Egg related parameters affecting fertility and hatchability in the Italian bantam breed Mericana della Brianza

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ABSTRACT

Local chicken breeds are a vital reservoir of gene resources and their conservation has a technical role related to the future development of the productive system, as well as a social-cultural role. The aim of this study was to evaluate the effects of egg weight, egg storage period and egg weight loss on hatchability of fertile eggs in the Italian bantam breed Mericana della Brianza. Fourteen females and eight males were kept in floor pens and divided in 8 families (1M:1 or 2F) during the reproductive season (March–June). Birds received a photoperiod of 14L:10D and were fed ad libitum. Egg production and egg weight were recorded daily. Eggs were divided in 4 weight groups: EW1 = <33 g, EW2 = 33–36 g, EW3 = 36–39 g and EW4 = >39 g. Eggs were stored at 18 °C and classified in 3 egg storage groups: ES1 = 0–4, ES2 = 5–9 and ES3 = 10–15 days. Egg weight loss was recorded and distributed in 5 different classes: EWL1 = <10%, EWL2 = 10–15%, EWL3 = 16–20%, EWL4 = 21–25%, EWL5 = >25%. Fertility, embryo mortality and hatchability were recorded. The mean values during the reproductive season were 82% fertility and 50% hatchability of fertile eggs. The best combination of fertility and hatchability values were recorded in EW2 and lower fertility was recorded in EW1 (P<0.05). Hatchability decreased under 50% after 10 day storage period before incubation and the best hatchability was recorded in EW1. The present results contribute to the knowledge on reproductive parameters necessary to improve the reproductive efficiency of this Italian breed within a conservation plan.

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1. Introduction

Livestock breeds are recognized as important components of world biodiversity because the genes and gene combinations they carry may be useful to agriculture in the future (IUCN, 1980). Specific breeds may possess great adaptability to different environmental challenges and livestock genetic conservation measures are, therefore, likely to remain focused on the maintenance of breeds. The major threat to rare and minority breeds is absorption into breeds with larger numbers of animals, usually by repeated use of males from the breed that is under threat because of small animal numbers (Bradley et al., 1994). Accordingly, the basic principle of breed conservation is the promotion of pure breeding (Stephen and Bradley Daniel, 1995). The development of a conservation program includes different activities: improvement on the knowledge of biological functions, conservation of typical morphological characteristics, development of selection strategies, control of inbreeding and, strategies for enhancing financial wellbeing through diffusion of the breed in local productive systems (Zanon and Sabbioni, 2001). In Italy, few conservation programs for avian breeds are in progress with the support of local institutions because their consistency today is dramatically decreased. With a broad literature
search, 90 Italian avian breeds were described, classified and dated from the last century. The majority of the breeds (61%) were classified as extinct, 13% as threatened, 17% as poorly dispersed and only 8% as still widely spread (Zanon and Sabbioni, 2001). It is clear that efforts for conservation of Italian avian breeds are urgently required, in particular for local chicken breeds that are good prospects for environmental adaptability and disease resistance as a vital reservoir of gene resources. Many factors such as desirable fertility and hatchability affect breeding program success. According to Meir and Ar (2008), it is well accepted that water loss through the shell during artificial incubation is one of the key factors that affects hatching success. Additionally, storage of hatching eggs is a necessary part of commercial incubation, even though storage length and conditions may influence post-ovipositional mortality of embryos (Lapão et al., 1999). Furthermore, the effects of age and egg size (egg weight) on production parameters have been studied (Wilson, 1991; Ulmer-Franco et al., 2010).

Preservation of local avian breeds plays an important role in safeguarding of animal biodiversity; it could have a relevant role in developing new high quality products for niche markets and also represents an important tool to preserve and support the rural economy in some marginal agricultural areas. The aim of the present study was to record the reproductive performance in a small population of Mericanella della Brianza (MDB) chickens. MDB is the unique Italian bantam breed with an official standard (FIAV, 1996) and was historically present in rural areas of the Lombardia region (North Italy). The standard of the breed explains the morphological characteristics typical of these poultry but no data are available on reproductive performance and growth parameters. In the present study, reproductive parameters have been recorded during the breeding season and different egg parameters known to be related to hatchability were studied. Egg weight, days of egg storage before incubation and decrease of egg weight during incubation were recorded and the effect on hatchability was studied.

2. Materials and methods

Chicken breeders of the Italian bantam breed Mericanella della Brianza were used. Fourteen females and eight males were available at the beginning of the reproductive season on March and housed in a controlled environment at the Poultry Unit, Animal Production Centre, University of Milan (Lodi, Italy). All birds, aged from 24 to 36 weeks, had white plumage and were acquired over a few reproductive seasons from a local breeder. The birds were divided into eight families, one male and one or two females from each family, and kept in floor pens (1 m × 3 m) under natural mating conditions. Birds received a photoperiod of 14L:10D and were fed ad libitum a standard commercial diet for chicken breeding flocks (2800 kcal ME/kg, 15% crude protein, 3% ether extract, 10.5% ash, 3.10% calcium). Egg production was recorded daily in each pen from March to June and the proportion of eggs/hen was calculated per week. Eggs were weighed, labeled with the date of oviposition and stored at 18 °C in an isolation room until setting, relative humidity (RH) was 70%. Egg storage was up to 15 days maximum to test the effect of egg storage on hatchability. Eggs were set every 2 weeks from March to May. Standard incubation parameters for hen eggs were used (Romboi and Marzoni, 2008). From Day 1 to 18 (Day 0= first day of incubation), incubation temperature was 37.7 °C, RH was 55% and eggs were automatically turned (incubator model 11FH, capacity 702 eggs, Maino, I). From Day 19 to 21, eggs were transferred into the hatchers (incubator model 1350XH, capacity 350 eggs, Maino, I) and kept at 37.2 °C and 80% RH. A total of six consecutive settings were performed with a total of 387 eggs. The number of eggs incubated for each setting was 71 in Setting 1, 40 in Setting 2, 79 in Setting 3, 58 in Setting 4, 57 in Setting 5 and 82 in Setting 6.

Fertility and early embryo mortality were recorded on the seventh day of incubation by candling, clear eggs and dead embryos were discarded. Clear eggs were opened to assess fertility and very early embryo mortality occurring within 48 h incubation was recorded. Fertility (%) was calculated on total settings. Egg weight was recorded on Day 18 before transfer into the hatchers and egg weight loss (EWL) during incubation was calculated. Hatchability was recorded and eggs for which there was no hatching were opened to record late embryo mortality. Hatchability and embryo mortality were calculated on fertile eggs. Embryo mortality was also grouped in classes according to the development of dead embryos and data have been presented and discussed in a previous paper (Cerolini et al., 2010).

Descriptive statistical parameters were calculated for egg weight, EWL, fertility, hatchability and embryo mortality. Egg weight was classified in four groups: EW1 = <33 g, EW2 = 33–36 g, EW3 = 36–39 g and EW4 = 40 g. Egg storage was classified in three groups: ES1 = 0–4 days, ES2 = 5–9 days and ES3 = 10–15 days. EWL was distributed in five different classes: EWL1 = <10%, EWL2 = 10–15%, EWL3 = 16–20%, EWL4 = 21–25%, EWL5 = >25%. Data on fertility, embryo mortality and hatchability were analyzed using the χ² test to determine results diverging from the null hypothesis; egg weight, egg storage and EWL classes were considered as frequency categories (SAS, 1999).

3. Results

Mean egg production recorded weekly during the reproductive period is reported in Fig. 1. Egg production was high
for nine consecutive weeks (period March–May), although with some variation from 46% to 30%; egg production subsequently progressively decreased in the following weeks and was less than 10% on Week 14 and 15 (end of June). In total, 443 eggs were laid in 15 weeks by 14 females (average 31.6 eggs/hen, ranging from 6.5 to 46 eggs/hen).

Mean egg weight was 33.9 ± 3.5 g (mean ± SD) with great variation from 23 to 45 g. The frequency of eggs according to the weight is reported in Fig. 2. The majority (51%) of eggs were within the range 33–37 g and very few eggs weighed less than 28 or more than 39 g. Mean egg weight did not show any variation during the reproductive period and very similar values were measured in the consecutive weeks of oviposition (data not shown).

The overall breeding performance of the local breed measured during the reproductive season was 82% fertility and 50% hatchability on fertile eggs. Even with highly acceptable fertility hatchability of fertile eggs was poor due to the great amount of embryo mortality. Fertility and hatchability were affected by egg related parameters.

The $\chi^2$ calculated from the frequency of fertile ($P<0.05$) and hatched eggs and the frequency of dead embryos ($P<0.01$) per each egg weight group was statistically significant. The proportion of infertile eggs recorded in EW1, egg weight <33 g, was 26% (fertility 74%) and different compared to the expected value ($P<0.05$). Greater fertility values, from 83% to 90%, were recorded in the other egg weight groups (Table 1). Greater embryo mortality was associated with lesser hatchability and was also recorded in EW1, corresponding to 66% and 34%, respectively, and both values were different ($P<0.01$) compared to the expected values (Table 1). With increasing egg weights, hatchability was improved and embryo mortality was reduced. The most desirable combined values for fertility and hatchability, corresponding to 83% and 62%, respectively, were recorded in EW2, including eggs from 33 to 36 g, even though no significant differences were detected with the expected values.

The $\chi^2$ calculated from the frequencies of hatched eggs and dead embryos per each egg storage group were statistically significant ($P<0.05$). A clear negative relationship between hatchability and egg storage, and a concomitant opposite trend between embryo mortality and egg storage was found (Table 2). The most desirable condition was recorded with eggs stored up to 4 days (ES1), corresponding to 58% hatchability and 42% embryo mortality. Hatchability was greatly reduced, although not significantly, to 38% and embryo mortality increased to 62% in ES3, corresponding to 10–15 day of storage before incubation (Table 2).

The $\chi^2$ calculated from the frequency of hatched eggs and dead embryos according to the EWL category were significant ($P<0.01$). The mean proportion of EWL during incubation (1–18 days) was 18.9 ± 7.2%. A negative relationship between hatchability and EWL was found (Table 3). Greater hatchability and lesser embryo mortality were recorded in EWL1 and EWL2; in particular, the most desirable hatchability, corresponding to 83%, was found in EWL1, corresponding to the lesser values of weight loss.

### Table 1

<table>
<thead>
<tr>
<th>Egg weight groups</th>
<th>Fertile eggs, %</th>
<th>Infertile eggs, %</th>
<th>DE, % on fertile eggs</th>
<th>HC, % on fertile eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>EW1 (&lt;33 g)</td>
<td>74 (90)</td>
<td>26 (32)$^b$</td>
<td>66 (59)$^b$</td>
<td>34 (31)</td>
</tr>
<tr>
<td>EW2 (33–36 g)</td>
<td>83 (93)</td>
<td>17 (19)</td>
<td>38 (35)</td>
<td>62 (58)</td>
</tr>
<tr>
<td>EW3 (36–39 g)</td>
<td>88 (92)</td>
<td>12 (13)</td>
<td>50 (46)</td>
<td>50 (46)</td>
</tr>
<tr>
<td>EW4 (≥39 g)</td>
<td>90 (26)</td>
<td>10 (3)</td>
<td>42 (11)</td>
<td>58 (15)$^b$</td>
</tr>
<tr>
<td>Total</td>
<td>82 (301)</td>
<td>18 (67)</td>
<td>50 (151)</td>
<td>50 (150)</td>
</tr>
</tbody>
</table>

$^a$ Values in the same column with superscripts are different from total value ($\chi^2$ test, $P<0.01$).

$^b$ Value in the same column with superscript is different from total value ($\chi^2$ test, $P<0.05$).

### Table 2

<table>
<thead>
<tr>
<th>Egg storage groups</th>
<th>DE, %</th>
<th>HC, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>ES1 (0–4 days)</td>
<td>42 (46)</td>
<td>58 (62)</td>
</tr>
<tr>
<td>ES2 (5–9 days)</td>
<td>48 (57)</td>
<td>52 (61)</td>
</tr>
<tr>
<td>ES3 (10–15 days)</td>
<td>62 (38)</td>
<td>38 (23)</td>
</tr>
<tr>
<td>Total</td>
<td>49 (141)</td>
<td>51 (147)</td>
</tr>
</tbody>
</table>

### Table 3

<table>
<thead>
<tr>
<th>Egg weight loss classes</th>
<th>DE, %</th>
<th>HC, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>EWL1 (&lt;10%)</td>
<td>17 (1)</td>
<td>83 (5)</td>
</tr>
<tr>
<td>EWL2 (10–15%)</td>
<td>33 (11)</td>
<td>67 (22)</td>
</tr>
<tr>
<td>EWL3 (16–20%)</td>
<td>54 (34)</td>
<td>46 (29)</td>
</tr>
<tr>
<td>EWL4 (21–25%)</td>
<td>53 (11)</td>
<td>48 (10)</td>
</tr>
<tr>
<td>EWL5 (&gt;25%)</td>
<td>85 (17)$^a$</td>
<td>15 (3)$^a$</td>
</tr>
<tr>
<td>Total</td>
<td>52 (74)</td>
<td>48 (69)</td>
</tr>
</tbody>
</table>

$^a$ Values in the same column with superscripts are different from total value ($\chi^2$ test, $P<0.01$).

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![Fig. 2. Distribution of egg weight in Mericanal della Brianza hens.](image)
(<10%). Hatchability decreased 46% and 48% in EWL3 and EWL4, respectively, and a greater decrease occurred to 15% in EWL5. As a consequence, a concomitant progressive increase in embryo mortality was found (Table 3). The proportions of hatched chicks and dead embryos recorded in EWL5 were different \( P < 0.01 \) compared to the expected values (Table 3).

4. Discussion

Preservation of local breeds is necessary to allow breeders to use these breeds as biodiversity “capital” and preservation is also useful for creation of new breeds in response to environmental changes such as diseases and changes in consumer demand (Woelders et al., 2006). The study and improvement of the reproductive function in local breeds are the first steps for development of a conservation program. In birds, both reproductive and incubation parameters have to be studied to improve breeding performance.

According to egg production, the most important reproductive period in the MdB breed is from March to May and the maximum rate of egg production was 46%. Despite acceptable fertility (82%), hatchability of MdB eggs was quite poor (50%) and largely affected by egg-related parameters.

Egg weight significantly affected fertility and hatchability and lesser weight eggs were associated with greater embryo mortality (66%) compared to heavy eggs (38%-50%). Lighter weight eggs were associated with greater embryo mortality, resulting in a lesser hatchability in the Cobb strain (Tona et al., 2001; Pedroso et al., 2005). The lesser hatchability as well as greater embryonic mortality with lighter weight eggs may be due to the smaller egg size. In small eggs there may be insufficient nutrients and pores, which could affect the embryo development and the hatching process; in fact embryonic metabolism, such as lipid utilization and respiration, increases with embryonic growth (McLoughlin and Gous, 1999). Furthermore, Zaniboni et al. (2006) described a different proportion in egg components in the MdB breed (albumen: 47.3% and yolk: 36.4%) compared to common eggs (albumen: 62% and yolk: 29%), and such differences may further explain the relationship between embryo mortality and egg weight in the present study.

Hatchability and chick quality are influenced by preincubation storage conditions, e.g. the egg storage time, temperature, humidity, gaseous environment, and the orientation and positional changes of the eggs (Reis et al., 1997). Long storage periods are associated with greater mortalities at the early stage (Boleli, 2003) and indicate a greater sensitivity of the embryo to metabolic disorders and genetic deviations at the initial stages of development (Bloom and Muscarella, 1998). Eggs could be classified as infertile just for this early mortality causing a difficult identification of the embryo and decreasing total fertility proportion. It is generally recognized that prolonged egg storage reduces hatchability (Becker, 1964; Antwi, 1993; Lapão et al., 1999) and has been associated with retardation of embryonic development and morphological changes in the blastoderm (Arora and Kosin, 1966a; Mather and Laughin, 1976, 1977, 1979). Early embryonic mortality resulting from dehydration during the early stages of incubation (Brake et al., 1993; Arora and Kosin, 1966b) was also associated with longer periods of egg storage before incubation (Lapão et al., 1999). Hatchability of eggs stored for longer periods (i.e. 9 or 12 days) was improved by decreasing storage temperature from 16.5°C or 15°C to 10°C (Ruiz and Lunam, 2002; Meijerhof et al., 1994).

The negative effect of longer periods of egg storage on hatchability but not on fertility has been confirmed with eggs from the MdB breed. According to the present results it is suggested that storage of MdB eggs up to 4 days before incubation is the most desirable duration of storage to obtain the most desirable hatchability and for a maximum of 9 days to increase the size of each hatching so as to sustain hatchability at rates of greater than 50%. Temperature and relative humidity during storage of MdB eggs have to be studied to prevent embryo mortality after long periods of storage before incubation.

Water loss is essential to prevent a relative increase in water content due to the formation of metabolic water (Barnett et al., 2004) and egg weight water loss during incubation is almost entirely due to water diffusion through the shell (Tona et al., 2001). Zakaria et al. (2009) provided evidence to support that egg weight loss may be explained by liberation of water as a result of deterioration of the albumen with subsequent passage of unbound water through the eggshell as influenced by variation in albumen quality due to flock age, storage time, and storage conditions (Brake et al., 1997; Lapão et al., 1999; Tona et al., 2001). Too great or too little water loss increases embryo mortality and decreases chick quality. Thus, maintaining a proper water budget during incubation ensures maximal hatchability and greater hatching quality (Meir and Ar, 1987a, 1987b, 1991). Maintenance of incubation water loss of 12–13% of initial egg mass until internal pipping time of the embryo, ensures proper distributions of osmotic and ionic concentrations in different compartments of the developing embryo, and a proper air cell volume under the shell for the pipping embryo to start lung respiration (Ar, 1991a, 1991b), thus supporting maximal hatchability and greater chick quality (Hulet et al., 1987; Meir and Ar, 1987b, 1991; French and Tullett, 1991). Hatchability of fertile eggs in Mericanel breeders was significantly affected by egg weight loss during incubation. The greatest hatchability, 83%, was associated with the least EWL (EWL1), corresponding to the range 8–10%. The progressive increase of EWL above the optimal value of 10% was associated with a constant progressive decrease in hatchability; however, hatchability was acceptable, 67%, even if EWL was increased from 10% to 15%. Meir and Ar (2008) found that a safe target water loss of 13–14% was most desirable for hatchability. Nevertheless, hatchability was acceptable over a wide range of water losses (9–16%) and decreased when water loss was less than 9% or greater than 16%. Water loss exceeding 20% of initial egg mass causes increased mortality and subsequent dehydration of the embryo, thus decreasing hatching success (Davis and Ackerman, 1987). Results of the present study confirm the marked decrease of hatchability with EWL >25%. Tona et al. (2001) and Zakaria et al. (2009) also found that the most desirable hatchability was when EWL
ranged from 10.9% to 11.1%. Many authors (Tullett, 1981; Christensen and McCorkle, 1982; Meir et al., 1984; Meir and Ar, 1987a, 1987b) suggested the presence of an optimum EWL range, under or above which egg hatchability is compromised. This phenomenon may be related to the dehydration state of the embryo during the latter days of incubation, making the hatching process difficult because of excessive water loss, or the embryo may drown in its amniotic fluid (Tona et al., 2001). The relatively greater water loss from the eggs affects not only hatchability but also chick quality and performance (Barnett et al., 2004); therefore, it is highly important to preserve the most desirable incubation conditions to obtain the optimal range for hatching of domestic fowl eggs, focusing on temperature and humidity conditions. For MdB eggs, it was discovered that there is a threshold in EWL, not a range, above which hatchability was greatly compromised suggesting that only excessive dehydration is a problem in the small size MdB eggs. The threshold is 10% for optimal results and 15% dehydration can occur with acceptable results still being obtained. Incubation conditions for Mericanel eggs have to be revised and adjusted to prevent excessive water loss during incubation and provide the required condition during artificial incubation. In MdB breeders, hatchability was reported to also be affected by the parents (Cerolini et al., 2010) suggesting the need for an accurate selection of the breeders and planning of the matings.

In conclusion, the present results suggest specific management of Mericanel eggs before and during incubation to optimize hatching of live chicks. Eggs of less than 33 g in weight should be discarded, storage time before incubation should not exceed 9 days and egg weight loss during incubation should not exceed 15% of initial egg weight. Further studies are suggested to optimize temperature and relative humidity values during egg storage and artificial incubation according to the specific egg characteristics of the Mericanel breed.

Genetic, reproductive and productive parameters should be known to program objective conservation plans based on scientific data (Cerolini et al., 2010). The present results provide knowledge for improving reproductive parameters of this local Italian breed.

Authors’ contributions

M. Madeddu drafted paper and contributed analyzing data. L. Zaniboni, M.G. Mangiagalli and C. Cassinelli participated in design of the study, handled the animals and collected data. S. Cerolini conceived of the study, participated in its design and coordination, performed the statistical analysis and helped to draft the manuscript. All co-authors provided inputs during final manuscript preparation. All authors read and approved the final manuscript.

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