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ACTIVITY OF HYDROLYTIC ENZYMES IN SEMEN OF ROOSTERS' (*Gallus domesticus)* DIFFERENT BREEDS

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The activity of eight hydrolytic enzymes in seminal plasma and cocks sperm of four commercial breeds (New Hempshire, Rhode Island Red, Plymouth Rock and Sussex) was compared. Sperm plasma contained significantly higher activity of all tested glycosidases and arylsulphatase. The highest level of activity was observed for N-acetylglucosaminidase both in seminal plasma (from 1656,8 to 2666,8 mU/ml of plasma) and sperm (from 13,7 to 35,8 mU/10^9 of spermatozoa). It has been shown that the level of enzymes activity is a breed-dependent feature and is not correlated with the concentration of spermatozoa.

Key words: ROOSTER, BREED, SEMEN, GLYCOSIDASES, ARYLSULPHATASE

The increasing use of artificial insemination (AI) in poultry industry emphasizes the need for good quality sperm distribution. A proper evaluation of semen prior to AI or storage is very important to avoid losses in fertility [1]. Because semen evaluation is extremely important for semen storage and AI, a rapid, economical, objective and strongly fertility predictive method of semen evaluation would be beneficial for the poultry industry protocols. Determination of enzyme concentration in semen has been practiced for a long time. These methods are simple, rapid and inexpensive to perform. On the other hand, they are prone to errors, therefore it is necessary to select an enzyme which appears only in sperm cells. Numerous enzymes have been determined in semen of several species, especially bulls and boars. They include aspartate-aminotransferase (AT-ase), fumarase, isocitratedehydrogenase, aconitase, arylsulphatases (AS), Na+/K+-ATPase, glutamic oxaloacetic transaminase (GOT), lactic dehydrogenase (LDH), cholinesterase, acid phosphatase and alkaline phosphatase [2]. No convincing results which would favor the use of enzyme in assessing pre- and post-thaw birds semen quality determination have been presented so far. Several hydrolytic enzymes of lysosomal origin have been found in the mammalian spermatozoon. They reside in the acrosome and are believed to aid the spermatozoon to penetrate the surrounding cells and zona pellucida of the egg before fertilization [3, 4]. Acid glycosidases [EC 3.2.1.] are a group of hydrolytic enzymes that catalyze the breakdown of terminal oligosaccharide units of glycoproteins and glycolipids. These enzymes are common in the male genital tract and semen of mammals [4, 5] and birds [6–9].

The aim of the study was to compare the activity of hydrolytic enzymes in semen of different breeds of cocks and correlate it with sperm concentration. It was the first attempt to determine the diagnostic validity of these markers as additional tools in classic semen evaluation.

Materials and methods

Four commercial breeds of roosters were used: New Hempshire (NH), Rhode Island Red (RIR), Plymouth Rock (PR) and Sussex (Sx). All birds were caged individually on the chicken breeding farm, under a photoperiod of 14 h light:10 h dark and fed with a commercial breeder's ration *ad libitum*. Semen was collected three times in March-April season by the abdominal method [10]. Individual ejaculates were combined during collection. Sperm concentration in each ejaculate was determined with a spectrophotometer according to Froman and Feltmann [11]. Freshly ejaculated semen (8 ml) was diluted in the ratio of 1:4 (v/v) using 0,9 % NaCl and centrifuged at 800 xg for 10 min at 4 °C to remove seminal plasma (supernatant). Sperm pellets were washed eight times by suspension in 8 ml of 0,9 % NaCl and centrifuged at 800 xg for 10 min. The washed spermatozoa were suspended in 0,5 % Triton X-100, incubated at 37 °C for 30 min and homogenized in a Teflon homogenizer. The homogenized

suspension was centrifuged at 14000 g for 30 min. The extraction procedure was repeated twice and the extracts were mixed (spermatozoa extract).

In seminal plasma and spermatozoa extracts the activity of the following acid hydrolases: N-acetyl-β-D-hexosaminidase (β-HEX), α-D-galactosidase (α-GAL), β-D-galactosidase (β-GAL), α-D-mannosidase (α-MAN), β-D-mannosidase (β-MAN), α-L-fucosidase (α-FUC), α-D-glucosidase (α-GLU) and arylsulphatase (ARYL) was determined. Enzymatic assays of glycosidase activity were performed according to the method described by Barrett and Heath [12] based on absorbance measurements at 400 nm (A 400 nm) of enzymatically released *p*-nitrophenol (*p*-NP) from suitable *p*-NP-glycosides (Sigma USA) at optimum pH (3,7 for β-GAL; 4,5 for β-HEX, α-MAN, β-MAN and α-GAL; 5,0 for α-FUC and α-GLU. For ARYL in the same procedure nitrocatechol sulphate was used as a substrate at pH 5,0 [12]. One unit (U) is defined as the enzyme activity hydrolyzing 1 μmol of substrate per min at 37 °C under the assay conditions. Enzyme activities are expressed in mU ml^{-1} (in seminal plasma) or in mU per 10^9 spermatozoa (in spermatozoa). Data was analyzed with the use of a one-way analysis of variance followed by Tukey's multiple range test. Relations between semen concentration and glycosidase activities were determined using the Spearman's correlation coefficient. Results were considered significant at $p<0,05$. Values are expressed as means ±SD. The statistical analyses were performed using Sigma-Stat 2.03 (SPSS Science Software Ltd., USA).

Results and discussion

The presence of all examined glycosidase activities was demonstrated in seminal plasma and spermatozoa (tab. 1), whereas ARYL activity was detected in seminal plasma only. The highest activity in seminal plasma was determined for β-HEX followed by α-MAN, β-GAL, β-MAN and ARYL activity. The most active enzymes in spermatozoa extracts were β-HEX, β-GAL and β-MAN. Such occurrence of acid glycosidases is typical for birds, similar results were obtained for turkey and gander semen [7, 8, 9].

Table 1

The activity of hydrolytic enzymes in cock seminal plasma (SP) and spermatozoa (S)

Enzyme activity SP [mU/ml of plasma] S [mU/10^9spermatozoa]		Breed of cocks			
		NH	RIR	PR	Sx
Semen concentration [10^9 spermatozoa/ml]		5,4 ± 1,0 *a*	5,1 ± 0,6 *a*	6,6 ±1,5 *b*	5,5 ± 0,3
NAG	SP	2368,0 ± 284,2 *a*	1656,8 ± 182,8 *b*	2486,0 ± 522,1 *a*	2666,8 ± 220,7 *a*
	S	25,4 ± 6,4 *a*	35,8 ± 2,5 *b*	29,4 ± 4,1 *a*	13,7 ± 3,1 *c*
α-MAN	SP	308,0 ± 61,6 *a*	164,4 ± 75,4 *b*	230,0 ± 92,1 *b*	336,0 ± 67,3 *a*
	S	0,1 ± 0,1	0,6 ± 0,2	0,4 ± 0,0	0,3 ± 0,1
β-GAL	SP	132,4 ± 10,7	116,4 ± 43,1	114,8 ± 43,6	168,0 ± 37,1
	S	2,9 ± 0,1	3,5 ± 0,8	2,8 ± 0,1	2,1 ± 0,1
β-MAN	SP	57,4 ± 1,6 *a*	77,7 ± 22,2 *a*	193,8 ± 73,6 *b*	112,7 ± 50,3 *a*
	S	3,4 ± 0,9 *a*	3,5 ± 0,8 *a*	2,8 ± 0,1 *a*	2,1 ± 0,1 *b*
α-FUK	SP	2,1 ± 0,3 *a*	2,8 ± 0,9 *a*	5,8 ± 2,0 *b*	3,1 ± 1,0 *a*
	S	0,05 ± 0,00 *a*	0,12 ± 0,02 *b*	0,06 ± 0,01 *a*	0,04 ± 0,01 *a*
α-GLU	SP	1,9 ± 0,2	1,9 ± 0,2	2,5 ± 0,6	2,2 ± 0,5
	S	0,03 ± 0,02 *a*	0,15 ± 0,01 *b*	0,10 ± 0,01 *b*	0,07 ±0,04 *b*
α-GAL	SP	1,9 ± 0,4	2,0 ± 0,2	2,2 ± 0,5	2,2 ± 0,4
	S	0,13 ± 0,01 *a*	0,22 ± 0,05 *b*	0,17 ± 0,02 *b*	0,12 ± 0,02 *a*
ARYL	SP	79,6 ± 0,5 *a*	62,8 ± 11,3 *a*	147,5 ±42,7 *b*	100,6 ± 17,4 *a*
	S	N.D	N.D	N.D	N.D

Note: N.D — not detected; a,b- means in rows with different letters are different ($p<0,05$)

There were breed-dependent differences ($p<0,05$) of some enzyme activities in semen. The most evident differences were observed for β-HEX in both seminal plasma and spermatozoa. The highest activity of this enzyme was noticed in seminal plasma of Sx birds, whereas the lowest for RiR ones. Some individual variations in enzyme activities were observed for all breeds of cocks, especially for β-

MAN. This recalls the results of previous studies on the fowl and turkey where inter-breed, inter-family and inter-species differences were found in other metabolic features of spermatozoa [6, 13, 14]. Inter-breed differences have also been demonstrated in the after-storage survival of spermatozoa in vitro [15].

Breed significantly affected sperm concentration ($P<0,05$). The PR birds had the highest value for sperm concentration followed by the Sx, NH and RIR (tabl. 1). The correlation coefficient between sperm concentration and enzyme activities in semen was low and not significant ($p>0,05$), except for α-MAN (tabl. 2).

Table 2

Relationships between sperm concentration and glycosidase activities in seminal plasma and spermatozoa from cocks semen

Enzyme/source	Relationship with number of spermatozoa (r;p)	
	Seminal plasma	Spermatozoa
β-HEX	0,429; 0,260	0,381; 0,321
α-MAN	0,857; 0,032*	0,238; 0,537
β-GAL	–0,381; 0,321	0,503; 0,182
β-MAN	0,381; 0,321	0,095; 0,794
α-FUK	0,190; 0,619	0,048; 0,885
α-GLU	0,563; 0,120	0,168; 0,662
α-GAL	0,476; 0,207	0,190; 0,619
ARYL	0,595; 0,102	—

Note: *statistically significant ($p<0,05$)

The results showed that there were some differences in breed which concerned sperm concentration and hydrolytic enzyme activities in semen. Among the most active glycosidases, due to this high activity in seminal plasma and spermatozoa, β-HEX seems to be the best marker enzyme for further testing of glycosidases usage in fowl semen quality validation.

Although the role of extracellular glycosidases high amounts in semen and their physiological role is unknown, there is increasing evidence that these enzymes play a significant role in the processes associated with fertilization i.e. maturation of spermatozoa during epididymal transit, altering of ejaculated spermatozoa fertilization potential and sperm-egg interaction (16, 17). Extracellular glycosidases might be merely of membrane during semen storage damaged acrosome. These factors justify the need for further exploration of precise enzymatic activity which may bolster traditional fertility examinations, suggest better cryopreservation strategies and provide additional insight into biochemical alternations between normal and abnormal semen.

Conclusions

Hydrolytic enzyme activities in cock semen were breed-dependent feature but it is slightly correlated with sperm concentration. Inter-breed differences in fertility phenomena are known, therefore it would be vital to discover whether or not a direct relationship between enzymes activity in semen and functional aspects of sperm activity exists. This validation of hydrolytic enzymes as markers of semen quality needs further studies.

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АКТИВНІСТЬ ГІДРОЛІТИЧНИХ ФЕРМЕНТІВ У СПЕРМІ ПІВНІВ РІЗНИХ ПОРІД

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Порівнювали наявність 8 гідролітичних ферментів у спермовій рідині і сперматозоїдах півнів 4 поширених порід (Нью Гемпшир, Род Айленд Ред, Плімут Рок та Сассекс). Високу активність всіх вивчених глікозідаз і арилсульфатази виявлено в спермовій рідині, а високий рівень активності N-ацетилглюкозамінідази — як у спермовій рідині (від 1656,8 до 2666,8 од./мл рідині), так і у сперматозоїдах (від 13,7 до 35,8 $mU/10^9$ од./мг білка). Показано, що рівень активності ферментів залежить від породи півнів і не корелює з концентрацією сперматозоїдів.

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АКТИВНОСТЬ ГИДРОЛИТИЧЕСКИХ ФЕРМЕНТОВ В СПЕРМЕ ПЕТУХОВ РАЗНЫХ ПОРОД

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Проведено сравнение наличия 8 гидролитических ферментов в семенной жидкости и сперматозоидах петухов 4 распространенных пород (Нью Гемпшир, Род Айленд Рэд, Плимут Рок и Сассекс). Высокая активность всех изученнных гликозидаз и арилсульфатазы обнаружена в семенной жидкости. Высокий уровень активности N-ацетилглюкозаминидазы выявлен как в семенной жидкости (от 1656,8 до 2666,8 ед./мл жидкости), так и в сперматозоидах (от 13,7 до 35,8 ед./10^9). Показано, что уровень активности изученных ферментов зависит от породы петухов и не коррелирует с концентрацией сперматозоидов.

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