## The paradigm of erythrocyte membrane lipidome in healthy dogs: first evaluation of the optimal interval values of a fatty acid cluster

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Membrane phospholipids and their saturated, monounsaturated and polyunsaturated fatty acids (FA) as hydrophobic components have fundamental structural and functional roles for cell life and homeostatic balance. The membrane lipidome profile of erythrocytes connects with metabolic and nutritional status of living organisms, providing information of the healthy conditions. In dogs, scarce and imprecise data are reported, lacking a systematic approach to get this important information.

A population of sixty-eight healthy dogs recruited from Italy, classifying the group for age, sex, size and other dog characteristics, was used to start the systematic investigation about membrane lipidome and health. In this study were included only dogs fed with commercial diet appropriate for life-stage requirements (growth vs adult maintenance) without any supplementation. The erythrocyte membranes were isolated from EDTA-treated blood and their FA composition was determined by Gas Chromatography, as previously described for humans. A cluster of 10 FA, comprised of saturated (SFA), monounsaturated (MUFA), polyunsaturated fatty acids [PUFA (omega-6 and omega-3)] was considered, reporting also the total FA contents (total SFA, total MUFA, total PUFA), the ratios between FA families (SFA/MUFA, omega-6/omega-3) and indexes of fatty acids (unsaturation index, peroxidation index, PUFA balance). The quantitation of the FA refers to this cluster, allowing the results of the analyses to be referred as % of one fatty acid in the cluster, giving a first evaluation of interval values in the cohort of healthy dogs, including 30 males (6 neutered) and 38 females (12 sterilized), aged from 2 to 156 months (median 41). The main FA of erythrocytes membrane were omega-6 PUFA (median 59.95%), SFA (35.56%) and MUFA (11.70%), while omega-3 fatty acids were present in minimal concentrations (1.75%). In particular, arachidonic, stearic, palmitic, linoleic and stearic acids comprised nearly 94% of total RBC membrane FA as already observed in other mammalian species.

Interesting differences were observed within sex: increased levels of palmitoleic acid in intact males (p<0.01), whereas intact females had lower dihomogamma-linolenic acid levels (p<0.05). In keeping with reported literature, an age-related increase in EPA was observed (p<0.01). Differences in lipidome RBC profiles were found for different bodyweights: increased SFA (p<0.01), MUFA (p<0.05) and decreased omega-6 PUFA (p<0.01) levels along with bodyweight. These findings indirectly show the different distribution of FA according to the characteristic breed metabolism.

The results of the present study suggest that erythrocyte fatty acid-based lipidomic analysis can be used as the methodology for evaluating health conditions in dogs.