National Rural Development Programme 2014-2022
Measure 10.2 – Biodiversity

Project: TuBAvI-2 (2021-2024)
REPORT ON THE ACTIVITIES PERFORMED DURING THE THIRD AND FOURTH YEAR

UniTO

The present report describes the activities performed from May 1st, 2023 to December 31st, 2024. The activities are described by Action, according to the original programme.

University of Turin performed activities within Actions 1, 2, 4 and 6. Activities within Action 1 were performed as a consultant for Partner UniPI and activities within Actions 2, 4, and 6 were performed as a consultant for UniMI.

Action 1 – Phenotypical characterisation of autochthonous breeds and species

During the **2023 season**, a growth trial was conducted on three Piedmont poultry breeds housed at the poultry farm of the Conservation Centre for the Enhancement of Local Avian Genetic Resources (CoVaGEN) at the University of Turin. The three breeds under study were Bionda Piemontese (BP), Bianca di Saluzzo (BS), and Millefiori Piemontese (MP).

For this study, a total of 1,163 eggs (569 BP and 460 BS from the previous generation reared at CoVaGEN and 134 MP from rural farms) were collected over 10 days and stored in a controlled environment at 15°C and 70% relative humidity (RH). They were then incubated at 37.8°C and 55% RH (Victoria I-9, Italy) in a commercial incubator. From these eggs, 843 chicks successfully hatched (426 BP, 318 BS, and 99 MP), and they were immediately sexed and vaccinated against Marek's disease, Newcastle disease, infectious bronchitis, Gumboro disease, avian encephalomyelitis, and avian pox. During the first week of life, vaccines against coccidiosis and Salmonella spp. were administered, while booster shots for Newcastle disease, encephalomyelitis, infectious bronchitis, and Gumboro disease were given within the first three months of life. At hatching, the average body weight of the chicks was 40.8±2.70 g and 41.1±3.08 g for BP males and females, 40.9±3.69 g and 38.9±3.64 g for BS males and females, and 36.3±2.85 g and 36.5±3.20 g for MP males and females. For the first 28 days, the chicks were housed in a controlled environment with automated ventilation and lighting systems. Infrared lamps were available during the first two weeks. The lighting schedule included 23 hours of light and 1 hour of darkness for the first three days, then gradually adjusted to reach 8 hours of darkness, simulating natural conditions in the experimental facility. At the beginning of the trial, on day 29, all animals were sexed by visual examination, weighed, and individually marked with wing tags. The selected chicks were transferred to the experimental farm and distributed into separate pens according to breed and sex (10 animals per pen; 6 replicates per sex for BP and BS and 4 replicates per sex for MP), totalling 128 BP (64 males and 64 females), 128 BS (64 males and 64 females), and 88 MP (44 males and 44 females) included in the study.

The dimension of the pens was 6 m x 1.5 m x 10 m (length, width, height) and rice hulls were used as litter. The building was equipped with a natural ventilation system, so the climatic conditions were comparable to the external environment. The environmental conditions from day 29 to day 182 (June to

November 2023) are shown in **Figure 1.1**. During the trial, the animals were fed *ad libitum* with a commercial diet for the first period (1-28 days; AMEn: 12.5 MJ/kg and CP: 22.0%, as-fed basis), a diet for the second period (29-88 days; AMEn: 12.8 MJ/kg and CP: 20.5%, as-fed basis), and a commercial diet for the third period (85-182 days; AMEn: 13.3 MJ/kg and CP: 18.0%, as-fed basis) (Fratelli Borello S.p.A., Bra, CN – Italy). Water was provided *ad libitum* throughout the study. The birds were monitored until they reached 182 days of age, at which point 15 males/breed were selected for slaughter performance evaluation (**Table 1.1**)

Figures 1.2 and 1.3 illustrate the live weight trends of the three Piedmont breeds, respectively for females and males. The graphical representation shows that MP exhibited a generally higher weight for both sexes starting from 113 days of age. BS displayed the lowest weights for both sexes, while BP showed intermediate values. Sexual dimorphism is clearly highlighted by the weight differences between males and females.

Figures 1.4 and 1.5 illustrate the average weight gain trends of the three Piedmont breeds, respectively for females and males. The graphical representation indicates that MP exhibited consistently higher values for both sexes when considering the overall periods from days 29-85, 86-181, and 29-181. BS displayed the lowest values for both sexes, while BP showed intermediate values. When analysing data for different biweekly periods, inconsistent fluctuations were observed, a natural phenomenon in intermediate-growing breeds.

Figures 1.6 and 1.7 illustrate the feed conversion ratio (FCR) trends of the three Piedmont breeds, respectively for females and males. The graphical representation shows that MP had more favourable values for both sexes. During periods 155-169 and 169-181, a significant deterioration was observed in BS and BP for both sexes, indicating a marked slowdown in growth beyond 150 days of age. In contrast, Millefiori Piemontese exhibited a more prolonged growth pattern, leading to more favourable FCR values even in the final phase of the observation period.

Table 1.1 presents the slaughter performance of males from the three Piedmont chicken breeds. MP exhibits a higher live weight and chilled carcass weight compared to BP and BS (3242 g, 3034 g, 2912 g and 2102 g, 1997 g and 1849 g, respectively). Nevertheless, the chilled carcass yield is higher for BP compared to MP and BS (65.8%, 64.8% and 63.4%). Significant differences were observed in breast weight and yield: in both cases, MP shows higher values than BP and BS (346 g, 298 g, 259 g and 16.4%, 14.9% and 13.9%).

After the growth trial, the fertility performance and oviposition rate of hens from the three Piedmont poultry breeds were evaluated. Starting from the first laying day in **September 2023** (BP: 21 weeks of age, BS: 22 weeks of age, MP: 26 weeks of age), the number and weight of each individual egg laid were recorded daily until the end of the first laying year. The birds mentioned above were divided into six genetic families (for BP and BS) or four families (for MP), with 1 male and 10 females per family. **From January 2024 to September 2024**, on monthly basis, once the laying trend had stabilized, 15 eggs/breed were sampled for the evaluation of macroscopic and qualitative parameters, including whole egg weight and its components (albumen, yolk, and shell), the Shape Index (ratio of egg height to width, expressed as a percentage), shell thickness (measured at the equator and at the sharp and blunt poles), shell colour (expressed in the CIELAB scale), and yolk colour (expressed in both the CIELAB and Roche scales) (**Figure 1.11).** To assess the fertility performance of the three Piedmont poultry breeds, two incubation tests were conducted at 46 and 56 weeks of age. In both trials, eggs were collected over 10 days and

stored at 15 °C and 70% RH. In the first evaluation, 235 BP eggs, 223 BS eggs, and 119 MP eggs from the previously established genetic families were incubated. For the second evaluation, 283 BP eggs, 238 BS eggs, and 166 MP eggs were incubated.

Figure 1.8 represents the trend of the laying rate (expressed as daily laying percentage) and the evolution of the average egg weight (expressed in grams) of BP hens starting from the first week of egg laying, corresponding to the 21st week of life.

Figure 1.9 illustrates the trend of the laying rate (expressed as daily laying percentage) and the evolution of the average egg weight (expressed in grams) of BS hens, starting from the first week of egg laying of the earliest breed, BP. As shown in the graph, BS started laying at 22 weeks of age.

Figure 1.10 shows the trend of the laying rate (expressed as daily laying percentage) and the evolution of the average egg weight (expressed in grams) of MP hens, starting from the first week of egg laying of the earliest breed, BP. According to the graph, MP started laying at 26 weeks of age. Unlike the other Piedmont poultry breeds, MP interrupted egg laying for three weeks following the first week of laying.

Figure 1.11 compares the fertility and hatchability trends of the BP, BS, and MP breeds at 46 and 56 weeks of age (26 and 36 weeks of laying). In the first incubation trial, the breed with the highest fertility rate was BP, followed by BS and MP (94.0%, 85.2%, and 63.9%, respectively). The same trend was observed for hatchability rates (BP: 91.4%, BS: 90.0%, MP: 78.9%). In the second incubation trial, the highest fertility rate was recorded in BP, followed by MP and BS (95.1%, 88.0%, and 87.4%, respectively). Nevertheless, BP demonstrated the highest hatchability rate (93.7%), followed by BS (93.3%) and MP (81.5%).

Table 1.2 presents the overall data related to egg quality. The macroscopic aspects of the eggs reveal some differences, particularly in MP, which differs from BS and BP in the following parameters: egg weight, shape index, and eggshell colour (for the three indices L*, a*, b*). However, for the remaining parameters, the eggs appear similar. The only differences observed between BS and BP are egg weight, eggshell weight, eggshell colour (limited to indices L* and a*), and yolk colour (limited to index b*).

Action 2 – Genetic characterization of breeds and species raised in Italy, including the use of genomic information

Task 2.1 Genetic characterization using microsatellite markers

Genetic characterization with microsatellite markers is planned to provide useful tools for the conservation of biodiversity in nucleus populations managed by PAs and in the semen Cryobank. Specifically, the following objectives are considered:

1) Identify male candidates for replenishment and semen production;
2) Characterize new genetic lines in breeds under conservation;
3) Characterize new chicken breeds.

Task 2.1.1 Characterization of male candidates

For the characterization of candidates for semen production, 20 subjects of the Ermellinata breed and 16 subjects of Robusta Lionata were analysed. For replenishment and management plans, candidates present in the conservation nuclei of UniMOL (Siciliana, 44), UniFI (Mugellese, 49; Valdarnese, 55; Valdarno, 49), UniTO (Bionda Piemontese, 62; Bianca di Saluzzo 58) and UniMI (Mericanel della

Brianza, 70) were characterized. A total of 423 subjects of the *Gallus gallus* species present in the conservation Centre were analysed. Additionally, 32 turkey subjects (genus *Meleagris*) were characterized.

Task 2.1.2 Identification of new genetic lines in the Modenese chicken breed

Twenty-three subjects belonging to the Modenese breed were genetically characterized to identify new genetic lines. To estimate the inbreeding rate, the average observed heterozygosity (Ho=0.41) was analysed, and median, standard deviation (SD), standard error (SE), maximum, and minimum values were reported. The Hardy-Weinberg equilibrium test shows a significant deviation from equilibrium only in some loci. The graph in Figure 2.1 shows the distribution of individual molecular inbreeding (H-ind) in the analysed population. The overall distribution follows a bell-shaped curve, with the distribution skewed to the right (generally high values of individual variability).

Task 2 2 Characterisation of polymorphisms of genetic markers in linkage with candidate genes (GAS)

The characterisation of the minisatellite region of Pax7 gene, candidate as a marker for selected productive traits, was performed through the evaluation of its polymorphism (number of alleles, allelic frequency, and genotypes) within the analysed breeds and across them.

The analysis was performed on 1131 individuals from 17 breeds, spread among 28 populations reared in different conservation centres or different generations from the same population. The results were summarised in a report which was made available for the download from the project website, on the page dedicated to the results obtained during the third-fourth year of the project, in the Action 2 section, consultant UniTO.

Action 4 – Estimation of genetic and genomic indices and reproductive management

Task 4.1 Selection of males for reproduction and semen production

Typing data were used to calculate the kinship matrix by evaluating the number of alleles shared for each pair of individuals belonging to the same breed. The average kinship among all breeders (P) and the average kinship with family lines (Pf) were then calculated.

Principal Coordinate Analysis (PCoA) was used to highlight the existing genetic variability in the sampled groups. The results were graphically represented (Figure 4.1) to make the genetic differences between the subjects evident. As an estimate of inbreeding, the average observed heterozygosity was analysed, along with the number of samples (N), number of different alleles (Na), number of effective alleles (Ne), Shannon Index (I), effective heterozygosity (He), and fixation index (F). Observed heterozygosity is very low (0.24-0.33) in Veneto populations, but the percentage of polymorphic loci is 73%, and the overall average kinship is also high. For male selection plans, the individual variability index (H-ind) and genetic distance (DPS) were estimated. Based on the results of the previously reported analyses, to maximize the selection of males, the individual variability indices (H-ind) and the degree of kinship (P or Pf) were combined into a new conservation index (IC) calculated as the difference between H-ind and P/Pf. The index ranges from 1 to -1, with high values indicating low inbreeding and greater genetic variability. Based on the IC index, individuals were identified with an index value above the population average.

Task 4.2 Reproductive management

For mating plans, the individual variability index (H-ind) and the average kinship index (P) concerning all analyzed subjects were estimated. Subjects with higher H-ind allow for the conservation of greater genetic variability, while those with lower P help to contain the increase in inbreeding. Matings were

made by calculating the average kinship of typed males with the subjects of each family present in the conservation nucleus.

The selection of breeders was carried out to cover the greatest existing variability, taking into account individual heterozygosity using the conservation index (IC). Matings were planned between subjects belonging to different bloodlines to minimize kinship between the male and the female group. For each male, the average kinship between the females in the group and the selected male was calculated to minimize the inbreeding rate (ΔF). The analysis results were reported in the genetic evaluation document sent to the UniMI coordinator and to the reference PA. The indices were published on the project's website at the page https://www.pollitaliani.it/en/actions/action-4/conservation-index/.

FIGURES and TABLES

Figure 1.1 - Temperature (°C) and Relative Humidity (%) Trends from June to November 2023

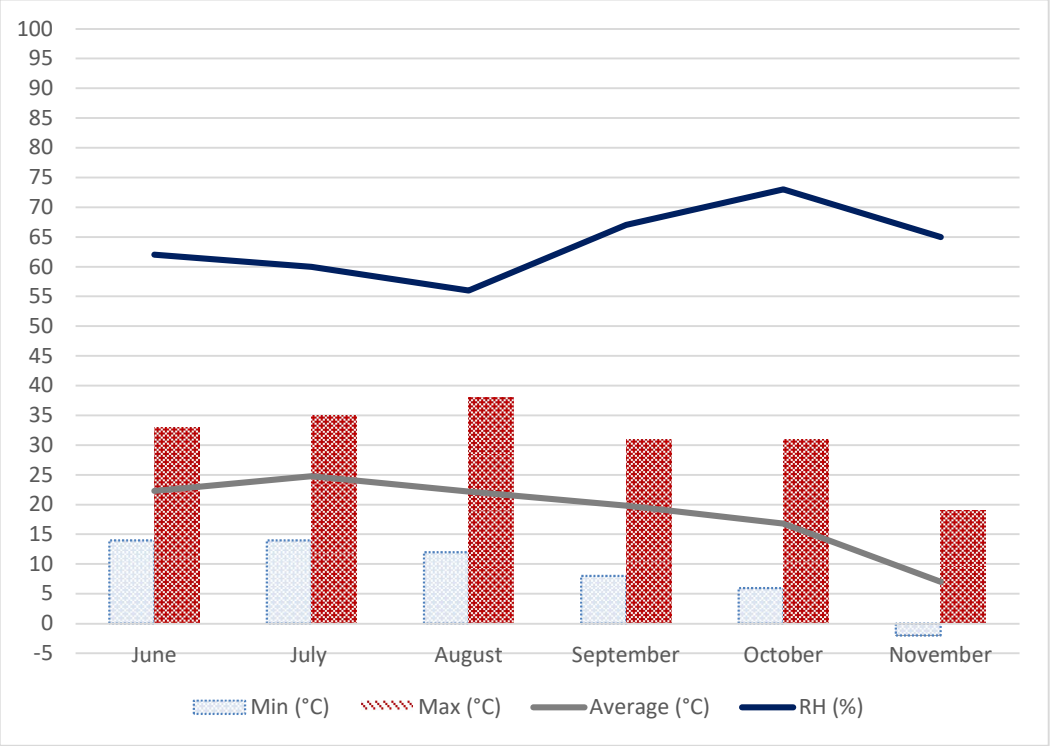

Figure 1.2 - Live Weight Trends of Female Bianca di Saluzzo (BS), Bionda Piemontese (BP), and Millefiori Piemontese (MP)

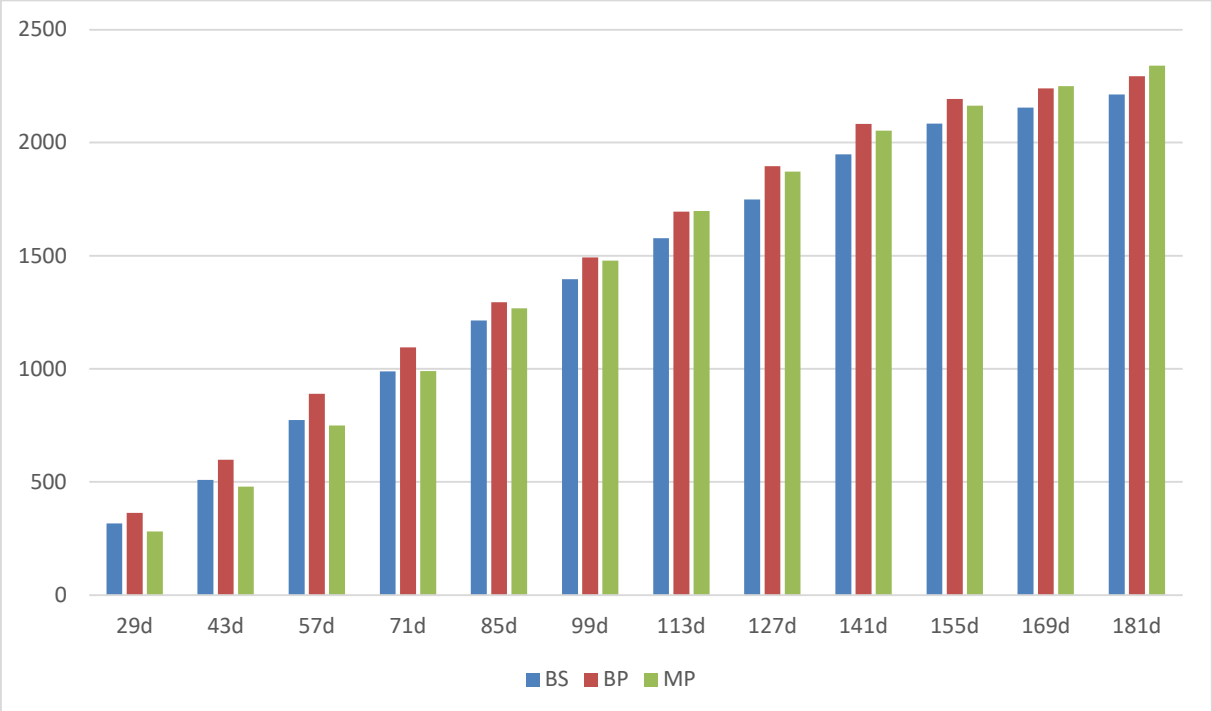

Figure 1.3 - Live Weight Trends of Male Bianca di Saluzzo (BS), Bionda Piemontese (BP), and Millefiori Piemontese (MP)

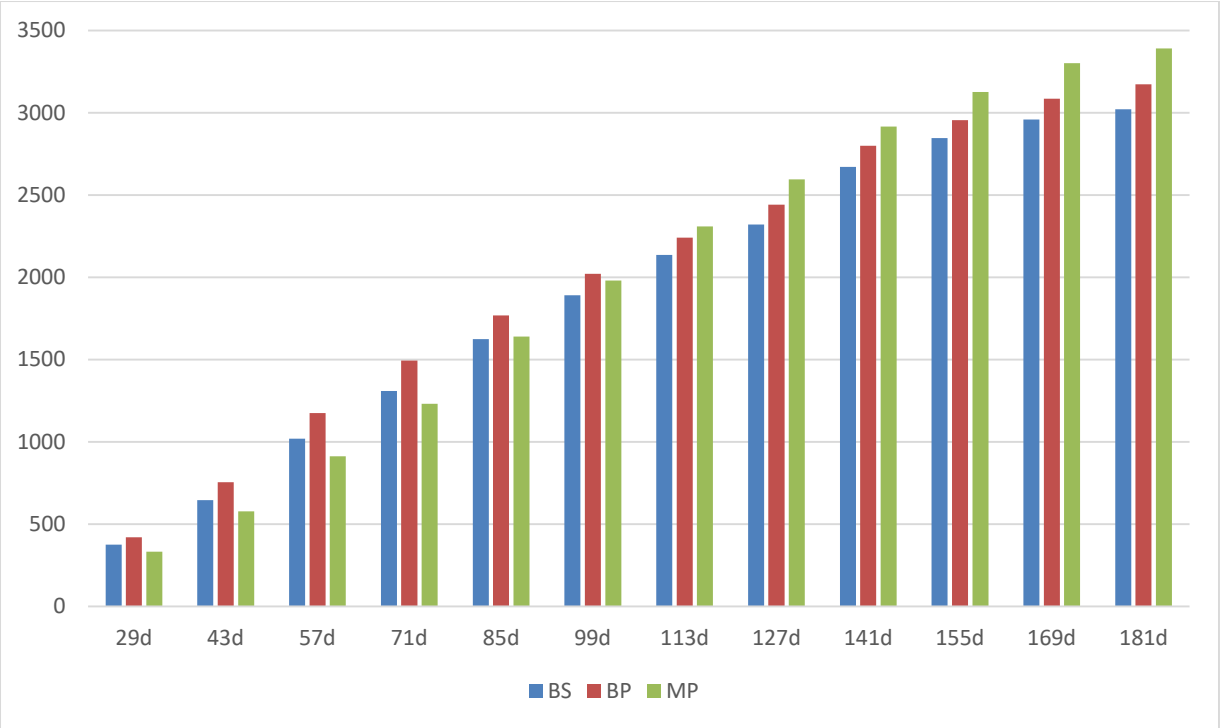

Figure 1.4 - Average Weight Gain Trends of Female Bianca di Saluzzo (BS), Bionda Piemontese (BP), and Millefiori Piemontese (MP)

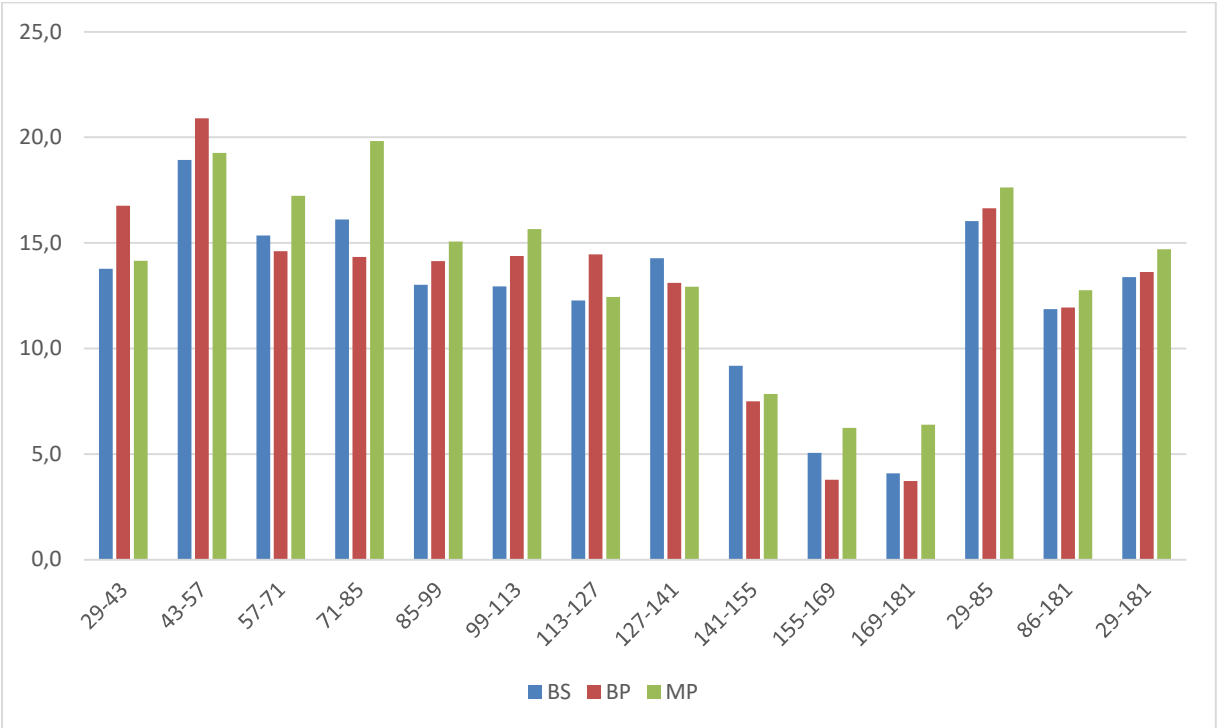

Figure 1.5 - Average Weight Gain Trends of Male Bianca di Saluzzo (BS), Biondа Piemontese (BP), and Millefiori Piemontese (MP)

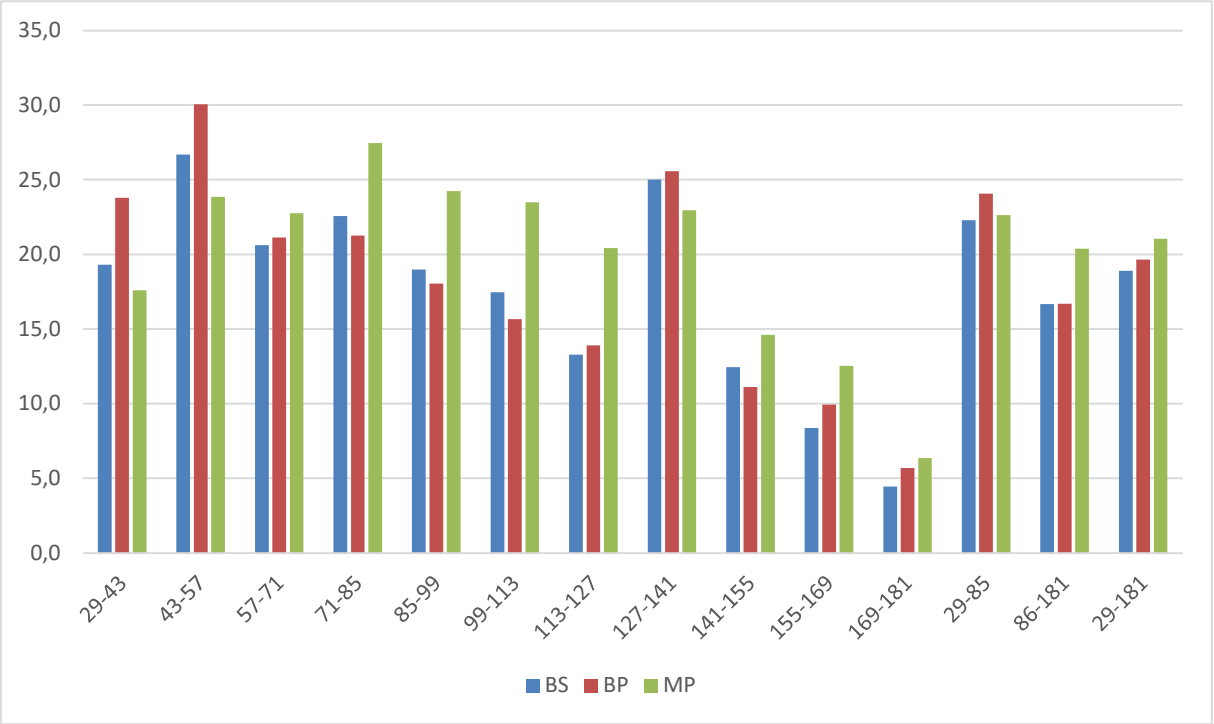

Figure 1.6 - Feed Conversion Ratio Trends of Female Bianca di Saluzzo (BS), Biondа Piemontese (BP), and Millefiori Piemontese (MP)

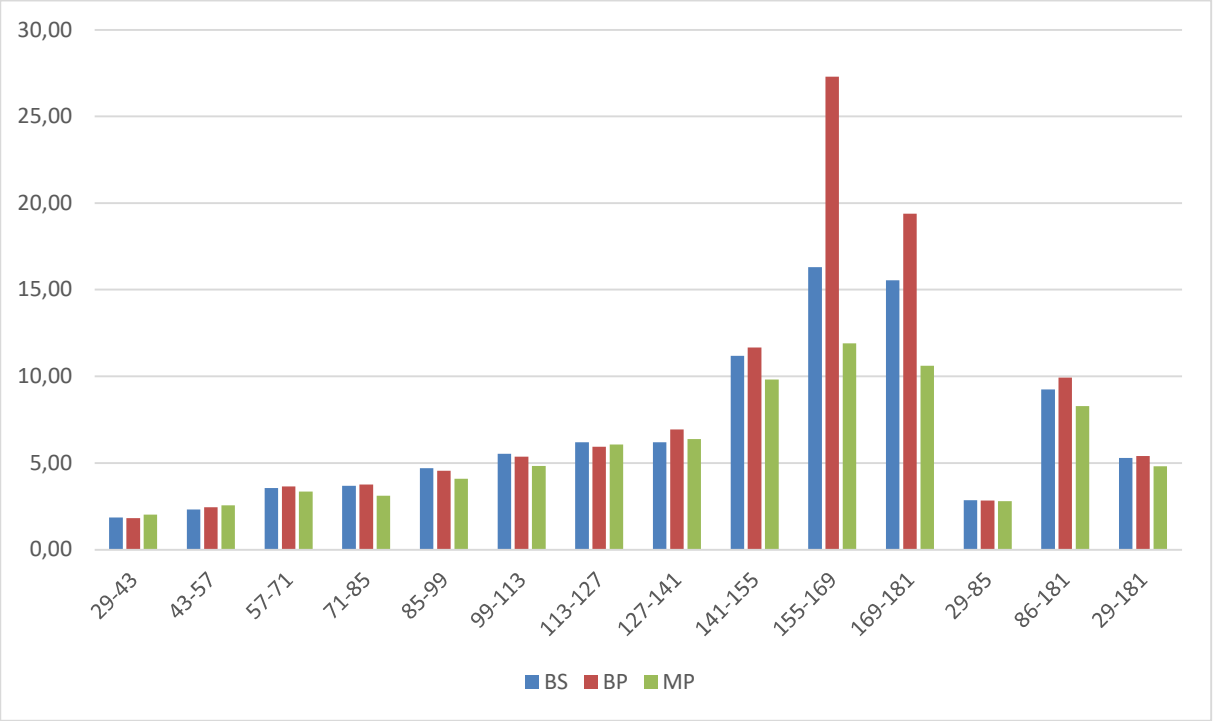

Figure 1.7 - Feed Conversion Ratio Trends of Male Bianca di Saluzzo (BS), Bionda Piemontese (BP), and Millefiori Piemontese (MP)

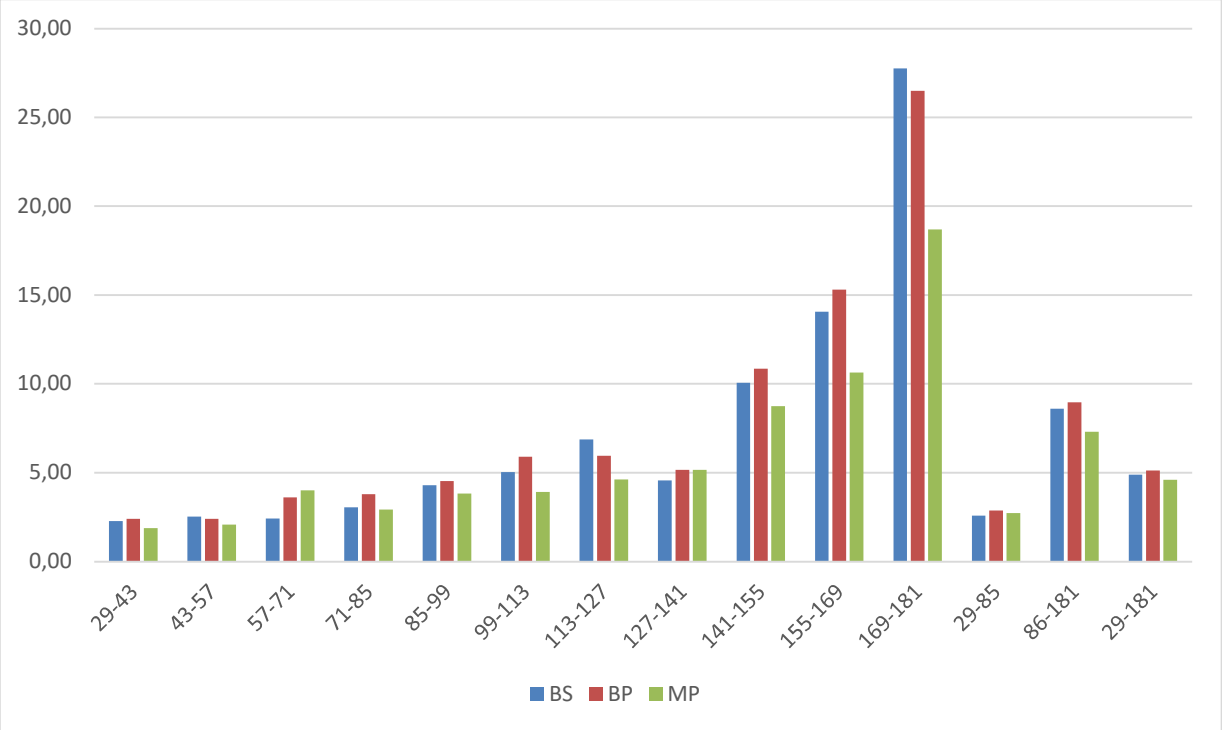

Table 1.1 – Slaughter performance of male Bianca di Saluzzo (BS), Bionda Piemontese (BP) and Millefiori Piemontese (MP) chickens

	BP	BS	MP	p-value	SEM
Live weight	3034 ab	2912 b	3242 a	0.039	125.6
Ready-to-cook carcass (g)	2031	1896	2129	0.069	9804
Ready-to-cook carcass yield (%)	66.9	65.0	65,6	0.374	1.383
Chilled carcass (g)	1997 ab	1849 b	2102 a	0.030	91.69
Chilled carcass yield (%)	65.8 a	63.4 b	64.8 ab	0.059	0.985
Breasts (g)	298 b	259 b	346 a	<0.001	17.94
Breasts yield (%)	14.9 b	13.9 b	16.4 a	<0.001	0.434
Thighs (g)	728	681	760	0.097	35.43
Thighs yield (%)	36.5	36.9	36.1	0.433	0.638
Testicles (g)	9.96 ab	10.3 a	6.42 b	0.021	0.629
Gizzard (g)	45.5 b	41.9 b	59.6 a	<0.001	3.779
Proventriculus (g)	8.71	8.89	8.81	0.966	0.671
Spleen (g)	4.33	4.65	5.07	0.498	0.625
Liver (g)	42.4	3.,4	45.9	0.161	3.344
Heart (g)	14.5	15.2	15.9	0.440	1.033
Intestine (g)	91.4	88.4	91.3	0.782	4.875
Abdominal fat (g)	45.9	54.8	35.0	0.349	13.48

Table 1.2 – Qualitative analysis of eggs from Bianca di Saluzzo (BS), Bionda Piemontese (BP), and Millefiori Piemontese (MP)

	BP	BS	MP	p-value	SEM
Egg weight (g)	59.8 b	60.2 a	59.0 c	0.050	0.501
Albumen weight (g)	34.7	35.0	34.0	0.087	0.455
Yolk weight (g)	18.7	18.8	18.5	0.285	0.188
Shell weight (g)	6.70 a	6.47 b	6.59 ab	0.025	0.080
Albumen yield (%)	58.0	58.1	57.7	0.609	0.471
Yolk yield (%)	30.8	31.1	31.2	0.672	0.188
Shell yield (%)	11.2 a	10.8 b	11.1 a	0.003	0.126
Shape Index	72.4 b	73.3 b	76.9 a	<0.001	0.498
Equatorial eggshell thickness (mm)	0.41	0.41	0.41	0.052	0.003
Blunt-end eggshell thickness (mm)	0.40	0.39	0.41	0.102	0.005
Sharp-end eggshell thickness (mm)	0.42	0.43	0.42	0.142	0.004
L* shell	81.83 a	80.42 b	78.16 c	<0.001	0.447
a* shell	4.87 c	5.63 b	6.75 a	<0.001	0.233
b* shell	15.40 b	15.90 b	19.79 a	<0.001	0.484
L* yolk	49.98 ab	50.25 a	49.15 b	0.025	0.399
a* yolk	2.83	2.77	2.50	0.548	0.319
b* yolk	45.47 b	47.47 a	45.14 b	<0.001	0.571
Yolk Roche scale	10	10	10	0.305	0.208

Figure 1.8 – Trend of laying percentage and evolution of egg weight in Bionda Piemontese (BP) hens

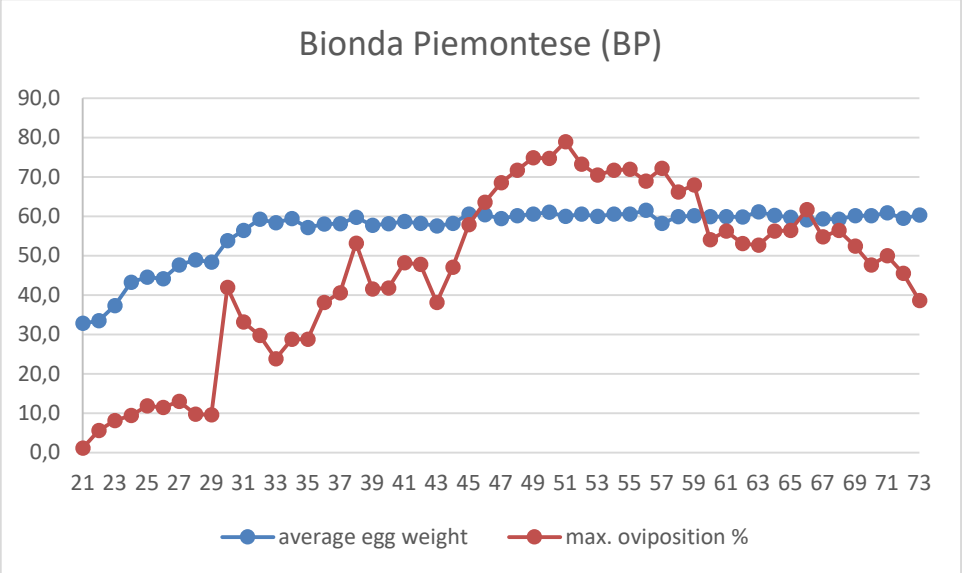

Figure 1.9 – Trend of laying percentage and evolution of egg weight in Bianca di Saluzzo (BS) hens

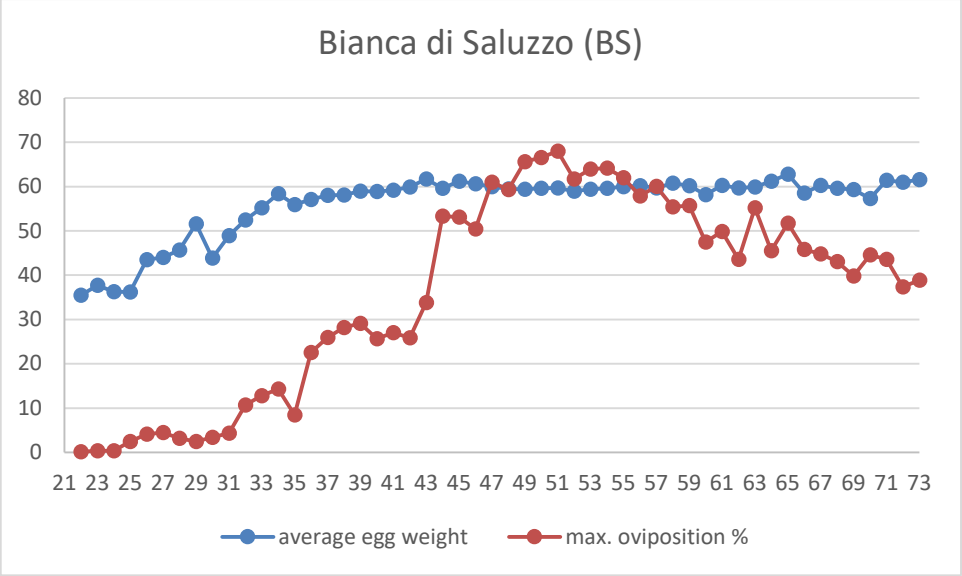

Figure 1.10 – Trend of laying percentage and evolution of egg weight in Millefiori Piemontese (MP) hens

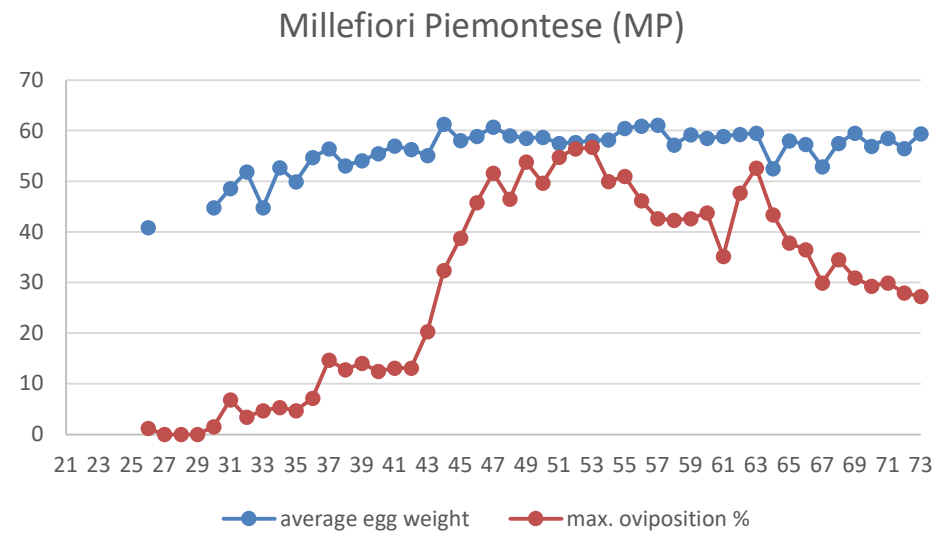

Figure 1.11 – Comparison of fertility and hatchability percentage in Bionda Piemontese, Bianca di Saluzzo and Millefiori Piemontese breeds at 46 and 56 weeks of age (26 and 36 weeks of oviposition)

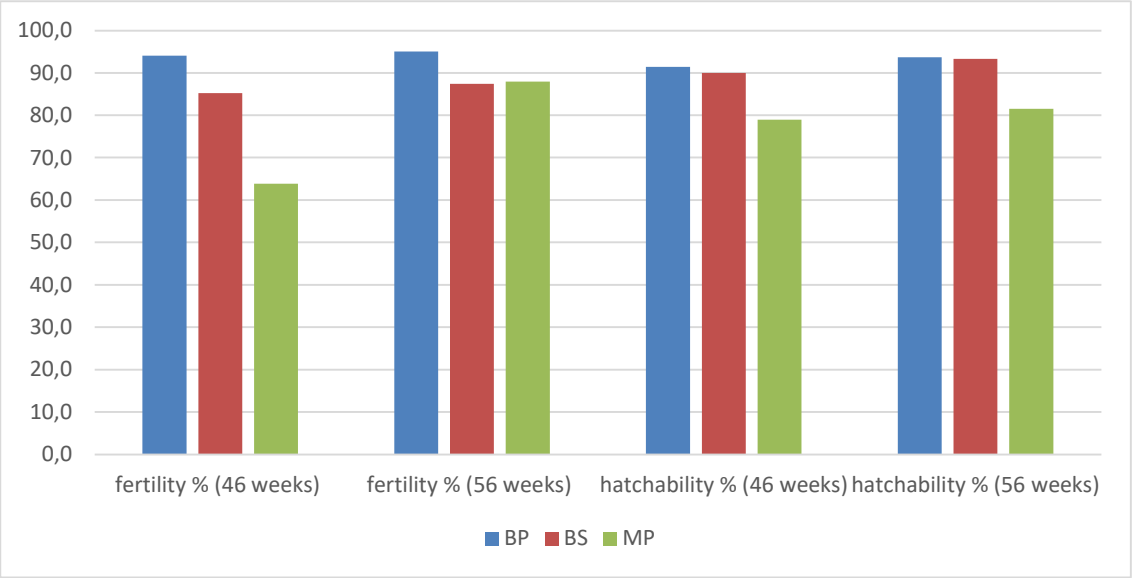

Figure 2.1 – Individual molecular inbreeding of Modenese breed

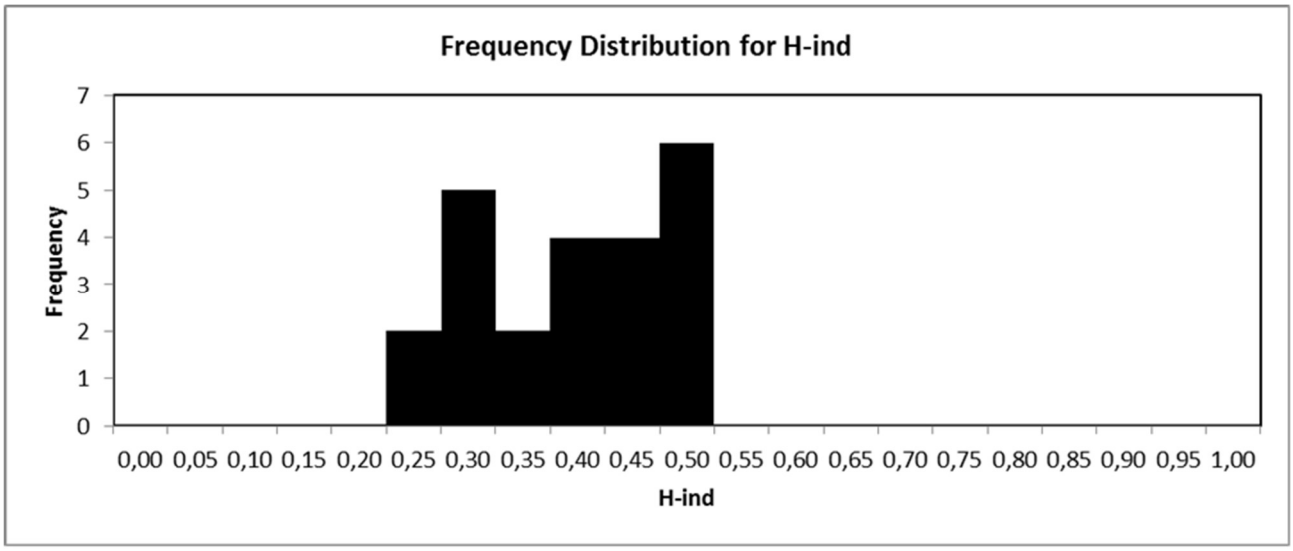

Figure 4.1 – Principal Coordinate Analysis (PCoA) in Modenese population