STATISTICAL EVALUATION OF SEMINAL PLASMA VS. BLOOD SERUM BIOCHEMICAL PARAMETERS IN BRUNA AND HOLSTEIN FRIESIAN BREEDING BULLS

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Abstract. A range of blood serum biochemical parameters (% protein, mg% fructose, IU - GOT, GPT and alkaline phosphatase enzyme activity) were analysed, as well as some seminal plasma biochemical parameters (% protein, mg% glucose, IU - GOT, GPT and alkaline phosphatase enzyme activity, mg% Ca, mg% P, mg% Mg) in two breeds of bulls, namely Bruna and Holstein Friesian. The t test revealed that the Friesian breed significantly differed from the Bruna breed with regard to seminal GOT activity (t=-3.175**), seminal alkaline phosphatase (t=2.848**), serum protein (t=3.813***), serum phosphorus and magnesium (t=-4.022**; -3.228**). Variability coefficients of enzymes activities were highly elevated in the Bruna breed (101.22% GPT and 83.76% alkaline phosphatase). Seminal enzymatic activities were not normally distributed to any of the breeds. In the Holstein Friesian breed, the significant correlations between seminal parameters ($p < 0.01$ and $p < 0.001$), were more numerous. Significant correlations between serum and seminal biochemical parameters, which were different from one breed to another, can be considered race characteristics. The same significant correlations between serum and seminal parameters that have been established in both breeds can be considered species characteristics, for example: serum protein-seminal protein (Bruna r=0.536**, p<0.01; Friesian r=0.673***, p<0.001), as well as serum alkaline phosphatase-seminal protein (Bruna r=-0.420*, p<0.05; Friesian r=-0.536**, p<0.01). Regressions revealed that in the Bruna breed the serum protein influenced 28% of the seminal protein variability, this percentage being 45% in the Friesian breed.

Keywords: blood serum parameters, Bruna breed, Holstein Friesian breed, seminal plasma parameters, statistical analysis.

Rezumat. Evaluarea statistică comparativă a parametrilor biochimici ai plasmei seminale şi ai serului sangvin la taurii de reproducţie din rasele Brună şi Holstein Friză. S-au analizat o serie de parametri biochimici ai serului sangvin (proteina %, fructoza mg%, activitatea enzimelor GOT, GPT si a fosfatazei alcaline UI) si a plasmei seminale (proteina %, glucoza mg%, activitatea enzimelor GOT, GPT si a fosfatazei alcaline UI, Ca mg%, P mg%, Mg mg%) la doua rase de tauri de reproducţie, Brună si Holstein Friză. Testul t a scos în evidenţă faptul că rasa Friză a diferit semnificativ de rasa Brună în privinţa activităţii GOT seminale (t=-3,175**), fosfatazei alcaline seminale (t=2,848**), proteinei serice (t=3,813***), fosforului şi magneziului seric (t=-4,022**; -3,228**). Coeficienţii de variabilitate la activităţile enzimatice seminale au fost extrem de crescuţi la rasa Brună, (101,22% GPT şi 83,76% fosfataza alcalină). Activităţile enzimatice seminale nu au fost distribuite normal, la niciuna dintre rase. Corelaţiile semnificative dintre parametri seminali ($p<0,01$ si $p<0,001$), au fost mai numeroase la rasa Holstein Friză. Corelaţiile semnificative dintre parametri biochimici, care au diferit de la o rasa la alta, pot fi considerate caracteristici de rasă. Numeroasele corelaţii semnificative dintre parametri serici si seminali care s-au stabilit la ambele rase, pot fi considerate caracteristici de specie, spre exemplu: proteina serică-proteina seminală (Brună r=0,536**, p<0,01; Friză r=0,673***, p<0,001), precum si fosfataza alcalină serică-proteină seminală (Brună r=-0,420*, p<0,05; Friză r=-0,536**, p<0,01). Regresiile au relevat faptul ca la rasa Brună proteina serică a influenţat variabilitatea proteinei seminale în proporţie de 28%, la rasa Friză acest procent a fost de 45%.

Cuvinte cheie: analiză statistică, parametri plasmei seminale, parametri serului sangvin, rasa Brună, rasa Holstein Friză.

INTRODUCTION

Monitoring the biochemical parameters of blood or other biological fluids is a powerful tool for assessing the status of domestic animals health. Knowing the physiological limits of these parameters variation is a major concern for specialists in veterinary medicine. The relationship between blood biochemical parameters and seminal plasma in breeding bulls is less studied in literature and may be relevant not only for overseeing the animal health status, but also for establishing the mechanisms by which a number of phenotypic factors (diet, animal growth conditions, ambient temperatures etc.) are reflected in the body fluids biochemical variables. The data provided by literature on the normal variation intervals of blood biochemical parameters are very different from one author to another. These seem to depend both on specific experimental conditions, as well as on the breed. For example, the blood serum total protein concentration reported by KANEKO et al. (1997) ranged from 67.4 to 74.6 g/l, and STOJEVIĆ et al. (2008) communicated an average of 85.16±6.31 g/l). Some of the serum parameters, for instance calcium and magnesium concentrations vary more closely between different authors: Ca (mg%): 9.48 (PAYNE & PAYNE, 1987), 9.72-12.4 (KANEKO et al., 1997); 9.6±1.6 (STOJEVIĆ et al., 2008); Mg (mg%): 2.51(PAYNE & PAYNE, 1987), 1.8-4.31 (KANEKO et al., 1997), 2.21 (FREERKING, 1979), 1.97±0.34 (STOJEVIĆ et al., 2008). The enzymes activities of bovine serum vary significantly with different authors, the values being generally characterized by high variation ranges: alkaline phosphatase (IU): 96.81±1.94 - 162.36±193.25 (DIKOVIC, 2017), 89.6±7.95 (AL-FARTOSI, 2010), 0.3-114.3 (ALLCROFT & FOLLEY, 1941); GPT (U/l): 11-40 (KANEKO et al., 1997), 35.98-47.7 (MAZZULLO et al., 2014), 19.40±0.30 (ELIAZAB, 2015); GOT (U/l): 78-132 (KANEKO et al., 2008), 95.21-130.3 (MAZZULLO et al., 2014) and 45.05±3.04 (ELIAZAB, 2015).

Numerous studies have associated biochemical parameters of sperm with its quality. However, the values of these parameters should be interpreted with caution, being in many cases a breed characteristic. For example, in a

previous study concerning seminal plasma of Bruna bulls we obtained seminal protein values of 6.32±1.67% (TAMBA-BEREHOIU et al., 2017), lower than those specified in literature for other breeds: 13.5 g/l for the Nelore breed, [ASSUMPÇÃO et al., 2005] and similar values 7.04%, obtained by other researchers (GRAHAM, 1978). The content of seminal plasma in fructose, generally presents values ranging from 460-730 mg% (KIRTON et al., 1964; CUPPS et al., 1969). Enzymatic activity of semen also varies widely. Transaminase activity, represented by the enzymes glutamate-oxalacetate-transaminase (GOT) and pyruvate glutamate transaminase (GPT), presents in the literature values from 166.72 IU and respectively 34.56 IU, reported by CHAUHAN & SRIVASTAVA (1973) in bison semen, to 594.25 GOT (IU) and 40.93 GPT (IU), reported for Holstein bulls semen by HUSSAIN et al., (2016). The activity of alkaline phosphatase in seminal plasma also recorded different values in various authors. CHENOWETH & LORTON (2014) which quotes sources in the literature, reported a value of 1687.5 IU, MURDOCH & WHITE (1968) reported a value of 1990 UI and in previous studies conducted by us on Bruna bulls, we found values of 234.09-8947.56 IU (TAMBA-BEREHOIU, 2017). Differences in literature can highlight the breed characteristics as well as the influences of experimental conditions on seminal enzymatic systems.

The purpose of the paper was to highlight the way the variation of some semen biochemical parameters, from breeding bulls of Bruna and Friesian races, correlated with the dynamics of bovine serum biochemical parameters.

MATERIAL AND METHODS

The experiment consisted in the determination of some seminal plasma and blood serum biochemical parameters, taken from Bruna and Friesian breeding bulls. In this regard, 26 ejaculates were collected from every breed in the month of September (temperate climate). The sperm collection was done with the artificial vagina. The breeding bulls were kept under similar conditions, had close ages (3 yeas), and were used in the same regime for reproduction based on artificial inseminations. Blood samples were taken shortly after sperm collection. Seminal plasma (supernatant) was obtained by semen centrifugation, 20 min. at 2500 rpm. Blood serum was obtained by centrifuging the coagulated blood for 20 minutes at 2500 rpm. Parameters of seminal plasma were analysed by the following methods:

- determination of total seminal protein by the biuret method (%). 0.1 ml of seminal plasma was sampled, to which 5 ml of biuret reagent was added. The colour develops in 30' and the sample was occasionally stirred. The optical density (O.D.) was read at spectrophotometer (λ=570 nm) against a 1% bovine albumin standard (KRUEZIGER et al., 2009);

- **determination of seminal fructose (mg%)**. 2 ml of deproteinized seminal plasma (with zinc sulphate and sodium hydroxide solutions) was filtered. It was added 2 ml of 0.1% resorcinol in alcohol and 30 ml of 30% HCl. The sample was incubated 10' at 80° C in a water bath. After one hour rest in the dark, O.D. at 540 nm was read, against a control containing fructose solution, instead of seminal plasma (JIN-CHUN LU et al., 2007);

- **determination of seminal GOT activity (IU).** The enzyme glutamate oxalacetate transaminase catalyses a transamination reaction, namely: L-aspartate + α-ketoglutarate ↔ L-glutamate + oxaloacetate reaction. To 0.1 ml of seminal plasma was added 0.5 ml of enzyme substrate in phosphate buffer at pH 7.4 (aspartic acid, α-ketoglutaric) and it was incubated for 60 min. at 37° C. Subsequently, 0.5 ml reagent of 1 mM 2,4-dinitrophenylhydrazine solution in 1N HCl was added. After stirring and resting for 20 min., 5 ml of 0.4 N NaOH was added. After another 5 min rest, O.D. at 546 nm was read (REITMAN et al., 1957);

- determination of seminal GPT activity (IU). Pyruvate-glutamate transaminase catalyses the L-alanine + α-ketoglutarate ↔ L-glutamate + pyruvate reaction. 0.1 ml of seminal plasma was incubated for 30 min. at 37° C, with enzyme substrate (alanine, α-ketoglutaric acid in phosphate buffer pH 7.4). It was added 0.5 ml of 1 mM 2,4-dinitrophenyl hydrazine, prepared in 1N HCl. After stirring, the sample rested for 20 min, then 5 ml of 0.4 N NaOH was added. The sample was read after 5 min. to a spectrophotometer at 546 nm (REITMAN et al., 1957);

- determination of seminal alkaline phosphatase activity (IU). The reaction is the following: para-nitro-phenyl phosphate is hydrolyzed under the action of phosphatases (Bessey-Lowry-Brock method) and phenol is released. Phenol is yellow in alkaline medium and its concentration can be determined spectrophotometrically. 0.1 ml of seminal plasma, and 2 ml added substrate (7.6 mM sodium p-nitrophenyl phosphate solution in glycol buffer pH 10.4) were incubated for 30 min at 37° C. It was added 10 ml of 0.1 N NaOH and O.D. was read on the spectrophotometer at 405 nm. The control contained 2 mM para-nitrophenol solution (GALINDO, 2010);

Blood serum parameters were analysed according to the following methods:

- determination of blood serum protein, GOT, GPT and alkaline phosphatase activities. The same methods as for the determination of seminal plasma biochemical parameters were used.

- determination of blood serum glucose (mg%). The blood glucose in the presence of ortho-toluidine forms, in acidic medium, a green-blue complex. The colour intensity is proportional to the glucose concentration. To deproteinized serum, 6% trichloroacetic acid solution was added. It was centrifuged. To 1 ml supernatant 3 ml of ortho-toluidine reagent was added. The colour was developing in 20 minutes on a water bath. The O.D was read spectrophotometrically at λ=610 nm. (COOPER et al., 1970, based on the original method of HULTMAN, 1959).

- complexometric determination of Ca in blood serum (mg%). 1 ml serum, diluted with 25 ml distilled water was basified with 0.2 ml NaOH 9 N. Murexide crystals were used as indicator and the solution was titrated with 0.01 N

EDTA, until the colour turns from pink to purple. The control contains a standard solution of 2% $CaCO_3$ (ELDJARN, 2009, based on the original method of RAVINDRANATH, 1981).

- determination of blood serum P (mg%). To 0.1 ml of serum, sodium pyrosulfite and borax solution, ammonium molybdate solution and ascorbic acid solution were added. The control ws prepared similarly but contained instead of serum, 0.1 ml of phosphorus standard. The mixtures was allowed to rest 15 minutes. Then 2.5 ml of sulfite and sodium carbonate solution was added. It was stirred and O.D. was spectrophotometrically read at λ=660 nm (FOGG & WILKINSON, 1957).

- determination of blood serum Mg (mg%). To 2 ml serum, was added with stirring: 0.5 ml of polyvinyl alcohol, 0.5 ml of yellow titanium and 1 ml of 7.5% sodium hydroxide. After 5 minutes the sample and control O.D. were spectrophotometrically read at λ=540 nm. The colour was stable about one hour (HEAGY, 1948).

The analysed parameters were subjected to computer-assisted statistical calculations, using the professional IBM SPSS Statistics Program. In this regard, computing of descriptive statistics, t-test, Shapiro-Wilk normality test, correlation coefficients and regressions have been performed.

RESULTS AND DISCUSSIONS

The descriptive statistics of seminal plasma and blood serum biochemical parameters in Bruna and Holstein Friesian breeds are shown in Table 1.

Table 1. Bruna and Friesian breeds seminal plasma and blood serum biochemical parameters.

Parameters	Mean	Std.Dev.	Minimum	Maximum	CV%
Seminal plasma Bruna breed					
1. Protein (%)	4.205	1.345	1.98	8.66	31.98
2. Fructose (mg%)	678.377	144.054	314.68	975.50	21.23
3. GOT (IU)	104.928	19.540	29.60	136.70	18.62
4. GPT (IU)	20.513	20.764	1.78	85.16	**101.22**
5. Alkaline Phosphatase (IU)	2804.373	2349.199	590.10	11330.44	**83.76**
Blood serum Bruna breed					
6. Protein (%)	4.718	0.637	3.96	6.69	13.50
7. Glucose (mg%)	42.071	11.455	21.97	69.23	**27.22**
8. GOT (IU)	41.333	7.062	26.19	56.12	17.08
9. GPT (IU)	18.427	9.765	1.78	35.89	**52.99**
10. Alkaline Phosphatase (IU)	34.481	22.284	9.29	87.22	**64.62**
11. Ca mg%	9.327	1.263	7.60	12.00	13.54
12. P mg%	5.270	1.019	3.64	7.37	19.33
13. Mg mg%	2.669	0.377	1.85	3.60	14.12
Seminal plasma Friesian breed					
1. Protein (%)	4.473	0.719	3.120	6.58	16.00
2. Fructose (mg%)	699.593	189.474	398.590	1059.42	**27.08**
3. GOT (IU)	88.655	17.351	51.030	137.54	19.57
4. GPT (IU)	15.075	10.430	0.880	46.61	**69.18**
5. Alkaline Phosphatase (IU)	5176.582	3538.217	1498.180	14777.84	**68.35**
Blood serum Friesian breed					
6. Protein (%)	5.489	0.811	4.000	7.18	14.77
7. Glucose (mg%)	33.307	12.538	7.400	57.12	**37.64**
8. GOT (IU)	38.257	7.562	24.260	58.32	19.76
9. GPT (IU)	16.763	6.655	1.810	31.60	**39.70**
10. Alkaline Phosphatase (IU)	27.308	11.975	5.370	56.16	**43.85**
11. Ca mg%	9.492	1.241	7.600	12.80	13.07
12. P mg%	4.216	0.864	2.790	6.25	20.49
13. Mg mg%	2.294	0.457	1.430	2.91	19.92

Source: Own calculation based on the experiment results.

The parameters of semen and blood serum were consistent with the results of different authors who have treated similar subjects (TAMBA-BEREHOIU, 2017; STOJEVIĆ et al., 2008; HUSSAIN et al., 2016 and others). At a first data analysis, the values of the GOT, GPT, and alkaline phosphatase enzymes activities, reached significantly higher levels in seminal plasma than in blood serum, in both breeds. Also, the seminal plasma and blood serum carbohydrates values are extremely different. Seminal fructose, compared to serum glucose, reached a value of about 16 times higher in the Bruna breed and 21 times higher in the Friesian breed. Metabolically speaking, serum glucose is regulated by insulin, whereas seminal fructose is not influenced by this hormone, so it can provide in large quantities additional energy to gametes, to maintain their mobility.

From the variability coefficients point of view, we can state that the enzymatic activities of GPT and alkaline phosphatase had extremely high values in both breeds, especially in the seminal plasma, but also in the blood serum (Bruna breed: 101.22% seminal GPT and 52.99% serum GPT; 83.76% seminal alkaline phosphatase, 64.62% serum

alkaline phosphatase; Friesian breed: 69.18% seminal GPT and 39.70% serum GPT; 68.35% seminal alkaline phosphatase, 43.85% serum alkaline phosphatase). Relatively high variability coefficients had also other parameters, in particular seminal plasma fructose and blood serum glucose in both breeds. However, the variability was noticeably lower in the Friesian breed than in the Bruna breed. The variability in GOT activity was moderate and at the same values in seminal plasma and serum, regardless of breed. Enzymatic activity of semen depends on many factors (breed, amount of substrate, enzyme activators, certain hormones, temperature, nutrition and even quantity of semen etc.), fact which explains the increased variability in these biochemical parameters. Extremely high variability suggested that these parameters are not normally distributed. Generally, extreme variability of enzyme parameters reflects their sensitivity to environmental factors. At the same time, the large variation ranges of enzymatic activity can be explained by enzymes involvement in mechanisms of maintaining the normal biological constants of some parameters.

The results of the Shapiro-Wilk test and the differences between mean and median of the non-normal distributed parameters are outlined in Table 2.

Table 2. The Shapiro-Wilk test of seminal plasma and blood serum biochemical parameters in Bruna and Friesian breeds.

Parameter	Bread	Mean	Median	Mean-median differences	W	p
Seminal protein (%)	Bruna	4.205	3.960	0.245	0.872**	0.004
Seminal GOT activity (IU)		104.928	107.505	- 2.577	0.807***	0.000
Seminal GPT activity (IU)		20.513	15.775	4.738	0.662***	0.000
Seminal alkaline phosphatase activity (IU)		2804.373	2071.000	733.373	0.764***	0.000
Serum protein (%)		4.718	4.580	0.138	0.873**	0.004
Serum alkaline phosphatase activity (IU)		34.481	30.240	4.24	0.898*	0.014
Seminal GPT activity (IU)	Friesian	15.075	12.055	3.02	0.871**	0.003
Seminal alkaline phosphatase activity (IU)		5176.582	4378.430	798.152	0.765***	0.000
Serum protein (%)		5.489	5.245	0.244	0.891**	0.010
Serum Mg (mg%)		2.294	2.375	- 0.081	0.904*	0.019

*significant at p<0.05; **very significant p<0.01; ***extremely significant p<0.001

Significant deviations from normality, especially in the activity of seminal and serum GOT, GPT and alkaline phosphatase enzymes, are observed, in both breeds.

Significant differences between breeds of the means and standard deviations of certain seminal and serum biochemical parameters, were tested using the t (Student) test (Table 3).

Table 3. The significance of means and standard deviations differences of biochemical parameters between breeds.

Parameter	Breed	Mean	t-value	p	Std.Dev.	F-ratio variances	p
Seminal GOT activity (IU)	Friesian	88.654	-3.175**	0.0025	17.350	1.268	0.556
	Bruna	104.928			19.539		
Seminal alkaline phosphat. activity (IU)	Friesian	5176.582	2.848**	0.0063	3538.217	2.268*	0.045
	Bruna	2804.373			2349.199		
Serum protein (%)	Friesian	5.489	3.813***	0.0003	0.811	1.616	0.236
	Bruna	4.718			0.637		
Serum glucose (mg%)	Friesian	33.307	-2.631*	0.0112	12.538	1.192	0.655
	Bruna	42.071			11.455		
Serum P(mg%)	Friesian	4.216	-4.022***	0.0002	0.864	1.390	0.416
	Bruna	5.270			1.019		
Serum Mg (mg%)	Friesian	2.294	-3.228**	0.0022	0.457	1.470	0.347
	Bruna	2.669			0.377		

*significant at p<0.05; **very significant p<0.01; ***extremely significant p<0.001

Standard deviations are significantly different between breeds (p<0.05), only for seminal alkaline phosphatase activity. Instead, the Friesian breed differs extremely significant from the Bruna breed, regarding serum protein and serum phosphorus (t=3.813***, respectively t=-4.022***). At the same time, between the Friesian and Bruna breeds there were very significant differences between seminal GOT activity (t=-3.175**), seminal alkaline phosphatase activity (t=2.848**) and serum Mg (t=-3.228**). Serum glucose is also significantly different between Friesian and Bruna breeds (t=-2.631*). All these differences customize racial characteristics and can influence the reproductive behavior of breeding bulls and the quality of the semen collected for artificial inseminations.

Within each breed, specific metabolic correlations were established between the biochemical parameters of seminal plasma and blood serum. Table 4 shows Pearson significant correlations between seminal and serum biochemical parameters in Bruna bulls (n=26), which are not found in Friesian bulls (n=26).

Table 4. Pearson correlations coefficients, Bruna breed (n=26).

Parameters	r/p	pGOT	pGPT	pAlk	sP	sPhos
sGlu (mg%)	r	0.416*				
	p	0.034				
sGPT (IU)	r				**-0.574****	
	p				0.002	
sAlk (IU)	r			**0.562****		
	p			0.003		
sPhos (mg%)	r	0.427*	0.411*			
	p	0.029	0.037			
sMg (mg%)	r					0.439*
	p					0.025

*significant at p<0.05; **very significant p<0.01; pGOT-plasma GOT, pGPT-plasma GPT, pAlk-plasma alkaline phosphatase, sP-serum protein, sGlu-serum glucose, sGPT-serum GPT, sAlk-serum alkaline phosphatase, sPhos-serum phosphorus, sMg-serum magesium.

Serum alkaline phosphatase activity correlated very significantly (p<0.01) with seminal alkaline phosphatase activity (r=0.562**) and seminal protein correlated with seminal GPT activity (p<0.01), very significant (r=-0.574 **). These correlations are explicable, it is about the correlations between serum and seminal proteins. The increase of the magnesium level simultaneous with that of phosphorus (present in phosphate-based biological systems) can be explained by the role of magnesium in the most biological processes involving phosphates: synthesis and use of macroergic compounds (ADP, ATP, AMP), synthesis of hydrogen and electron transporters (di- and triphosphoropyridine nucleotides, DPN, TPN,), phosphorylation mechanisms, activation of glycolytic enzymes, bones metabolism (activation of enzymes such as ATP-aze, alkaline phosphatase, pyrophosphatase etc.). Therefore, it is not excluded that the relationship between the two parameters is part of a metabolic balance. In the Friesian breed, there have been also noticed a number of significant correlations between seminal and serum biochemical parameters that do not appear in Bruna breed (Table 5).

Table 5. Pearson correlations coefficients, Friesian breed (n=26).

Parameters	r/p	pP	pGOT	pGPT	sP	sAlk
pGPT (IU)	r		0.580**			
	p		0.002			
sGOT (IU)	r				-0.392*	
	p				0.047	
sAlk (IUI)	r		-0.425*		**-0.536****	
	p		0.030		0.005	
sMg (mg%)`	r	-0.527**	-0.392*	-0.456*	-0.880***	0.506**
	p	0.006	0.048	0.019	0.000	0.008

*significant at p<0.05; **very significant p<0.01; ***extremely significant p<0.001; Pp-plasma protein, pGOT-plasma GOT, pGPT-plasma GPT, sP-serum protein, sGOT-serum GOT, sAlk-serum alkaline phosphatase, sMg-serum magesium

It is observed that serum magnesium established significant positive and negative correlations with both seminal parameters, e.g. protein (r=-0.527**, p<0.01), GOT activity (r=-0.392*, p<0.05) and GPT activity (r=-0.456* , p<0.05) as well as with other serum parameters, such as protein (r=-0.880***, p<0.001) and alkaline phosphatase activity (r=0.506**, p<0.01). Serum magnesium exists in three forms: free or ionic (about 55% of total), bound to serum proteins (about 30%) and complexed with phosphate or citrate ions (15%) (FAVUS, 2006). The data in literature, concerning the relationship between serum proteins and magnesium suggest that serum magnesium levels are independent of that of albumin (EVANS et al., 1988). Furthermore, most authors consider that the serum magnesium level is not even a true indicator of the body total magnesium content (JAHNEN-DECHENT et al., 2012). Magnesium balance is determined by the absorption from the gastrointestinal tract and also, by the renal excretion. To these phenomena, bones depletion can be added under certain conditions (KANEKO et al., 2008). Numerous studies have shown, that besides the role of the diet, significant influence on the serum magnesium value is played by certain animal stressors like: transport, unfavourable thermal conditions, effort, reproductive exploitation, water regime or age (RAYSSIGUIER et al., 2013). Given the numerous factors involved in modulating the relationship between magnesium and serum proteins, we believe that the identified correlation can not be explained exclusively on the basis of available data in this paper. Seminal GOT activity also significantly correlated with seminal GPT activity (r=0.580**, p<0.01) and serum alkaline phosphatase activity (r=-0.425*, p<0.05). The serum protein established significant correlations with other serum enzymes, GOT (r=-0.392*, p<0.05) and alkaline phosphatase (-0.536**, p<0.01).

We have identified a number of significant correlations that are common to both races, but with different degrees of significance (Table 6).

Table 6. Similar correlations in both breeds.

Specification	r/p	Breed	pP	pGOT	sAlk
sP	r	Bruna	**0.536****	**-0.525****	**-0.420***
	p		0.005	0.006	0.033
	r	Friesian	**0.673*****	**0.389***	**-0.536****
	p		0.000	0.049	0.005

*significant at p<0.05; **very significant p<0.01; ***extremely significant p<0.001; Pp-plasma protein, pGOT-plasma GOT, sP-serum protein, sAlk-serum alkaline phosphatase

It was noted that the serum protein is the parameter which correlated positive with seminal protein, to both breeds (Bruna r=0.536**, p<0.01; Friesian r=0.673***, p<0.001) and also with serum GOT for Bruna and Friesian breeds (r=-0.525**, p<0.01; respectively r=0.389*, p<0.05). The activity of serum alkaline phosphatase established with seminal protein significant negative correlations in both breeds (Bruna r=-0.420*, p<0.05; Friesian r=-0.536**, p<0.01). The correlations in Table 6 can be considered mostly species characteristics, because they occurred between the same biochemical parameters in both breeds.

It is useful to observe regressions between blood serum and seminal plasma parameters, in each breed (probability level of p<0.01 and p<0.001). Figure 1 shows these regressions in Bruna breed (p<0.01).

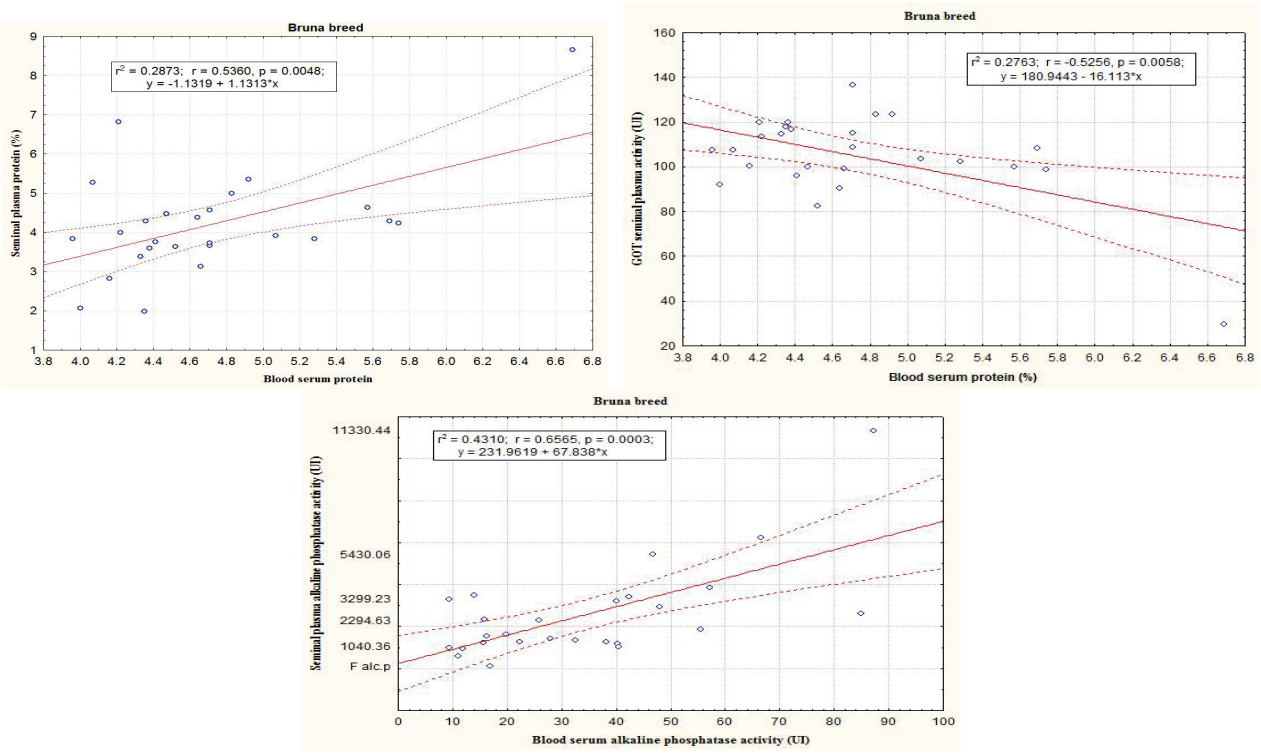

Figure 1. Blood serum – seminal plasma biochemical parameters regressions in Bruna bulls.

Serum protein determined 28% (r^2=0.287) of seminal protein variation and 27% of seminal GOT (r^2=0.276) variation. In contrast, the influence of serum alkaline phosphatase was noticeable on the increase of seminal alkaline phosphatase activity (43%), the coefficient of determination being increased in this case (r^2=0.431).

Figure 2 shows the regressions between blood serum and seminal plasma parameters in Friesian bulls breed.

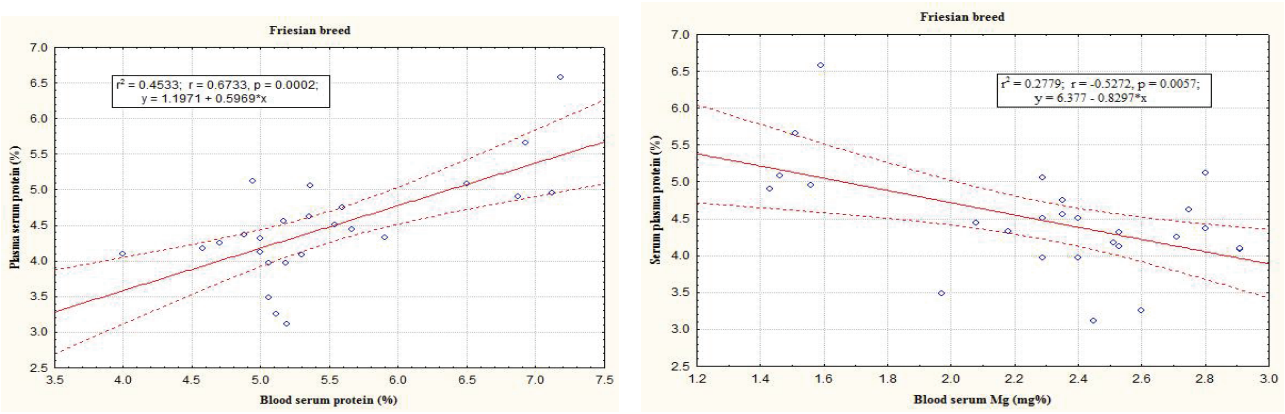

Figure 2. Blood serum - seminal plasma biochemical parameters regressions in Friesian bulls.

In Friesian breed, serum protein influences 45% of the seminal protein value (r^2=0.453). Seminal protein variability, or decrease in its concentration, is due to the blood serum Mg value, in proportion of 27.7% (r^2=0.277).

Biochemical analyses have reflected that the level of semen enzymes activities, namely GOT, GPT, and alkaline phosphatase, was dependent on how the blood serum protein varied. It had also been observed that the biochemical parameters have established stronger correlations in Friesian breed compared to Bruna breed and in larger

numbers. We must mention that the semen collection periods and various other factors, that is to say: temperatures, nutrition and reproductive exploitation, may partially modify the biochemical status of the two breeds semen.

CONCLUSIONS

The Friesian breed was significantly different from the Bruna breed, with respect to the values of seminal GOT (t=-3.175**), seminal alkaline phosphatase (t=2.848**), serum protein (t=3.813***), serum glucose (t=2.631*), serum phosphorus (t=-4.022***) and serum magnesium (-3.228**). Seminal GPT and alkaline phosphatase activities in particular, but also serum GPT and alkaline phosphatase activities, were extremely high in both breeds.

The Bruna breed revealed the highest coefficients of variability, eg: 101.22% seminal GPT and 83.76% alkaline phosphatase. The variability of seminal plasma and blood serum biochemical parameters was generally lower in Friesian breed than in Bruna breed.

The Shapiro-Wilk test demonstrated that not all the parameters had a normal distribution. The values of seminal GOT, GPT, alkaline phosphatase activities ($p<0.001$), phosphorus ($p<0.05$) and protein ($p<0.01$) deviated significantly from normal distribution in the Bruna breed. In the Friesian breed, seminal GPT ($p<0.01$) and alkaline phosphatase activities ($p<0.001$) as well as serum protein ($p<0.01$) showed significant deviations from normal distribution.

Pearson correlations between blood serum and seminal plasma parameters, which did not coincide to both breeds, at the probability levels of $p<0.05$ $p<0.01$ and $p<0.001$, were numerous. These correlations could be considered breed characteristics. Correlations between seminal biochemical parameters were more frequent in the Friesian breed and showed higher levels of significance. The same situation also emerged between biochemical parameters of blood serum. Certain correlations between the same biochemical parameters of blood serum and seminal plasma have occurred in both breeds. Thus, the serum protein correlated with seminal protein (Bruna r=0.536**; Friesian r=0.673***), with seminal GOT activity (Bruna r=-0.525**; Friesian r=0.389*) and with serum alkaline phosphatase activity (Bruna r=-0.420*; Friesian r=-0.536**), constituting species characteristics.

Regressions between serum and seminal parameters have revealed to what extent the serum parameters have influenced the seminal parameters. In Bruna breed, the serum protein influenced 28% of the seminal protein variability, 27% of the seminal GOT activity variability and serum alkaline phosphatase activity influenced the increase in seminal alkaline phosphatase activity by 43%. In Friesian breed, serum protein affects 45% of the seminal protein variability. Also, seminal protein varied, depending on the amount of serum Mg, in a proportion of 27.7%.

The results obtained by analysing the blood serum and seminal plasma of two breeds, Bruna and Holstein Friesian, highlighted some of the variability factors as well as the relationships patterns and the reciprocal influences of serum and semen biochemical parameters.

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Received: March 30, 2018
Accepted: May 06, 2018