Semen traits in cornish roosters during the process of physiological ageing

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Abstract

In this paper, we determined the evolution of ejaculate volume, sperm concentration, motility and viability along with the evolution of the activity of some enzymes (TGO, alkaline phosphatase, ATPase and lactic dehydrogenase) in seminal plasma of Cornish roosters at 30, 42, 57 and 63 weeks of age. The monitored parameters showed a specific evolution that characterizes the physiological ageing process in Cornish roosters. Thus, the volume of ejaculate showed a significant decrease (P = 0.011) from 0.39 to 0.32 microliters. The number of sperm per ejaculate decreased from 0.86 to 0.29x109 (P = 0.002). Motility decreased from 69% to 41% (P = 0.001) and sperm viability decreased from 89 to 74% (P = 0.00). The percentage of abnormal spermatozoa decreased not significantly, from 96 to 94%, some types of abnormalities increasing and others decreasing in frequency during the monitoring period. The activity of the monitored enzymes characterized the decrease of the anaerobic metabolism of spermatozoa and the increase of the membrane permeability of the spermatozoa in the physiological ageing process.

Keywords*: rooster, semen feature, ageing process*

Introduction

Modern technologies of breeding and exploitation of hens face, among other things, a number of reproductive problems. These are generated by a multitude of factors: environmental, nutritional, technological, caused by biological material, etc. As for the biological material, these problems are caused by either females or males. In the case of males, one of the most common and chronic problems is that of fertility. The issue of cock fertility is also supported by many factors, among which age is one of the most relevant. In most breeds of hens, the ageing speed of the males is not synchronized with the period of technological exploitation of the hens, the roosters usually ahead of the hens. Cornish inbred chicken strains are used as male parental genetic lines to produce broiler hens. Genetic categories of Cornish inbred strains are subjected to high selection pressure. Cornish breed is particular due to the side effects of a high selection pressure for growth rate, conformation and constitution. They have led to a decrease in the reproductive performance of males of this breed. Knowing these ageing effects upon the sperm traits, especially in older roosters, about to be removed from selection programs, can be a support for specialists in preventing the decline in reproductive performance of this breed. The present paper aims to determine the evolution of sperm properties (ejaculated volume, density, motility, viability, and spermatozoon abnormalities) and enzymatic chemical composition of the semen plasma in Cornish roosters during their technological exploitation.

Material and method

The research was carried out on industrially raised Cornish roosters in order to obtain male parents for tetralinear hybrids of meat (broiler). A number of 20 roosters aged 20 weeks with an average weight of 2.24 kg were accommodated in individual boxes of 0.7 x 0.7 m, connected to the automatic watering system of the hall. The feeding was done manually with a commercial feed recipe containing as main ingredients by %: wheat 40.2, barley 31.4, oats 10.6, soybean meal 5.5,

grass meal 2.2, fish meal 5.5, dicalcium phosphate 0.7, limestone 0.9, vitamin and mineral premix 0.6. The roosters benefited of artificial lighting, after a light program, of 16.5 light hours (from 5:30 to 22) from 23 to 63 weeks of age. Experimental analyses were performed at the ages of 30, 42, 57 and 63 weeks.

The ejaculates were obtained according to the method described by Bunaciu *et al.* (1978) and were collected in transparent glass graduated collection tubes; volumes were recorded directly in the tube immediately after collection at the lower margin of the semen meniscus and were expressed in µL. Sperm motility was assessed by a wet preparation technique using a Nihon Kohden optical microscope on a warmed plate. Motility was estimated by direct observation of spermatozoa in at least five fields, using 400x magnification and a lowered condenser to disperse the light. Motility was expressed here as the percentage of all spermatozoa showing progressive movements. Nonprogressive spermatozoa with other patterns of movement were not considered in this category. Sperm count was determined using a hemocytometer with a Nihon Kohden optical microscope. For this assessment, fresh semen samples were diluted (1:200) and fixed using neutral Hancock's solution (62.5 mL of 37% formaldehyde, 150 mL of 1% saline, 150 mL of sodium phosphate buffer, and 500 mL of double-distilled water) and a Potain pipette. The results were expressed as the number of spermatozoa per mL. Viability of the spermatozoa was evaluated by eosin–nigrosin staining (Merck, Darmstadt, Germany) according to Kondracki *et al.* (1968). The results were expressed as the percentage of all spermatozoa classed as viable. The spermatozoa abnormality frequency and types were also analyzed. For enzymatic assays, the sperm samples were diluted 1: 3, using an equal volume of 0,9% NaCl solution. The diluted samples were allowed to equilibrate for one hour at 4°C, then centrifuged at 2000 rpm in Janetzky centrifuge using glass centrifuge ampoules for one hour to separate sperm from seminal plasma. The two sperm components, seminal plasma and spermatozoa, were extracted into ampoules, separated by decantation and frozen at a temperature of -20°C for subsequent dosing. The enzymatic activity was determined according to the methods cited by Manta *et al.* (1966) thus: TGO activity was determined by the Rertman and Frankel's method and was expressed in units/L (one unite is the µg pyruvate liberated / mL / mg prot / 37°C; the activity of acid phosphatase from seminal plasma was determined by the Walter and Schutt method and expressed in units/L; one unit is the quantity of µg phosphate liberated / mL / mg prot / min / 60°C; ATPase activity was determined by the Zilversmith and Davis method and was expressed in units/L; one unit is the nmol phosphate liberated / mL / mg prot / min / 42°C; LDH activity was expressed in International Units (IU). The data obtained were centralized using the Excel 2010 program and the statistical processing was performed using the GraphPad program for Windows, version 8.0.2, GraphPad Software, Inc. The correlations of the age of the roosters and the sperm features were analysed by Pearson correlation coefficient. The significance between the groups was analysed by ANOVA and the the differences were considered significant for values of $P \leq 0.05$.

Results and discussions

The results regarding the evolution of the ejaculate volume according to the age of the roosters are presented in Table 1. The analysis of these data shows that the volume of ejaculate increased from the age of 30 to 42 weeks. From the age of 42 to 57 weeks, ejaculate values decreased significantly ($P = 0.013$). Expressed as a percentage and related to the value from 42 weeks, considered 100%, these decreased values were 71%. The evolution curve of this physiological parameter of sperm presents a particular relative bell shape with a peak located somewhere in the 42-week-old area.

Table 1

Evolution of ejaculate volume in Cornish roosters according to age

Item	Rooster age				Mean of period	P
	Week. 30	Week.42	Week. 57	Week. 63		
Ejaculate volume	$0.39^{\#}$± 0.04	0.44±0.10	0.35± 0.019	$0.23^{\#}$± 0.12	0.35± 0.12	0.013
Spermatozoa number ($x10^9$/ mL of semen)	2.22^{a}±0.11	2.16±0.09	2.28±0.56	1.28^{a}±0.32	1.98±0.87	0.011
Spermatozoa number (10^9/ ejaculate)	0.86c±0.11	$0.95^{b;c}$±0.09	0.79±0.56	0.29^{b}±0,32	0.69±0,87	0.002
Sperm motility (%)	69.2±6.5	68.72±12.3	51.3^{d}±7.9	41.3^{d}±6.6	57.6±8.8	0.001
Sperm viability (%)	89.7±5.6	86.5±11.0	79.5^{e}±6.5	74.4^{e}±3.5	82.0±3.9	0,000
Normal spermatozoa (%)	96.3± 12.5	96.0± 6.9	95.7± 8.5	94± 4.5	96.2± 6.9	0.065

Legend :
- values are expressed as mean ± standard error of mean;
- P value was calculated based on the HSD test, *honest significant difference;*
- values with the same exponent in the same row are significantly different.

Sperm density initially (at 30 weeks) showed values between 2.22 and 2.42 x109 per mL. Subsequently, at the age of 42 weeks, the values showed an increase of 30.57%, with a statistically significant increase percentage (P = 0.011). By the age of reformation (53 weeks), spermatogenesis decreased, with sperm density decreasing to 1.28 x109 / mL at 63 weeks.

Sperm motility presented a plateau on the age limits from 30 to 42 weeks. After the age of 42 weeks up to 63 weeks the breed lost off its sperm motility. In total, the breed showed a significant decrease in sperm motility during the monitored age (P < 0.001) of an amounts to 30.4%.

Sperm viability (defined as the percentage of sperm whose nucleus did not stain with eosin-nigrosin) had an average value of 74.4%. This biological parameter of sperm also showed a specific evolution during the monitored period, decreasing statistically significant (P < 0.01) during the monitored period, a value of of 25.2%.

Data regarding the volume of ejaculate in domestic bird species are revealed by the literature, respectively Bunaciu *et al.* (2009) as well as Bunaciu and Dojană (1982) and Dumitrescu (1978). Values for large roosters can reach as much as 1 mL according to the data from these authors. From the analysis of the Pearson correlation coefficient it results that the ejaculate volume in our monitored roosters correlates negatively with rooster age (r values showed oscillations from -0.52 to -0.81) but it remains significantly elevated until 63 weeks of age. Thus, we can say that the values of ejaculate volume in adult roosters are related to body weight and also influenced by age. Jarinkovičová *et al.* (2012) studied ejaculate samples from roosters from three breeding lines: Barred Plymouth Rock (BPR), Sussex Light (SU) and Rhode Island Red. Significant differences in ejaculate volume were reported by line: the highest ejaculate volume was reported at the BPR line (0.66 cm3) and the lowest at the SU line (0.46 cm^3, P ≤ 0.01). Changes in ejaculate volume have been reported in connection with the intensity of male use in artificial insemination by Bunaciu *et al.* (1979), the method of sperm collection by Bunaciu *et al.* (1992), the introduction of various vitamin supplements in the diet, for example vitamin E revealed by Bălăceanu *et al.* (2019)

and Sima *et al.* (2019). In an experiment of Elagib *et al.* (2016) on Leghorn White (var. Bovans) roosters of two different ages (1 and 2 years) to study the effect of age and season on sperm characteristics, the authors found that there was a significant difference ($P < 0.05$) between two age groups in terms of sperm ejaculation volume: 0.22 ± 0.02 and 0.29 ± 0.25 mL, which is in line with the results we obtained on the Cornish monitored breed. On the other hand, the authors did not report any significant differences in spermatozoa concentration. The summer season caused a significant reduction in sperm volume by 8.7% in one-year-olds and in the spermatozoa concentration of 2-year-olds by 19.7%. Tabatabaei *et al.* (2010) conducted an experiment to study the characteristics of sperm in local Iranian broiler roosters (grandparents) aged between 26 and 45 weeks and found a decrease in sperm concentration as they age.

Motility is, along with the number of spermatozoa, one of the most important morphological parameters of sperm. Sperm motility generally reflects the viability of a spermatozoa population. The positive correlation between spermatozoa motility and fertilization capacity has been demonstrated in many species, although this correlation is not absolute. Spermatozoa motility is a functional measurement of the spermatozoa themselves. Important metabolic pathways are involved in regulating spermatozoa motility: calcium, the cAMP-dependent protein kinase pathway, kinases and phosphokinases, reactive oxygen species but also cell volume and plasma sperm osmolarity, as it was revealed by Pereira *et al.* (2017). One of the factors that significantly changes sperm motility in the artificial insemination industry is thinners and cryoprotectants, according to Baguio and Capitan (2018). In a study on White Cornish roosters from 23 to 57 weeks of life, Sima *et al.* (2019) found that the percentage of sperm motility remained unchanged statistically ($P = 0.19$), which would argue for the possibility of expanding the exploitation of these breeds in industrial conditions at least until the age of 57 weeks of life. Technological conditions, vitamin deficiencies can be the cause of significant decreases in motility. This observation acquires concrete practical significance given that many breeders claim significant direct economic losses induced by the need to replace old roosters with younger roosters.

Along with motility, sperm viability is the basic parameter for assessing the fertilizing qualities of sperm. Like motility, sperm viability is influenced by a complex of factors. One paper that deals with the effect of age on sperm viability in roosters is that of Tabatabaei *et al.* (2010). The authors conducted a study on native broiler roosters (parents) aged 26, 34 and 43 weeks. The authors reported a decrease in sperm viability by about eight percent from 26 to 34 weeks of age and another eight percent from 34 to 43 weeks of life. No particular explanation was provided for this process other than the simple effect of the phenomenon of physiological ageing: "the exact reason for the decrease in sperm quality through ageing is not clear."

Surprisingly, the percentage of morphologically normal sperm showed no significant changes during the monitored period (Figure 1). However, it is important to remember the changes in the type of anomalies during the life of roosters. The evolution of the normal percentage of sperm in the ejaculate was revealed by Siudzinska and Lukaszewicz (2008) in a study performed on several cock breeds of different sizes: Green-Legged Partridge, Black Minorca, White Crested Black Polish, and Italian Partridge aged six months. The authors revealed values between 70.5 and 69.1%, which is in agreement with the results of our research on the Cornish breed. Only one breed, the Italian Partridge, which is a light-sized breed, had a much lower percentage, at 54.0%. Edens and Sefton (2009) also noted the absence of significant differences in sperm count and testicular histological structure related to the age of roosters from the age of 32 to 42 weeks. The results were confirmed by Bunaciu *et al.* (1987) on other rooster lines.

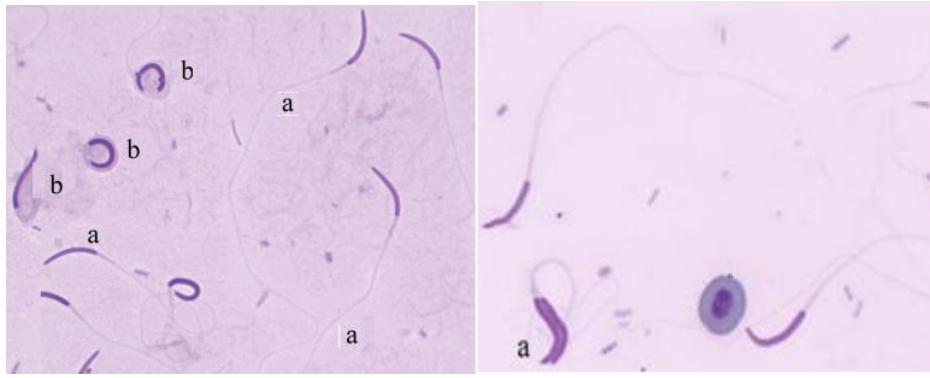

Figure 1 Sperm abnormalities in a 42-week-old Cornish rooster.
Left: a - normal spermatozoon; b - spermatozoon with protoplasmic drop (immatures); right: a - double-headed spermatozoon (eosin - nigrosin stain; ob. x90)

Morphological abnormalities of sperm were systematized into two broad categories: head and acrosome abnormalities (one category), and intermediate piece abnormalities and tail abnormalities (including end piece abnormalities, the second category.) The main head abnormalities were represented by: absence of head, double head, bent head, hook-shaped head, oversized head (thick, swollen, bumpy), undersized head, knotted head (Figure 1). The main anomalies of the acrosome were represented by: absence of acrosome, swollen acrosome), rounded acrosome and hook-shaped acrosome. It was found a total percentage of 2.8%. They were also the most common types of abnormalities form young to advanced ages. It was found that the percentage of sperm with head and acrosome abnormalities is negative and very intensely correlated with age, decreasing from young to advanced ages (r between -0.94 and -0.99). The presence of protoplasmic drop was also observed only until the age of 42 weeks. However, the percentage of sperm with a spiral tail increased from 0 to 1.5%.

Table 2

Evolution according to age of some semen enzymes in Cornish roosters

Enzyme	Age of the roosters				Mean on total period	r
	30 weeks	42 weeks	57 weeks	63 weeks		
TGO (U/L)	$33.3^{a}\pm2.2$	34.2±2.2	26.0±3.0	$18.1^{a}\pm2.9$	27,75±2,22	-0.88
Alkaline phosphatase (U/L)	1450.0±43.4	1540.0±16.9	$1311.2^{b}\pm2.0$	$111.31\pm^{b}13.0$	1353.3±15,0	-0.82
ATP-ase (U/L)	57.6± 6.4	33.3±3.5	$55.6^{c}\pm4.3$	$50.4^{c}\pm4.3$	49.8±3.0	-0.11
Lactic dehydrogenase (IU)	1200±36	$1065^{d}\pm65$	1190±76	$954^{d}\pm43$	1102,2±32	-0,57

Note:
- values are expressed as mean ± standard error of mean;
- values with the same exponent in the same row are significantly different;
- r = Pearson's correlation coefficient.

For comparison, we specify the research conducted by Bunaciu et al. (1987, 1978) on three breeds of turkeys: White breed large type, White small type and Bronze breed: the results showed

that only in one of the breeds the percentage of dead and abnormal sperm increases as the males advance in the breeding season, respectively, while in the other two monitored breeds, the correlation with age was weak.

The seminal plasma activity of the investigated enzymes revealed a general tendency of inverse correlation with the age of the roosters (Table 2, negative r values for all the investigated enzymes). A close correlation with age showed TGO (r = -0.88) and LDH (r = -0.57). The source of plasma TGO can be considered to be sperm, which can lose intracellular enzyme by increasing the permeability of plasmalemma. Excessive decrease in LDH activity may be related to decreased intensity of aerobic carbohydrate metabolism, specific to the ageing process. According to Singer *et al*. (1980), alkaline phosphatase activity in semen showed an increasing trend with increasing sperm count, revealing increased membrane permeability of sperm, as in the case of TGO. According to Dumitru and Dinischiotu (1994), the activity of acid phosphatase seems to be correlated with the intensity of the sperm harvesting process since the activity of this enzyme was higher in Mini Rock roosters that were subjected to a more frequent sperm harvesting regime. On the other hand, as shown by the data presented by Dinischiotu *et al*. (1982), the evolution of ATP-ase activity in sperm is a function of sperm fertility, the authors proposing the determination of this enzyme as a test for assessing the fertilizing capacity of spermatozoa.

Conclusions

The ageing process in Cornish roosters is characterized by profound changes in the biological parameters of sperm. Ejaculate volume, sperm density, motility and viability decrease noticeably while the percentage of normal spermatozoa do not change significantly. However, at the age of 63 weeks, the sperm of these roosters still retain properties comparable to those of 53 weeks, which would make it possible to extend the period of keeping these roosters in the herd up to 63 weeks of life.

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