Determination of Semen Characteristics and Their Effect on Hatch Results in White Layer Sire Lines

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ABSTRACT: This study was carried out on four fast-feathering white layer pure lines (Black, Blue, Brown and Maroon lines) which have been produced by pedigreed since 1995 at Poultry Research Institute (Ankara, Turkey). At the institute, fast feathering cockerels and slow feathering hens are mated for white layer hybrid production. The aim of the study was to determine the line with the best performance according to the hatching traits and to use it as sire line for hybrid production. For this purpose, sperm characteristics, seminal and blood plasma testosterone and 17β-oestradiol hormone levels, fertility and hatchability of eggs from four lines were determined. A total of 117 cockerels, 1019 hens and 16310 eggs were used in this three-year study. Significant genotype effects were observed on ejaculate volume, egg fertility, hatchability and semen and blood plasma testosterone concentrations ($p<0.05$). Hatchability of the Blue and Brown lines was higher than that of the Black and Maroon lines ($p<0.05$). Heritability coefficients of fertility (sire + dam) were estimated for the Black, Blue, Brown and Maroon lines, with the values reaching 0.29 ± 0.18, 0.59 ± 0.18, 0.11 ± 0.07 and 0.11 ± 0.06, respectively.

Keywords: Fertility, Hatchability, Pure line, Sperme characteristics, White Layer

Beyaz Yumurtacı Baba Hatlarında Sperma Özelliklerinin ve Bu Özelliklerin Kuluçka Sonuçlarına Etkisinin Belirlenmesi

ÖZ: Bu çalışma 1995 yılından beri Tavukçuluk Araştırma Enstitüsü'nde (Ankara, Türkiye) pedigrili olarak yetiştirilen ve hızlı tüylenme özelliği gösteren beyaz yumurtacı dört hat (Black, Blue, Brown ve Maroon hatları) üzerinde yapılmıştır. Enstitüde hızlı tüylenen horozlar ile yavaş tüylenen tavuklar çiftleştirilerek, beyaz yumurtacı hibrit genotipler üretilmektedir. Çalışmanın amacı, kuluçka sonuçlarına göre en iyi hattın belirlenmesi ve bunun hibrit üretiminde baba hattı olarak kullanılmasıdır. Bu amaçla dört hatta semen özellikleri, seminal ve kan plazma testosteron ve 17β-östradiol hormon düzeyleri ile yumurtalarda döllülük ve kuluçka randımanı belirlenmiştir. Üç yıl devam eden bu çalışmada toplam 117 horoz, 1019 tavuk ve 16310 yumurta kullanılmıştır. Genotipin; ejakülat hacmi, yumurta döllülük oranı, kuluçka randımanı ile semen ve kan plazma testosteron konsantrasyonları üzerine etkisinin istatistiki olarak önemli olduğu gözlenmiştir ($p<0.05$). Blue ve Brown hatlarında kuluçka randımanı, Black ve Maroon hatlarına göre daha yüksek olmuştur ($p<0.05$). Döllülüğün kalıtım derecesi (baba + ana) Black, Blue, Brown ve Maroon hatları için sırasıyla 0.29 ± 0.18, 0.59 ± 0.18, 0.11 ± 0.07 ve 0.11 ± 0.06 olarak tahmin edilmiştir.

Anahtar Kelimeler: Döllülük oranı, Kuluçka randımanı, Sperma özellikleri, Beyaz yumurtacı

INTRODUCTION

The most important reproductive traits of poultry are; fertility, hatchability, embryonic development, semen characteristics and age at first egg (1). Generally reproductive capacity is expressed as hatchability influenced by various genetic and non-genetic factors (2). The importance of semen evaluation in poultry breeding, which selects breeding males or routinely monitors reproductive performance, was well recognised (3). The traditional evaluation of poultry semen quality is mainly based on monitoring motility, viability, spermatozoa concentration, semen morphology and acrosomal integrity (4). Fertility in breeder flocks can be improved by using cockerels with high sperm quality.

Measurement of hatchability in breeder flocks matches that of bird fertility which in turn, is not only the responsibility of female birds. Male birds contribute more to total flock fertility, as a single male fertilises 10–11 female birds in a typical breeder flock. Hence, male fertility is one of the first limiting factors for achieving maximum hatchability. In fertility, semen quality as well as hormones are effective. Some researchers stated that reproductive performance was measured by judging physical traits, such as comb size and shank length, as physical appearance was determined by testosterone (T) production by the testes (5). Since 1985, a number of studies have described enzyme linked immunosorbent assays (ELISA) using microtiter plates as alternative to radioimmunoassay for measuring concentrations of mammalian steroid T and 17β-oestradiol (E2) (6-8).

In a population undergoing selection, superior families with regard to a selected trait contribute more offspring to the next generation. Egg production and hatching traits must be considered to produce better quality and more chicks from the parental stock. This study was conducted to determine egg production and hatchability traits of four White Leghorn sire lines to identify the line with the highest fitness. The objective in using parental lines was to produce high numbers of hatching eggs with living embryos. Hens play a critical role toward producing eggs and providing spermatozoa with a suitable microenvironment in the

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sperm storage tubules of the oviduct. Egg production and other reproductive traits of hens are economically important in the poultry industry.

This study aimed to determine the principal spermatological characteristics and their effects on hatching traits in white layer cockerels at the Ankara Poultry Research Institute. Blood and seminal plasma E2 and T concentrations were also determined. Selecting superior sires and dams from the population based on high fertility and embryonic survival and hatchability can lead to genetic improvement of these traits.

MATERIAL and METHODS

Animals and housing
This study was approved by the Ethical Committee of Poultry Research Institute, Ankara (Number: 2014/02) and carried out between 2014 and 2017 years. Animal material of the study included 45-month-old fast-feathering white layers hens and cockerels from the Black, Blue, Brown and Maroon lines. These lines are used as sire lines, and sex can be determined in day-old hybrid chicks according to feathering rate when mated with slow feathering dam genotypes. Birds were kept in individual battery-type cages under a day length of 15 h light and fed ad libitum with formulated rations according to the NRC (9).

Sperm evaluation
In this study, ejaculate volume, sperm concentration, motility and vitality traits of cockerels were evaluated before they were used for artificial insemination (AI) according to the technique described by Tekin (10).

Ejaculate volume
Semen ejaculate volume was macroscopically evaluated immediately after collection and recorded directly from the semen collection tube. Semen volume was expressed as cubic centimetres (cm³).

Sperm concentration
Sperm concentration was expressed as the number of cells per ml of semen. After dilution with distilled water at 1: 400 ratios, sperm cell concentration was determined by hemocytometer method, using a microscope with DFC 290 digital camera (DME 13595xxx; Leica, Wetzlar, Germany) and was recorded as $\times 10^9$/ml.

Sperm motility
One drop of diluted semen was placed on a slide and covered with a glass cover slip. Sperm motility was estimated by microscopic observation at 400× magnification. Motility was expressed as the percentage of motile spermatozoa with moderate to rapid progressive movement. At least three microscopic fields were examined for each sample.

Sperm vitality
Using a micropipette, one drop of semen and eosin-nigrosin stain was placed on a pre-warmed (37°C) glass slide and gently mixed. A thin smear was prepared on another glass slide and viewed under a microscope at 400× magnification. The proportions of live (eosin-impermeable) and dead (eosin-permeable) spermatozoa in samples were assessed based on 200 counted cells (11).

Determination of seminal and blood plasma T and E2 concentrations
Semen samples were centrifuged at 2000 g for 10 min at 4°C and stored at −20°C until measurement of T and E2 concentrations. After semen collection, blood samples were obtained from the vena ulnaris, using heparinised syringes (EDTA K3, Hema & Lab., Ankara, Turkey). Blood was centrifuged (MIPRO, MPS-1000, Protek Lab Group, Taipei, Taiwan), whereas blood plasma was stored under the same conditions as seminal plasma. Seminal and blood plasma T and E2 concentrations were determined using an ELISA method described by Oxford Biomedical Research Inc.

Data recording for egg production traits
Data for the following egg production traits of hens were recorded. Age at first egg (AFE): AFE was the age at which the hen laid her first egg. Body weight at first egg (BWFE): hens were weighed individually on the first egg-laying day and recorded as BWFE. Egg weight (EW): the mean consecutive values (g) of three eggs at weeks 28, 32 and 36. Egg number (EN): total egg number produced by an individual hen over 43 weeks.

Artificially insemination and incubation
Feathers around the cloaca of each cockerel were clipped to minimise semen contamination during artificial insemination (AI). A single ejaculate of semen was collected from each cockerel twice per week by the abdominal massage method (12). Firstly, semen was diluted with saline solution (0.9% sodium chloride; Polifarma Pharmaceutical Industry, İstanbul, Turkey) at a ratio of approximately 50%. Then, nine hens were artificially inseminated with fresh semen of one male in the afternoon using 1 ml pellucid plastic syringes (Hayat tıbbi aletler, İstanbul, Turkey). Two days after the first insemination, eggs were collected 18 days. The wing numbers of the sire and dam were written on the eggs by pencil. According to the Institute's breeding program, it is necessary to have approximately 5 full sister at the same age in order to make accurate genetic estimation. Therefore, hatching eggs are collected for 18 days. All eggs were collected in the morning; cracked and dirty eggs were discarded and stored in a cold air depot for 18 days at 12°C and 80% relative humidity. The eggs were pre-warmed prior to incubation. At the 18th day of incubation, the eggs were candled. The eggs determined as fertile were transferred to the hatchers. Eggs with no embryo development were broken and classified as early dead embryos or infertile. The following incubation traits were investigated: Fertility was determined as the ratio of the number of fertile eggs to the set number of total eggs. Embryonic mortality was determined as the ratio of the number of dead embryos to the set number of fertile eggs. Hatchability was calculated by two methods:

- The number of chicks hatched as a percentage of all eggs set.
- The number of chicks hatched as a percentage of the fertile egg set

Statistical analyses
The data were analysed using the GLM procedure of Minitab (13), according to the following model:

$$y_{irxv} = \mu + s_i + dr(s_i) + b_x + e_{irxv}$$

Where y_{irxv} is the record of the vth progeny of the rth female mated to the ith male in the xth year, μ is the common mean, s_i is the effect of the ith male (i = subscript for male), $dr(s_i)$ is the fixed effect of the rth female, which is mated to the ith male (r = subscript for female), bx is the fixed effect of the year (x = subscript for year), e_{irxv} is the random error, and e is assumed as N (0, θ²).

Significant differences between means were determined by Tukey's test, and considered at p<0.05. Multiple linear regression analysis was employed to study the relationship between the dependent variable hatchability and other independent variables (14). Fertility and hatchability data were transformed into angles [angle = (arc sin)] prior to analysis (15). Untransformed values were displayed in the tables to aid in visualising results. The regression model for the data set with 11 explanatory variables is as follows: $y_i = b_0 + b_1x_{1j} + b_2x_{2j} ….+ b_{rj} + ε_i$

RESULTS and DISCUSSION

Since the reproductive performance of the different lines was compared in this article, the performances of the cokerels as well as the hens were taken into account. Table 1 presents the means and standard errors for the egg production traits measured in the current study. Significant differences were observed in AFE, BWFE, EN and EW among the lines (p<0.05). High egg production and egg weight were recorded in the Brown line (149.30 eggs and 60.79 g, respectively). The Black line reached AFE later (142.94 days) than the other lines with heavier body weight (1399.80 g) than the other lines. As shown in Table 2, body weight at sexual maturity age of cockerels, ejaculate volume and sperm motility varied from 1837.20 g

to 2021.40 g, 0.63 cm³ to 0.84 cm³ and 68.97 % to 75.17 %, respectively, and according to this traits significant differences were calculated among the lines (p<0.05).

No significant differences were observed in sperm concentrations and live sperms among the lines, as displayed in Table 3. However, fertility, hatchability of fertile eggs and hatchability traits varied significantly among the lines (p<0.05). Although no significant difference was observed between the Brown and Blue lines, the highest fertility, hatchability of fertile eggs and hatchability were recorded in the Brown line (87.85%, 92.16% and 80.97%, respectively). Fertility of the Brown line was followed by that of the Blue line (85.84%), Maroon line (83.97%) and Black line (80.86%). In the Brown line hatchability of fertile eggs was high (93.65%) but hatchability of set eggs was low (75.73 %). The highest hatchability of fertile egg rate was observed in Blue Line (94.14%), and the lowest in Maroon Line (89.80%) (p<0.05).

Blood plasma E2 and T concentrations of all over the lines were lower than those in seminal plasma as showed in Table 4. Significant variations in blood and seminal plasma T concentrations (p<0.05) were observed among the lines. The highest T value was measured as 0.14 in the blood and 0.22 ng/ml in the seminal plasma of the Blue line. The highest E2 value totalled 0.41 in the blood and 0.85 ng/ml in the seminal plasma of the Maroon and Brown lines, respectively.

Heritabilities with standard errors were estimated from sire (s), dam (d) and sire plus dam (s + d) components (Table 5). Hatchability and fertility of h²d in the Black and Blue lines were higher than h²s values, whereas the opposite held true for the Brown and Maroon lines.

Table 1. Egg production traits of four pure lines of 43 week laying period ($\overline{X} \pm s\overline{x}$)

Lines	N	AFE (day)	BWFE (g)	EN	EW (g)
Black	256	142.94±0.32[a]	1399.80±7.36[a]	144.95±0.43[b]	58.45±0.19[c]
Blue	259	141.52±0.24[b]	1319.50±8.12[b]	145.51±0.34[b]	59.71±0.23[b]
Brown	256	140.21±0.48[c]	1377.30±8.89[a]	149.30±0.45[a]	60.79±0.22[a]
Maroon	248	139.16±0.34[c]	1314.80±8.54[b]	148.25 ±0.73[a]	58.44±0.22[c]

[a-c]Means within columns with different letters are significantly different (p<0.05), N: Number of Hen; AFE: Age of first egg; BWFE: Body weight at first egg; EN: Egg number; EW: Egg weight.

Table 2. Body weight at sexual maturity age, sperm volume and motility of cockerels ($\overline{X} \pm s\overline{x}$)

Lines	Number of cockerels	Body weight at sexual maturity age of cockerels (g)	Ejaculate volume (cm³)	Motility (%)
Black	29	2021.40±24.90[a]	0.63±0.03[b]	68.97±1.25[b]
Blue	29	1837.20±31.10[b]	0.74±0.04[ab]	73.97±1.15[ab]
Brown	30	1896.70±35.30[b]	0.84±0.04[a]	75.17±1.45[a]
Maroon	29	1840.00±26.20[b]	0.82±0.03[a]	71.21±1.85[ab]

[a-b]Means within columns with different letters are significantly different (p<0.05)

Table 3. The mean semen characteristics of cockerels ($\overline{X} \pm s\overline{x}$)

Lines	Sperm concentration (×10⁹ cells/ ml)	Live sperm (%)	Fertility (%)	Hatchability of fertile eggs (%)	Hatchability of set eggs (%)
Black	3.34±11	84.66±1.18	80.86±1.33[b]	93.65±1.11[a]	75.73±1.57[b]
Blue	3.21±94	84.18±0.80	85.84±1.05[a]	94.14±0.92[a]	80.81±1.08[a]
Brown	3.11±86	85.04±0.85	87.85±0.80[a]	92.16±0.87[a]	80.97±0.94[a]
Maroon	3.02±78	86.38±0.79	83.97±1.59[ab]	89.80±1.24[b]	75.41±1.42[b]

[a-b]Means within the same column with different letters are significantly different (p<0.05)

Table 4. Testosterone and estradiol concentrations in blood and seminal plasma of   cockerels ($\overline{X} \pm s\overline{x}$)

Lines	Blood plasma estradiol (ng/ml)	Seminal plasma estradiol (ng/ml)	Blood plasma testosterone (ng/ml)	Seminal plasma testosterone (ng/ml)
Black	0.15±0.01	0.60±0.11	0.07±0.01[b]	0.17±0.01[b]
Blue	0.09±0.01	0.84±0.20	0.14±0.01[a]	0.22±0.01[a]
Brown	0.19±0.02	0.85±0.42	0.04±0.01[b]	0.18±0.01[ab]
Maroon	0.41±0.18	0.73±0.30	0.06±0.01[b]	0.21±0.01[ab]

[a-b]Means within the same column with different letters are significantly different (p<0.05)

Table 5. Estimated heritability for hatchability and fertility ($\overline{X} \pm s\overline{x}$)

Traits	Heritability	Black	Blue	Brown	Maroon
Hatchability	h^2s	0.16±0.14	0.12±0.09	0.22±0.16	0.22±0.16
	h^2d	0.17±0.05	0.57±0.37	0.14±0.05	0.09±0.02
	h^2s+d	0.14±0.09	0.28±0.17	0.18±0.08	0.11±0.08
Fertility	h^2s	0.02±0.01	0.11±0.06	0.22±0.14	0.23±0.16
	h^2d	0.56±0.37	0.21±0.12	0.12±0.02	0.08±0.03
	h^2s+d	0.29±0.18	0.59±0.18	0.11±0.07	0.11±0.06

h^2: Heritability; s: Sire; d: Dam.

Table 6. Estimated regression coefficient of hatchability with other traits

Traits	Coef	SE Coef	p
Ejaculate volume (cm³)	-0.018	0.004	0.186
Sperm concentration (×10⁶/ml	0.013	0.001	0.021*
Blood plasma estradiol (ng/ml)	-0.002	0.006	0.698
Seminal plasma estradiol (ng/ml)	-0.006	0.002	0.007*
Blood plasma testosterone (ng/ml)	0.011	0.003	0.775
Seminal plasma testosterone (ng/ml)	0.007	0.004	0.896
B. weight at sexual maturity age of cockerels (g)	0.015	0.007	0.382
Live sperm (%)	0.059	0.011	0.418
Fertility (%)	0.738	0.061	0.001*
Motility (%)	0.103	0.059	0.041*

*Means within the same column with different letters are significantly different (p<0.05)

Coefficient of hatchability with other traits was analysed by multiple linear regression models (Table 6). These models provide the amount by which the dependent variable (hatchability %) increased when one independent variable (studied trait) was changed by one unit, and all other independent variables were held constant. As independent variables, sperm concentration (×10⁹/ml), seminal plasma E2 (ng/ml), fertility (%) and motility (%) exerted significant effects on hatchability (p<0.05). However, the remaining independent variables caused no changes (p>0.05). The calculated coefficients were generally positive. Thus, hatchability and associated traits increased together. However, the estimated coefficients for ejaculate volume, blood plasma and seminal plasma E2 levels were negative.

Breeding enterprise aims to achieve higher frequency of hatching suitable eggs, increased fertilisation rate and decreased embryonic mortality, which are accompanied by higher percentage of hatched healthy chicks. Semen qualities of cockerels determine the fertility of male chickens, whereas females contribute the eggs (16). AI studies generally examine cockerel semen quality (17). Evaluation of semen quality characteristics of cockerels provides essential information about their reproductive traits; semen quality characteristics have also been reported to be major determinants of fertility and hatchability of eggs. Cockerel semen evaluation is an essential step when assessing breeding durability of any male fowl, whereas the relationship between semen volume, sperm motility and sperm are very important in semen evaluation. These parameters, to a large extent,

determine fertility potential of semen (14, 18). The most important semen characteristics comprise sperm concentration, viability and motility, which agree with our study results (19). In our study, hatchability was positively affected by sperm concentration, fertility and motility but negatively affected by seminal plasma E2 concentration (p<0.05). Important semen characteristics for successful egg fertilisation are sperm concentration, viability and motility (20). Some researcher stated that a single insemination dose of 125 × 10⁶ total sperm/hen results in maximum fertility (21). Insemination doses >100 × 10⁶ total sperm causes no increase in the number of sperm stored in the oviduct (22). In our study, mean sperm concentration per cockerel totalled 3174.23 × 10⁶, and considering that one cock's sperm was administered to nine hens, mean insemination dose measured 352.69 × 10⁶ per hen. Thus, sperm number per AI was approximately three times more than the required amount according to Brillard and Mcdaniel (21). Increasing volume of semen inseminated from 0.05 ml to 0.07 ml resulted in non-significant increase in fertility in White Leghorns (23). In our study, no significant difference in sperm concentration or live sperm ratio was observed, but fertility and hatchability significantly differed among the four lines (p<0.05). According to these results, the highest semen volume and sperm concentration were insufficient for increased fertility and hatchability. There is a positive correlation between sperm straight line velocity and sperm mobility which is generally evaluated by swimming capacity of sperm (24). When sperm mobility is weak, and a large number of sperms are either dead or immobile,

they will be unable to either reach or penetrate the egg; thus, our study showed that motility significantly influenced fertility and hatchability ($p < 0.05$).

Cockerel body weight is an indicator of semen volume and concentration. Heavier poultry breeds possess larger testes and produce more sperm cells during spermatogenesis, resulting in higher semen concentration (25). However, in our study, heavier cockerels produced lower volumes of ejaculate with lower sperm concentration. Body weight affected semen volume, as the cockerels in the Black line were heaviest at sexual maturity, (2021.40 ± 40 g) but recorded lower ejaculate volumes compared with those in the Brown and Maroon lines.

Ejaculate volume, egg fertility, hatchability and semen and blood plasma T concentrations were dependent on cockerel strain. Brown and Maroon lines were characterised by higher than average semen volume but lower fertility and hatchability in the Maroon line. Minimum and maximum mean ejaculate volumes (0.63 and 0.84 cm³, respectively) were observed in the Black and Brown lines. The ejaculate volumes measured in this study is higher than the findings of Jarinkovicova et al. (26), who reported ejaculate volumes of 0.66, 0.46 and 0.55 cm³ in Barred Plymouth Rock, Sussex Light and Rhode Island Red, respectively. Semen volume, sperm concentration and duration of fertility in chickens measured 0.5–0.8 ml, 3.5×10^9 sperm/ml and 8 days, respectively (27). Mean ejaculate volumes of Malaysian Red jungle fowl, domestic chickens and Bantam chickens totalled 0.33, 0.29 and 0.10 cm³, respectively (28). By contrast, a relatively high volume (0.72 cm³) was noted in Brown Leghorn (29). Overall mean ejaculate volume of cockerels reached 0.7 ml in different poultry breeds (30, 31) and this measurement agrees with our study value of 0.75 ml. The percentage of live sperm recorded during semen collection of the four lines totalled 84.18%–86.38%, a range which was considered moderate, and no significant difference was detected among the lines ($p > 0.05$).

The most important reproductive traits in poultry breeding include fertility and hatchability of eggs, but heritability of these traits is generally low (32). In our study, estimated heritabilities for fertility and hatchability estimated between 0.02–0.59 and 0.09–0.57, respectively, and heritability estimated for hatchability was almost the same as that of fertility. Heritability estimated for fertility and hatchability in chickens were 0.06 and 0.13 (28) respectively, indicating that non-genetic factors pose more considerable influence on these traits. Some researchers who have studied fertility and hatchability before us had predicted lower heritability than our study (33, 34). Fertility and hatchability are binary traits in nature: egg is either fertile or not, and similarly, embryos either hatch or fail to do so (35). In our study, estimated heritability from sire and dam components for fertility estimated between 0.02–0.23 and 0.08–0.56, respectively, which are similar to those (0.09–0.31) reported by other researchers (36).

Fertility and hatchability serve as major determinants of profitability in the hatchery enterprise and are more vital when keeping parental stocks to establish high-producing crossbreeds (18). Genotype significantly affects different hatchability and egg production traits. The three most important semen characteristics necessary for egg fertilisation include sperm concentration, viability and motility. Fertility is negatively affected by defects that occur in any of these characteristics. Fertility of male

chickens in a breeder flock bears more economic importance than that of females because male chickens are responsible for fertilising eggs originating from a number of females (37). In this study, fertility of eggs laid between the four breeds differed, and this result may be attributed to genetic factors. These results agree with the findings that reported a fertilisation rate of 94.0% in White Leghorn and 90.0% in Rhode Island Red (38). Cockerel body weight is important when assessing breeding flock performance. A significant negative correlation is observed between body weight and reproductive performance of males (39, 40). Males must reach a minimum body weight typical for a given breed, strain or type before being used for breeding (41, 42). This finding is contrary to the reported negative effect of body weight on semen production (37, 43). However, some researchers reported a positive correlation between body weight and semen volume when cockerels were 48 weeks of age (39). Maximum embryonic mortality and infertility occur in large-sized eggs, followed by medium- and small-sized egg groups (44). According to Landauer (45) studies hatchability reached maximum level at an EW of around 55 g, whereas some researchers determined that embryonic mortality increased as EW increased (46). On the other hand, selection for high EW featured lower reproductive fitness than lines selected for other egg production traits (47). In our study, when other research findings were considered, EW totalled 58.44–60.79 g, which was considered a medium weight range.

A number of hormones control sexual maturity, semen production and the behaviour connected with reproduction, aggression and stress in male laying breeders. In the present study, blood and seminal plasma T and E2 concentrations were determined, and variability between cockerels was evaluated. However, males may possibly exhibit normal T production but low fertility and produce very few chicks. A sperm should possess a number of vital characteristics for a bird to be a successful breeder. Fertilisation requires sufficient semen quality for sperms to reach and penetrate the egg (22). Using a competitive protein binding assay, a research on 1- or 2-year-old cocks yielded a mean plasma T level of 0.24 µg/100 ml in healthy White Leghorn chickens. Five-month-old cocks at the onset of pubescence presented a mean T level of 0.12 µg/100 ml (48). Another study reported a seminal plasma T concentration of 1.57 ± 0.17 ng/ml for Leghorn cockerels (49). In our study, seminal T levels measured 0.17–0.22 ng/ml, which was lower than those reported by some researchers as 0,24 and 1,57 ng/ml (48, 49). Progesterone was the most predominant among reproductive steroid hormones quantified in cockerel seminal plasma, with a range of 1.85 ng/ml to 4.89 ng/ml, T was present in lower concentrations and ranged from 0.01 ng/ml to 3.71 ng/ml (50). Progesterone treatment significantly decreases the ability of sperms to reach and penetrate the egg; thus, males that secrete more progesterone into seminal plasma can possess decreased capability to fertilise eggs (50).

An egg must first be fertilised to hatch, and flocks with high fertility show the best potential for excellent hatchability (51). Sperms with higher motility move in more straight-line path than those with lower motility (52). In a previous research, sperm motility of Rhode Island Red cocks was determined between 68.97% and 73.97% (53); these findings agree with our study findings, which ranged from 71.21% to 75.17%.

A multiple linear regression model was used to describe how hatchability relates to other traits. The regression coefficients suggested that a one unit decrease in ejaculate volume, blood plasma E2 or seminal plasma E2 concentrations increased hatchability by 0.018%, 0.002% and 0.006%, respectively. However, when sperm concentration, blood plasma T, seminal plasma T or body weight at sexual maturity age of cockerels increased by one unit, live sperm (%), fertility (%), motility (%) and hatchability increased by 0.013%, 0.011%, 0.007%, 0.015%, 0.059%, 0.738% and 0.103%, respectively. Minimum and maximum values of these parameters and all other independent variables were held constant in this model.

At the Ankara Poultry Research Institute, a research was also conducted to determine suitable cross combinations by crossing white egg layer parents in different combinations in previous study (54). According to results the said research, the Brown line cross combination was deemed superior to other combinations in terms of egg production traits and thus supported our research findings. Results suggest that the Brown line or Brown line combinations genotypes have better egg production results and hatchability traits than those of the other genotypes and will be suitable as sire line.

CONCLUSIONS

The Black line exhibited the lowest semen volume, indicating that the Brown and Blue lines possessed better hatch results compared with those of the Black and Maroon lines. This may be due to the fact that sperm concentration of Black Line is higher than that of Maroon Line. Characterising cockerel semen before AI is an important initial starting point, as cockerels with acceptable sperm motility, high ejaculate volume and total sperm concentration must be selected to obtain high fertility rates. In this study, ejaculate volume, sperm concentration and percentage of live sperm varied widely. Semen parameters of the sire line cockerels all recorded comparable values with that of Black, Blue, Brown and Maroon lines, following the microscopic evaluation. However, cockerels with a high mobility, liveability and concentrations of sperm must be selected for breeding purposes to obtain high fertility.

ACKNOWLEDGEMENT

REFERENCES

1. **Szwaczkowski, T.,** 2003. Use of mixed model methodology in poultry breeding, estimation of genetic parameters. Poultry Genetics, Breeding and Biotechnology. Georgia.
2. **Machal, L., Zatloukal, M., Stanislav, J.S., Molnarova, Z.,** 2003. The process of embryonic mortality in seven initial laying strains during incubation. *Arc Anim Breed*. 46: 483-489.
3. **Cheng, F.P., Guo, T.J., Wu, T.J., Lin, T.E., Ursem, P.J.F., Colenbrander, B., Fung, H.P.,** 2002. Annual variation in semen characteristics of pigeons (Columba livia). *Poult Sci*. 81: 1050-1056.
4. **Bansal, A.K., Cheema, R.S.,** 2014. Analysis of sperm and relationship between conventional sperm parameters and hypo-osmotic swelling test/acrylamide penetration assay – crossbred cattle bulls. *Adv Appl Sci Res*. 6: 39–44.
5. **Mcdaniel, C.D., Hannah, J.I., Parker, H.M., Smith, T.W., Schultz, C.D., Zumwalt, Z.D.,** 1998. Use of a sperm analyzer for evaluating broiler breeder males. 1. Effects of altering sperm quality and quantity on the sperm motility index. *Poult.Sci*. 77, 888-893.
6. **Elder, P.A., Lewis, J.G.,** 1985. An enzyme-linked immunosorbent assa! (ELIS.41 for plasma testosterone. *J Stero Bioch*, 12: 635-638.
7. **Marcus, G.J., Durnford, R.,** 1988. Estradiol assay by microtitre plate enzyme immunoassay. *J Steroid Biochem*. 29: 207-212.
8. **Nash, J.P., Davail-Cuisset, B., Bhattacharyya, S., Suter, HC., Le Menn, F., Kirne, D.E.,** 2000. An enzyme linked immunosorbant assay (ELISA) for testosterone, estradiol, and 17,20 β-dihydroxy-4-pregnen-3-one using acetylcholinesterase as tracer, application to measurement of diel patterns in rainbow trout (Oncorhynchus mykiss). *Fish Phys Biochem*. 22: 355-363.
9. **NRC.,** 1994. National Research Council. Nutrients requirements of poultry. 9th ed Washington DC. National academic press.
10. **Tekin, N.,** 1994. Spermanın muayenesi ve değerlendirilmesi. (in) Evcil hayvanlarda reprodüksiyon sun'i tohumlama doğum ve infertilite. Ed, E.Alaçam e., I. Baskı. 69-79. Dizgievi, Konya.
11. **Lukaszewicz, E., Jerysz, A., Partyka, A., Siudzinska, A.,** 2008. Efficacy of evaluation of rooster sperm morphology using different staining methods. *Res Vet Sci*. 85: 583-588.
12. **Burrows, W.H., Quinn, J.P.,** 1937. The collection of spermatozoa from the domestic fowl and turkey. *Poult Sci*. 26: 19-24.
13. **Minitab Inc., 2016** Minitab 16 version for windows, State College PA 1680-3008, Pennsylvania.
14. **Peters, S.O., Omidiji, E.A., Ikeobi, C.O.N., Ozoje, M.O., Debambo, O.A.A.,** 2004. Effect of naked neck and frizzled genes on egg traits, fertility and hatchability in local chicken. 9th Annual conference of animal science association of Nigeria.
15. **Duzgunes, O., Kesici, T., Gurbuz, F.,** 1993. İstatistik Metotları I, II. baskı, Ankara Üniversitesi Ziraat Fakültesi Yayınları. Ankara.
16. **Liu, S.J., Zheng, J.X., Yang, N.,** 2008. Semen quality factor as an indicator of fertilizing ability for geese. *Poult Sci*. 87: 155-159.
17. **Siudzinska, A., Lukaszewicz, E.,** 2008. The effect of semen extenders and storage time on sperm morphology of four chicken breeds. *J App Poult Res*. 17: 101-108.
18. **Peters, S.O., Shoyebo, O.D., Ilori, B.M., Zoje, M.O.O., Ikeobi, C.O.N., Adebambo, OA.,** 2008. Semen quality traits of seven strain of chickens raised in humid tropics. I J Poult Sci. 7: 949-953.
19. **Mellor, S.,** 2001. Selecting males by sperm quality. *World Poultry*, 17: 32-34.

20. **Parker, H.M., Yeatman, J.B., Schultz, C.D., Zumwalt, C.D., McDaniel, C.D.,** 2000. Use of a sperm analyzer for evaluating broiler breeder males. 2. Selection of young broiler breeder roosters for the sperm quality index increases fertile egg production. *Poult.Sci.* 79: 771-777.

21. **Brillard, J.P., Mcdaniel, G.R.,** 1986. Influence of spermatozoa numbers and insemination frequency on fertility in dwarf broiler breeder hens. *Poult Sci.* 65: 2330–2334.

22. **Mcdaniel, C.D., Bramwell, R.K., Howarth, J.R.,** 1997. Development of a novel fluorescence technique for quantifying the total number of spermatozoa stored in the uterovaginal junction of hens. *J Rep Fert.* 109: 173–179.

23. **Hughes, B.L., Holleman, K.A.,** 1976. Efficiency of producing White Leghorn hatching eggs. *Poult Sci.* 55: 2383-2388.

24. **Froman, D.P., Feltmann, A.,** 2000. Sperm mobility: phenotype in roosters (*Gallus domesticus*) determined by concentration of motile sperm and straight line velocity. *Biol Reproduc.* 62: 303-309.

25. **Adeyemo, G.O., Longe, O.G., Adejumo, D.O.,** 2007. The reproduction performance of breeder cocks fed cottonseed cake-based diets. *Int J Poult Sci.* 6: 140-144.

26. **Jarinkovicova, L., Machal, L., Machal, J., Filipcik, R., Tumova, E., Horsky, R.**, 2012. Relationship of ejaculate quality and selected biochemical parameters of blood in cockerels of three laying lines. *Czech J Anim Sci.* 57: 370-376.

27. **Smyth, J.R.,** 1968. The artificial insemination of farm animals. Rutgers University Press, New Brunswick.

28. **Malik, A., Haron, A.W., Yusoff, R., Nesa, M., Bukar, M., Kasim, A.,** 2013. Evaluation of the ejaculate quality of the Red jungle fowl, domestic chicken, and Bantam chicken in Malaysia. *Turk J Vet Anim Sci.* 37: 564–568.

29. **Hrncar, C., Barborova, L., Bujko, J.,** 2013. The quality of ejaculate in cocks of Brown Leghorn, Oravka and Brahma breeds. J Anim Sci Biotech. 46: 66–69.

30. **Tuncer, P.B., Kinet, H., Özdoğan, N., Demiral, O.Ö.,** 2006. Denizli horozlarında bazı spermatolojik özelliklerin değerlendirilmesi. *Erciyes Üniversitesi Veteriner Fakültesi Dergisi.* 3: 37-42.

31. **Tuncer, P.B., Kinet, H., Özdoğan, N.,** 2008. Evaluation of some spermatological characteristics in Gerze cocks. *Ankara Üniversitesi Veteriner Fakültesi Dergisi.* 55: 99-102.

32. **Falconer, D.S., MacKay, T.F.C.,** 1996. Introduction to Quantitative Genetics. 4th ed. Long SciTech. New York. 1996.

33. **Sapp, R.L., Rekaya, R., Misztal, I., Wing, T.,** 2004. Male and female fertility and hatchability in chickens, A longitudinal mixed model approach. *Poult.Sci.* 83:1253–1259.

34. **Bennewitz, J., Morgades, O., Preisinger, R., Thaller, G., Kalm, E.,** 2007. Variance Component and Breeding Value Estimation for Reproductive Traits in Laying Hens Using a Bayesian Threshold Model. *Poult Sci.* 86: 823–828.

35. **Sewalem, A., Johansson, K.,** 2000. Egg weight and reproductive traits in laying hens, estimation of direct and maternal genetic effects using Bayesian approach via Gibbs sampling. *Anim Sci.* 70: 9–16.

36. **Beaumont, C., Millet, N., Le Bihan-Duval, E., Kipi, A., Dupuy, V.,** 1997. Genetic parameters of survival to the different stages of embryonic death in laying hens. *Poult Sci.* 76: 1193-1196.

37. **Wilson, H.R., Piesco, N.P., Miller, E.R., Nesbeth, W.G.,** 1979. Prediction of the fertility potential of broiler breeder males. *W Poult Sci J.* 35: 95-118.

38. **Islam, M.S., Howlinder, M.A.R., Kabir, F., Alam, J.,** 2002. Comparative assessment of fertility and hatchability Barred Plymouth Rock, White Leghorn, Rhode Island Red and White Rock hen. *Int J Poult Sci.* 1: 85-90.

39. **Harris, G.C., Benson, J.A., Sellers, R.S.,** 1980. The influence of day length, body weight and age on the production ability of breeder cockerels. *Poult Sci.* 63: 1705-1710.

40. **Lukaszewicz, E., Kruszynski, W.,** 2003. Evaluation of fresh semen and frozen-thawed semen of individual ganders by assessment of spermatozoa motility and morphology. *Theriogenology.* 59: 1627-1640.

41. **Lisowski, M., Bednarczyk, M.,** 2005. Effects of tamoxifen dose and nutrition scheme during growth on stimulation of the reproductive system in Cornish breed cocks. *Folia Biologica (Kraków).* 53: 1–6.

42. **Goger, H.,** 1993. Kuluçkacılıkta gözlenen embriyo ölümlerinin nedenlerinin belirlenmesiyle ilgili önemli konular. *Teknik Tavukçuluk Dergisi.* 78:3-9.

43. **Galal, A.,** 2007. Predicting semen attributes of naked neck and normally feathered male chickens from live performance traits. *I J Poult Sci.* 1: 36–42.

44. **Iqbal, J., Khan, S.H., Mukhtar, N., Ahmed, T., Pasha, RA.,** 2016. Effects of egg size (weight) and age on hatching performance and chick quality of broiler breeder. *J App Anim Res.* 44: 54–64.

45. **Landauer, W.,** 1961. Hatchability of Chicken Eggs as Influenced by Environment and Heredity, *The Storrs Agricultural Experiment Station Monograph.* 1: 68–137.

46. **Hagger, C., Steiger-Stafl, D., Marguerat, C.,**1986. Embryonic mortality in chickens eggs as influenced by egg weight and inbreeding. *Poult Sci.* 65: 812–814.

47. **Sewalem, A., Johansson, K., Carlgren, A.B., Wilhelmson, M., Lillpers, K.,** 1998. Are reproductive traits impaired by selection for egg production traits in laying hens? *J Anim Breed Gen.* 115: 281–297.

48. **Schröcksnadel, H., Bator, A., Frick, J.,** 1971. Plasma testosterone level in cocks and hens. *Steroids.* 18: 359–365.

49. **Zeman, M., Kosutzky, J., Bobakova, E.,** 1986. Testosterone concentration in the seminal plasma of cocks. *Br Poult Sci.* 27: 261–266.

50. **Anderson, E.M., Navara, K.J.,** 2011. Steroid hormone content of seminal plasma influences fertilizing ability of sperm in White Leghorns. *Poult Sci.* 90: 2035–2040.

51. **Eslick, M.L., McDaniel, G.R.,** 1992. Interrelationships between fertility and hatchability of eggs from broiler breeder hens. *J App Poult Res,* 1:156–159.

52. **Vizcarra, J.A., Ford, J.J.,** 2006. Validation of the sperm mobility assay in boars and stallions. *Theriogenology.* 66:1091-1097.

53. **Machal, L., Krivanek, I.,** 2002. Indicators of semen quality of rooster of three paternal layer lines and specific conductivity of the semen. *Acta Vet Brno.* 71: 109–116.

54. **Kamanli, S., Boga, A.G., Durmus, I.,** 2016. Comparison of two-crossing combinations in terms of some performance and egg quality characteristics in white egg layer parents. *J Poult Res.* 13: 1-4.