



## Serum Vitamin A and Vitamin E Levels of Growing Lambs Infected or Not with Gastrointestinal Nematodes and Fed a Diet Containing Clinoptilolite

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**ABSTRACT** : The objective was to assess the concentrations of Vitamins A and E in blood of growing lambs infected or not with gastrointestinal nematodes (GIN) and fed a diet containing clinoptilolite. Twenty-four male lambs were used. A 2×2 factorial design consisting of two feeding treatments (B and Z) and two levels of parasitic status, infected (I) and uninfected (U) was used. Lambs were randomly assigned to one of four (n = 6), groups: BU (basal-uninfected), BI (basal-infected), ZU (zeolite-uninfected) and ZI (zeolite-infected). Lambs of groups BI and ZI were infected with a single dose of 15,000 L<sub>3</sub> larvae of GIN. Blood samples were collected from individual animals at the start of the experiment and, thereafter, at 15-day intervals. The average blood serum vitamin A and vitamin E, concentration in lambs (mean±SD) was 0.25±0.090 µg/ml and 1.59±0.769 µg/ml, respectively. Lambs fed Z diet had higher values of vitamin A (p<0.001), but lower values of vitamin E (p<0.01) when compared with those fed B diet. (**Key Words** : Nutrition, Clinoptilolite, Nematodes, Vitamin A, Vitamin E, Sheep)

### INTRODUCTION

The importance of vitamins as dietary components in animal nutrition was identified in the early 1900s. All vitamins have diverse functions and are significant for animal health and performance. Their importance in ruminant nutrition is well documented (Ames, 1958; NRC, 1985; Annicchiarico and Taibi, 2004). In ruminants, amongst the different vitamins only vitamins A and E must be provided exclusively from the diet (NRC, 1985). Vitamin A is necessary for all cellular division and differentiation and plays a key role in regulation of keratinisation and immune cell function. On the other hand, vitamin E is a biological antioxidant in mammalian cell membranes,

providing protection against free radicals. In sheep, several studies have documented the beneficial effects of vitamins A and E in general health, disease resistance and performance (e.g. Rooke et al., 2004; Bendich, 2004; Debier et al., 2005). Hence, the experimental evidence from a wide variety of disciplines has resulted in vitamins A and E being added to commercial concentrates for many years (Annicchiarico and Taibi, 2004).

However, evidence regarding the interaction between parasitism with gastrointestinal nematodes (GIN) and nutrition on serum vitamin A and E concentrations in sheep or other ruminants is scarce. Gastrointestinal parasitism is considered a major issue in sheep production systems because it is related with reduced animal productivity and compromised animal welfare (Sykes and Greer, 2003; Hoste et al., 2005; Liu et al., 2005). The common approach towards control of parasitic infections relies heavily on anthelmintic drugs, but recently much research effort has been directed towards various alternatives to control GIN of ruminants (Coop et al., 1995; Hoste et al., 2005). One such alternative has been the use of clinoptilolite (a natural zeolite) in the diet of growing lambs infected or not with gastrointestinal nematodes (Deligiannis et al., 2005). This latter study showed that a diet containing clinoptilolite

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Received May 29, 2009; Accepted December 24, 2009

reduced significantly the parasitic burden of infected lambs. It should be noted here that the European Commission has provisionally authorised the use of clinoptilolite of volcanic or sedimentary origin as an additive in feedstuffs for farm animals (European Commission Regulation 2200/2001).

## MATERIALS AND METHODS

### Animals and management

The study was carried out using blood samples from lambs used in the experiment described in detail by Deligiannis et al. (2005). Twenty-four entire male lambs of the Karagouniko breed were used. At the commencement of the experiment the average body weight (BW) of lambs was 20.0 (SD 0.98) kg. All lambs were housed in individual pens, with straw for bedding, in a sheep shed that was naturally ventilated. Each individual pen measured 1×1.5 m and was equipped with feed boxes for concentrate and hay as well as a plastic trough providing a constant supply of fresh water. The lambs had been vaccinated against different types of clostridia and, on arrival to the experimental unit, they were de-wormed with a single dose of albendazole (7.5 mg/kg; Albendazole, Veterin<sup>®</sup>, S.A.). All lambs were free from trematodes and tapeworms.

Lambs were given free and continuous access to a nutritionally non-limiting pelleted concentrate that was either a basal diet (B) or a “zeolite diet” (Z). The latter (Z) was formulated by supplementing B with clinoptilolite at a level of 3%. The concentrate pellets (3 mm) contained 191 g crude protein (CP)/kg DM and 11 MJ metabolisable energy (ME)/kg DM. Both diets were formulated to be relatively high in protein and energy content in order to support the potential growth of lambs; they were also non-limiting in minerals and vitamins (NRC, 2001; Infascelli et al., 2005). The lambs were given free and continuous access to fresh water. In addition to the pelleted concentrate, each lamb received a daily allowance of about 100 g of unchopped lucerne hay, containing 162 g CP/kg DM and 7.8 MJ ME/kg DM, to ensure normal rumen function. Intake of concentrates was recorded in the morning of the same day each week.

### Natural zeolite

The zeolitic material used was a Clinoptilolite-rich tuff that was obtained from the Palaeogene zeolite-rich tuffs of the Evros area, North-Eastern Greece. Clinoptilolite is a naturally occurring zeolite in Greece, which, in common with the other natural or synthetic zeolites, belongs to a family of crystalline aluminosilicate minerals. They have a three-dimensional, porous structure consisting of SiO<sub>4</sub> and AlO<sub>4</sub> tetrahedra joined by shared oxygen atoms that are responsible for specific cation exchange capacity. The latter,

combined with the porosity of clinoptilolite, provides many of its very useful properties (Kantiranis et al., 2002). The tuff was crushed in an industrial crusher and the 0.8 mm fraction was homogenised. The mineralogical composition showed that it contained 88% calcium rich clinoptilolite, 5% plagioclase-feldspar, 4% mica and clay minerals and 3% quartz.

### Experimental design

Immediately after the acclimatization period, which lasted for 21 days, the lambs were randomly assigned to one of four (n = 6) treatment groups, taking into account their BW (average BW: 24.0 (SD 1.67) kg). A 2×2 factorial design consisted of two feeding treatments (B and Z) and two levels of parasitic status, infected (I) and uninfected (U) was used. The first two groups were comprised of lambs that were fed on the basal diet (B) and were either uninfected or infected with GIN, hence called BU and BI, respectively. The other two groups were comprised of lambs that were fed on the “zeolite diet” (Z) and were either uninfected or infected with GI nematodes, hence called ZU and ZI, respectively. Lambs of groups BI and ZI were infected with a single dose of 15000 L3 larvae of GI nematodes (3 ml×5000 L3 larvae of *Haemonchus*, 20.0% *Teladorsagia*, 23.3%, *Trichostrongylus*, 43.3% *Cooperia* 10.0% and *Oesophagostomum-Bunostomum* 3.4%). The L3 larvae originated from coprocultures of faeces obtained from sheep naturally infected with a mixture of nematodes. The *Trichostrongylus* species used were: *T. colubriformis*, *T. axei* and *T. vitrinus*.

### Blood sampling and analytical procedures

Blood samples were collected from each individual animal in all the experimental groups at four time points (14, 28, 42 and 56 days after the infection with GIN nematodes). The blood was obtained by jugular vein puncture in a vacuum glass tube using a tube holder with 20-gauge needle. Following the clotting of blood, the tubes were then centrifuged at low speed for serum separation. The separated serum was transferred into plastic vials and placed into a deep freezer for further biochemical analysis.

Vitamin E in serum was determined by a fluorimetric method (Hansen and Warwick, 1969) using an Hitachi F2000 fluorometer. Vitamin A in serum was measured by a colorimetric method (Roels and Trout, 1972) using an Hitachi 2000 spectrophotometer.

### Statistical analysis

All statistical analyses were performed using Genstat version 5 (Lawes Agricultural Trust, 1993). Data on serum concentrations of vitamin A and E were analysed separately by an analysis of variance (ANOVA) model for repeated

**Table 1.** Serum vitamin A and vitamin E concentration in lambs infected or not with gastrointestinal nematodes and fed a diet with or without zeolite

	Infected		SED	p	Feeding treatment		SED	p
	Yes	No			Basal	Zeolite		
Vitamin A (µg/ml)	0.266	0.235	0.0219	NS	0.210	0.291	0.0219	***
Vitamin E (µg/ml)	1.523	1.652	0.1318	NS	1.838	1.337	0.1318	**

NS: Not significant. \*\* p<0.01, \*\*\*p<0.001.

measurements. Also, a two-way ANOVA with two levels of feeding (basal and zeolite) and two levels of parasitic status (infected and uninfected) was used to test for the effects of diet, infection and their interaction. In order to evaluate possible association of either vitamin A or E levels with the parasitic status of lambs, the analysis was restricted to two groups; data from lambs infected with GIN were compared with those without infection using Students t-test.

### RESULTS

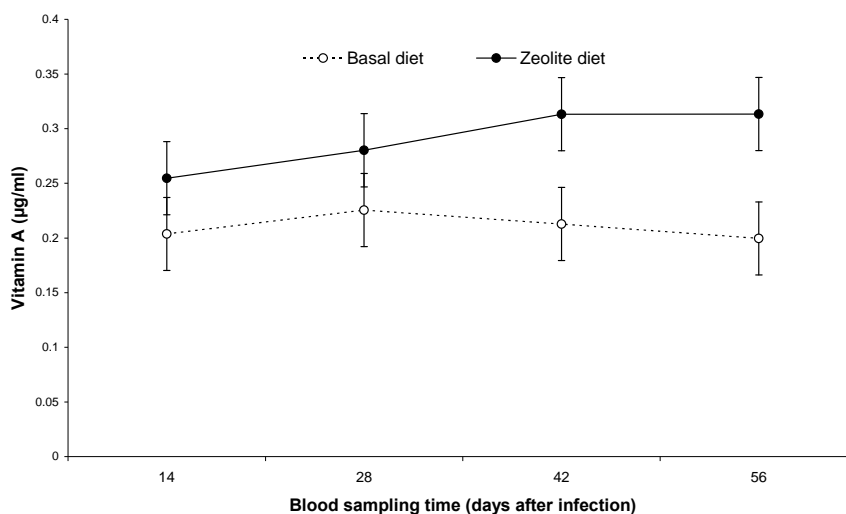
Throughout the experiment no clinical signs or disease were observed, indicating that a subclinical parasitism was achieved following the initial infection with GIN. The overall mean (±SD) of blood serum vitamin A concentration in lambs was 0.25±0.090 µg/ml and the average serum vitamin E concentration was 1.59±0.769 µg/ml.

Table 1 shows the average concentration of vitamins A and E in blood serum of lambs according to parasitic status and feeding treatment. The concentration of both vitamins was affected significantly by feeding treatment; lambs fed the zeolite diet had higher values of vitamin A (p<0.001), but lower values of vitamin E (p<0.01) when compared with those fed the basal diet. The parasitic status of lambs did not seem to have any significant effect on either vitamin A or vitamin E concentration (Table 1).

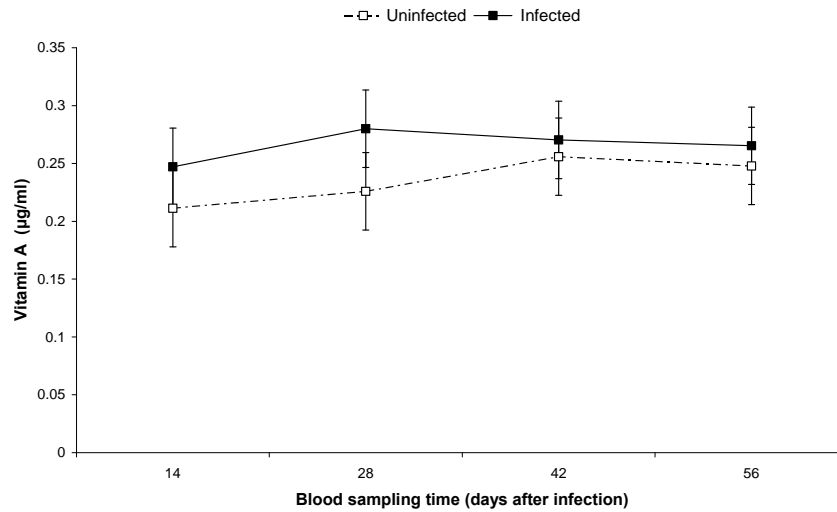
Figure 1 shows the relationship of serum vitamin A

concentration and feeding treatment with days from infection, irrespective of the parasitic status of lambs. At the time of allocation of lambs to treatments and for the first two samplings the serum concentrations of vitamin A were similar between groups. However, after the third sampling (day 42 post-infection), the concentration of vitamin A had risen significantly in lambs fed the zeolite diet, but decreased in those fed the basal diet. The average concentration of vitamin A in the serum of lambs in relation to parasitic status and days from infection, irrespective of feeding treatment, is presented in Figure 2; the parasitic status of lambs had no significant effect on serum concentration of vitamin A.

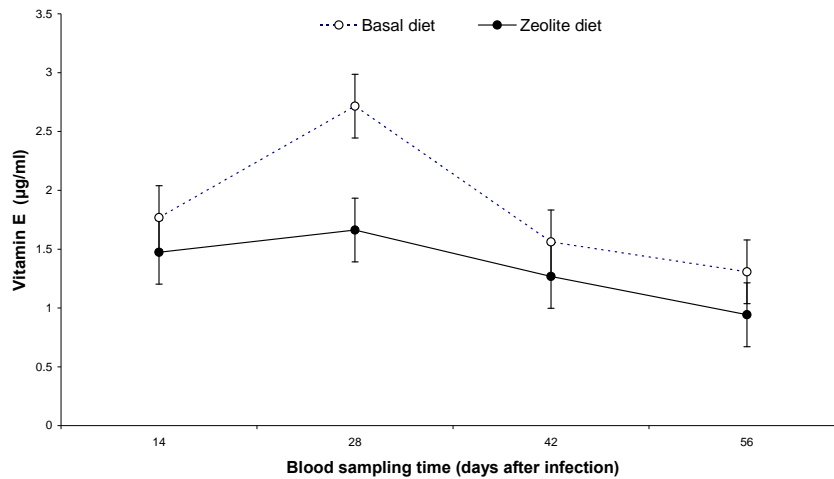
Figure 3 shows the average concentration of vitamin E in blood serum of lambs grouped according to feeding treatment. The concentration of vitamin E in blood serum was not significantly different between lambs fed the zeolite or the basal diet; the only exception was a significant increase of vitamin E concentration in blood samples obtained from lambs fed the basal diet at day 28 after the start of the experiment. Moreover, no significant differences in serum vitamin E concentration, over time, were found between lambs infected or not with GIN (Figure 4). Here, higher levels of vitamin E were found in uninfected lambs up to day 28 from the start of the experiment. However, that was later reversed without the differences in vitamin E concentrations being statistically significant (Figure 4).



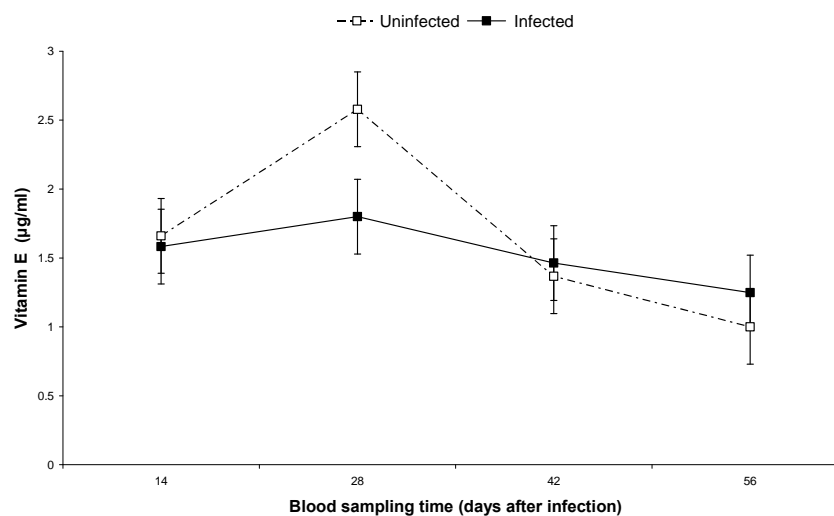
**Figure 1.** Blood serum concentration of Vitamin A in growing lambs in relation to feeding treatment and time.



**Figure 2.** Blood serum concentration of Vitamin A in growing lambs in relation to parasitic status (infected or not with gastrointestinal nematodes) and days post infection.



**Figure 3.** Blood serum concentration of Vitamin E in growing lambs in relation to feeding treatment and time.



**Figure 4.** Blood serum concentration of Vitamin E in growing lambs in relation to parasitic status (infected or not with gastrointestinal nematodes) and days post infection.

## DISCUSSION

The objective was to assess the changes in blood concentrations of two selected vitamins, vitamin A and vitamin E, in growing lambs and to study the effects and interactions between clinoptilolite supplementation in the diet and infection with GIN. Studies on interactions between host nutrition and GIN infections in sheep are abundant in the literature. The dominant view is that improvement in host nutrition can enhance the ability to regulate the GIN population as well as the ability to withstand the negative effects of GIN infection, while maintaining a reasonable level of production and therefore reduce the reliance on chemotherapy to control GIN parasitism (VanHoutert and Sykes, 1996; Sykes and Greer, 2003; Walkden-Brown and Eady, 2003; Hoste et al., 2005).

Considering that growing lambs are more prone to parasitism, their feeding during growth is very important for performance and general health. Major nutrients, such as energy and protein, play an important role in the susceptibility of animals to disease and particularly to infection with GIN. A balanced supply of micronutrients, such as vitamins A and E, is also important, as deficiencies of the latter have been associated with increased incidence of diseases. The risk of specific clinical signs and general health disorders in growing lambs deficient in vitamin A and/or vitamin E is well documented (NRC, 1985; Hatfield et al., 1999; Rooke et al., 2004; Infascelli et al., 2005). One of the major actions of vitamin A is increasing resistance to infection, which is very important in the case of parasitism with GIN (Koski and Scott, 2001). However, there is no evidence in the literature regarding the significance of either vitamin A or vitamin E concentration in blood serum as a result of parasitic challenge. To our knowledge any association between vitamin A and/or vitamin E and zeolite diet in lambs parasitized or not with GIN has not been reported. Our hypothesis was that GIN could affect the nutritional status.

Our hypothesis that parasite infection with GIN could affect the serum concentration of either vitamin A or E arises from evidence in early studies that nutrition has a strong influence on host susceptibility to GI parasite infection in domestic ruminants. Many of the negative effects of GIN parasitism observed in ruminants result from the disruption of nutrient metabolism (Coop and Kyriazakis, 1999; Coop and Kyriazakis, 2001; Sykes and Greer, 2003). Certain nutrients, including some of the vitamins are involved, in an enterohepatic circulation that depends on the integrity of the liver, the biliary tract and intestine for maintenance of the circulatory body pool (Rosenberg and Bowman, 1982). Hence, our hypothesis was that GI parasites could also affect the above process and

subsequently the nutritional - vitamin status of the host.

Recently, the effect of feeding clinoptilolite in blood metabolites has been investigated in dairy cows by Katsoulos et al. (2005a, b). Katsoulos et al. (2005a) monitored the serum concentrations of b-carotene and vitamins A and E, following long-term dietary supplementation of clinoptilolite. They concluded that the average values of both vitamins remained within reference ranges without being affected by the supplementation of clinoptilolite in the diet.

In the present study, the feeding treatment (basal or zeolite diet) contributed most to the variation in the concentrations of vitamin A and E in blood serum. The results showed that supplementation of the diet with clinoptilolite resulted in increased values of vitamin A in blood serum, but decreased the concentration of vitamin E. The latter observation suggests that clinoptilolite feeding has a negative influence on the vitamin E status of growing lambs. The mechanism of action of clinoptilolite is unknown, but most likely it affects the absorption and metabolism of vitamin E, or it may bind the vitamin molecules. The fact that zeolites are used as buffers in ruminant diets implies that they may induce physiological or biochemical changes in the gastrointestinal tract of ruminants (Mumpton and Fishman, 1977; Pond, 1993; Ramos and Hernandez, 1997).

The finding that infection with GIN did not have any significant effect on the serum concentration of either vitamin A or vitamin E is interesting because of the scarcity of relevant information for sheep. As shown in Figure 2 the serum concentration of vitamin A declined over time but remained unaffected by the parasitic status of lambs. Similarly, in the case of vitamin E a reduction was observed over time (Figure 4), which was greater in uninfected animals but not statistically significant when compared with the infected group. Interpretation of the above data suggests to us that the likelihood of detecting differences in serum concentration of vitamins in growing lambs depends both on the difference between levels of parasitism and the potential of clinoptilolite to enhance resistance to infection from GIN. In conclusion and in the context of this experiment, further studies are required to clarify the role of clinoptilolite feeding in the effective control of GIN parasitism in sheep as well as its consequences for vitamins and minerals, digestion and other competing physiological functions.

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