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# The Influence of the *Dictyocaulus filaria* on the Phospholipid and Fatty Acid Composition in the Blood of Sheep

Gulmira Musyrmanovra BASTARBEKOVA, Abdurachim Ergashevich KUCHBAEV, Djaloliddin Azimovich AZIMOV
Institute of Zoology of Uzbek Academy of Sciences, 1, Niyazov Str., Tashkent, 700095 the Republic of UZBEKISTAN

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**Abstract:** This paper provides results of an analysis of phospholipids (PL) and their fatty acid (FA) content in the blood of sheep infected by the nematode *Dictyocaulus filaria*. Significantly high level of arachidonic acid in the lysophospholipids, and low levels of docozangexenic acid in the phospholipids were obtained, which indicate structural and functional disorders of cellular membranes in blood during dictyocaulosis. These disorders are regarded as metabolic criteria for an assessment of the relation between parasite and host.

**Key Words:** sheep, blood, nematode, phospholipid, fatty acid, healthy and infected sheep, *Dictyocaulus filaria*

## Koyun Kanındaki Fosfolipid ile Yağ Asidi İçeriğine *Dictyocaulus filaria* Solucanının Etkisi

**Özet:** Bu çalışmada *Dictyocaulus filaria* ile enfekte olan koyunlarda fosfolipid ile yağ asidi içeriği analizinin sonuçları verilmektedir. Lisofosfolipidlerdeki arakidonik asitte önemli artışlar elde edilirken fosfolipidlerdeki dokozangeksenik asitte düşüş kaydedilmiştir. Bu durum, diktiyokaulosis sırasında kandaki hücresel ince zarların yapısal ve işlevsel bozukluklarının işaretidir. Bu bozukluklar, asalakla konuğun arasındaki ilişkinin değerlendirilmesinde metabolik ölçüt olarak sayılmaktadır.

**Anahtar Sözcükler:** koyun, kan, solucan, fosfolipid, yağ asidi, sağlıklı ve enfekte olan koyunlar, *Dictyocaulus filaria*

## Introduction

Parasites not only cause specific local tissue reaction, but also bring about general physiological and biochemical reactions of considerable variance in hosts, which are necessary for long-term existence of the parasites (1). A peculiarity of the way of life of parasites, including *D. filaria*, is connected not only with the necessity to adapt to the host organism, but also with the ability to obtain many readily available metabolites which are synthesized by the host organism (2).

The helminths are thought to affect structural and functional characteristics of the host cellular membranes (3).

Therefore, phosholipids (PL) and fatty acid (FA) composition can serve as estimates of structural-functional description in the parasite-host system, as shown in Kreps (4), and play a role in the adaptation of an organism to its environment.

The aim of the study was to show changes in PL and their FA composition in the blood of sheep upon infection by the nematode *Dictyocaulus filaria* parasitizing in the lumen of the respiratory track.

## Materials and Methods

Blood of healthy sheep and of those infected by the nematode *D. filaria* were obtained at the Tashkent meat plant. The extraction of lipids from blood was carried out as described by Folch et al. (5). Division of phosholipids into individual fractions was conducted by thin-layer chromatography on silica gel in a system of solvents chlorophol-metanol 28% ammonia (13:7:1 by volume) (6). The quantitative content of separate fractions of PL was estimated by the concentration of phosphorus taken off from the stained areas on the plate, and expressed as a percentage of the total of all fractions of PL, according to the method of Vaskovsky et al. (7). For the identification of the composition of FA content, PL was exposed to methylation for 25-30 minutes at 80°C with methanol containing 1% sulphuric acid (8). The total amount of methyl ethers of the FA of PL was analyzed by

chromatography (LHM-80). Identification of PL fractions and their FA was conducted with standards, by addition of individual FA, and then compared with literature data (9, 10). The quantitative assessment of the content of other fatty acids was the peak area on the chromatograph calculated by the multipication of the peak height by its width at half-height. The content of separate fatty acid fractions was expressed as a percentage of the sum of all fatty acids in the sample. The results of over 6 measurements conducted in the same conditions were averaged out. The experimental data were processed statistically by Student's t-test.

**Results and Discussion**

The results of the comparative study on the composition of PL in blood of healthy sheep and those infected by the nematode D. filaria are shown in Table 1.

The data show that the basic mass of PL in the blood of healthy sheep is represented by phosphatidylcholine (PC), the proportion of which reaches 60%. One of the massive fractions in the PL composition tissue of the majority of invertebrate and vertebrate animals, including sheep, is phosphatidylethanolamine (PE), the content of which ranges from 16 to 30%, but in some cases is even higher (4). The total content of PC and PE is 82.7%. The total contents of the other PLs–cardiolipin (CL), sphingomyelin (SM), phosphatidylserine (PS), lisophosphatidylcholine (LPC) and lysophosphatidylethanolamine (LPE)–constitute a small part (13.3%), with the exception of phosphatidylinozitol and phosphatidic acid, which is in agreement with findings for other vertebrate animals (11, 12). In the blood of sheep, phosphatidylinozitol and phosphatidic acid are found in negligible amounts (<0.05%). The most characteristic index of the composition of PL in the blood of animals is considered to be the ratio of amino-containing PLs (PE, PS, LPE) to choline-containing PLs (PS, SM, LPC). For the blood of healthy sheep this ratio is 0.4. Upon infection of the blood of sheep by the nematode *D. filaria*, changes in the quantitative content of individual PL fractions (Table 1) are revealed. The CL content was 1.9-fold higher, and the PS content was 1.4-fold higher than in healthy sheep, while the PC content was 1.2-fold higher. LPC, a product of PC degradation, was observed to be 3.4-fold higher. A higher quantitative content of LPC against the lower PC indicates an activation of phospholipase $A_2$ upon the infection of the sheep blood. It is known that the endogenous phospholipase of membranes plays a role in the process of adaptation of an organism to constant environmental changes, and under pathology or stress contributes to the formation of "latent" damage to membranes (13).

A high content of LPC in the composition of PL is one of the factors causing inhibition of phosphorylation, as well as membrane-coherent enzymes and a disturbance in the structural integrity of membranes (14,15). The content of PLs such as SM and PE is affected to a lesser degree. A similar phenomenon was observed in an investigation conducted by Sidorov et al. (3). The ratio of amino-containing PLs to cholin-containing PLs in the blood of sheep during infection reaches 0.6, which is notably higher than in healthy sheep.

Thus, our facts enable us to conclude that parasitizing by the nematode *D. filaria* causes essential changes in the quantitative content of separate fractions of PL in the blood of sheep, especially LPC, bringing about disturbances of lipid-albumen interaction and damage to the structural integrity of the membranes.

The results of gas-liquid chromatography of the FA compositon of PL of healthy sheep and those infected by *D. filaria* are given in Table 2.

We identified 13 FAs in the general PL of the sheep blood.

The major fatty acids of the phospholipid of healthy sheep, are myristic acid, oleic, stearic acid and arachidonic

Table 1. Changes in the phospholipid composition of sheep blood infected by *Dictyocaulus filaria* (% of the total PL, $\bar{x}\pm Sx$).

| Phospholipids | In sheep blood samples | |
|---|---|---|
| | healthy (n=12) | infected (n=13) |
| Phosphatidylcholine | 60.0±1.2 | 49.2±1.1 |
| Phosphatidylethanolamine | 22.7±1.0 | 24.2±0.9 |
| Cardiolipin | 3.2±0.4 | 6.1±0.7 |
| Sphingomyelin | 3.1±0.3 | 3.7±0.4 |
| Phosphatidylserine | 5.2±0.6 | 7.4±0.6 |
| Phosphatidoylinozitol | Traces | Traces |
| Phosphatidic acid | Traces | Traces |
| Lysophosphatidylcholine | 1.0±0.2 | 3.7±0.4 |
| Lysophosphatidylethanolamine | 0.8±0.3 | 1.0±0.5 |
| Unidentified phospholipid | 3.8±0.6 | 4.7±0.4 |

P=0.05

Table 2. Fatty acid composition of common PLs in the blood of healthy sheep and those infected by the nematode *Dictyocaulus filaria* ($\bar{x}\pm S_{\bar{x}}$% from the sum of the faaty acids).

| Fatty acids | Healthy sheep | Infected sheep |
|---|---|---|
| Lauric acid (12:0) | 1.3±0.3 | 1.8±0.5 |
| Myristoleic acid (14:1) | 1.8±0.1 | 1.4±0.3 |
| Myristic acid (14:0) | 23.6±0.8 | 19.6±0.9 |
| Palmitoleic acid (16:0) | 1.2±0.2 | 1.0±0.4 |
| Palmitic acid (16:0) | 1.4±0.4 | 1.2±0.2 |
| Linoleic acid (18:2) | 0.4±0.4 | 0.2±0.2 |
| Oleic acid (18:1) | 29.2±0.8 | 25.0±0.7 |
| Stearic acid (18:0) | 18.3±0.6 | 18.9±0.4 |
| Nonadecenic acid (19:1) | 1.4±0.5 | 1.1±0.2 |
| Nonadecanic acid (19:0) | 1.4±0.4 | 0.9±0.1 |
| Arachidonic acid (20:4) | 7.5±0.2 | 14.8±0.5 |
| Docozangexaegenic acid (22:4) | 3.9±0.7 | 8.2±0.6 |
| Docozagexagen acid (22:6) | 6.0±0.3 | 3.5±0.4 |
| Unidentified fatty acids | 3.6±0.3 | 3.2±0.2 |
| Saturated FA | 46.0±0.6 | 42.4±0.9 |
| Unsaturated FA | 51.4±0.8 | 55.2±0.7 |

Note: the first number after the acid name stands for the number of the carbon atom in the hydrocarbon chain, and the second stands for the number of the double bond.

acid, the total content of which reaches 78.6%. The FA composition of PL in the blood of sheep, as well as in other vertebrate and invertebrate animals (16-17), is characterized by a high degree of unsaturated acids: oleic acid, arachidonic acid, and docozagexenic acid, whose total reach 42% of the total of all unsaturated FAs. It is known that the composition of FA of structural PL determines to a considerable degree the functional properties of the membrane, which are characterized by a higher content of polyunsaturated FA of PL (3). Among FAs in the blood of healthy sheep, arachidonic acid and docozangexenic acid (20:4 and 22:6) are recorded in a greater quantity and the total of all polyunsaturated FA constitutes over 38% (Table 2). Saturated FAs are mainly represented by myristic acid and strearic acid, the total of which consitutes 91% of all saturated FA. The ratio of unsaturated to saturated FA of PL in the blood of healthy sheep is 1:1.

In sheep infected by *D. filaria*, the qualitative composition of (*PL FA) in blood does not differ from that of healthy sheep, but differs in the quantity of separate FAs observed, namely, myristic acid, oleic, arachidonic acid, docozantetraenic acid and docozagexagenic acid, these changes being interrelated. In particular, the content of myristic acid decreases 1.2-fold, oleic acid 1.1-fold and docozangexenic acid 1.7-fold. At the same time, the content of one of the massive fractions of fatty acids, arachidonic acid and docozantetraenic acid, increase 1.9-fold and 2.1-fold, respectively.

This study shows that polyunsaturated long-chain fatty acids are not subject to change, but short-chain fatty acids, both saturated and unsaturated, are subject to change. In this case, a resistance of these fractions to the infection by the nematodes *D. filaria* is manifested.

Khyulderom et al. (18) showed a blocking action of arachidonic acid to the permeability of intercellular contacts in cells. A mechanism of the disconnecting action of arachidonic acid consists of a direct interaction of the arachidonic acid with the contact canal of cells. It is suggested that a rise in the concentration of arachidonic acid reduces intracellular pH and increases concentrations of $Ca^{2+}$, and causes inhibition of interplasmic exchange of low-molecular substances (ions and metabolites) between contacting cells, which may be one of the exogenic physiological regulators of contact permeability. Of all the rich range of FA, docozangexenic acid has long attracted the attention of investigators, so far as it is recorded in sufficient quantities in the lipids of actively moving animals, in particular fishes possessing high metabolite levels (19). Therefore, this acid was called the "acid of adaptation". It is well known that enzymes fitted in the lipid matrix are in the lipid environment, which is different in its content from other parts of membranes, as polunsaturated acids, particularly docozangexenic acid, form a considerable part of the fatty acids of these layers (19). Therefore, a decrease in the concentration of docozangexenic acid can significantly change the activity of membrane-linked enzymes.

Furthermore, the intensity of the modification of the structural organization of the membranes exemplified in PL and their fatty acids may cause changes in the conformational and physico-chemical properties of membranic albumens-enzymes in the sheep exposed to infection by the nematode *D. filaria*. The aggregate of structural changes taking place in the cellular membranes of the blood of these sheep, such as an increase in the concentration of arachidonic acid and a decrease in the concentration of docozagexenic acid of general phospholipids, as well as an increase in the concentration of lyzophosphatidylcholine, may bring about a

malfunction of the structural and functional characteristics of the cellular cells of the host tissue, which can have a negative effect on the vital functions of the blood fraction and the organism as a whole.

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