_3 and T_4 due to Zn deficiency was related to thyroid lesions (Gupta *et al.*, 1997). It appears likely that Zn is required for biological functioning of T_3 and its related receptors indicating that T_3 and Zn play important roles in growth and also via interacting with the somatotrophic axis at multiple levels (Hedley *et al*, 2001). These finding together with the present study may support that supplemental vitamin A with Zn is a metabolic requirement for higher thyriod activity enhancing productive performance of sheep.

In conclusion, the present study showed that combined supplementation of vitamin A with Zn appreciably exerted beneficial effects than each of vitamin A or Zn alone in enhancing growth performance of male lambs under summer conditions. These lambs received supplemental vitamin A with Zn exhibited more favorable signs in their physiolgical reactions than those received each of vitamin A or Zn alone, indicating a synergistic relationship between both nutrients and reflecting superiority of their efficient metabolic activities. Further study may be needed to determine this useful combined effect of both nutrients on reproductive performance of those male lambs.

REFERENCES

Abd El-Rahim, M.I.; El-Gaffary, M.N.; Tawfeek, M.I.; El-Kelawy, H.M.; Rawia, S.A.; and Abd El-Rahim, M.I. (1995): Effect of supplementation with different levels of Zn on growth performance, nutrient digestibility, mineral metabolism, blood constituents, organ histipathology and reproductive efficiency in NZW rabbita. Egypt. J. Rabbit Sci. 5:11-31.

Bersin, N.I. (1988): Interrelation between vitamin A and zinc in animals. Vestnik Sel'skohozaistvennoi Nauki, 1: 106-111.

Blunt, M.H. (1975): The Blood of Sheep: Composition and Function. Springer-Verlag, Berlin. Heidelberg. New York.

Bondi, A. and Sklan, D. (1984): Vitamin A and carotene in animal nutrition. Prog. Food Nutr. Sci., 8:165-91.

Chew, B.P. (1987): Vitamin A and ß-carotene on host defence. J. Dairy Sci., 70: 2732.

Chew, B.P. (1993): Role of carotenoids in the immune response. J. Dairy Sci., 76: 2804-2811.

Dacie, S.J. and Lewis, S.M. (1991): Practical hematology. 7th Ed., Churchill Livingstose. Pages 118-127.

Dennert, G. (1984): Retinoids and the immune system immuno-stimulation by vitamin A Pages 373-390 in the retinoids. M.B. Sporn, A.B. Roberts and D.C. Goodman, ed. Academic Press, NY, USA.

Duncan, D.B. (1955): Multiple range test and multiple F-test. Biometrics, 11: 1-42.

El-Barody, M.A.A.; Rabie, Z.B.H. and El-Feel, F.M.R. (1993): Productive and reproductive responses of pregnant Egyptian buffaloes to vitamin A injection during summer. Minia J. Agric. Res. & Dev., 15: 717-733.

El-Masry, K.A.; Youssef, H.M.; Abdel-Samee, A.M.; Maria, I.F.M. and Metawally, M.K. (1998): Effects of supplemental Zn and vitamin A on some blood biochemical and immune indices related to growth performance in growing calves. First international conference on animal production and health in semi-arid areas, El-Arish, Egypt, 1-3 Sept., 130-151.

Gaynor, P.J.; Montgomery, M.J. and Holmes, C.R. (1988): Effect of zinc chloride or zinc sulphate treatment of protein supplement on milk production. J. Dairy Sci. 71: 2175-2180.

Gupta, R.P.; Verma, P.C. and Garg, S.L. (1997): Effect of experimental zinc deficiency on thyroid gland in guinea-pigs. Ann. Nutr. Metab., 41(6): 376-381.

Harper, H.A.; Rodwell, V.W. and Mayes, P.A. (1979): Review of physiological chemistry. 17th Ed., Lange Medical Puplications, Los Altos, California, USA. Pages 147-151.

Hatfield, P.G.; Swenson, C.K.; Kott, R.W.; Ansotegui, R.P.; Roth, N.J. and Robinson, B.L. (2001): Zinc and copper status in ewes supplemented with sulphate- and amino acid- complexed forms of zinc and copper. J. Anim. Sci., 79: 261-266.

Hedley, C.F.; Kristen, E.G.; Krishna, G.; Chunli, H. and Steven, A.Z. (2001): Actions and interactions of thyroid hormone and zinc status in growing rats. J. Nutr., 131:1135-1141.

Ishibashi, M.; Ushinohama, K; Kamimura, S. and Hamana, K. (1999): Blood concentrations of growth hormone, insulin-like growth factor-1, thyroid hormone and vitamin A in Japanese Black calves with retarded growth. J. Jap. Vet. Med. Assoc. 52: 427-430.

Kegley, E.B. and Spears, J.W. (1995): Immune response and erformance of sheep fed supplemental zinc as zinc oxide or zinc methionine. Sheep and Goats Res. J., 11 (3): 127-131.

Kincaid, R.L. (1999): Assessment of trace mineral status of ruminants: A review, Proceedings of the American Society of Animal Science,

Kralik, A.; Eder, K. and Kirchgessner, M. (1996): Influence of zinc and selenium deficiency on parameters relating to thyroid hormone metabolism. Horm. Metab. Res., 28(5): 223-226.

Kumar, M.V.; Sunvold, G.D. and Scarpace, P.J. (1999): Dietary vitamin A supplementation in rats: Suppression of leptin and induction of UCP1 mRNA. J. Lipid Res., 40: 824-829.

Mayland, H.F., Kramer, T.R. and Johnson. W.T. (1987): Trace elements in the nutrition and immunological response of grazing livestock. In: Proceedings, Grazing Livestock Nutrition Conference. Univ. of WY., Jackson Hole. July 23-24, 1987

McDowell, L.R.; Conrad, J.H. and Hembry, F.G. (1993): Mineral for Grazing Ruminants in Tropical Regions., pp: 42

McDowell, L.R. (1995): Minerals in Animal and Human Nutrition. Academic Press, Inc. NY.

Mehrez, A.E.F. (1989): Effect of vitamin A and its derivatives on reproduction and milk production in cattle. M.Sc. Thesis. Fac. Agric., Mansoura Univ.

Michal, J.J.; Heirman, L.R.; Wong, T.S.; Frigg, M. and Volker, L. (1994): Modulatory effect of dietary beta-carotene on blood and mammary leukocytes function in periparturient dairy cows. J. Dairy Sci. 77: 1408-1421.

Morley, J.E.; Gordon, J. and Hershman, J.M. (1980): Zinc deficiency, chronic starvation, and hypothalamic-pituitary-thyroid function. Am. J. Clin. Nutr., 33(8): 1767-1770

Neeld, J.B. and Pearson, W.N. (1963): Macro and micromethods for the determination of serum vitamin A using trifluroacetic acid. J. Nutr., 79: 454.

Nonnecke, M.A.; Fowler, M.A. Pesch, B.A.; Miller, B.L.; Horst, R.L.; Johnson, T.E.; Perry, H.B.; Housken, D.E. and Harp, J.A. (2000): Effect of dietary vitamin A (VA) and E (VE) on function and composition of circulating leukocyte populations from milk replacer-fed neonatal calves. J. Dairy Sci., 83, Suppl.1.

Nockels, C.F. and Blair, R. (1996): Antioxidants improve cattle immunity following stress. Anim. Sci. & Tech., 62: 59-68.

NRC, (1985): Nutrient requierments of sheep. 6th Ed., Washington, D.C. National Academy Press. Pages 22-23.

Parul, C. and Keith, P.W. Jr (1998): Interactions between zinc and vitamin A: an update Am. J. Clin. Nutr. 68 (Suppl): 435-441.

Pond, W.G.; Church, D.C. and Pond, K.R. (1995): Basic animal nutrition and feeding. 4th Ed. Jhon Wiley & Sons. New York. USA. Pages 223-229.

Prasad, T. and Kundu, M.S. (1995): Serum IgG and IgM responses to sheep red blood cells (SRBC) in weaned calves fed milk supplemented with Zn and Cu. Nutr., 11(5): 712-715.

Rahman, M.; Wahed, M. and Fuchs, G. (2002): Synergistic effect of zinc and vitamin A on the biochemical indexes of vitamin A nutrition in children. Am J Clin Nutr; 75: 92-98.

SAS, (1992): SAS/STAT Guide for personal computers, SAS Inst., Cary. N.C. USA.

Sewell, H.B. (1993): Vitamins for Beef Cattle. Department of Animal Sci. Curators of the University of Missouri, USA.

Soliman, E.B. (2002): Effect of vitamin A supplementation on some physiological reactions of ewes and their male lambs during suckling period. Assuit Vet. Med. J., 47: 67-79.

Soliman, H.B.; Abdelrahim, A.I.; Zakia, A.M. and Shommein, A.M. (1988): Zinc deficiency in sheep: Field cases. Trop. Anim. Health Prod. 20: 47-51.

Shankar, A.H. and Prasad, A.S. (1998): Zinc and immune function: the biological basis of altered resistance to infection. Am. J. Clin. Nutr., 68 (suppl): 447–463.

Somers, M. and Underwood, E.J. (2003): Studies of zinc nutrition in sheep. 2. The influence of zinc deficiency in ram lambs upon the digestibility of the dry matter and the utilization of the nitrogen and sulphur of the diet. Aust. J. Agric. Res., 20: 899-903

Tjoelker, L.W.; Chew, B.P. and Tanaka, T.S. (1988a): Bovine vitamin A and ß-carotene intake and lactational status. 1. Responsiveness of peripheral blood polymorphonuclear leukocytes to vitamin A and ß-carotene challenge in vitro. J. Dairy Sci., 71: 3112-3119.

Tjoelker, L.W.; Chew, B.P. and Tanaka, T.S. (1988b): Bovine vitamin A and ß-carotene intake and lactational status. 2. Responsiveness of mitogen-stimulated peripheral blood lymphocytes to vitamin A and ß-carotene challenge in vitro. J. Dairy Sci., 71: 3120-3127.

Topping, D.L.; Clark, D.G. and Dreosti, I.E. (1981): Impaired thermoregulation in cold exposed zinc deficient rats. Nutr. Rep. Int., 24: 643-648.

Underwood, E.J. (1977): Zinc. In: Trace Elements in Human and Animal Nutrition 4th Ed., Academic press, NY, USA.

Table 1: Constituents of concentrate feed mixture and its feeding values on dry matter basis.

Constituents	DM (%)	CP (%)	CF (%)	TDN (%)	ME (Mcal/kg)	β-carotene (mg/kg)	Zinc (mg/kg)
Corn yellow (30 %)	30	3.03	0.66	26.1	0.95	0.6	4.20
Wheat bran (30 %)	30	3.00	3.39	21.3	0.77	0.9	34.20
Rice bran (31 %)	31	4.37	3.97	22.94	0.83	-	8.99
Cotton seed meal (6%)	6	2.71	0.72	3.90	0.15	-	3.72
Limestone (2 %)	2	-	-	-	-	-	-
Salt (1 %)	1	-	-	-	-	-	-
Total	100	13.11	8.74	74.24	2.70	1.5	51.11

Table 2: Effects of vitamin A and Zn supplementation on growth performance of male lambs (mean ± SEM).

	Treatments				
Parameters	Control	T1	T2	T3	± SEM
IBW (kg)	17.35	17.50	17.30	17.99	0.97
FBW (kg) *	26.10 b	27.65 b	27.80 b	30.57 a	1.35
DWG (kg/day) **	0.125 c	0.145 b	0.150 b	0.1797 a	3.35
Relative growth rate (%)	40.5 b	45.1 b	46.4 b	51.8 a	2.77
FI (kg/head/day)	1.37	1.39	1.41	1.47	0.07
FC (kg feed/kg gain) *	10.96 a	9.58 b	9.40 b	8.18 c	0.66

a,b,c means within the same row having different superscripts significantly different (* P<0.05 and ** P<0.01).
T1 = Vitamin A, T2 = Zn, T3 = Vitamin A with Zn.
IBM = Initial body weight, DWG = Daily weight gain, FBW = Final body weight, FI = Feed intake, FC = Feed conversion.

Table 3: Effects of vitamin A and Zn supplementation on thermal, cardiorespiratory responses and hematological parameters (mean ± SEM).

	Treatments				
Parameters	Control	T1	T2	T3	± SEM
R.T (° C)	39.40	39.38	39.60	39.54	0.04
R.R (r.p.m)	47.55	48.45	50.33	53.00	1.45
P.R (pulse/min.)	62.05	61.32	63.20	63.65	2.50
RBC ($x10^6/mm^3$)	9.80	10.50	9.50	10.60	0.42
Hb (g/dl) **	9.70 b	11.20 a	9.20 b	11.40 a	0.73
PCV (%)	31.5	32.0	29.9	32.5	1.05
MCH (µ/µg) *	9.80 b	10.67 a	9.67 b	10.75 a	0.12
MCV (Cu/µ)	32.1	30.5	31.5	30.7	1.56
MCHC (%) *	30.8 b	35.0 a	30.77 b	34.6 a	1.30

a,b means within the same row having different superscripts significantly different (* P<0.05 and ** P<0.01).
T1 = Vitamin A, T2 = Zn, T3 = Vitamin A with Zn
RT=Rectal temperature, RR=Respiration rate, PR=Pulse rate, RBC=Red blood cells, Hb=Hemoglobin, PCV=Packed cell volume, MCH= Mean corpuscular hemoglobin, MCHC= mean corpuscular hemoglobin concentration

Table 4: Effects of vitamin A and Zn supplementation on total leucocytes count and its differential cell percentages (mean ± SEM).

	Treatments				
Parameters	Control	T1	T2	T3	SEM
Total leucocytes ($x10^3/mm^3$) *	7.35 b	7.80 b	7.85 b	8.45 a	0.25
Neutrophils (%) **	35.80 a	28.65 b	32.0 a	27.05 b	0.15
Eosinophils (%)	3.70	3.40	3.50	3.30	0.07
Basophils (%)	1.0	1.0	1.0	1.0	0.00
Lymphocyes (%) *	55.90 c	63.45 ab	60.00 b	67.25 a	2.64
Monocytes (%)	3.60	3.50	3.50	3.65	0.05

a,b,c means within the same row having different superscripts significantly different (* P<0.05 and ** P<0.01).
T1 = Vitamin A, T2 = Zn, T3 = Vitamin A with Zn

Table 5: Effects of vitamin A and Zn supplementation on plasma biochemical constituents (mean ± SEM).

	Treatments				
Parameters	Control	T1	T2	T3	± SEM
Glucose (mg/dl)	53.50	51.85	54.35	59.85	4.40
Total protein (g/dl) *	7.22 c	7.90 b	8.30 b	9.20 a	0.22
Albumin (g/dl) *	3.30 b	3.90 a	3.80 a	4.00 a	0.08
Globulin (g/dl) **	3.92 c	4.00 c	4.50 b	5.20 a	0.11
Total lipids (mg/dl)	120.3	118.0	125.2	127.5	5.41
Vitamin A (µg/dl) *	13.46 b	16.45 a	13.90 b	17.75 a	0.75
Zn (µg/dl) **	70.5 b	73.5 b	85.3 a	90.9 a	4.45
T_3 (ng/ml) ***	1.33 c	1.55 b	1.69 b	1.95 a	0.08

a,b,c means within the same row having different superscripts significantly differen (* P<0.05, ** P<0.01, *** P<0.001).
T1 = Vitamin A, T2 = Zn, T3 = Vitamin A with Zn