

*National Rural Development Programme 2014-2022*  
*Measure 10.2 – Biodiversity*

*Project: TuBAVI-2 (2021-2024)*

**REPORT ON THE ACTIVITIES PERFORMED DURING THE THIRD YEAR**

*UniMI*

The report describes the activities performed from 01.05.2023 to 31.03.2024. The activities are described by Action, according to the original programme.

**Action 1 – Phenotypical characterisation of autochthonous breeds and species**

*Task 1.1 – Phenotypic characterisation of Mericanel della Brianza (MB) chickens*

The reproductive period of the Mericanel della Brianza chickens selected during the second year was still ongoing in May 2023 and was concluded in July. Oviposition performance data were described in the previous activity report and a constant decline in egg production was observed in the period May-July 2023. Eggs set in May were incubated to select new breeders; the following average values were registered: fertility = 82.6%, hatchability/incubated eggs = 71.5%, hatchability/fertilised eggs = 86.4%. Total 190 chicks were hatched, individually marked on hatch.

Chicks were reared in a floor pen with controlled environment during the growing period; at 40 days of age birds were sexed and males and females reared separately from then on. The chicks underwent vaccination programme as follows: a) Marek disease, at 1 day of age; b) Newcastle disease, at 1, 4, and 20 weeks of age; c) salmonellosis, at 1, 7, and 18 weeks of age. Body weight was registered in 86 sample birds on 30, 60, 127, 142, and 190 days of age to monitor the growth curve; at 190 days of age, average body weight was 966 g (DS=110 g) in males and 769 g (DS=108 g) in females, in accordance with the standard body weight of the breed. In July, all males (n=82) were sampled (feathers) and genotyped in order to set mating plans according to individual variability index (external service UniTO). Female breeders were selected according to kinship and to the numbers of birds available for each family line. Overall, 48 female and 6 male breeders were selected (together with further 10 spare males). Sexual maturity occurred on 21 weeks of age and weekly oviposition rate progressively increased from 1 to 23% in the period October-December 2023.

Selected breeders were organised into 6 familiar lines, reared in floor pens with controlled environment starting from January 2024; moreover, additional 10 selected males were reared. Data on familiar lines are reported in Table 1.1. The reproductive period is ongoing; planned activities include: a) daily recording of egg production (number and weight) per family line, b) individual morphological characterisation, c) incubation of eggs in the period April-May to monitor fertility and hatchability of family lines. The weekly oviposition rate (%) recorded so far increased from 19% to 39% during January and then has remained constant during the following months.

*Task 1.2 Phenotypic characterisation of Modenese (MO) chickens*

During 2023, new Modenese (MO) birds were found in fancy farms and new adults (13 females and 7 males, 180 days of age) were housed to the Poultry Center on October 2023. A quarantine period was observed to run sanitary tests, then rearing started in December. The birds were individually marked, weighted, and sampled (feathers). Samples were sent to the Molecular Genetics Laboratory (UniTO) for microsatellite marker genotyping in order to analyse individual variability and kinship. Analyses identified 3 familiar lines (Figure 1.1) and were used to set a mating plan to reduce consanguinity. Data on familiar lines in reproduction from March 2024 are reported in Table 1.2. Each familiar line is reared in a floor pen with controlled environment. Morphological characterisation of individual breeders showed homogeneous qualitative traits, consistent with wheaten gold colour: yellow shanks, white ear-lobe, orange/yellow eyes, red simple comb with 5-6 spikes, falling to one side in the hen; average values of quantitative morphological traits are reported in Table 1.3. Occasional oviposition was registered during November and December 2023, and a constantly increasing weekly oviposition

rate (4% to 46%) has been observed starting from January until now. Like MB breed, reproductive data recording is still ongoing, and hatching of the progeny for the selection of young breeders is expected in June.

*Task 1.3 Phenotypic characterisation of Nero d'Italia (NI) and Brianzolo (Br) turkeys*

**Brianzolo breed (BR)** - The reproductive period of the three couples of Brianzolo (BR) breed, started during the second year, continued during May-July 2023 in controlled environment. Oviposition was constant in the period March-July 2023, after a period of sexual rest (see Report on the activities of the second year); oviposition rate was low, not higher than 29%, and individual egg production was 25 eggs/16 oviposition weeks. All breeders were moved to outdoor enclosures from August to December 2023; occasional oviposition was recorded during this period only in September and October. Eggs set during the period April-May 2023 were incubated, fertility was 96%, embryonic mortality 21%, and hatchability/incubated eggs 75%, corresponding to 18 hatched poults.

Poults, marked and weighted on hatch, were reared in floor pens with controlled environment up to adult age. Poults underwent vaccination for Newcastle disease at 1, 4, and 19 weeks of age. Body weight was registered on 4, 10, 16, 21, 30, 33, and 35 weeks of age and average registered values are reported in Table 1.4 for males and females. Body weight from hatch to 16 weeks of age was very similar in both sexes, while males showed a higher body weight increase in the following weeks. Birds were moved to outdoor enclosures in the period August-December 2023 and then again to controlled environment in the following months. Mortality rate during the growing period was 11% (2 birds), then 5 females and 11 males reached adult age. Sexual maturity in females occurred on 30-31 weeks of age, in December.

At the beginning of March 2023, familiar lines were organised: 1 group with 3 females hatched in 2022 (TV), and 2 groups with 5 females hatched in 2023 (TG, 2 females in one group and 3 females in the other). It is planned to mate TV females in turn with each of the 3 males hatched in 2022, and TG females with males hatched in 2023, randomly chosen, and changed every third week. It is planned to set eggs three times along the period April-May 2024 in order to select young breeders. Reproductive traits recording is ongoing and will be completed in July 2024. Along the period January-March, weekly oviposition rate of TG females increased constantly from 6% to 80%, while TV females started oviposition in March, with an average weekly oviposition rate of 26%.

All the reared turkeys were sampled (feathers) in November 2023. Samples were sent to the Molecular Genetics Laboratory (UniTO) for microsatellite marker genotyping in order to analyse population structure and kinship.

**Nero d'Italia breed (NI)** - A new group of Nero d'Italia turkeys (n=10) was housed to the Poultry Center on July 2023, in order to set a nucleus population. A quarantine period was observed to run sanitary tests, then rearing started in September. All the birds were marked, sexed, then males and females were parted and reared in outdoor enclosures in the period September-December, and in floor pens in controlled environment in the following months. Body weight was registered on 98, 126, 160, 190, and 220 days of age; growth curve for males (n=3) and females (n=7) is reported in Figure 1.4. Sexual maturity of females occurred on 45 weeks of age in April and oviposition traits recording is ongoing. At the beginning of March, 3 family lines were organised, each made of 2-3 females and 1 male; it is planned to rotate the males from one familiar line to another every third week. In the period April-May, three egg settings are planned in order to select new breeders.

All the birds were sampled (feathers) in November. Samples were sent to the Molecular Genetics Laboratory (UniTO) for microsatellite marker genotyping in order to analyse population structure and kinship.

*Task 1.4 Phenotypic characterisation of semen in chicken and turkey breeds*

**Proteome** – The functional informatic analysis of shared and exclusive proteins of different chicken breed semen was concluded during the third year. Preliminary results will be presented in a poster at the European Poultry Congress (Valencia, Spain) in June 2024. Complete results are being organised to prepare a paper to be submitted for the publication to an open access specialised journal of the field.

**Lipids** – Verification and organisation of the laboratory data produced in previous months were concluded in 2023. At present, statistical analysis of final data is being planned, in order to obtain results on any difference among breeds in lipid composition of the semen. It is planned to present the data in a final report.

**Semen production and quality** - During the third year, semen production was evaluated in MO chickens and in BR and NI turkeys. Males from different breeds, reared as described in task 1.2 and task 1.3, were used for semen collection in the period December-March, before the familiar lines were organised. Semen collection was routinely performed twice weekly and semen quality was regularly evaluated. Average values of qualitative parameters recorded in individual ejaculates for MO and BR breeds are reported in Table 1.5. NI turkeys did not produce ejaculates on milking for semen collection.

## **Action 2 - Genetic characterisation of Italian autochthonous breeds and species using also genomic tools**

*Task 2.1 - Genetic characterisation using microsatellite markers*

See UniTO report.

*Task 2.3 Sequencing of genome in Meleagris gallopavo species*

Genomic data were obtained in May 2023 and statistical analysis was performed in the following months. Final results have been organised and presented as a scientific report for the publication on Plos One. The review of the manuscript is still ongoing.

## **Action 4 - Estimation of genetic and genomic indices and reproductive management**

*Task 4.1 - Selection of males for reproduction and production of semen*

See UniTO report.

*Task 4.2 - Reproductive management*

See UniTO report.

## **Action 8 – Collection of biological samples and germplasm**

*Task 8.2 Collection of germplasm for the Semen Cryobank*

The Italian Semen Cryobank of Autochthonous Chicken and Turkey Breeds was implemented and semen collected from the breeds described in Action 1 task 1.4 was stored in liquid nitrogen. Semen doses were prepared according to the cryopreservation procedure for chicken semen described in the Cryobank SOP. Due to the small volume of MO chicken and BR turkey ejaculates, semen doses were produced with breed semen pools. Overall, 149 doses of MO breed semen and 26 doses of BR breed semen were stored.

## **Action 10 – Communication and dissemination activities**

Several communication and dissemination activities were performed and are briefly described:

- Breed technical data sheets, *Meleagris gallopavo* species, were planned, produced and published online. The form structure was planned in accordance with all PAs, who provided the data to fill in. The forms were published online in [www.pollitaliani.it](http://www.pollitaliani.it) and a print version is available as *booklet* pdf.
- Breed technical data sheets, *Gallus gallus* species, were updated with colour specific data (Mugellese and Livorno breed).
- The website [www.pollitaliani.it](http://www.pollitaliani.it) was regularly updated, especially in the pages dedicated to: publications, participation to scientific or dissemination events; new pages were added on the second annual meeting and on the results of the activities performed during the second year.

- The preparation of a poster is ongoing, displaying chicken and turkey breeds conserved by the project TuBAvI-2. The poster will be provided to the PAs and to the consultants UniTO and ANCI, for the distribution to the audience in dissemination events.
- Scientific articles on open access journals, including a cooperative article by all the PAs, and communications in national and international conventions were published, as reported on the web page [pollitaliani.it/publications](http://pollitaliani.it/publications) and as listed below.

Publications:

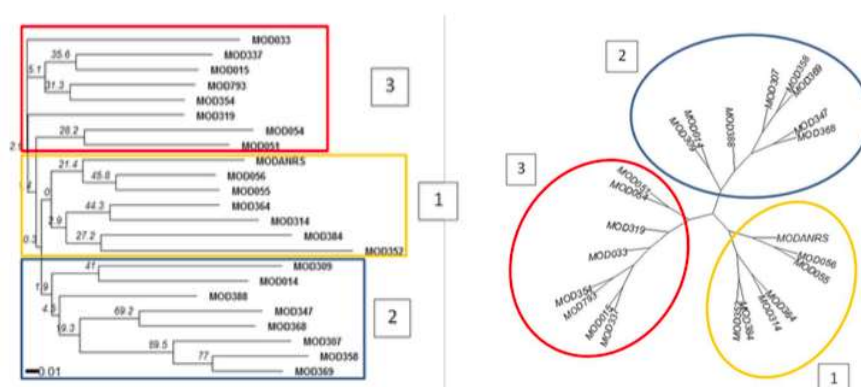
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- Madeddu M, Zaniboni L, Marelli SP, Di Iorio M, Iaffaldano N, Mangiagalli MG, Cerolini S (2023) Variability in semen freezability within an Italian chicken breed. ASPA 25th Congress, Monopoli (Bari, Italy), June 13-16, 2023. *Italian Journal of Animal Sciences*, vol. 22, supplement 1, oral presentation O115.
- Madeddu M, Zaniboni L, Marelli SP, Tognoli C, Di Iorio M, Iaffaldano N, Mangiagalli MG, Cerolini S (2023) Egg production traits during ageing in the chicken Italian breed Mericanel della Brianza. ASPA 25th Congress, Monopoli (Bari, Italy), June 13-16, 2023. *Italian Journal of Animal Sciences*, vol. 22, supplement 1, poster P120.
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- Marelli SP, Madeddu M, Tognoli C, Mangiagalli MG, Zaniboni L, Iaffaldano N, Di Iorio M, Cerolini S Bird reactivity and semen production in the Pepoi and Robusta Maculata Italian chicken (*Gallus gallus domesticus*) breeds. *European Symposium on Poultry Welfare*, June 26-29, 2023, Praga (Repubblica Ceca)
- Cerolini S Advancement in conservation programs of Italian poultry genetic resources for rural development 25<sup>th</sup> Congress of the Association for Animal Science and Productions (ASPAs 2023), June 13-16, 2023, Monopoli (Bari, Italy), invited lecture.

## FIGURES AND TABLES

**Table 1.1 – Data of the familiar lines of Mericanel della Brianza breed in reproduction in 2024: number of birds, average body weight of females, average body weight and individual variability index (H-Indiv) for males, familiar molecular kinship (PM)**

Familiar line	Number of birds		Body weight (g)		H-indiv	PM
	males	females	males	females	males	
A	1	8	1194	726	0,577	0,54
BC	1	10	1090	844	0,462	0,57
DEF	1	9	986	809	0,577	0,54
G	1	2	966	810	0,654	0,51
5	1	9	1060	886	0,577	0,56
6	1	10	1040	707	0,538	0,54

**Figure 1.1 – Graphic representation of genetic distances among the Modenese breed birds that identify three family lines**



**Table 1.2 – Data of the familiar lines of Modenese breed in reproduction in 2024. Body weight and H-Indiv for females are average values of the family line. (H-indiv: individual variability index; PM: familiar molecular kinship)**

Familiar line	Number of birds		Body weight (g)		H-indiv	H-indiv	PM
	male	females	male	females	male	females	
1	1	4	2660	2146	0,31	0,46	0,49
2	1	3	2490	2247	0,50	0,38	0,56
3	1	6	2240	2328	0,35	0,43	0,53

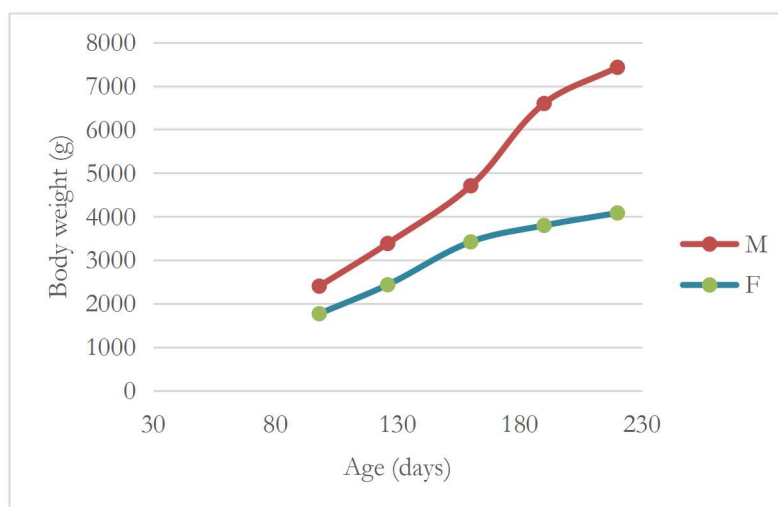
**Table 1.3 - Quantitative morphological parameters in Modenese breeders**

Parameter	Males		Females	
	Average	SD	Average	SD
Body weight (g)	2330	193	2253	283
Body length (cm)	45,4	1,99	40,0	2,00
Shank length (cm)	9,75	0,42	8,27	0,60
Shank diameter (cm)	1,10	0,15	0,82	0,10
Shank circumference (cm)	4,71	0,27	4,07	0,17
Chest circumference (cm)	35,6	2,93	35,5	2,82
Wing span (cm)	48,6	1,27	42,1	2,19

**Table 1.4 – Body weight (Average±SD) of male and female Brianzolo turkeys during the growing period**

Age (weeks)	Males	Females
hatch	47.5 ± 1.05	47.2 ± 2.59
4	265 ± 51.8	218 ± 11.3
10	927 ± 79	888 ± 5.66
16	1566 ± 170	1241 ± 68
21	3013 ± 370	2088 ± 145
30	4650 ± 362	2895 ± 300
33	5005 ± 202	2780 ± 36
35	5120 ± 14	3330 ± 127

**Figure 1.2 – Growth curve of male and female turkeys of the Nero d'Italia breed**



**Table 1.5 – Quali-quantitative parameters of the semen of Modenese (MO) breed chickens and Brianzolo (BR) breed turkeys**

Parameter*	Breed	
	MO	BR
Volume (mL)	0,27 ± 0,09	0,07 ± 0,04
Concentration (x10 <sup>9</sup> /mL)	2,31 ± 0,50	8,00 ± 1,97
IM (%)	80,1 ± 9,62	91,8 ± 2,09
Motilità (%)	55,6 ± 19,1	72,2 ± 27,2
MP (%)	9,27 ± 6,80	20,7 ± 14,1
VCL (µm/s)	45,0 ± 11,9	64,1 ± 30,8
VSL (µm/s)	17,3 ± 7,24	27,4 ± 12,2
VAP (µm/s)	27,5 ± 9,25	42,2 ± 19,0
LIN (%)	37,6 ± 8,17	42,8 ± 8,87
STR (%)	64,6 ± 3,67	65,0 ± 7,08
WOB (%)	60,1 ± 5,95	65,7 ± 6,84
ALH (µm)	3,19 ± 0,37	3,32 ± 0,87
BCF (Hz)	7,29 ± 1,10	7,51 ± 0,86

\*IM: sperm membrane integrity, MP: progressive motility, VCL: curvilinear velocity, VSL: linear velocity, VAP: vectorial mean velocity, LIN: VSL/VCL, STR: VSL/VAP, WOB: VAP/VCL, ALH: amplitude of lateral head displacement, BCF: beat cross frequency.