

Effect of Parenteral Administration of Vitamin B to Goats on Performance, Lice (Phthiraptera) Infestations and Cellular Immunity^[1]

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Abstract

This study was carried out on 20 of 8-10 months old Anatolian black goats which were healthy or *Linognathus africanus* infested (n=10 each). Each goat was housed individually all along the study. Five of ten louse-infested goats were administered vitamin B complex (Benefor enj) intramuscularly at a dose of 40mL/goat. Remaining goats (n=5) were not given vitamin B complex. The other goats that have not been infested with *L. africanus* were grouped exactly the same as above. All the animals (n=20) were first sensitized and then measured for hypersensitivity reaction in terms of cellular immune response. Infested goats given vitamin B complex were shown a decrease in *L. africanus* infestation. The other 5 number of infested goats that not given vitamin B complex were detected with an increase in the louse infestation. At the end of the trial, the higher live weight was recorded in healthy goats than those not administered vitamin B complex.

Keywords: *Linognathus africanus*, Vitamin B, Performance, Cellular immunity, Goat

Keçilerde Parenteral Verilen B Vitamininin Performans, Bit (Phthiraptera) Enfestasyonları ve Hücresel İmmünite Üzerine Etkisi

Özet

Bu araştırma 20 adet 8-10 aylık, *Linognathus africanus* ile enfeste (n=10) ve sağlıklı (n=10) erkek kıl keçilerinde yapıldı. Her bir keçi çalışma süresince bireysel bölmelerde ayrı ayrı beslendi. Bitli olan 10 adet keçinin 5 tanesine vitamin B kompleksi (Benefor enj) 40mL/keçi dozunda kas içi yolla uygulandı. Diğer 5 adet bitli hayvana vitamin B kompleksi uygulanmadı. Bitsiz 10 adet keçi ise aynı dozda vitamin B kompleksi uygulanan ve uygulanmayan olmak üzere iki gruba ayrıldı. Tüm hayvanlar (n=20) önce duyarlılaştırıldı ve ardından aşırı duyarlılık reaksiyonu ile hücresel immün cevap yönünden değerlendirildi. Çalışma sonunda, bitli olup vitamin B kompleksi uygulanan 5 adet keçide *L. africanus* enfestasyonunda önemli derecede azalma görülürken, vitamin B kompleksi uygulanmayan diğer 5 adet keçide ise bit enfestasyonunda artış gözlemlendi. Deneme sonunda en yüksek canlı ağırlık bit enfestasyonu olmayan ve B vitamini verilmeyen gruptaki keçilerden elde edildi.

Anahtar sözcükler: *Linognathus africanus*, B Vitamini, Performans, Hücresel immünite, Keçi

INTRODUCTION

Infestation of louse is called *pediculosis* or *phthirapteriasis*^[1]. Louse infestation is the most common in winter and early spring. The rate of infestation is higher in animals with poor body structure than animals with good body composition^[2-9]. Lice cause significant decline in productivity and major economic losses worldwide^[1,10-13]. It has been reported that in Turkey there are 109 lice

species belonging to a large number of genus that morphologically diagnosed in birds and mammals up to day. Among these mammalian lice, 20 lice species belonging to 8 genera in the Anoplura, 8 lice species belonging to 3 genera in Ischnocera and 2 lice species belonging to 2 genera in Amblycera were identified^[14].

Konya is a pioneering region of national goat breeding with the number of 260.577 goats^[15]. Huge economic



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loses in the yields of meat, milk and wool are caused by lice infestations in goats every year. Lice are known to cause weakening in animals by diminishing body conditions of animals. This causes great damage not only to the country's economy but also to its animal health. Millions of dollars of drugs are used every year depending on *pediculosis* throughout the World [12,16,17].

Despite not enough, studies have been conducted on lice infestations in various regions of the country [18-20]. In a study from the Region Van, a positive lose rate of 57.60% was found in examined goats. Infestations caused by the species of *Bovicola caprae*, *B. crassipes*, *B. limbata*, *L. africanus*, *L. stenopsis* were reported as being 52.51%, 7.56%, 6.39%, 17.81%, 15.71%, respectively. Additionally, it was emphasized that the infestation was more common in winter months with the rate of 40.16%. By some other studies carried out in Turkey, the rate of lice infestations in goats was found to vary between 57.60% and 82.10% [21,22]. The lice on sheep can cause itching because of intense irritation, secondary bristle and wool loss by destroying nerve endings in the skin. However, small ruminants carrying few lice do not show clinical signs. This parasite also causes restlessness, weak condition, low efficiency and leads to friction and thickening of the skin caused by the itching [16,17]. Blood-sucking lice may penetrate through the mouth organelles and bleed. Serum leaked from wound cause the wool to become dirty. These wounds attract flies and predispose the infested goats to myiasis [23,24].

The purpose of this research is to measure relationship between the lice infestation, cellular immune response and performance in goats which vitamin B complex were administered parenterally and thus indirectly determine the effect of vitamin B complex on host resistance to infestation.

MATERIAL and METHODS

The ethic committee document related to this study was taken from the Ethics Committee of the Experimental Animal Production and Research Center of the Veterinary Faculty of Selcuk University with the number of Decision 2016/16.

This research was carried out between 21 January and 21 March 2016. The research material consisted of 20 goats obtained from a private flock in Konya region and from which lice collected. All the goats from the same breed (hair goat) and gentle (male) were placed to individual compartments one by one in sheep unit of Prof. Dr. Hümeyra Özgen Research and Application Farm, Faculty of Veterinary Medicine, Selcuk University.

Firstly, animals were weighed in the experiment, then were divided into two groups in which each group has 10 goats. Infested animals were put into the first group. The first group were further divided into two groups, consisting

of one given the vitamin B complex via parenteral route (n=5), and the other (n=5) not given vitamin B complex. The same trial procedure was also applied to the second group including 10 animals.

Parasitological Examination

Flotation, Sedimentation and Barmen-Wetzel techniques were applied to all animals before starting to trial. Positive animals for the presence of other parasites were only included in the study provided that treatment completed.

At first, 10 goats infested with lice were macroscopically examined. Later, the hairs on the back, neck, tail and foot parts were collected by scraping into a plastic cuvette with an appropriate comb. White-barrel-shaped eggs were identified by appearing on the bristles of dark hairy goats. Identification of lice, nymphs and ovaries was performed by macroscopic and microscopic examinations [16]. In addition, the hair in the form of pinches was taken from these areas by using scissors. These bristles were put in nylon bags separately and the protocol numbers were noted.

Afterwards all samples were brought to the laboratory of the Parasitology, Veterinary Faculty of Selcuk University. Lice and eggs fallen into a white plastic cuvette were collected and taken into bottles containing 70% alcohol. The lice were fixed on slides with Canadian Balsam followed by a treatment in 10% KOH to make them transparent. Lice were identified by examining in binocular light microscope in accordance with the relevant literature. Data for performance, condition and parasite presence were evaluated in goat groups with and without vitamin B complex.

Vitamin B complex used in this study includes 100 mg Thiamin HCl (Vit. B), 5 mg Riboflavin (Vit. B2), 10 mg Pridoxin HCl (Vit. B6), 100 mcg Cyanocobalamin (Vit B12), 100 mg Niasin and 10 mg D-Panthenol per 1 mL. Five goats in first group infested with lice and other 5 in second group without infestation were injected with vitamin B complex intramuscularly (dose 5 mL/goat/week; 8 times, totally 40 mL/goat) and performance values were evaluated.

Determination of Nutrition Performance

At the beginning of the study, all the goats, taken to the experiment were weighed using an electronic weighing machine on an empty stomach in the morning. During the experiment, weights were measured four times for two months, once an every two weeks. The live weights as well as live weight gains were determined.

Individual feeding program was applied in the trial. Every goat was nourished with 4% of its weight concentrated feed. Roughage was given to the animals as *ad libitum*.

Immunological examination

For the immune measurements, all the goats were first sensitized and then measurements for delayed type allergy

reaction were performed. For this purpose, a method was used with modifications [25]. On the 50th day of the experiment, one side of necks of all the goats were shaved 15 cm x 15 cm in dimensions and topical application of 0.2 mL 90% (in alcohol) DMSO (Dimethylsulfoxide; Sigma D2650) was applied to the center of this field. Three minutes later, the same area was again topically applied with 0.2 mL of 5% DNCB (1-Chloro-2,4-dinitrobenzene; Sigma-Aldrich-237329) (wt/vol solvent alcohol). This sensitization was repeated in the same manner and to the same spots after 2 days (the 52nd day of the experiment).

The skin thicknesses of the two points (10 cm apart from each other) in sensitized area were measured at 57th day of trial. 0.1 mL as control to first spot (4:1 acetone:oil) of 0.1% acetone (Sigma Aldrich 320110) + olive oil (Sigma C8392) and 5% DNCB as test to second spot were injected. Sensitivity determination was recorded by measuring skin thickness at 24 h.

Statistical Analysis

Anderson Darling normality test was first applied to analyze distribution of the samples then Paired sample t test was used. P values of weight gain and skin thickness for all groups less than 0.05 ($P < 0,05$) were considered as statistically significant.

RESULTS

By the parasitological examination of the lice from infested group, all the lice collected from all parts of the body were found to be *L. africanus*, a blood-sucking louse species (Fig.1, Fig. 2). In the second subgroup of infested group and the goats from which the vitamin B complex has not been applied to, lice infestation was observed to increase at the end of the study. At the beginning of the work, the average number of lice counted was 25 in the 10 cm² area, while at the end it was 50 from the same area.

Significant losses of wool amounts and levels of irritations were observed due to severe infestations in goats without vitamin B complex of the infested group.

Skin thicknesses were recorded before and after sensitization as well as following testing by using digital calipers (Fig. 3).

Based on groups, degrees of hypersensitivity reactions and values measured afterwards are shown in Table 1. When skin thickness of control site compared with

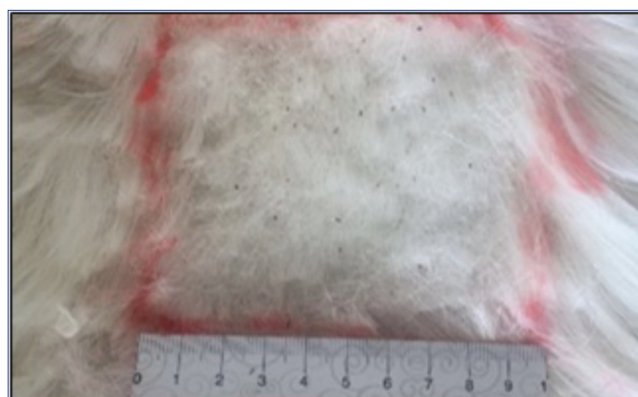


Fig 1. *Linognathus africanus*

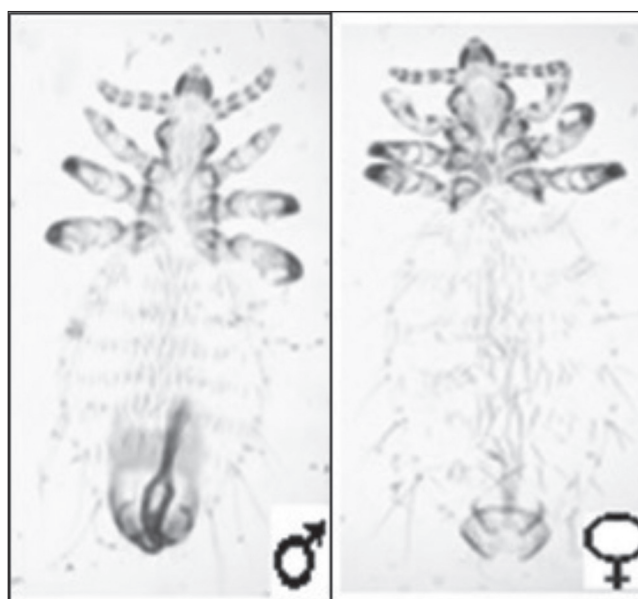


Fig 2. *Linognathus africanus*, original, microscopic appearance

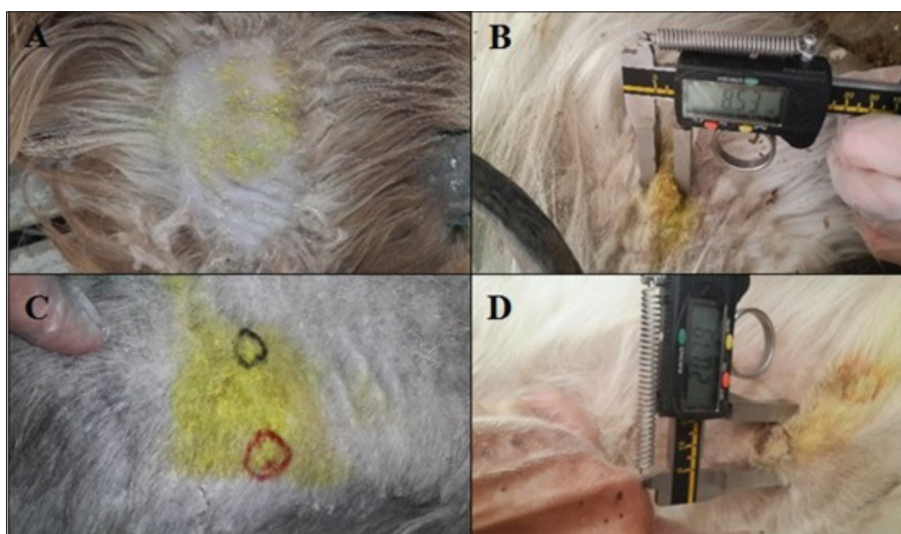


Fig 3. Normal, sensitized and test skin thickness measurements, A- Shaved skin, skin ready for sensitization, B- Sensitized skin, C- Control and test injections, D- Test reading at 24th h

Table 1. Effect of vitamin B application on hypersensitivity reaction in healthy and lice-infested goats

Skin Thickness (ST)		Groups*			
		1	2	3	4
Before sensitization ST		3.89±0.63	4.13±0.45	3.49±0.36	3.78±0.75
Before ID application ST		6.71±0.77	8.55±1.09	7.62±2.78	8.31±2.36
24 h after ID application ST	Control ST	9.72±1.80	9.89±1.79	7.55±2.02	7.85±1.58
	Test ST	17.98±1.93	19.51±2.26	19.89±1.96	21.43±4.19
	Test ST-Control ST	8.26±2.41	9.62±1.94	12.34±1.77	13.58±4.02

* 1: Lice infested, vitamin B complex applied, 2: Lice infested, vitamin B complex unapplied, 3: Non-lice infestation, vitamin B complex applied, 4: Non-lice infestation, vitamin B complex unapplied

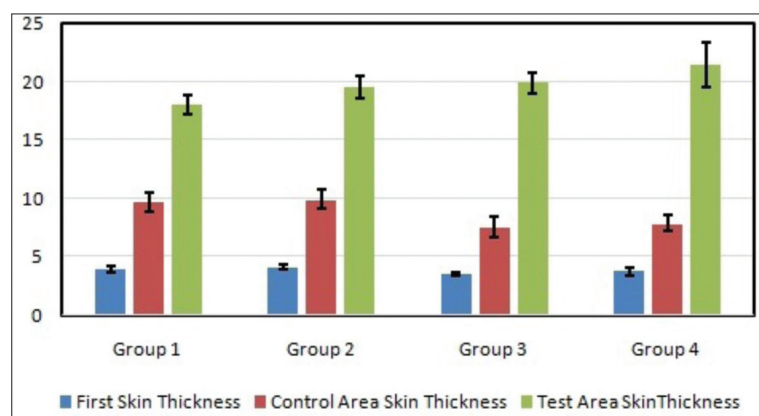


Fig 4. Statistically comparison of each group skin thickness

Fig 5. Statistically comparison of each group at the beginning and end of the study

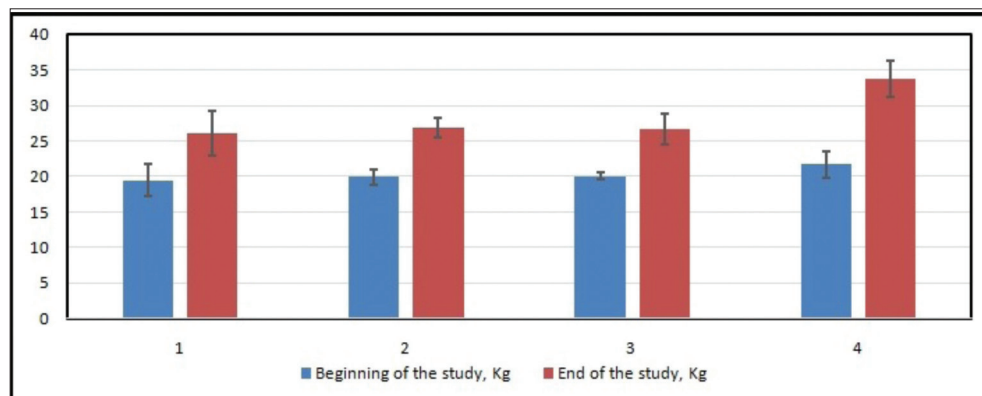


Table 2. The live weight averages obtained at the end of the study

Days	Lousy		Not Lousy	
	1 (Vitamed, Kg)	2 (Without Vitamin, Kg)	3 (Vitamed, Kg)	4 (Without Vitamin, Kg)
26.01.2016	19.50	19.92	20.02	21.68
09.02.2016	21.62	20.14	22.22	24.20
23.02.2016	23.48	22.80	23.66	28.44
12.03.2016	24.16	24.38	24.70	29.26
23.03.2016	26.08	26.86	26.72	33.72
Average	22.97	22.82	23.46	27.46

those of first (for sensitization) or second (for trial) injections all determined to be statistically significant ($P < 0.05$). However, no differences were seen between the

groups with or without louse infestations ($P > 0.05$). This is also the same case for the groups of vitamin supplementation ($P > 0.05$) (Fig. 4).

The measurements of nutrition performances are presented in *Table 2* and *Fig. 5*.

DISCUSSION

Single-sex goats were used and no gender comparisons were made in this study, since type of goat gender has been reported as not one of the determining factors for spreading of lice [2,9,22,26,27].

L. africanus toxins cause paralysis in hair roots and lead to alopecia resulting in development of suppurative lesions by disrupting skin integrity. However, no dermatitis occurrence was not detected in this study.

Two sides of neck are the body regions recommended for the measurement of cellular immunity using allergic skin tests [28]. In addition, as the primary region where the lice are localized on the host and in cases where the number of lice is low, both sides are suggested as suitable sides. In this trial, these regions were preferred for intradermal injection.

The delayed type of hypersensitivity reactions are used to assess cellular immune response. IVth type of hypersensitivity reactions is an *in vivo* test and has found clinical use in animals [29-31]. It has long been known that the vitamin B complex is one of the major factors in the development of resistance to ectoparasites in animals [32,33]. Various studies have been made to explain this effect of vitamin B [34,35]. At present study, the development of the hypersensitivity reaction induced by DNCB was examined in both healthy and *L. africanus* infested goats. The adaptation of the method to the goats was clinically determined to develop a hypersensitivity reaction in the goats. Of vitamin B complex applied goats, the skin thickness of *L. africanus* infested goats (17.98±1.93 mm) was found to be lower than that measured by healthy goats (19.89±1.96 mm) (P>0.05).

A similarity was the case for the groups which did not receive vitamin B complex (19.51±2.26, 21.43±4.19 mm) (P>0.05) (*Fig. 4*). These findings may lead to the conclusion that *L. africanus* infestation had an effect on suppressing the development of cellular immune response. On the other hand, with the planning of new trials with more goats and different types of lice infestations in the future, it would then be possible to determine statistically meaningful conclusions.

It is known that various toxins of blood-sucking lice cause local paralysis and alopecia by reaching the hair roots [16]. Although the *L. africanus* lice are not deeply established on surface skin, it is suggested that migration of cells which affect the skin and subcutaneous tissues contributing to the formation of cellular granulomas (primarily T cells) to the region was inhibited by lice. Lice may cause this effect either locally via their secretions on wool to reach the skin

or by a systemic way following absorption of secretions leading to T cell differentiation or even by both ways.

At the beginning of the trial, live weights were determined as 19.50, 19.92, 20.02 and 21.68 kg in the groups. At the end of the experiment, the average live weights obtained from the same groups were as 22.97, 22.82, 23.46 and 27.46 kg, respectively. At this time of the study, the highest live weight was obtained from a group which no lice infestation occurred and no vitamin applied (P<0.05). In this particular group, the increase in live weight can be due to the absence of lice-infestation and injection stress.

In the first group of the infested animals applied with vitamin B complex, there was a decrease in the number of lice at the end of the experiment while the lice had already been removed in the goat number 3. This was interpreted as the possibility that the application of the vitamin B complex would likely remove the stress factors in the goat and reduce the intensity of the lice infestation.

It was concluded that the DNCB hypersensitivity reaction can be used when cellular immunity measurement is needed in *L. africanus* infested goats, but this should be interpreted with caution and the recommendation of applying alternative tests or methods should be taken into account. In addition, when the vitamin B complex was administered in dose of 40 mL/goat by parenteral route (IM) in *L. africanus* infested goats, cellular immune system of the host and development of lice are negatively influenced. Vitamin B complex in goat breeding is concluded to be recommended parenterally at the dose used in this study.

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