

# An innovative approach to assessing the integral parameters of the hybrid pig blood for the methodological support of animal husbandry development

Sergei Yu. Zaitsev\*, and Oksana A. Voronina

Federal Research Center for Animal Husbandry named after Academy Member L.K. Ernst, Dubrovitsy 60, Podolsk Municipal District, Moscow Region, Podolsk 142132, Russia

**Abstract.** The main aims of research were: to measure the surface tension (ST) values of hybrid pig blood; to establish the relationships between these ST-values and obtained biochemical parameters of the same blood samples. All studied animals (n=43) were healthy and grown at the feeding stations (Russia). The ST-values (measured using PAT-device) of the pig blood were obtained at initial (n=30) and final (n=13) points of animal fattening. The following correlations between eST at final (or initial) point of pig fattening and the biochemical parameters were obtained: +0.80 (+0.19) with the ratio of albumins to globulins (A/G), -0.39 with globulins (-0.38) with phospholipids, +0.32 (+0.40) with the “de Ritis” coefficient, -0.52 (+0.35) with Cl. The correlations between eTA at same fattening points and the biochemical parameters were obtained: +0.32 with A/G ratio, +0.18 with globulins, +0.36 with phospholipids, +0.28 (+0.17) with the “de Ritis” coefficient, -0.32 (+0.21) with Mg, +0.35 with Fe, +0.30 with Cl, +0.31 (+0.34) with the ratio of calcium to phosphorus (Ca/P). Such tendency indicated the stabilization of pig physiological-biochemical status during their fattening. The authors recommended the following eST (42-46 mN/m) and eTA (10-30 mN·m<sup>-1</sup>·s<sup>-½</sup>) blood parameters as reference values for further applications in husbandry.

## 1 Introduction

There are many well-known physical-chemical methods for estimation of the physiological-biochemical status (PBS) of humans [1,2] and animals at various physiological conditions [3,4]. It is well-known that the majority of these methods are almost the same traditional biochemical methods that were developed for a long time ago and used up to now [3,4]. For about fifty years the measurements of the dynamic surface tension (ST) of various biological fluids were carefully studied and the automatic devices were developed using tensiometry approach [1-4]. The ST measurements were successfully applied for PBS evaluation of healthy humans for about 30 years [1, 2]. The initial works of some international groups at the ST measurements of human biological liquids (blood, urine, etc.) are summarized in a

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\* Corresponding author: [s.y.zaitsev@mail.ru](mailto:s.y.zaitsev@mail.ru)

few reviews and books [1,2]. These studies carried out for the healthy men and women at different ages, as well as for patients with some illness [2]. Since that studies, a lot of research have been fulfilled in these directions, but here we could discuss only recent ones [5-8]. A relatively “simple and low-cost device” was designed and fabricated for measuring the blood ST by “pendant drop method” [5]. Using this instrument, the ST value of human blood “is inferred using drop shape factor method and image analysis technique at various experimental conditions” [5]. It was interesting that the ST values of the blood samples of healthy humans strongly correlated with the temperature of these samples [5] that was in good agreement with the literature data [1, 2]. It was interesting that the ST values of the “female blood” were remarkably different from those of the “male blood” [5]. Remarkably, that the only negligible effects of patient ages on ST values were found. This research [5] supported the ST reference data of blood samples at various conditions that were found earlier [1, 2].

A design of “microfluidic system for screening disease based on physical properties of blood” can be considered as the further development in the ST field [6]. The authors [6] proposed this system as “novel point of care systems for large-scale screening of health of the population residing in resource-limited areas of low- and middle-income countries with a view to obtaining data at a community level as a rationale to achieve better public health outcomes” [6]. This system measuring the following physical properties of various samples (“like normal, tuberculous and anemic blood samples” [6]): 1) the blood viscosity values were measured using “a power-law model coupled with the Rabinowitsch-Mooney correction for non-Newtonian shear rates developed in a steady laminar Poiseuille flow”; 2) the ST values were measured “by solving the Young-Laplace equation for pendant drop shape hanging on a vertical needle” [6]. The authors [6] concluded that the “major advantage of this system was low-cost, as well as its simplicity and portability” [6]. Some Polish scientists [7] did not find pronounced difference in ST values between the blood samples taken from healthy men and women. This seems in contrast to the previous findings [1-4]. Moreover, these authors indicated “no correlation between viscosity and surface tension of blood at 37°C” [7].

Numerous optical methods recently used for advanced ST measurements in the field of various medical applications that was summarized in the comprehensive review [8]. The authors [8] insisted that ST measurements of biological fluids “suffered several flaws including lack of dynamic control and required a direct contact with the samples” [8] and concluded that the proposed optical method “offers a good opportunity in fields such as medical and diagnostic analysis for monitoring applications” [8]. Evaluation of serum ST is an innovative approach to assess the integral parameters of the animal blood in order to support the animal husbandry development [9-12].

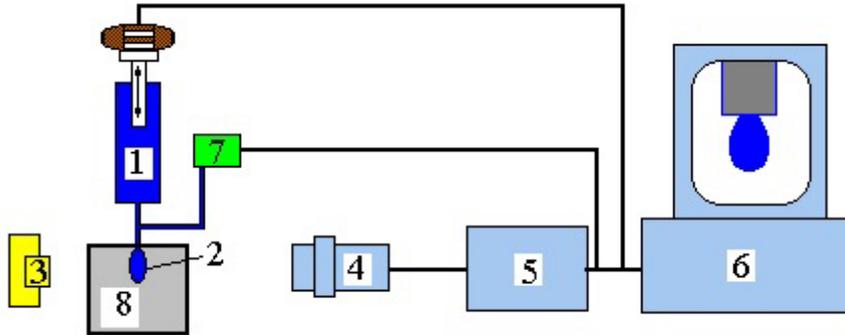
Recently, our group [4, 9-12] has used ST measurements of some biological fluids (blood, milk, etc.) from various animals for their PBS estimation. Up to begin of our work there were no comprehensive ST data of animal blood and their correlations with the most important biochemical parameters. In the field of science and practice of farm animals the ST investigations can give an important information on PBS of these organisms, as well as for general monitoring of animals, especially at large farms, etc. [10-14] including those of the same hybrid pigs [15,16]. Pork is one of the key husbandry areas in world livestock production [17], and in the 21st century it determines positive dynamics in all domestic livestock breeding in terms of the volume and quality of meat produced [18-20].

The main aims of this work were the following: to measure the ST values of the hybrid pig blood; to establish the relationships between these ST values and obtained biochemical parameters of the same blood samples, as well as to propose the obtained ST data as reference values for further applications in husbandry.

## 2 Materials and Methods

The studies were carried out on hybrid boars (n=43 total animals) grown at the feeding stations of the selection-hybrid center (SHC, Russia). All animals were divided onto the 2 groups: 1) at initial point (setting) of pig fattening (n=30), 2) at final point (withdrawing) of pig fattening (n=13), respectively. All studied animals were clinically healthy. The samples of blood serum were collected at initial and final points of pig fattening. The major experiments were fulfilled in the Farm Animal's Physiology and Biochemistry Department (Federal Research Center for Animal Husbandry named after Academy Member L.K. Ernst) during January-April 2021. 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".

Measurement of such ST parameters [4] as the equilibrium surface tension (eST) and extrapolated tilt angle (eTA) were carried out using a PAT\_1P device (so called "Topfen-Blasen-Profiltenziometer") and treated as the "ST-time tensiograms" [4] using the "ADSA program" [1]. The PAT\_1P device is using so-called "hanging drop" method, which adapted for measuring of various biological liquids [1-4]. The advantages of the method of "hanging drop" [1-4] 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 lifetime drops (from 10 to 10000 s) that complements the maximum pressure in a bubble method that we used before [9-11]. As an example, a scheme of our PAT\_1P ("Sinterface Technologies", Germany) instrument is shown at the Figure 1.



**Fig. 1.** The principle concept of the ST measurements by tensiometer PAT\_1P. The macro-dosing system 1, the drop of the biological liquid 2, light source 3, the camera lens 4, an analog-digital converter 5, a computer system 6, a micro-dosing system 7, thermostatic cell 8 (adapted from [5]).

The experimental error of the measurement of surface tension by the "hanging drop" method is about 0.1 mN/m. [5]). The main parameter of the droplet hanging on the capillary tip is its volume. The larger a volume of the drop, the more it is different from a spherical shape. The Laplace equation describes the mechanical equilibrium as balance of forces acting on the drop. Excessive pressure in the liquid drop placed in another liquid or air is determining curvature radii ( $R_1$  and  $R_2$ ) and the surface tension at the interface:

$$\sigma \left( \frac{1}{R_1} + \frac{1}{R_2} \right) = \Delta P \quad (1)$$

where  $\sigma$  - surface tension,  $\Delta P$  - pressure difference between the phases (excessive pressure).

In this case, the magnitude of pressure difference can be expressed as a linear function of the drop height :

$$\Delta P = \Delta P_0 + \Delta \rho g z \quad (2)$$

where  $\Delta P_0$  - the difference between the hydrostatic pressure in the plane  $z = 0$ ,  $z$  - vertical coordinate,  $\Delta \rho$  - difference between the densities of the two bulk phases,  $g$  - gravitational acceleration.

Capillary forces tend to make a spherical droplet, whereas gravity, on the contrary, tends to draw a drop along the vertical axis. Thus, if the surface tension is known, the droplet shape (the curvature radii  $R_1$  and  $R_2$ ) can be defined by “the Laplace equation” [5]. Neumann A. W. and co-workers proposed a method, called “Axisymmetric Drop Shape Analysis (ADSA)” [5], in which the droplet form is automatically analyzed, optimized and compared with the Laplace theoretical profile. Thus, the surface tension values can be calculated from the geometrical parameters of the drop [5]. Some examples of the ST measurements of animal blood plasma and serum by the maximum bubble pressure and hanging drop methods are presented below.

The measurements of the major biochemical parameters of the hybrid pig blood were fulfilled at automatic biochemical analyzer “Chem Well” (“Awareness Technology”, USA) using reagents from “Spinreact” (Spain), “Analyticon” (Germany) and “Diacon” (Russia) by standard procedures published elsewhere [12]. The following biochemical parameters [12] were obtained: total amount of globulins (G), the ratio of albumins to globulins (A/G), total phospholipids (PL), the ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT) as so called de Ritis coefficient (AST/ALT), total magnesium (Mg), total iron (better “ferrum” as Fe), total chloride (better “chlorine” as Cl), the ratio of calcium to phosphorus (Ca/P). The experimental data were statistically treated by Excel and “R” programs.

### 3 Results

The main directions of this work were the following: to measure the ST parameters of the pig blood serum; to compare them and to find any correlations between these ST and some biochemical parameters of the pig blood serum; to present the obtained ST values as reference data for further applications in husbandry and biomedical fields. The authors choose the equilibrium surface tension (eST) and extrapolated tilt angle (eTA) as the main ST parameters at setting and withdrawing points of pigs’ fattening. The following biochemical parameters of the blood serum of hybrid pigs were measured: total amount of globulins (G), the ratio of albumins to globulins (A/G), total phospholipids (PL), the ratio of aspartate aminotransferase (AST) to alanine aminotransferase (ALT) as so called de Ritis coefficient (AST/ALT), total magnesium (Mg), total iron (better “ferrum” as Fe), total chloride (better “chlorine” as Cl), the ratio of calcium to phosphorus (Ca/P). These data are the most important for estimation of the pigs’ PBS during their growth and fattening [4,12], especially for Russia, as a country with highly intensive pig farming.

#### 3.1 Measurements of the surface tension and biochemical parameters of pig blood

The surface tension parameters (such as eST and eTA) and some biochemical parameters of pig blood (Table 1), were obtained for the first time.

**Table 1.** The surface tension parameters and biochemical parameters of pig blood (at initial point of their fattening).

№	G, g/L	A/G, mM	PL, mM	AST/ ALT, r.u.*	Ca/P, r.u.*	Mg, mM	Fe, mkM	Cl, mM	eST, mN/m	eTA, mN·m <sup>-1</sup> ·s <sup>-½</sup>
1	32.18	1.25	1.85	0.88	0.67	0.95	26.82	133.92	43.24	13.37
2	29.68	1.23	1.69	1.34	0.71	0.74	23.25	121.21	45.21	17.45
3	24.82	1.68	1.39	1.26	0.76	0.66	46.49	123.02	44.14	16.73
4	27.62	1.26	1.67	1.31	0.46	0.58	17.88	104.41	44.23	17.24
5	23.62	1.80	1.43	1.16	0.57	0.37	24.14	119.39	45.18	-4.11
6	22.76	1.68	1.54	1.30	0.74	0.67	23.25	125.29	44.69	17.93
7	22.91	1.51	1.67	2.10	0.65	0.75	17.88	114.40	45.06	18.97
8	28.40	1.44	1.22	1.43	0.65	0.69	22.35	127.11	45.59	35.54
9	28.71	1.25	1.98	0.91	0.71	0.68	20.56	89.39	42.73	12.81
10	28.30	1.18	1.45	0.85	0.95	0.73	25.93	91.47	42.98	3.08
11	25.87	1.45	2.28	0.77	0.59	0.52	30.40	89.39	43.83	15.62
12	27.82	1.38	1.41	0.97	0.56	0.75	21.86	100.62	42.31	12.12
13	30.45	1.27	1.87	0.60	0.71	0.66	17.88	89.39	43.46	13.75
14	38.08	1.20	1.76	1.20	0.64	0.90	40.23	96.05	44.88	16.21
15	26.77	1.36	1.74	1.53	0.65	0.68	20.56	88.15	43.65	12.63
16	30.72	1.09	1.56	1.30	0.59	0.68	33.08	86.07	44.27	9.86
17	30.31	1.10	2.06	2.12	0.79	0.70	36.66	96.05	44.07	12.32
18	25.11	1.49	1.87	0.99	0.73	0.56	38.44	95.63	43.80	10.69
19	33.09	0.92	2.11	0.57	0.79	0.57	26.82	97.71	44.31	12.04
20	29.41	1.24	2.21	1.09	0.64	0.61	34.87	100.62	43.57	15.35
21	28.79	1.25	2.26	0.80	0.67	0.55	26.82	103.53	42.87	19.24
22	31.29	1.16	1.54	0.76	0.67	0.41	21.46	95.63	45.17	7.33
23	32.74	1.12	1.48	1.07	0.56	0.75	29.50	102.70	44.56	12.69
24	29.96	1.15	1.54	1.18	0.62	0.91	22.35	94.38	44.59	11.01
25	31.01	1.09	1.89	0.79	0.70	0.76	20.56	98.96	42.18	8.82
26	42.80	1.02	1.76	1.94	0.91	0.92	28.44	100.20	44.68	10.78
27	29.48	1.24	2.04	1.91	1.01	0.79	42.91	111.84	44.09	11.01
28	22.41	1.56	1.48	1.57	0.66	0.51	22.35	104.78	43.94	12.22
29	26.70	1.38	1.78	1.12	0.70	0.64	33.08	103.11	44.50	10.37
30	34.89	1.10	1.87	1.36	0.94	0.79	31.29	107.69	43.56	11.69

Notes: r.u.\* - relative units

The average eST and eTA data (Table 1) of the blood serum at initial point of hybrid pigs' fattening were the following:  $44.04 \pm 0.16$  mN/m and  $13.16 \pm 1.16$  mN·m<sup>-1</sup>·s<sup>-½</sup>, respectively. It is interesting that the standard deviations of the eST and eTA data were the following: 0.88 mN/m and 6.33 mN·m<sup>-1</sup>·s<sup>-½</sup>, respectively. The last values had the following meaning: the low

standard deviation in the case of eST indicated that the values tend to be close to the average eST data (also called the “expected value”) of the set, while a high standard deviation in the case of eTA indicated that these values are spreading out over a wider range. The authors recommended to use the eST and eTA (Table 1) data in the range of 42-46 mN/m and 10-30  $\text{mN}\cdot\text{m}^{-1}\cdot\text{s}^{-1/2}$ , respectively, as reference for the blood serum evaluation at initial point of hybrid pigs’ fattening.

The average values (Table 1) of the following biochemical parameters of the blood serum at initial point of pigs’ fattening were:  $1.30\pm 0.04$  - for the A/G ratio,  $29.22\pm 0.81$  g/L - for total globulins,  $1.75\pm 0.05$  g/L - for total phospholipids,  $1.20\pm 0.07$  - for the de Ritis coefficient (AST/ALT),  $0.68\pm 0.03$  mM - for the total magnesium (Mg),  $27.60\pm 1.41$  mkM - for the total iron (better “ferrum” as Fe),  $103.7\pm 2.3$  mM - for the total chloride (better “chlorine” as Cl),  $0.70\pm 0.02$  - for the ratio of calcium to phosphorus (Ca/P), respectively. The obtained data are in agreement with the found literature data for these animals [10-12]. These average values of the major biochemical parameters have been used for correlation analysis with the obtained eST and eTA data, presented in the part 3.2 below.

The major biochemical and ST parameters of hybrid pigs’ blood (Table 2) at final point of their fattening were obtained for the first time.

**Table 2.** The surface tension parameters and biochemical parameters of pig blood (at final point of pig fattening).

№	G, g/L	A/G, mM	PL, mM	AST/ ALT, r.u.*	Ca/P, r.u.*	Mg, mM	Fe, mkM	Cl, mM	eST, mN/m	eTA, $\text{mN}\cdot\text{m}^{-1}\cdot\text{s}^{-1/2}$
31	32.18	1.21	1.48	1.89	0.69	0.63	25.03	100.33	45.45	15.61
32	36.61	1.10	2.50	1.40	0.58	0.61	34.87	110.77	42.51	19.31
33	39.68	0.94	1.65	1.51	0.69	0.57	20.56	100.33	43.74	21.29
34	34.33	1.15	2.37	0.94	0.79	0.65	22.35	106.68	43.12	12.69
35	36.90	1.08	2.00	1.05	0.81	0.89	31.29	103.96	44.39	10.85
36	35.40	1.24	2.50	2.40	0.67	0.61	25.93	112.13	44.06	20.39
37	33.61	1.28	1.43	1.20	0.65	0.67	23.25	106.23	42.73	5.98
38	41.76	0.87	1.58	1.54	0.39	0.58	21.46	103.96	43.61	5.70
39	38.96	1.07	1.48	0.61	0.71	0.56	25.03	110.77	43.75	18.55
40	37.11	1.10	1.09	1.19	0.65	0.67	13.41	106.23	42.86	10.24
41	36.19	0.92	1.37	1.34	0.86	0.54	25.93	102.59	43.88	13.55
42	29.75	1.65	1.37	1.29	0.65	0.59	24.14	101.23	43.95	13.38
43	43.75	1.03	1.54	1.43	0.78	0.64	27.72	108.95	42.48	19.63

Notes: r.u.\* - relative units

The average eST and eTA data (Table 2) of the blood serum at final point of hybrid pigs’ fattening were the following:  $43.58\pm 0.23$  mN/m and  $14.40\pm 1.46$   $\text{mN}\cdot\text{m}^{-1}\cdot\text{s}^{-1/2}$ , respectively. These eST and eTA values were in the range of the reference data for the blood serum evaluation for hybrid pigs’ fattening. It is interesting that the standard deviations of the eST and eTA data were the following: 0.84 mN/m and  $5.28$   $\text{mN}\cdot\text{m}^{-1}\cdot\text{s}^{-1/2}$ , respectively. As we discussed above a low value of the standard deviation in the case of eST indicated that the values “gathering” close to the average eST value of the set, while a high standard deviation in the case of eTA indicated that the values are relatively far from the “expected eTA value”.

The average values (Table 2) of the following biochemical parameters of the blood serum at final point of pigs' fattening were:  $1.13 \pm 0.20$  - for the A/G ratio,  $36.63 \pm 1.06$  g/L - for total globulins,  $1.72 \pm 0.13$  g/L - for total phospholipids,  $1.37 \pm 0.12$  - for the de Ritis coefficient (AST/ALT),  $0.63 \pm 0.02$  mM - for the total magnesium (Mg),  $24.69 \pm 1.44$  mkM - for the total iron (better "ferrum" as Fe),  $105.7 \pm 1.1$  mM - for the total chloride (better "chlorine" as Cl),  $0.69 \pm 0.03$  - for the ratio of calcium to phosphorus (Ca/P), respectively. These average values of these biochemical parameters have been used for correlation analysis with the obtained eST and eTA data, presented in the part 3.4 below.

It is important to highlight that the general protein, lipid and mineral parameters at final point of pigs' fattening were almost the same as those at setting one. Thus, the small changes in the eST and eTA parameters at these two points of pigs' fattening were mainly due to the same small changes in the "protein and lipid" content of the pigs' blood. These average values of the major biochemical parameters have been used for correlation analysis with the obtained eST and eTA data, presented in the part 3.2 below.

### 3.2. Correlations between the surface tension and biochemical parameters of pig blood

It was interesting to study correlations between the ST and biochemical parameters of the pigs' blood at initial point of their fattening (table 3), because it gives the most valuable information for PBS estimation.

**Table 3.** Correlations between the surface tension and biochemical parameters of pig blood (at initial point of pig fattening).

Numbers	Parameters	eST, mN/m	eTA, $\text{mN} \cdot \text{m}^{-1} \cdot \text{s}^{-1/2}$
1	A/G	0.19	0.07
2	Globulins	0.01	-0.04
3	Phospholipids	-0.38	-0.01
4	AST/ALT	0.40	0.17
5	Mg	-0.11	0.21
6	Fe	0.07	-0.05
7	Cl	0.35	0.34
8	Ca/P	-0.11	-0.16

The most significant correlations between eST at initial point of pigs' fattening and the biochemical parameters were obtained: +0.19 with the A/G ratio, -0.38 with phospholipids, 0.40 with the de Ritis coefficient (AST/ALT), 0.35 with Cl (Table 3). It is important to highlight that only a few extremely weak correlations were found between eST parameters (at initial point of pigs' fattening) and all studied biochemical parameters: +0.01 with globulins, -0.11 with Mg, +0.07 with Fe, -0.11 with the ratio of calcium to phosphorus (Ca/P) (Table 3).

The most significant correlations between eTA parameters (at initial point of pigs' fattening) and the following biochemical parameters were obtained: +0.17 with the de Ritis coefficient (AST/ALT), +0.21 with Mg, +0.34 with Cl (Table 3). In contrast, mainly weak correlations were found between eST parameters (at initial point of pigs' fattening) and the other studied biochemical parameters (Table 3). Such tendency indicated the stabilization of pigs' PBS during their growth and fattening that can be useful for estimation of pig productivity, nutrition, etc.

It was important to compare the correlations between the ST and biochemical parameters of the blood serum of hybrid pigs at final point of their fattening (Table 4) and further comparing with those at setting one (Table 3).

**Table 4.** Correlations between the surface tension and biochemical parameters of pig blood (at final point of pig fattening).

Numbers	Parameters	eST, mN/m	eTA, mN*m <sup>-1</sup> *s <sup>-½</sup>
1	A/G	0.80	0.32
2	Globulins	-0.39	0.18
3	Phospholipids	-0.08	0.36
4	AST/ALT	0.32	0.28
5	Mg	0.08	-0.32
6	Fe	0.04	0.35
7	Cl	-0.52	0.30
8	Ca/P	0.12	0.31

The most significant correlations between eST at final point of pigs' fattening and the biochemical parameters were obtained: +0.80 with the A/G ratio, -0.39 with globulins, 0.32 with the de Ritis coefficient (AST/ALT), -0.52 with Cl (Table 3). It is important to highlight that only a few extremely weak correlations were found between eST parameters (at initial point of pigs' fattening) and all studied biochemical parameters: -0.08 with phospholipids, +0.08 with Mg, +0.04 with Fe, +0.12 with the ratio of calcium to phosphorus (Ca/P) (Table 4).

In contrast, the numerous significant correlations between eTA parameters (at final point of pigs' fattening) and the biochemical parameters were obtained: +0.32 with the A/G ratio, +0.18 with globulins, +0.36 with phospholipids, +0.28 with the de Ritis coefficient (AST/ALT), -0.32 with Mg, +0.35 with Fe, +0.30 with Cl, +0.31 with the ratio of calcium to phosphorus (Ca/P) (Table 4). It is important to highlight that no extremely weak correlations were found between eST parameters (at initial point of pigs' fattening) and all studied biochemical parameters (Table 4). These correlations are supporting the general knowledge about a stabilization of pigs' metabolic pathways during their growth [4,13,14]. The obtained tendencies indicated an importance of both ST parameters (eST and eTA) in estimation of pigs' PBS status during their fattening.

## 4 Conclusions

Thus, all the ST parameters (measured using a PAT\_1P device) of the pigs' blood were obtained for the first time. The following eST and eTA parameters were recommended as reference values for animal practice: 42-46 mN/m and 10-30 mN·m<sup>-1</sup>·s<sup>-½</sup>, respectively. The following correlation coefficients between eST at final (or initial) point of pigs' fattening and the biochemical parameters were obtained: +0.80 (+0.19) with the A/G ratio, -0.39 with globulins (-0.38) with phospholipids, +0.32 (+0.40) with the de Ritis coefficient (AST/ALT), -0.52 (+0.35) with Cl. The correlations between eTA at final (or initial) point of pigs' fattening and the biochemical parameters were obtained: +0.32 with the A/G ratio, +0.18 with globulins, +0.36 with phospholipids, +0.28 (+0.17) with the de Ritis coefficient (AST/ALT), -0.32 (+0.21) with Mg, +0.35 with Fe, +0.30 with Cl, +0.31 (+0.34) with the ratio of calcium to phosphorus (Ca/P). Such tendency indicated the stabilization of pigs' PBS during their growth and fattening that can be useful as reference values for further applications in husbandry.

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## Conflicts of Interest

The authors declare no conflict of interest.

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