Evaluation of serum 25-hydroxy vitamin D3 levels in goat kids naturally infected with *Giardia duodenalis*

*Giardia duodenalis* ile doğal enfekte oğlaklarda serum 25-hidroksi vitamin D₃ düzeylerinin değerlendirilmesi

**ABSTRACT**

The aim of this study was to determine 25-hydroxy vitamin D₃ levels in diarrhoeic goat kids. For this purpose, blood sample withdrawn from 10 diarrhoeic goat kids (Group I) and 10 healthy goat kids (Group II). Diagnosis of giardiasis was performed with multiple methods including microscopic examination and rapid test kits. Mean 25-hydroxy vitamin D₃ levels were determined as 33,37 ng/mL in diarrhoeic goat kids and 86,78 ng/mL in control and that is found to statistically significant (p<0.001). Consequently, it was convinced that giardiasis-related intestinal malabsorption may cause severe reduction in vitamin D levels and vitamin D supplement should be administered proper and sufficient doses due to anti-microbial effect with anti-giardial medication in treatment regime.

**Keywords:** Goat, vitamin D, deficiency, giardiasis

**ÖZET**

Bu çalışmada ishallı oğlaklarda 25-hidroksi vitamin D₃ seviyelerinin belirlenmesi amaçlandı. Bu amaçla 10 ishallı (grup I) ve 10 sağlıklı (grup II) oğlakta kan örnekleri toplandı. Giardiasizin tanısında mikroskobik muayene ve hızlı tanı test kriterlerinden yararlandı. Ortalama 25-hidroksi vitamin D₃ seviyeleri ishallı grupta 33,37 ng/mL iken sağlıklı grupta 86,78 ng/mL olarak ölçüldü ve bu farklılık istatistiksel olarak anlamlı bulundu (p<0.001). Sonuç olarak giardiasis ilişkili intestinal malabsorbsiyonların D vitamin seviyelerin vice oğlaklarda şiddetli azalmalar neden olabileceği ve anti-giardial sağlamlık yanında antimiokrbiyel etkilerinden dolaylı vitamin D takviyelerinin uygun ve yeterli dozda sağlaştırma rejimlerine ekleneği kanaatini oluşturdu.

**Anahtar Kelimeler:** Keçi, vitamin D, yetmezlik, giardiasis

**INTRODUCTION**

Especially suitable geographical conditions for goat breeding increases the rearing and spreading of this species in Turkey (Günlü and Alaşahan, 2010). On the other hand, improper hygiene standards may result within the interaction of some zoonotic disease between humans and animals (Matilla et al., 2018) as like giardiasis. Several forms of *Giardia duodenalis* involving seven assemblages (A–G) and assemblage E zoonotic for small ruminant (Jafari et al., 2014) have been implicated. Its zoonotic potential creates danger for human especially in child (Al-Habsi et al., 2017; Horton et al., 2019).

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Giardiasis is caused by Giardia spp. commonly *G. duodenalis* as a protozoan flagellate agent and it might infect multiple hosts that have a wide range of organism including mammals, birds and amphibians. Furthermore, giardiasis has a common prevalence in goat kids as like other ruminant (Santin et al., 2007; Geurden et al., 2008; Squire et al., 2017; Santin, 2020). Diarrhoea, weight loss and mortalities might be occurred in goat kids (O’Handley and Olsen, 2006). In this context enteric parasite *G. duodenalis* causes growth retardation related to diarrhea in young animal unlike adults, of which disease especially possess subclinical process (Lalle et al., 2005; O’Handley and Olson, 2006; Ural et al., 2017).

Vitamin D synthesis exist through two ways including skin in that cholecalciferol as also known Vitamin D₃ formed by photolyzes of epidermal 7-dehydrocholesterol with absorbed ultraviolet light and ergocalciferol as known Vitamin D₂ proceed from a dietary steroid. After transferred into circulation, both compounds are hydroxylated to 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D that is active metabolite of vitamin D in liver and kidney via consecutively enzymatic reaction (MacLaughlin et al., 1982; Webb and Holick, 1988). Vitamin D is synthesised in both humans and ruminant skin (Holick et al., 1979; Hymøller and Jensen, 2010), contrary to dog and cats only required with diet (How et al., 1994). Like large and small ruminants can be able to produce Vitamin D by mostly their skin and nutritional way (Hidiroglou and Karpinski, 1989; Kovács et al., 2015; Nemeth et al., 2017).

According to this study authors, giardiasis one of the most common and unminged aetiological agents of diarrhoeic young small ruminant. Commonly distribution and molecular characterization of agent has been researched in many studies (Santin et al., 2007; Geurden et al., 2008; Jafari et al., 2014; Al-Habsi et al., 2017; Squire et al, 2017; Horton et al., 2019; Santin, 2020). Indeed, vitamin D levels in goat kids infected with giardia was questioned. For this purpose, we aimed to determine 25- hidroxy vitamin D₃ levels in diarrhoeic goat kids.

**MATERIALS AND METHODS**

The animal of the study was formed within10 diarrhoeic goat kids (Group I) and 10 healthy goat kids (Group II) from commercial goat farm located in Aydın province. Both groups were selected from different gender and 15-35-day old age.

Diagnosis of giardiasis consisted of both the microscopic examination of Giardia cysts/trophozoites and rapid test kits (Anigen Rapid Bovid-5 Ag Test Kit, Bionote Lab.) principled with ELISA used to determine the antigen of the parasite (125 cysts and above in 100 μL feces) (Olson et al., 2010) in fecal samples collected rectally. Additionally, fecal samples collected from the healthy group with no symptoms of diarrhea were subjected to similar procedures with Group I for detecting negative status of giardiasis.

Blood samples were withdrawn from *Vena jugularis* to serum tubes (BD Vacutainer® Plus Serum Tüpleri, Becton, Dickinson and Company, New Jersey, ABD) for once were centrifuged at 3000 RPM for 10 min and separated serum. Following this process, serum 25- hidroxy vitamin D₃ levels were measured by commercial test kits working with immune fluorescence method (Beijing Savant Biotechnology Co., China). After completed of study, standard anti-giardial treatment protocol was applied to Group I.

Obtained data were evaluated whether the descriptive statistics showed normal distribution. Differences between the groups were determined with Mann-Whitney U test. All analysis was realized with SPSS programme (SPSS 22.0, IBM) and p<0.05 were considered statistically significant.
Ethical statement

This study was approved by local ethics committee of Aydın Adnan Menderes University with number of 2013/076.

RESULTS

Diarrhoeic all goat kids were found to be infected with *G. duodenalis* as a result of fecal microscopic examination and rapid test (Figure 1). Additionally, weight loss was seen in all diarrhoeic animals. There were no clinical findings were observed except weight loss and diarrhea. The main body temperature, respiratory rate and heart rate were 39.2\(^\circ\)C, 38 breaths per minute, 82 beats per minute, respectively.

There were statistically significant results related to 25-hidroxy vitamin D\(_3\) levels of healthy (Group II) and infected goat kids (Group I) (Table 1).

| Group                        | n   | X ± SD           | p value |
|------------------------------|-----|------------------|---------|
| Healthy (Group I) goat kids  | 10  | 86.78±21.30 ng/mL| p<0.001 |
| Infected (Group II) goat kids| 10  | 33.37±16.31 ng/mL|         |

\(a,b\): Values within a column with different superscripts are statistically different (p<0.05).

DISCUSSION

Giardiasis in small ruminant has generally unminded aetiological agents for subclinical diarrhea process and related study has mostly about its molecular characterization. On the other hand, vitamin D levels involved in many physiological functions was questioned in infected goat kids. For this purpose, we aimed
to determine 25-hydroxy vitamin D₃ levels in diarrhoeic goat kids.

Decreased appetite, fecal formation and mild depression were observed in the seventh-eighth days after post inoculation in goat kids experimentally infected with giardiasis (3x10⁶ giardia inoculated). The same study was found to goat kids were abnormally defecation over an average six-day period (Koudela and Vitovec, 1998). Our study realized in naturally infected animals under field conditions was determined that goat kids were not depressed even though decreased appetite and deterioration in fecal formation. It was stated that feces formation was softer for more than five days according to goat keepers’ anamnesis.

The study performed in northern India reported that the prevalence of G. duodenalis was 33.8% in goats close contact with humans. And also it was stated that zoonotic assemblages A and B was predominant in the same study unlike others declared of non-zoonotic assemblage E in goats (Utaaker et al., 2017). Analyzed of related study, it is seen that farm keeper, veterinarians and relevant stakeholders are at risk. In our study, it was recommended to taking preventive measures under the control of both farm keepers and related stakeholders.

As interestingly phrynoderma rarely seen pattern of follicular hyperkeratosis in 6-year-old boy attributed with chronic giardiasis takes an important place in literatures. It was associated with vitamin deficiency (A and C) and essential fatty acids. In the same study was stated that vitamin deficiency occured by intestinal malabsorption as a result of this chronic parasiter intestinal infection (Girard et al., 2006). It was thought that vitamin D deficiency may be associated with malabsorption due to chronic giardiasis although it has not been proven clearly based on the similar hypothesis. Similarly, one of the results of diarrhea was reported to accompany vitamin D deficiency in human medicine (Abed et al., 2014). In a recent study including some of the authors, vitamin D levels of diarrhoeic lambs infected with G. duodenalis, were found to be lower than healthy ones (Camkerten et al., 2019). Indeed, mentioned hypothesis of Girard et al. (2006) is supported by our results that mean vitamin D levels were determined as 33.37 ng/mL in diarrhoeic goat kids and 86.78 ng/mL in control as like our previous study (Camkerten et al., 2019) in lambs.

**CONCLUSION**

Consequently, it was convinced that giardiasis-related intestinal malabsorption may cause severe reduction in vitamin D levels and vitamin D supplement should be administered proper and sufficient doses with anti-giardial medication in treatment regime.

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