Methodological approach to the tensiometrical analysis of the blood serum samples of Duroc pigs

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Abstract. The aim of this work was to compare the dynamic surface tension (DST) with biochemical parameters of the blood serum of Duroc pigs (BSDP). BSDP samples were taken after various times of pig fattening: 65 days (Group 1), 72 days (Group 2), 84-89 days (Group 3), 91-100 days (Group 4). Our approach is consisting in the BSDP-DST-measurement using all four modes of BPA-tensiometer: M1) "Standard experiment"; M2) "Experiment at constant lifetime"; M3) "Accelerated experiment"; M4) "Quick scan". Here, we have worked out all the modes mentioned above for determining the BSDP tensiometric parameters. The BSDP-DST parameters obtained for groups 3 and 4 (84-89 and 91-101 fattening days, respectively) were very high and close in numbers. (from 73.4-74.6 mN/m for ST\textsubscript{a} to 58.1-60.5 mN/m for ST\textsubscript{e}, respectively). These BSDP-DST parameters were significantly higher (7-13\%) at extremely low interface "life-times" (0.01-0.1 s), as compared to those for groups 1 and 2 (65 and 72 fattening days, respectively). In contrast, the BSDP-DST parameters obtained for groups 1 and 2 were low (from 66.1-71.4 mN/m for ST\textsubscript{a} to 56.9-57.9 mN/m for ST\textsubscript{e}, respectively). These data (in combination with other physical-chemical methods) can be used for the fundamental data set and monitoring the pig growth, health, productivity, etc.

1. Introduction

The major biochemical processes in the mammal’s organisms are depending on the quality of biological liquids. Evaluation of the general properties and features of the animal biological liquids (blood plasma and serum, urea, milk, etc.) is an important in fundamental (surfactant properties, bionterfaces, interactions, etc.) and applied (such as estimation of the "animal health and productivity, diagnostics and treatment of various diseases, the nutrition utilization by the animal, the quality of milk and meat production, etc.") aspects [1-3]. The blood plasma (serum) of humans or animals can be considered the most complicated colloid system among all known natural liquids. Since there are numerous complexes of various biologically active compounds (BAC) in plasma it is reasonable to consider blood as a "super-complex colloid system" (SCCS). The author [4] proposed the term SCCS in order to highlight the coexistence of the different BAC complexes with particular hierarchy in their "structure-property" relationships. Examples are a few types of the plasma lipoprotein "particles" (simply named lipoproteins) consisting of the major natural lipids (triacylglycerols, phospholipids, cholesterol, etc.) and particular proteins (so-called apoproteins with general names ApoA, ApoB, ApoC, ApoE or special names B-48, C-II, etc.) [2, 3].

There have been attempts to consider such complexes in terms of supramolecular systems [4-7].
well-known definition of supramolecular chemistry by J.-M. Lehn [5] is "chemistry beyond the molecule, bearing on the organized entities of higher complexity that result from the association of two or more chemical species held together by intermolecular forces". Therefore, the fields of supramolecular chemistry [5-7] and medical chemistry [8] are overlapping in some aspects. Steed J.W. and Atwood J.L. considered these as "the supramolecular chemistry of life" and "biological inspiration for supramolecular chemistry" [8]. It is important to highlight that the structures and the major functions of lipoproteins dealing with the metabolism of cholesterol, triacylglycerols and some proteins are so complicated that it is difficult to treat the set of all such particles and their "structure-property" relationships in terms of supramolecular systems [3, 4]. Moreover, the existing terminology of supramolecular systems seems to reflect better initial molecular events, such as molecular recognition and interactions (of the antigen-antibody, hormone-receptor or drug–receptor types, etc.) [9]. In contrast, the "joint continuous variation" (JCV) of molecular events results in association and dissociation of various BAC complexes that are typical for natural objects such as blood [3, 4].

Blood plasma is a "liquid part" of human or animal blood without blood cells. Serum is the plasma from which the clotting proteins (such as fibrinogen) have been removed. It contains dissolved proteins (serum albumins, various globulins, etc.), electrolytes (Na⁺, Ca²⁺, HCO₃⁻, Cl⁻, etc.) and other BAC (glucose, lipids, hormones, clotting factors, etc.) [1-3]. Plasma and serum also serve as the major biological liquid for laboratory or clinical diagnostics. That is why the comprehensive analysis of some colloid properties of human and animal biological liquids including the study of the dynamic surface tension and the lipid-protein content has become important. Traditionally, the diagnostics methods and approaches are developed more advanced for humans as compared to animals. This is particularly true for the interfacial methods and approaches that will be briefly described below.

Recently the powerful techniques of the dynamic surface tension (DST) measurements have been developed and successfully applied to various biological liquids [4, 10-14]. The recent "adjumps" of such a general method of blood diagnostics is of particular importance for productive and domestic animals, for diagnostics in veterinary medicine and some biomedical applications [15, 16]. There have been numerous attempts to estimate the static surface tension of human blood serum or plasma samples, but noticeable differences connecting with the age and gender; statistically reliable correlations with total lipids, serum cholesterol and some enzymes levels; with plasma free hemoglobin levels, cardiopulmonary bypass and oxygenator type have not been found [17-19].

The most comprehensive and successful studies of human biological liquids (blood, urine, etc.) were conducted in the Donetsk Medical University, Ukraine [10, 11] with DST devices and technology from "SINTERFACE Technologies GmbH" and MPI Colloids and Interfaces, Germany [20]. The DST measurements of human blood samples (done in Ukraine and Germany [10, 11, 20]) have shown particular correlations with blood biochemical parameters. My personal observations during the work with these groups and the data published allowed me to conclude that such DST data can be the most important and reliable reference values for all other researchers, including my own group. The authors [10, 11] found small but pronounced differences in the DST values of blood of women and men of different ages. The digital DST parameters from σ₀ to σ₂ (will be defined below as SIGMA₀ - SIGMA₂) were slightly higher for women, whereas λ - the tilt of the tensiogram inclination (will be defined below as LAMBDA) was significantly higher for men. The digital DST parameters for children of different sexes were practically identical, but higher than for adults, especially σ₀ values. In contrast, the tilt of the tensiogram inclination was higher for men than those for children (both boys and girls) and significantly higher than those for women. These effects are due to remarkable differences of blood plasma components: protein, lipid, carbohydrate, salt components for children and adults of different sex and age [10, 11]. Thus, for biomedical applications it is important to take into consideration the following: the reference DST values are different for adults of both sexes, whereas for children independent of gender the average values can be used as references.

It is important to highlight that for children, it is necessary to study both major liquids (blood and urine) as "health indicators", whereas for adults, it is sufficient to investigate the DST blood parameters (depending on the anamnesis). Actually, there were stronger (no matter, positive or negative)
correlations between DST and biochemical parameters in the blood as compared to the urine. That is why the blood study is more often used and will be discussed below. For example, for healthy adults the $\alpha_0$ parameter has positive correlations with the concentration of blood $\alpha_1$-globulins, $\beta_2$-globulins, fibrinogen, glucose, potassium, sodium, total and ionized calcium, $\sigma_1$ – with glucose and all electrolytes, $\sigma_2$ - with the concentration of total protein, albumins, $\beta$-globulins, HDL (high density lipoproteins) and total calcium. However, negative correlations are observed for: $\sigma_0$ - with $\alpha_2$-globulins, IgG and IgA, $\sigma_1$ – with the level of IgG and IgM, chlorides, triglycerides, total cholesterol, LDL (low density lipoproteins) and VLDL (very low density lipoproteins); $\sigma_2$ – with the content of triglycerides, cholesterol, phospholipid, LDL, urea and chlorides [20-22].

Thus, the DST analysis of human blood is not only of fundamental interest for chemists and biologists, but it has also applied significance for an early diagnosis of pathological processes and controls for therapeutic treatment [23-31].

It is important to emphasize prior to the beginning of our work [32] there were almost no reliable correlations between the DST measurements and the most important biochemical parameters of animal blood. In the field of veterinary science and practice the DST investigations can give valuable information on an early estimation of the physiological-biochemical status of the organism, for general inspection of cattle before vaccination (immunization) or slaughter, for "quick separation” of healthy and ill animals in the case of infection, etc. [33, 34]. There were a few Ph.D.-theses prepared in my research group on some of the above mentioned topics and these results were published mainly in Russian [35-37].

The aim of this work was to compare the dynamic surface tension (DST) parameters of the blood serum of Duroc pigs (BSDP) by four approaches that consisted in the multiple check of measurement modes of all BSDP DST parameters.

2. Materials and Methods

2.1. Materials

The samples of pig blood serum were collected during boars fattening. The major experiments were fulfilled in the Chemistry Department (Russian State Agrarian University - Moscow Agricultural Academy named after K.A. Timiryazeva) with materials from Farm Animal’s Physiology and Biochemistry Department (Federal Research Center for Animal Husbandry named after Academy Member L.K. Ernst) during September-November 2020. All experiments were carried out in the "compliance with legal and ethical standards and requirements for keeping animals used in scientific research, as well as in the selection of samples of animal biomaterial animals" and approved by "Bioethics Commission of the Federal Research Center for Animal Husbandry named after Academy Member L.K. Ernst”.

The blood serum samples for the further measurements of the dynamic surface tension (DST) parameters of these liquids were taken after various times of pig fattening: 65 days (Group 1), 72 days (Group 2), 84-89 days (Group 3), 91-100 days (Group 4).

2.2. Methods

The biochemical parameters of animal blood serum sample were determined using a "ChemWell" automatic biochemical analyzer (Awareness Technology, USA) with reagents of "Analyticon Biotechnologies AG" (Germany) and "Spinreact" (Spain). The following biochemical parameters: the concentration of total protein (biuret method), albumin (colorimetric method), cholesterol and TG (enzyme-colorimetric method). The following ratios and parameters: A/G and the concentration of globulins were determined by calculation.

Measurements of such DST parameters [38-40] as the current surface tension values (SIGMA$_0$ at $t$→$0$, SIGMA$_\text{max}$ at $t$→$\text{max}$, etc.) were carried out using a BPA device (so called "Tensiometer") [40]. The obtained data were treated as the "DST vs. time dependence" or, simply – "tensiogram" [15] using the "ADSA program” [1] and the tilt angles at the initial and final parts of the tensiogram (TA$_0$ at $t$→$0$ and
TA_m at t→max, respectively) were extrapolated. The BPA device is using so-called "air bubble" method, which adapted for measuring of various biological liquids [33]. The advantages of the method of "hanging drop" [1, 2] are as follows: a small volume of liquid to be analyzed (less than 0.5 ml), the simple and convenient temperature control of samples, a wide range of measurements "surface lifetimes" (from 0.01 to 100 s) that complements the maximum pressure in a "hanging drop" method at PAT_1P ("Sinterface Technologies", Germany) that we used before [9-11, 33].

Types of experimental procedures. A method for measuring dynamic surface tension with BPA, in addition to measuring and calculating dynamic surface tension, includes automatic testing of measurement conditions, correcting aerodynamic and viscous resistance.

BPA allows you to carry out experiments in one of four modes: M1 mode or "Standard experiment"; M2 mode or "Experiment with a given constant lifetime"; M3 mode or "Accelerated experiment"; M4 mode or "Quick scan". We have worked out all the above modes for determining the tensiometric parameters of the blood serum of Duroc pigs (data - in the parts 3 and 4 below).

3. Results
The methodological approach is consisting in the multiple check of measurement modes of the dynamic surface tension (tensiometric) parameters (of the blood serum of Duroc pigs), i.e. all the possibilities briefly mentioned above and summarized in the Tables 1 and 2.

Table 1. Measurements of the dynamic surface tension (DST) parameters of the pig blood serum by BPA (average values) by the following modes: M1 – "Standard experiment"; M2 - "Experiment with a given constant lifetime"; M3 - "Advanced experiment"; M4 - "Quick scan".

| Parameters | 0.01, s | 0.1, s | 1.0, s | 2.0, s | 3.0, s | 4.0, s | 5.0, s |
|------------|---------|---------|---------|---------|---------|---------|---------|
| Device     | 72.6    | 72.2    | 71.63   | 71.54   | 71.46   | 71.47   | 71.39   |
| control    |         |         |         |         |         |         |         |
| M1         | 74.8    | 73.0    | 72.33   | 68.08   | 65.88   | 64.00   | 62.61   |
| M2         | 74.4    | 74.2    | 72.17   | 74.0    | 74.3    | 74.6    | 74.8    |
| M3         | 70.16   | 67.51   | 65.31   | 63.33   | 62.40   | 61.35   | 61.35   |
| M4         | 72.19   | 69.58   | 66.20   | 63.33   | 62.40   | 61.35   | 61.35   |
|            | a       | a       | a       | a       | a       | a       | a       |

* - these data impossible to measure with this mode (regime).

Table 2. Measurements of the dynamic surface tension (DST) parameters of the pig blood serum by BPA (average values) by the following modes: M1 – "Standard experiment"; M2 - "Experiment with a given constant lifetime"; M3 - "Advanced experiment"; M4 - "Quick scan".

| Parameters | 6.0, s | 7.0, s | 8.0, s | 9.0, s | ST_w, s | TA_0 | ST_m, s | TA_m |
|------------|--------|--------|--------|--------|---------|------|---------|------|
|            | at min.| at min.| at max.| at max.| r.u.    | r.u. | r.u.    | r.u. |
| Device     | 71.36  | 71.33  | 71.30  | 71.29  | 72.39   | 0.83 | 71.27   | 0.15 |
| control    |        |        |        |        |         |      |         |      |
| M1         | 61.88  | 61.71  | 61.3  | 61.2  | 76.55   | 4.12 | 51.55   | 25.84|
| M2         | a      | a      | a      | a      | 79.56   | 7.07 | 66.69   | 5.69 |
| M3         | a      | a      | a      | a      | 74.69   | 4.27 | 57.02   | 14.75|
| M4         | 60.94  | 60.94  | 60.94 | 60.94 | 74.65   | 5.46 | 54.36   | 16.36|

* – these data impossible to measure with this mode (regime).

The further measurements of the DST parameters of the blood serum were fulfilled using the pig samples taken after various times of pig fattening: 65 days (Group 1), 72 days (Group 2), 84-89 days (Group 3), 91-100 days (Group 4).
Table 3. Measurements of the dynamic surface tension (DST) parameters of the pig blood serum by BPA (average values) by the mode M1.

| Parameters  | 0.01, s | 0.1, s | 1.0, s | 2.0, s | 3.0, s | 4.0, s | 5.0, s |
|-------------|---------|--------|--------|--------|--------|--------|--------|
| Device control | 74.8 ±0.4 | 74.5 ±0.4 | 73.9 ±0.4 | 74.1 ±0.4 | 73.6 ±0.4 | 73.7 ±0.4 | 73.9 ±0.4 |
| Group 1     | 74.5 ±4.3 | 72.7 ±3.7 | 68.8 ±3.3 | 65.5 ±2.7 | 63.6 ±2.9 | 62.1 ±2.3 | 60.8 ±2.3 |
| Group 2     | 71.9 ±4.1 | 70.1 ±2.9 | 67.3 ±3.4 | 63.8 ±2.2 | 61.1 ±2.4 | 60.0 ±2.4 | 59.2 ±2.4 |
| Group 3     | 75.1 ±4.4 | 74.8 ±4.2 | 72.1 ±3.4 | 69.5 ±2.9 | 67.1 ±2.4 | 65.8 ±2.4 | 63.8 ±2.1 |
| Group 4     | 75.4 ±4.5 | 74.9 ±4.3 | 72.2 ±3.3 | 68.8 ±2.8 | 66.3 ±2.2 | 64.4 ±2.1 | 63.1 ±2.1 |

Table 4. Measurements of the dynamic surface tension (DST) parameters of the pig blood serum by BPA (average values) by the mode M1.

| Parameters  | 6.0, s | 7.0, s | ST_{at min.} | TA_{at min.} | ST_{at max.} | TA_{at max.} |
|-------------|--------|--------|---------------|---------------|---------------|---------------|
| Device control | 73.6 ±0.4 | 73.4 ±0.4 | 74.9 ±0.5 | 0.77 ±0.06 | 73.3 ±0.3 | 0.21 ±0.02 |
| Group 1     | 59.9 ±2.8 | 59.4 ±2.8 | 71.4 ±3.1 | 1.41 ±0.23 | 57.9 ±2.5 | 1.24 ±0.13 |
| Group 2     | 59.3 ±2.6 | 58.1 ±2.7 | 66.1 ±2.9 | 2.09 ±0.25 | 56.9 ±2.4 | 1.18 ±0.12 |
| Group 3     | 61.6 ±2.9 | 61.2 ±2.9 | 73.4 ±3.5 | 1.51 ±0.24 | 60.5 ±2.7 | 1.08 ±0.11 |
| Group 4     | 61.8 ±2.8 | 61.4 ±2.7 | 74.6 ±3.8 | 1.42 ±0.23 | 58.1 ±2.6 | 1.30 ±0.21 |

It was important to highlight that the DST parameters of the blood serum obtained for the groups 3 and 4 (long fattening, respectively) were significantly higher (7-13%), especially at extremely low "lifetimes" of the interfaces (0.1 s and ST_{0} ), as compared to the group 2 (72 days of fattening). It was interesting that the DST parameters of the blood serum obtained for the groups 3 and 4 (long fattening, respectively) were almost the same (Tables 3 and 4). The DST parameters of the blood serum obtained
for the groups 3 and 4 (long fattening, respectively), were only slightly higher (3-5%), especially at extremely low "life-times" of the interfaces (0.1 s and STn) as compared to the group 1 (65 days of fattening). These changes of the DST parameters of the blood serum obtained for the groups 3 and 4 (long fattening, respectively) were only slightly higher (about 2-4%) at relatively high "life-times" of the interfaces (6-7 s and STin) as compared to the groups 1 and 2 (65 and 72 days of fattening, respectively).

4. Discussions

The methodological approach is briefly mentioned above and all the details of the measurement modes of the DST parameters of the pig blood serum will be discussed below.

**Mode M1, Standard Experiment.** The experiment in the M1 mode allowed us to use the standard capabilities of the BPA-1R and obtain data on the dynamic surface tension in the largest possible range of the surface "life" time (up to 100 seconds). A standard experiment begins by measuring the dynamic surface tension with a minimum life time of 10 ms. The flow rate changes in small steps and the required waiting cycles for the set mode. Each point is considered as a separate experiment in the standard mode; its measurement lasts from 20 to 30 minutes.

**Mode M2, Experiment with a preset (constant) life time of the interface.** Experiment with constant life time of the interface (Mode M2) is a procedure for measuring the dynamic surface tension for the life time of bubbles, set by us in advance, when the pneumatic system of the device keeps the air flow rate through the capillary constant during the entire experiment cycle. The M2 mode is intended for use for continuous measurements in technological processes, as well as in process control tasks. Mode M2 takes into account the dynamic components of resistance and viscosity (the last parameters must be applied in the each case of using this mode).

**Mode M3, Experiment with increasing gas flow rate.** When measuring blood samples from pigs, as well as when measuring some other solutions (containing surfactants), copious foaming occurs (directly during the measurement process). This effect significantly distorts the obtained data (since it leads to a decrease in the concentration of the surfactant in the sample) or during the measurement the vessel with the solution may overflow with foam, with an excessively large amount of which the process itself is stopped by the device. To reduce the formation of foam during the experiment on measuring blood samples from pigs, we used the M3 mode, in which the experiment can be carried out in the reverse sequence (i.e., so that the gas flow rate reached maximum values only at the final stage of measurements). In contrast to the standard mode M1, the measurement in this mode (mode M3) begins with the maximum "lifetime" of the interface. The maximum "lifetime" of the interface measured in this experiment is about 5 seconds. The rest of the settings (for example, the correction of aerodynamic resistance or viscosity) are completely analogous to those (corrections) in the standard experiment (mode M1).

**M4 Mode, Accelerated Experiment.** In terms of its parameters (settings, corrections), the accelerated experiment in the M4 mode is fully consistent with the standard one (the M1 mode). The main difference is that the duration of the accelerated experiment is almost 5 times less than that of the standard one (but not less than 7 minutes). By setting the maximum "blowdown" time limit of 10 seconds, in the M1 mode, it is possible to use a faster change in the air flow rate through the measuring capillary.

**Comparison of the DST and biochemical parameters of the pig blood serum.**

It was important to compare the obtained DST and the major biochemical parameters of pig’s blood (at various duration of their fattening) because of the dependences between these parameters that were found for another animals.

It is interesting that the average values of the biochemical parameters of the blood serum for the groups 3 and 4 (at 84-89 and 91-101 days fattening, respectively) were very close: 69.8±1.5 g/L and 69.9±1.3 g/L - for total protein; 38.2±1.1 g/L and 37.5±1.1 g/L - for albumins (A); 31.6±1.2 g/L and 32.4±1.2 g/L - for globulins (G); 1.24 and 1.19 - for A/G ratio; 0.90±0.04 g/L and 0.93±0.05 g/L - for triglycerides; 2.25±0.06 mM and 2.22±0.05 mM – for cholesterol, respectively. Again, the DST
parameters of the blood serum obtained for the groups 3 and 4 (long fattening) were almost the same (Tables 3 and 4) and relatively high (from 73.4-74.6 mN/m for ST₀ to 58.1-60.5 mN/m for STₘ ).

The average values of the following biochemical parameters of the blood serum at 65 days (short) fattening were the following: 75.5±1.5 g/L - for total protein, 42.6±1.1 g/L - for albumins (A), 32.9±1.2 g/L - for globulins (G), 1.31 - for A/G ratio, 0.29±0.02 g/L - for triglycerides, 2.04±0.07 mM – for cholesterol, respectively. The DST parameters of the blood serum obtained for this group 1 (Tables 3 and 4) were relatively low (from 71.4 mN/m for ST₀ to 57.9 mN/m for STₘ ).

In contrast, the average values of the following biochemical parameters of the blood serum at 72 days fattening were the following: 77.3±1.7 g/L - for total protein, 42.0±1.2 g/L - for albumins (A), 35.3±1.1 g/L - for globulins (G), 1.23 - for A/G ratio, 0.28±0.01 g/L - for triglycerides, 2.46±0.08 mM – for cholesterol, respectively. The DST parameters of the blood serum obtained for this group 2 (Tables 3 and 4) were extremely low (from 66.1 mN/m for ST₀ to 56.9 mN/m for STₘ ).

It is found a general strong dependence between an increase in the protein content (not only total protein, but also - albumins, globulins and the ratio between these fractions) in pig blood and a decrease of the DST parameters of this liquid (i.e. an opposite correlation). This is reasonable from the general colloid views because blood serum proteins can be considered as strong surfactants, which adsorbing relatively fast at the interface. In contrast, only moderate dependence between the lipid content (triglycerides, cholesterol) and DST parameters is found, probably, because of the less amount of lipids in the blood serum as compared to proteins. Nevertheless, lipids are also very active surfactants adsorbing at the interface. Thus, the major biochemical parameters of pig’s blood (at various duration of their fattening) are correlating with the obtained DST parameters of this liquid. The authors recommended to use the obtained DST data as reference values for the blood serum evaluation of Duroc pig’s fattening.

5. Conclusions
The methodological approach is consisting in the multiple check of measurement modes of the dynamic surface tension (DST) parameters of the blood serum of Duroc pigs (BSDP). A particular construction of tensiometer (BPA) allowed to carry out experiments in one of four modes: M1 mode or "Standard experiment"; M2 mode or "Experiment with a given constant lifetime"; M3 mode or "Accelerated experiment"; M4 mode or "Quick scan". Here, we have worked out all the modes mentioned above for determining the BSDP tensiometric parameters. The BSDP DST parameters obtained for groups 3 and 4 (at 84-89 and 91-101 days fattening, respectively) were extremely high and similar (from 73.4-74.6 mN/m for ST₀ to 58.1-60.5 mN/m for STₘ ). These BSDP DST parameters were significantly higher (7-13%), especially at extremely low "life-times" of the interfaces (0.1 s and ST₀ ), as compared to those for groups 1 and 2 (65 and 72 days of fattening). It was interesting that the BSDP DST parameters obtained for the groups 3 and 4 were almost the same. In contrast, the BSDP DST parameters obtained for groups 1 and 2 were extremely low (from 66.1-71.4 mN/m for ST₀ to 56.9-57.9 mN/m for STₘ ). These data in combination with other physical-chemical methods can be used for monitoring the pig growth and health; quality of animal products; etc.

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Conflicts of Interest
The authors declare no conflict of interest.

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