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

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
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 ×10⁹/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).

**Semen samples**

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 unaris, using heparinised syringes (EDTA K3, Hema & Lab., Ankara, Turkey). Blood was centrifuged (MIPRO, MPS-1000, Protex 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**

Fathers 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, Istanbul, 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:

\[ y_{ijrv} = \mu + s_i + dr_j + b_k + e_{ijrv} \]
Where $y_{iwm}$ is the record of the $w^{th}$ progeny of the $r^{th}$ female mated to the $i^{th}$ male in the $m^{th}$ year, $\mu$ is the common mean, $s_i$ is the effect of the $i^{th}$ male ($i = \text{subscript for male}$), $d_{r}(s)$ is the fixed effect of the $r^{th}$ female, which is mated to the $i^{th}$ male ($r = \text{subscript for female}$), $b_x$ is the fixed effect of the year ($x = \text{subscript for year}$), $\theta_{wr}$ is the random error, and $\epsilon$ is assumed as $N(0, \theta^2)$.

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 $[\text{angle} = (\text{arc sin})]$ 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: $y_i = b_0 + b_1x_1 + b_2x_2 + ... + b_nx_n + \epsilon$.

**RESULTS and DISCUSSION**

Since the reproductive performance of the different lines was compared in this article, the performances of the cockerels 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 eggs and 60.79 g, respectively. The Black line reached the highest E2 value totalled 0.41 in the blood (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.

Herditabilities with standard errors were estimated from sire ($s$), dam ($d$) and sire plus dam ($s + d$) components (Table 5). Hatchability and fertility of $h^2d$ in the Black and Blue lines were higher than $h^2s$ 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 ($\bar{X} \pm s\bar{X}$)**

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

Means within columns with different letters are significantly different ($p<0.05$).

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

<table>
<thead>
<tr>
<th>Lines</th>
<th>Number of cockerels</th>
<th>Body weight at sexual maturity age of cockerels (g)</th>
<th>Ejaculate volume (cm³)</th>
<th>Motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>29</td>
<td>2021.40±24.90a</td>
<td>0.63±0.03c</td>
<td>68.97±1.25b</td>
</tr>
<tr>
<td>Blue</td>
<td>29</td>
<td>1837.20±31.10b</td>
<td>0.74±0.04eb</td>
<td>73.97±1.15ab</td>
</tr>
<tr>
<td>Brown</td>
<td>30</td>
<td>1896.70±35.30b</td>
<td>0.84±0.04a</td>
<td>75.17±1.45a</td>
</tr>
<tr>
<td>Maroon</td>
<td>29</td>
<td>1840.00±26.20b</td>
<td>0.82±0.03a</td>
<td>71.21±1.85ab</td>
</tr>
</tbody>
</table>

Means within columns with different letters are significantly different ($p<0.05$).

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

<table>
<thead>
<tr>
<th>Lines</th>
<th>Sperm concentration ($\times 10^8$ cells/ml)</th>
<th>Live sperm (%)</th>
<th>Fertility (%)</th>
<th>Hatchability of fertile eggs (%)</th>
<th>Hatchability of set eggs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>3.34±11</td>
<td>84.66±1.18</td>
<td>80.86±1.33a</td>
<td>93.65±1.11a</td>
<td>75.73±1.57b</td>
</tr>
<tr>
<td>Blue</td>
<td>3.21±94</td>
<td>84.18±1.08</td>
<td>85.84±1.05a</td>
<td>94.14±0.92a</td>
<td>80.81±1.08a</td>
</tr>
<tr>
<td>Brown</td>
<td>3.11±86</td>
<td>85.04±0.85</td>
<td>87.85±0.80a</td>
<td>92.16±0.87a</td>
<td>80.97±0.94a</td>
</tr>
<tr>
<td>Maroon</td>
<td>3.02±78</td>
<td>86.38±0.79</td>
<td>83.97±1.59ab</td>
<td>89.80±1.24b</td>
<td>75.41±1.42b</td>
</tr>
</tbody>
</table>

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 (X ± s̅).

<table>
<thead>
<tr>
<th>Lines</th>
<th>Blood plasma estradiol (ng/ml)</th>
<th>Seminal plasma estradiol (ng/ml)</th>
<th>Blood plasma testosterone (ng/ml)</th>
<th>Seminal plasma testosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>0.15±0.01</td>
<td>0.60±0.11</td>
<td>0.07±0.01</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>Blue</td>
<td>0.09±0.01</td>
<td>0.84±0.20</td>
<td>0.14±0.01</td>
<td>0.22±0.01</td>
</tr>
<tr>
<td>Brown</td>
<td>0.19±0.02</td>
<td>0.85±0.42</td>
<td>0.04±0.01</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Maroon</td>
<td>0.41±0.18</td>
<td>0.73±0.30</td>
<td>0.06±0.01</td>
<td>0.21±0.01</td>
</tr>
</tbody>
</table>

*a, b, abMeans within the same column with different letters are significantly different (p<0.05)

Table 5. Estimated heritability for hatchability and fertility (X ± s̅).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Heritability</th>
<th>Black</th>
<th>Blue</th>
<th>Brown</th>
<th>Maroon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatchability</td>
<td>h²s</td>
<td>0.16±0.14</td>
<td>0.12±0.09</td>
<td>0.22±0.16</td>
<td>0.22±0.16</td>
</tr>
<tr>
<td></td>
<td>h²d</td>
<td>0.17±0.05</td>
<td>0.57±0.37</td>
<td>0.14±0.05</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td></td>
<td>h²s+d</td>
<td>0.14±0.09</td>
<td>0.28±0.17</td>
<td>0.18±0.08</td>
<td>0.11±0.08</td>
</tr>
<tr>
<td>Fertility</td>
<td>h²s</td>
<td>0.02±0.01</td>
<td>0.11±0.06</td>
<td>0.22±0.14</td>
<td>0.23±0.16</td>
</tr>
<tr>
<td></td>
<td>h²d</td>
<td>0.56±0.37</td>
<td>0.21±0.12</td>
<td>0.12±0.02</td>
<td>0.08±0.03</td>
</tr>
<tr>
<td></td>
<td>h²s+d</td>
<td>0.29±0.18</td>
<td>0.59±0.18</td>
<td>0.11±0.07</td>
<td>0.11±0.06</td>
</tr>
</tbody>
</table>

h²: Heritability; s: Sire; d: Dam.

Table 6. Estimated regression coefficient of hatchability with other traits

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

*aMeans 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 (=10⁶/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 sperm/hen results in maximum fertility (21). Insemination doses >100 × 10⁶ total sperm/hen results in increased fertility (23). 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). 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). 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). 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). Insemination doses >100 × 10⁶ total sperm causes no increase in the number of sperm stored in the oviduct (22).
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 ± 49 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 Jarić-Nikolić et al. (29), 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 × 10⁸ 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%.

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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.

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