

Some Biochemical Parameters and Vitamins Levels in the Hair Goats Naturally Mix-Infested with Endo and Ectoparasities (Lice (*Linognathus africanus*) and *Trichostrongylidae* sp.)

¹Pinar Tanritanir, ⁴Nalan Ozdal, ²Cennet Ragbetli, ³Ibrahim Yoruk,

⁵Ebubekir Ceylan and ⁴Serdar Deger

¹Van School of Health, ²Van Health Services Vocational College,

³Department of Chemistry, Faculty of Art and Science,

University of Yuzuncu Yil, Van, 65080, Turkey

⁴Department of Parasitology, ⁵Department of Internal Medicine,

Faculty of Veterinary Medicine, University of Yuzuncu Yil, Van, 65080, Turkey

Abstract: The aim of this study was to investigate the changes in the blood serum concentration of some biochemical parameters and lipid-soluble vitamin levels in the goats naturally infested with lice and *Trichostrongylidae* sp. before and after treatment with moxidectin and effects of moxidectin treatment on these blood parameters. In this study, 20 hair goats naturally infected with lice (*Linognathus africanus*) and *Trichostrongylidae* sp. and 10 healthy hair goats (control) were used as material. Blood samples were collected from infested and control groups. Serum samples were separated and analysed for biochemical parameters with autoanalyser and some vitamins with high performance liquid chromatography. Animals in infested group were treated with ecto-endoparasitic drug (moxidectin) and in 8 and 15 days after treatment blood samples were again collected to determine some vitamins and biochemical parameters. As a results, concentrations of retinol, sodium, potassium, calcium and albumin were lower ($p < 0.05$) and activity of alanine aminotransferase was higher ($p < 0.05$) in infected goats than in the controls. There was no statistically significant difference in the concentration of vit D₃, tocopherol, total protein, glucose and activity of aspartate aminotransferase and alkaline phosphatase between infected and control group.

Key words: Hair goat, lice, *Trichostrongylidae*, vitamins (retinol, tocopherol, vit. D₃), biochemical parameters

INTRODUCTION

Blood parameters of haematological and biochemical of the animal exposed to both ectoparasites and endoparasites revealed significant differences. In studies conducted on animal with endoparasites, it was reported that levels of serum protein, serum globulin, seruloplasmine, vitamin A, C, E and B₁₂ together with some enzyme and mineral changed significantly. Moreover, this studies reported that parasitic infestations predispose animal to vitamin and mineral deficiency (Deger *et al.*, 1997).

External parasitic infestations cause severe health problems in livestock that may be accompanied by a decrease in some blood biochemical parameters, blood trace elements and mineral levels (Deger *et al.*, 2002; Aatish *et al.*, 2007). Lice can be a problem for goat producers, especially during the winter months. Lice infestations have been associated with blood loss

(Shemanchuk *et al.*, 1960), reduced calf birth weights (Khan and Schaalje, 1985), behavioural changes such as excessive scratching and decreased weight gains (Gibney *et al.*, 1985; Devaney *et al.*, 1992). Heavy louse infestations may cause pruritus, alopecia, excoriatio and self-wounding (Colebrook and Wall, 2004), uneasiness, anorexia and cachexia (Dede *et al.*, 2003). Severe infestation with sucking lice may cause anaemia, which was severe enough to be the cause of death (Shemanchuk *et al.*, 1960; Otter *et al.*, 2003). Changes in some haematological parameters (erythrocyte count, mean cell volume, hematocrit, hemoglobin concentration, leukocyte counts, serum albumin and globulin concentration, total serum protein) in animals with lice were reported (Davisa and Williams, 1986; Devaney *et al.*, 1992; Otter *et al.*, 2003).

Moxidectin is a synthetically-derived macrocyclic lactone molecule from the milbemycin group that is commercially available in several formulations to treat

parasitic infections and ectoparasite infestations of cattle, dogs, horses, sheep and goats (Burgu and Karaer, 2005).

The aim of this study was to investigate the changes in the blood serum concentration of some biochemical parameters and lipid-soluble vitamin levels in the goats naturally infested with lice and *Trichostrongylidae* sp. before and after treatment with moxidectin and effects of moxidectin treatment on these blood parameters.

MATERIALS AND METHODS

In this study, 20 hair goats naturally infected with lice (*Linognathus africanus*) and *Trichostrongylidae* sp. and 10 healthy hair goats (control) were used as material.

Goats were examined for the presence of ectoparasites and gathered ectoparasites from them were preserved in 70% alcohol. Identification and preparation of ectoparasites were made according to related literature (Tuff, 1977; Palma, 1978).

Goats faeces were analysed with fülleborn's flotation technique for cestode, nematode eggs and protozoa oocyst, by sedimentation technique for trematode egg and by baermann wethzel technique for protostrongylidae larvae. Egg counts in the 1 g fecal samples were also determined by the modified McMaster technique. Moreover, faecal cultures were done, to differentiate at genus level of trichostrongylid nematodes. Blood smears were prepared from animals to investigate blood protozoa.

Infested animals were treated with moxidectin. This group was subcutaneously administered 0.2 mg kg⁻¹ of moxidectin (Cydectin, Fako).

Blood samples were collected from infested and control groups. Serum samples were separated and analysed for biochemical parameters (Sodium, potassium, calcium, total protein, albumin, glucose, Alkaline

Phosphatase (ALP), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by autoanalyser (Modular PP, Roche/Hitachi, Japan) and some vitamins (retinol, tocopherol and vit D₃) by High Performance Liquid Chromatography (HPLC, Agilent-1100, Gemany) method (Zaspel and Csallany, 1983; Reynolds and Judd, 1984; Miller and Yang, 1985). After treatment with moxidectin, blood samples of goats were again collected to determine some biochemical parameters and vitamin levels in 8 and 15 days of treatment. The results were analysed using Duncan's test.

RESULTS

According to ectoparasitological examination, goats were found to be infested heavily with *Linognathus africanus*. In the examination of parasitological faecal analyses, *Nematodirus*, *Ostertagia* and *Trichostrongylus* sp. were observed in parasitological faecal examination. The mean Fecal Egg Count (FEC) in goats infected with trichostrongylid nematodes was 1185±68 g. There were no *Trematode*, *cestode* eggs and *protostrongylidae* larvae in faeces. Similarly no blood parasites were found in blood smear. In clinical examination, hair loss, reducing wool quality, cachexia, itching, uneasiness, lack of appetite were present in the infested goats.

Vitamins and biochemical parameters in infested and control group before and after treatment are given in Table 1.

Retinol, sodium, potassium, calcium and albumin concentrations, decreased significantly (p<0.05), while the activity of ALT increased (p<0.05). There was no significant change in the levels of vit D₃, tocopherol, total protein, glucose and activity of AST and ALP in any group.

Table 1: Some vitamins and biochemical parameters in goats infested with lice and *Trichostrongylidae* sp. before and after treatment and control group

Parameters	Infested group			
	Control group	Before treatment	After treatment	
			8 days	15 days
Vit D ₃ (µg mL ⁻¹)	0.024±0.0023	0.029±0.0033	0.025±0.0024	0.024±0.0030
Tocopherol (µg mL ⁻¹)	1.80±0.10	2.00±0.16	2.00±0.12	1.89±0.15
Retinol (µg mL ⁻¹)	0.52±0.07	0.24±0.02*a	0.28±0.02*a	0.41±0.03*
Sodium (mmol L ⁻¹)	148.00±1.77	137.06±0.84*a	141.40±2.13*a b	148.33±0.60
Potassium (mmol L ⁻¹)	5.08±0.17	3.93±0.12*a	4.44±0.10*b	4.39±0.09*
Calcium (mg dL ⁻¹)	9.64±0.24	8.63±0.26*	8.63±0.25*	9.12±0.11
Total Protein (g dL ⁻¹)	7.30±0.14	6.82±0.18a	6.66±0.22a	8.28±0.22*
Albumin (g dL ⁻¹)	3.14±0.10	1.80±0.06*a	1.75±0.05*a	2.91±0.03
Glucose (mg dL ⁻¹)	46.89±2.75	43.33±0.99	47.00±1.04	46.33±1.29
AST (U L ⁻¹)	96.70±8.81	87.17±2.38	100.60±4.22	95.67±5.35
ALT (U L ⁻¹)	21.00±2.47	30.89±1.11*	29.70±1.58*	32.00±1.22*
ALP (U L ⁻¹)	235.80±56.27	139.44±56.23	162.22±99.5	54.78±8.99
N	10	20	20	20

*: Different than control (p<0.05); a: different than 15 day (p<0.05); b: different than before treatment (p<0.05)

DISCUSSION

Because of deterioration of intercellular permeability in *Trichostrongylosis*, pepsinogen transfers to blood and blood plasmae proteins transfers to abomasum lumen. Loss of plasma protein cause hypoalbuminemi and this brings about weakening in muscle and decrease in wool quality, affecting metabolism (Soulsby, 1986). Some haematological and biochemical parameters (total protein, albumin) and some trace elements (Cu, Fe) levels were observed to be in a significant decrease in sheep with gastrointestinal parasites (Kozat *et al.*, 2006). While albumin levels increased, total protein, globulin, amilaz, chlorine levels in animals with *Trichostrongylosis*. Decreases in of phosphorus and magnesium concentrations and increases in levels vitamin B₁₂ were not significant in these animals (Ayaz *et al.*, 2007). No significant differences in serum albumin concentration of calves infested with low levels of lice and internal nematodes were detected, while significant increases in total serum protein concentration were detected (Devaney *et al.*, 1992). In this study, decreases in levels of albumin in infested goats were found to be statistically important. Moreover, total protein levels in the infested goats were lower than uninfested goats but this decrease was not found statistically important. In the animals, undergoing this treatment strategy, the total protein levels increased in 15th day and such an increase was found to be statistically significant compared to the controls. Weakening in muscle and decrease in wool quality in the infested goats may be depended on reduction in albumin level.

The deficiency of macro elements with parasitic diseases causes clinical disorder, loss of yield and death. Because of inflammation in gut wall in gastro-intestinal parasitosis, absorption of some vitamins and minerals was changed (Soulsby, 1986). Ayaz *et al.* (2007) reported that levels of potassium and calcium in sheep with independently infected *trichostrongylosis*, *fascioliasis*, *dicroceliasis*, *metastrongylosis* and *Hydatic kist* did not show a statistically significant change. In the same study, it was found that although, levels of sodium in sheep with *metastrongylosis* were higher than controls, sheep with *trichostrongylosis*, *fascioliasis*, *dicroceliasis*, *metastrongylosis* and *Hydatic kist* did not show a statistically significant difference. It was reported that calcium, copper, zinc and phospor concentrations in lice infested sheep and goats were significantly lower than uninfested animals (Deger *et al.*, 2002; Dede *et al.*, 2003). In this study, calcium, sodium and potassium concentrations in infested goats were lower ($p < 0.05$) than uninfested animals and decrease in calcium levels is coherent with some studies mentioned above.

In a study conducted on sheep infected with *Fasciola* sp. and *Trichostrongylidae* sp., it is reported that this parasites bring about oxidative stress accordingly lipid peroxidation and concentration of antioxidant matter preventing destructive effect of this decrease (Dede *et al.*, 2000). It was reported that vitamin E levels decline in goats infected with parasites (*Trichostrongylidae* sp. + *Protostrongylidae* sp. + *Eimeria* sp. + *Babesia* sp.), while vitamin C, retinol and β -carotene concentrations were not statistically different between groups (Dede *et al.*, 2002). In addition, a decrease in the concentration of vitamin A in animals infected with parasites has been reported (Das *et al.*, 1996; Hautvast *et al.*, 1998). Deger *et al.* (2001) found that concentration of vitamin E and β -carotene in sheep infected with babesiosis were lower than uninfested sheep and concentration of vitamin C and retinol did not show a statistically significant change. In this study, we observed that concentration of retinol was lower ($p < 0.05$) in goats infested with *Linognathus africanus* and *Trichostrongylidae* sp. than the control group although there was no statistically significant difference in the concentration of vit D₃ and tocopherol. These findings indicate that the concentrations of vitamins mentioned above are affected by the types of parasites and the hosts they invade.

The differences in activities of some liver enzymes (AST, ALT, GGT, ALP) in serum is generally indicator of some pathological changes of tissue and organ (Karagul *et al.*, 2000). In this study, although, ALP and AST activities did not show a statistically significant change, ALT activity in infested goats was higher than that of control group. But this ALT activity was in normal values for goats, according to the references of Karagul *et al.* (2000). In this study, the finding that liver enzymes did not increase significantly is an indication that both these parasites and the drug used did not have any impact on liver.

It is reported that both *trichostrongylosis* and lice infestations are prevalent of a high percentage in goats in the world and Turkey (Dumanli *et al.*, 1995; Akkaya, 1998; Kusiluka *et al.*, 1998; Mazyad and Helmy, 2001). As a result of patological disturbance occurred by gastro-intestinal parasites, disorders in feed consumption, protein metabolism and liquid balance depending on absorption defect in bowels (Soulsby, 1986). External parasitic infestations are a cause of severe health problems in livestock that may be accompanied by alteration in the blood parameters such a decrease in blood trace element, mineral levels (Deger *et al.*, 2002; Aatish *et al.*, 2007) and blood loss (Shemanchuk *et al.*, 1960). Especially, decreases in albumin levels cause loss

of efficiency and weakening of immune system of body. Pandita and Ram (1990) found that digestibility coefficients for nutrients (except nitrogen-free extract), nutrient balances, blood constituents (except white blood cells) and weight gains were significantly higher in non-infested goats than goats infested with ectoparasites (tick, mites and lice). When endoparasitic and ectoparasitic effects combine in the same animal these problems become more complicated. Devaney *et al.* (1992) also indicated that low density louse infestations (peak louse index of 125; counted on 122 sites) did not have an impact on weight gain, but that there was an interaction between louse infestations and gastrointestinal nematode infections that influenced weight gain.

Although, there is a lot of study about effect on blood parameters of endoparasitic and ectoparasitic diseases in animals, changes in the vitamin and biochemical parameters caused by especially louse in goats has not been examined thoroughly.

Consequently, alterations in some vitamins and biochemical parameters in goats infested with *L. africanus* and *Trichostrongylidae* sp. were investigated and statistically evaluated. According to the results, concentrations of retinol, sodium, potassium, calcium and albumin were lower and concentrations of ALT were higher in infected goats than in the controls. These blood parameters went back to normal levels to take into consideration both parameters in the control group and levels quoted references for goats after the infested goats were treated with moxidectin. There was no statistically significant difference in the concentration of vit D₃, tocopherol, total protein, glucose, AST, ALP between infected and control group.

ACKNOWLEDGEMENT

The authors would like to thank Dr. Semiha Dede for her opinion on the biochemical subject.

REFERENCES

- Aatish, H.U., Z. Sindhu, Z. Iqbal, A. Jabbar and Z. Tasawar, 2007. Prevalence of Sheep Mange in District Dera Ghazi Khan (Pakistan) and Associated Hematological/Biochemical Disturbances. *Int. J. Agric. Biol.*, 9 (6): 917-920. DOI: 1560-8530/2007/09-6-917-920. www.fspublishers.org/ijab/past-issues/IJABVOL_9_NO_6/25.pdf.
- Akkaya, H., 1998. The investigations on the Trichostrongylid nematodes of the Hair goats slaughtered in Istanbul. *Acta Parasitologica Turcica*, 22 (1): 77-87. http://apps.isiknowledge.com/full_record.do?product=CABI&search_mode=GeneralSearch&qid=10&SID=T15f3BPpJnegKDhGEHm&page=1&doc=1.
- Ayaz, E., A. Ertekin, N. Ozdal and Z. Tas, 2007. Some biochemical parameters in sheep infected with endoparasites (*Fasciola* sp., *Dicrocoelium dendriticum*, hydatid cysts, *Trichostrongylidae* and *Protostrongylidae*). *Acta Parasitologica Turcica*, 31 (1): 57-61. PMID: 17471414. http://www.tparazitolog.org/pdf/pdf_TPD_233.pdf.
- Burgu, A. and Z. Karaer, 2005. The treatment in parasitic diseases in veterinary medicine. Turkish Society for Parasitology Press No.: 19. Meta Basim, Bornova, Izmir, pp: 21-63. ISBN: 975-94646-6-7.
- Colebrook, E. and R. Wall, 2004. Ectoparasites of livestock in Europe and the Mediterranean region. *Vet. Parasitol.*, 120: 251-274. DOI: 10.1016/j.vetpar.2004.01.012.
- Das, B.S., D.I. Thurnham and D.B. Das, 1996. Plasma α -tocopherol, retinol and carotenoids in children with *Falciparum malaria*. *Am. J. Clin. Nutr.*, 64: 94-100. <http://www.ajcn.org/cgi/reprint/64/1/94>.
- Davisa, D.P. and R.E. Williams, 1986. Influence of hog lice, *Haematopinus suis*, on blood components, behavior, weight gain and feed efficiency of pigs. *Vet. Parasitol.*, 22 (3-4): 307-314. DOI: 10.1016/0304-4017(86)90119-6.
- Dede, S., Y. Deger, S. Deger and M. Alkan, 2000. Determination of the status of lipid peroxidation and antioxidants in sheep infected with certain endoparasites (*Fasciola* sp. + *Trichostrongylidae* sp. + *Eimeria* sp.). *Acta Parasitologica Turcica*, 24: 190-193. http://apps.isiknowledge.com/full_record.do?product=CABI&search_mode=GeneralSearch&qid=38&SID=T15f3BPpJnegKDhGEHm&page=1&doc=1.
- Dede, S., Y. Deger, T. Kahraman, S. Deger and M. Alkan, M. Cemek, 2002. Oxidation products of nitric oxide and the concentrations of antioxidant vitamins in parasitized goats. *Acta Vet. Brno.*, 71: 341-345. http://vfu-www.vfu.cz/acta-vet/vol71/pdf/71_341.pdf.
- Dede, S., Y. Deger and S. Deger, 2003. Serum profile of calcium, phosphore, copper and zinc in healthy and lice infested goats. *Indian Vet. J.*, 80: 150-151. http://apps.isiknowledge.com/full_record.do?product=CABI&search_mode=GeneralSearch&qid=47&SID=T15f3BPpJnegKDhGEHm&page=1&doc=6.
- Deger, Y., A. Gul, A. Bildik, S. Dede, F. Yur and S. Deger, 1997. Alterations on some blood parameters and vitamin C levels on infected dogs with parasites. *Acta Parasitologica Turcica*, 21 (2): 195-198. http://apps.isiknowledge.com/full_record.do?product=CABI&search_mode=GeneralSearch&qid=51&SID=T15f3BPpJnegKDhGEHm&page=1&doc=1.

- Deger, Y., S. Dede, T. Kahraman, S. Deger, N. Ormanci and K. Bicek, 2001. Determination of lipid peroxidation, nitric oxide oxidation products and the antioxidant status in sheep infected with babesiosis. T. Parasitol. Derg., 25 (1): 25-27. http://apps.isiknowledge.com/full_record.do?product=CABI&search_mode=GeneralSearch&qid=55&SID=T15f3BPpJnegKDhGEHm&page=1&doc=1.
- Deger, Y., S. Dede and S. Deger, 2002. Serum copper, zinc, calcium concentrations in lice infested sheep. Biol. Trace Element Res., 88: 87-90. DOI: 10.1385/BTER:88:1:87.
- Devaney, J.A., T.M. Craig, L.D. Rowe, C. Wade and D.K. Miller, 1992. Effects of low levels of lice and internal nematodes on weight gain and blood parameters in calves in central Texas. J. Econ. Entomol., 85 (1): 144-149. <http://www.ingentaconnect.com/content/esa/jee/1992/00000085/00000001/art00023>.
- Dumanli, N., E. Koroglu, Z. Erdogmus, M. Angin and H. Yilmaz, 1995. Lice (*Phthiraptera* sp.) on goats in the vicinity of Elazig. Turk. J. Vet. Anim. Sci., 19: 291-295. http://apps.isiknowledge.com/full_record.do?product=CABI&search_mode=GeneralSearch&qid=1&SID=T26dP72piC9o5ea@hej&page=5&doc=43.
- Gibney, V.J., J.B. Campbell, D.J. Boxler, D.C. Clanton and G.H. Deutscher, 1985. Effects of various infestation levels of cattle lice (*Mallophaga: Trichodectidae* and *Anoplura: Haematopinidae*) on feed efficiency and weight gains of beef heifers. J. Econ. Entomol., 78: 1304-1307. PMID: 4078141.
- Hautvast, J.L., J.J. Tolboom, C.E. West, E.M. Kafwembe, R.W. Sauerwein and W.A. Van Staveren, 1998. Malaria is associated with reduced serum retinol levels in rural Zambian children. Int. J. Vitam. Nutr. Res., 68: 384-388. http://grande.nal.usda.gov/ibids/index.php?mode2=detail&origin=ibids_references&throw=384496.
- Karagul, H., A. Altintas, U.R. Fidanci and T. Sel, 2000. Clinical Biochemistry. Medisan Publ. 45. 1st Edn. Ankara, Turkey, pp: 157-419. ISBN: 975-7774-42-1.
- Khan, M.A. and G.B. Schaalje, 1985. Chlorpyrifos for control of the short-nosed cattle louse, *Haematopinus eurysternus* (Nitzsch) (*Anoplura: Haematopinidae*) during winter. Can. J. Comp. Med., 49: 361-365. <http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1236193&blobtype=pdf>.
- Kozat, S., N. Yuksek, Y. Goz and I. Keles, 2006. Serum Iron, Total Iron-Binding Capacity, Unbound Iron-Binding Capacity, Transferrin Saturation, Serum Copper and Hematological Parameters in Pregnant Akkaraman Ewes Infected with Gastro-Intestinal Parasites. Turk. J. Vet. Anim. Sci., 30: 601-604. <http://journals.tubitak.gov.tr/veterinary/issues/vet-06-30-6/vet-30-6-15-0512-4.pdf>.
- Kusiluka, L.J.M., D.M. Kambarage, L.J.S. Harrison, C.J. Daborn and R.W. Matthewman, 1998. Causes of morbidity and mortality in goats in Morogoro district, Tanzania: The influence of management. Small Rum. Res., 29: 167-172. DOI: 10.1016/S0921-4488(97)00110-7.
- Mazyad, S.A. and M.M. Helmy, 2001. Studies on lice infesting goats in North Sinai. J. Egypt Soc. Parasitol., 31 (2): 511-516. PMID: 11478450.
- Miller, K.W. and C.S. Yang, 1985. An isocratic high-performance liquid chromatography method for the simultaneous analysis of plasma retinol, α -tocopherol and various carotenoids. Anal. Biochem., 145: 21-26. DOI: 10.1016/0003-2697(85)90321-5.
- Otter, A, D.F. Twomey, T.R. Crawshaw and P. Bates, 2003. Anaemia and mortality in calves infested with the long-nosed sucking louse (*Linognathus vituli*). Vet. Rec., 153 (6): 176-179. PMID: 12934729. <http://veterinaryrecord.bvapublications.com/cgi/content/abstract/153/6/176>.
- Palma, R.L., 1978. Slide-mounting of lice: A detailed description of the canada balsam technique. N.Z. Entomol., 6 (4): 432-436. http://www.ento.org.nz/nzentomologist/free_issues/NZEnto06_4_1978/Vol%206-4-432-436.pdf.
- Pandita, N.N. and S. Ram, 1990. Control of ectoparasitic infestation in country goats. Small. Rum. Res., 3 (4): 403-412. DOI: 10.1016/0921-4488(90)90021-W.
- Reynolds, S.L. and H.J. Judd, 1984. Rapid procedure for the determination of vitamins A and D in fortified skimmed milk powder using high-performance liquid chromatography. Analyst, 109: 489-492. DOI: 10.1039/AN9840900489.
- Shemanchuk, J.A., W.O. Haufe and C.O.M. Thompson, 1960. Anemia in range cattle heavily infested with the short-nosed sucking louse, *Haematopinus eurysternus* (Nitz.) (*Anoplura: Haematopinidae*). Can. J. Comp. Med. Vet. Sci., 24 (5): 158-161. <http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=1582707&blobtype=pdf>.
- Soulsby, E.J.L., 1986. Helminths, Arthropods and Protozoa of Domesticated Animals. 7th Edn. Baillere Tindall, London, pp: 216-373. ISBN: 0-7020-0820-6.
- Tuff, D.W., 1977. A key to the lice of man and domestic animals. Tex. J. Sci., 28 (1-4): 145-159. http://apps.isiknowledge.com/full_record.do?product=CABI&search_mode=GeneralSearch&qid=63&SID=T15f3BPpJnegKDhGEHm&page=1&doc=1.
- Zaspel, B.J. and S. Csallany, 1983. Determination of α -tocopherol in tissues and plasma by high-performance liquid chromatography. Anal. Biochem. 130: 146-150. DOI: 10.1016/0003-2697(83)90661-9. PMID: 6869795.