

National Rural Development Programme 2014-2022

Measure 10.2 – Biodiversity

Project: TuBAvI-2 (2021-2024)

REPORT ON THE ACTIVITIES PERFORMED DURING THE SECOND YEAR

PA UniMOL

The present report describes the activities performed from May 1st, 2022, to April 30, 2023. The activities are described by action, according to the original programme.

Action 1 – Phenotypical characterisation of autochthonous breeds and species

Task 1.1 - Phenotypic characterization of a Siciliana breed conservation nucleus

Siciliana (SI) breeders were organized into 3 families and raised at the Di Iorio Donato's farm, which has been previously agreed. All animals were raised outdoors with access to night shelters, following standard guidelines for poultry farming.

Egg production was recorded daily. Figure 1.1 shows the oviposition curve. From the recorded data it is possible to notice a progressive increase in the percentage of egg-laying starting from the last week of March until reaching the peak (73%) in May. After the peak period, the oviposition rate was constant with values close to 60%, then a progressive reduction was observed until it stopped completely. Oviposition was then absent for about five consecutive weeks and resumed from the second half of December. Additionally, the average weight of the eggs was weekly recorded along with the percentage of egg deposition.

The data obtained show a progressive increase in the average weight of the egg, with minimum values of about 49 g and maximum values of 54,5 g; the average weight recorded during the period under consideration was 52,2 g (Figure 1.2).

Three incubations of SI breed eggs were carried out to verify the level of fertility and hatching; the recorded results are shown in Table 1.1. The fertility and hatchability values recorded by family are displayed in Table 1.2. The breeders of families (n. 29) and the donors used for cryobank purposes (n. 11) were subjected to morphometric measurements according to FAO guidelines (2012); in Table 1.3 are reported the results obtained.

Task 1.2 - Evaluation of the productive characteristics of Siciliana breed chickens at a social farm located in Puglia

As part of this task, 85 SI breed chicks born during 2022 were raised at a social farm located in Foggia – Apulia region (Cooperativa Emmaus). This farm promotes the preservation of animal biodiversity within a community of social utility and thanks to this project a small rural breeding of SI has been created. The social farm serves as an example of a rural production system that is suitable for local breeds. Individual live weight, feed conversion ratio and mortality were recorded monthly on male and female subjects. Table 1.4 shows the mean weights expressed as means \pm standard deviation starting from the first day up to 180 days. The results obtained are in line with the growth performance recorded in the group maintained during the previous project. The mortality rate recorded up to six months was 2.3%. At 180 day of age, 24 birds (12 birds/sex) were weighed and slaughtered. Carcass and the main cuts weight were recorded. Males had a higher slaughter weight, carcass weight and yield than females; while, a higher breast yield was found in females compared to males (+ 2.9%) (Table 1.5). As part of the activities for the turnover of the SI breed conservation nucleus, 44 individuals (30 females and 14 males) were phenotypically selected. 3-4 intact whole growing feathers were collected from each subject and sent to the Animal Molecular Genetics Laboratory of the University of Turin (consultant for UniMI) for genetic analyses.

Task 1.3 e 1.4 - Phenotypic characterization of Romagnolo (RO) and Ermellinato di Rovigo (ER) turkeys

During 2022, 10 RO and 6 ER turkey breeds were purchased from private farms located in Emilia-Romagna. To increase the number of animals available, from April 2023, incubation activities of fertile eggs of both breeds were started.

Task 1.5 - Phenotypic characterization of Bianca di Saluzzo chicken breed

The activities related to this task will be carried out during the fourth step of the project (year 2024).

Task 1.6 - Phenotypic characterization for the assessment of innate immunity

The objective of the task is to monitor immune resilience on a panel of 7 native breeds of chicken and 2 of turkey by measuring some markers of innate immunity: complement, IgY and lysozyme. During the period May 2022-April 2023 was monitored the innate immunity in 7 chicken breeds: Livorno (LI) and Ancona (AN) - (UniPG); Mugellese (MG) and Valdarnese bianca (VB) - (UniFI); Bionda piemontese (BP) and Bianca di Saluzzo (BS) - (UniTO); Siciliana (SI) - (UniMOL).

For each breed, blood samples were collected from 5 males and 5 females; after coagulation (2 hours at room temperature), the samples were centrifuged at 3000 rpm for 20 minutes, the serum obtained from each subject was aliquoted in 3 or 4 eppendorf and stored at -20 °C. The quantitative analysis of the lysozyme C (LYZ) was carried out by a competitive enzyme immunoassay technique (ELISA kit), while for the determination of the Complement Component 3 (C3) and IgY was used a quantitative sandwich ELISA kit. Subsequently, the optical density was measured by at 450 nm using the ELISA reader Tecan - Infinite® M Nano. In Table 1.6 are shown the results obtained; considering the GLM procedure used to determinate the effects of the considered variables (breed and sex) on the immunological markers evaluated, a significant effect of breed for all considered parameters emerged, while the sex effect was significant for LYZ and C3. Significantly higher values of LYZ (ng/mL) were obtained in males of the BP breed compared to males and females of the all other breeds considered, excepted for males of the BS and LI breeds. Higher values of C3 (µg/mL) were recorded for SI breed and males of BP and BS breeds ($p < 0.05$). Finally, regarding the IgY, the highest concentrations (mg/mL) were obtained for females of SI breed compared to females and males of MG, VB, BS and BP breeds, and male of LI breed ($p < 0.05$). Knowledge of the analysed parameters allows to have preliminary data on the immune status of the breeds considered.

Task 1.7 - Phenotypic characterization of fresh semen

The assessment of fresh semen quality was carried out for the following chicken breeds: SI, Livorno bianca (LB), Livorno nera (LN), MG e VB. The following qualitative-quantitative parameters were evaluated: sperm volume and concentration, cellular membrane integrity (by flow cytometry) sperm total motility, progressive motility, and kinetic parameters (by CASA system with SCA software).

In Tables 1.7 and 1.8 are reported the results obtained from the assessment of fresh semen quality of the native chicken breeds considered.

Aliquot of seminal plasma and spermatozoa of the SI breed were prepared and stored at -20°C to evaluate the protein and lipid profile, which will be performed in an integrated manner with the PA UniMI.

Action 8 - Collection of biological material and germplasm

Task 8.1 - Germplasm collection in the Semen Cryobank of Italian Poultry Breeds

During the reference period the Cryobank of the Semen of Italian Poultry Breeds was implemented with semen doses of the chicken breeds indicated in the *task 1.7*. The seminal material was frozen using the procedure described in the cryobank SOP for the *Gallus gallus* species. The number of donors and

semen doses stored for each breed is reported in the table 8.1. Semen quality was also evaluated after the freezing/thawing process and the preliminary data obtained are reported in the table 8.2.

Action 10 - Accompanying actions: information actions, dissemination and preparation of thematic technical reports and technical-scientific reports

The activities carried out include, the participation in the updating of the project website, the creation of the technical data sheet of the Sicilian breed, the participation in a trade fair event and the production of the following scientific reports:

- 1) Di Iorio M., Iaffaldano N., Rusco G., Antenucci E., Madeddu M., Zaniboni L., Marelli S.P., Cerolini S. Phenotypic characterization of semen production and quality in Italian chicken and turkey breeds. (accepted for 25th ASPA Congress).
- 2) Di Iorio M., Rusco G., Antenucci E., Lerza L., Zaniboni L., Madeddu M., Iaffaldano N. Italian semen cryobank of autochthonous chicken breeds: the case study of Siciliana breed. (accepted for 25th ASPA Congress).
- 3) Mannelli F., Franzoni A., Galigani I., Nistri V., Daghio M., Scicutella F., Marzoni M., Minieri S., Salvucci S., Cerolini S., Iaffaldano N., Schiavone A., Cassandro M., Castellini C., Buccioni A. Characterization of morphological, reproductive, and productive performances of Mugellese breed: an update of knowledge. *Poultry Science*, 102, 102259.
- 4) Tavaniello S., Iaffaldano N., Peng M., Grassi G., Di Iorio M., Antenucci E., Palazzo M., Maiorano G. (2023). Carcass and meat quality traits of Siciliana chickens, a native Italian breed. EAAP Regional Meeting 2023, 26-28 April, Nitra (Slovakia).

FIGURES AND TABLES

Figure 1.1 – Oviposition rate measured in Siciliana hens during March 2022 – April 2023

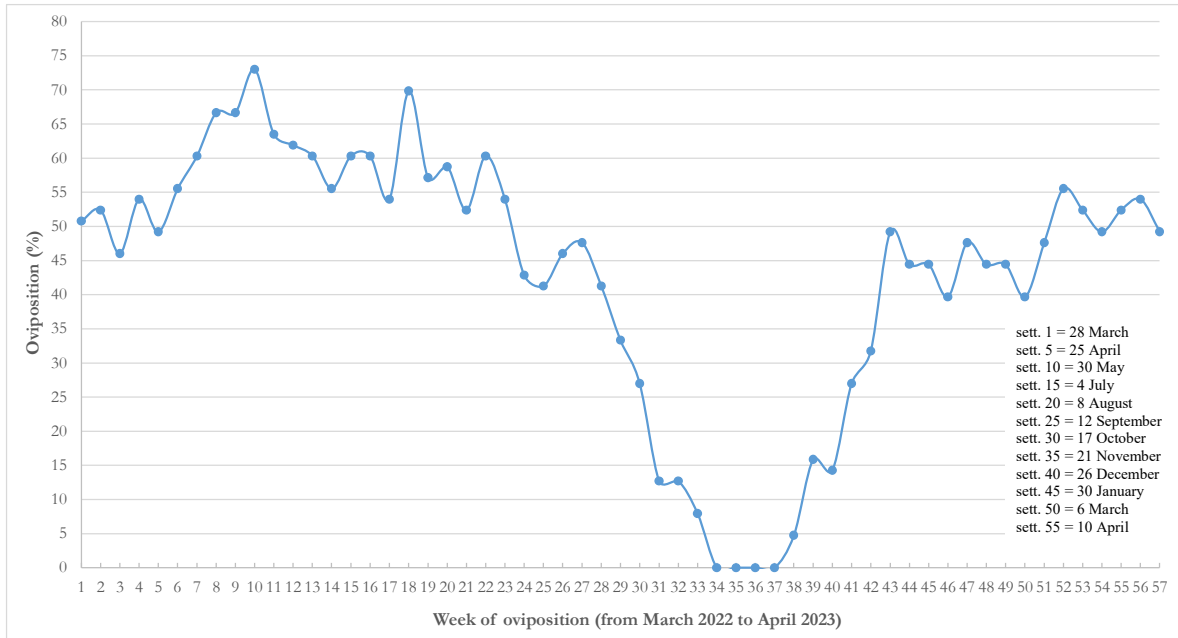


Figure 1.2 – Average egg weight recorded in Siciliana hens during March 2022 – April 2023

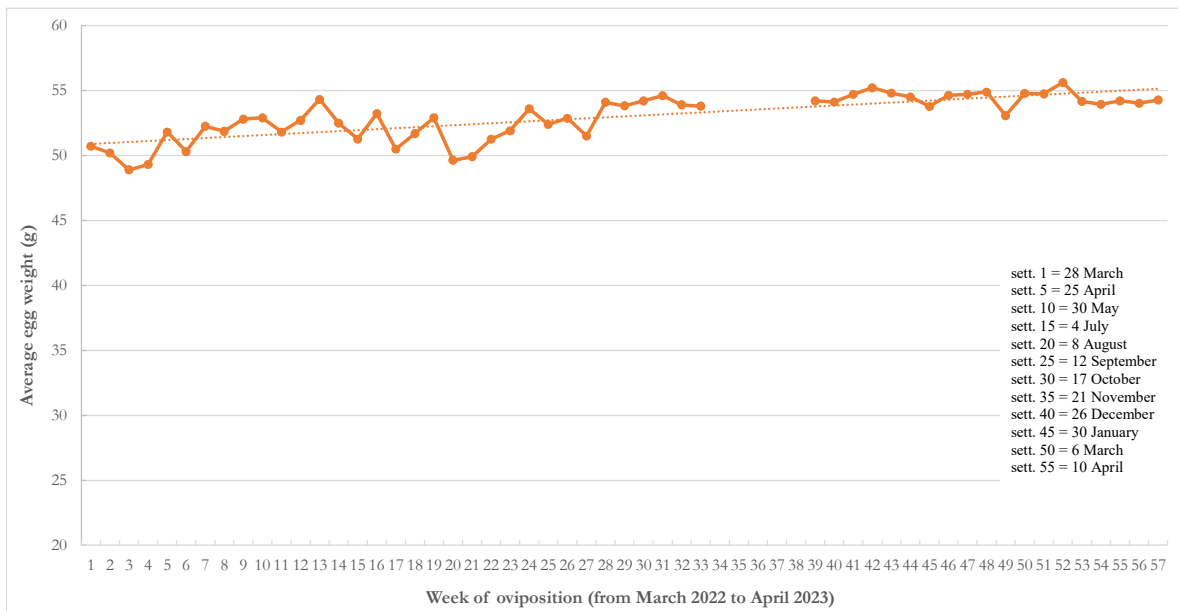


Table 1.1 – Artificial incubation parameters recorded in the Siciliana breed

Incubation	Incubated n°	Fertile n°	Died n°	Hatched n°	Fertile %	Died %	Hatched % on total	Hatched % on fertile
1	24	23	3	20	95.8	13.0	83.3	87.0
2	56	48	7	41	85.7	14.6	73.2	85.4
3	76	57	12	45	75.0	21.1	59.2	78.9
Total	156	128	22	106	82.1	17.2	67.9	82.8

Table 1.2 – Reproductive characteristics by family group of the Siciliana breed

Family	Hens (n.)	Fertility (%)	Hatchability (%)
A	9	87.0	95.7
B	9	82.1	67.4
C	8	76.1	85.7

Table 1.3 – Morphological characters measured in Siciliana breeders

Character	Males		Females	
	Mean ± SD	Min - max	Mean ± SD	Min - max
<i>Body weight (g)</i>	1,883.6 ± 365.8	1,452 - 2,702	1,428.2 ± 114.4	1,209 - 1,678
<i>Body length (cm)</i>	40.8 ± 1.5	37.6 - 43.8	35.7 ± 1.2	33.5 - 37.2
<i>Chest circumference (cm)</i>	32.1 ± 1.8	30.2 - 37.1	30.4 ± 1.2	28.5 - 34.2
<i>Shank length (cm)</i>	7.7 ± 0.4	6.9 - 8.2	6.6 ± 0.5	5.6 - 7.2
<i>Shank circumference (cm)</i>	4.3 ± 0.3	3.8 - 4.8	3.3 ± 0.2	2.9 - 3.5
<i>Wing span (cm)</i>	46.2 ± 1.3	42.2 - 47.5	38.6 ± 1.3	35.9 - 40.8

Table 1.4 – Growth performance of Siciliana chicken breed raised at social farm

Average weight (g)	Sex	
	Males Mean ± SD	Females Mean ± SD
<i>1st day</i>	35.8 ± 2.3	33.1 ± 2.2
<i>1st month</i>	275.1 ± 22.8	230.0 ± 27.3
<i>2nd month</i>	612.8 ± 55.4	498.1 ± 64.4
<i>3rd month</i>	951.7 ± 51.1	724.3 ± 73.7
<i>4th month</i>	1,255.0 ± 79.6	941.5 ± 117.1
<i>5th month</i>	1,450.4 ± 123.7	1,182.9 ± 145.3
<i>6th month</i>	1,483.3 ± 152.0	1,217.7 ± 132.4
Average FCR	9.3	8.8

Table 1.5 – Carcass yield and the main cuts recorded in Siciliana chickens

	Sex		<i>p</i> -Value
	Male Mean ± SD	Female Mean ± SD	
<i>Slaughter weight (g)</i>	1,511.8 ± 37.7 ^a	1,214.2 ± 35.2 ^b	<i>p</i> < 0.000
<i>Carcass weight (g)</i>	1,065.2 ± 34.6 ^a	779.8 ± 26.3 ^b	<i>p</i> < 0.000
<i>Carcass yield (%)</i>	70.3 ± 0.9 ^a	64.1 ± 0.5 ^b	<i>p</i> < 0.000
<i>Breast weight (g)</i>	128.8 ± 3.8 ^a	117.3 ± 6.1 ^a	<i>p</i> = 0.127
<i>Breast yield (%)</i>	12.2 ± 0.5 ^b	15.1 ± 0.7 ^a	<i>p</i> = 0.003
<i>Legs weight (g)</i>	281.2 ± 18.9 ^a	185.2 ± 9.9 ^b	<i>p</i> < 0.000
<i>Legs yield (%)</i>	26.2 ± 1.3 ^a	23.7 ± 0.9 ^a	<i>p</i> = 0.140

Table 1.6 – Markers of innate immunity measured in autochthonous chicken breeds

Breed	Sex	Markers		
		Lysozyme ng/mL	Complement µg/mL	IgY mg/mL
<i>Siciliana</i>	M	331.5 ± 70.3 ^{c-g}	272.1 ± 30.3 ^{ab}	14.3 ± 1.6 ^{a-d}
	F	264.9 ± 145.5 ^{f-h}	329.1 ± 46.0 ^a	19.0 ± 0.7 ^a
<i>Ancona</i>	M	625.0 ± 36.4 ^{b-e}	113.3 ± 31.5 ^{c-e}	14.5 ± 1.9 ^{a-d}
	F	256.4 ± 28.7 ^{f-h}	60.5 ± 5.2 ^e	15.9 ± 1.9 ^{abc}
<i>Livorno</i>	M	805.5 ± 185.5 ^{ab}	94.0 ± 3.2 ^{de}	9.8 ± 1.6 ^{de}
	F	143.0 ± 26.3 ^{gh}	46.0 ± 18.1 ^e	17.4 ± 1.9 ^{ab}
<i>Mugellese</i>	M	506.4 ± 88.2 ^{c-e}	192.4 ± 31.1 ^{bc}	13.1 ± 2.2 ^{b-d}
	F	30.7 ± 4.6 ^b	188.3 ± 38.3 ^{bc}	9.4 ± 1.0 ^{de}
<i>Valdarnese bianca</i>	M	426.5 ± 69.1 ^{d-f}	157.0 ± 47.2 ^{cd}	11.1 ± 1.9 ^{c-e}
	F	134.6 ± 69.4 ^{gh}	113.8 ± 8.5 ^{c-e}	10.8 ± 1.3 ^{c-e}
<i>Bianca di Saluzzo</i>	M	766.5 ± 86.0 ^{ab}	287.4 ± 21.3 ^a	7.3 ± 1.3 ^e
	F	513.8 ± 77.2 ^{c-e}	170.1 ± 13.5 ^{cd}	9.2 ± 1.8 ^{de}
<i>Bionda piemontese</i>	M	920.8 ± 55.2 ^a	315.2 ± 42.3 ^a	9.1 ± 1.9 ^{de}
	F	653.2 ± 118.2 ^{b-d}	189.8 ± 20.6 ^{bc}	10.4 ± 1.9 ^{c-e}
<i>Breed effect</i>		$p = 0.000$	$p = 0.000$	$p = 0.000$
<i>Sex effect</i>		$p = 0.000$	$p = 0.004$	$p = 0.051$

^{a-h}Values within a column reporting different superscript letter differ significantly at $p < 0.05$; values expressed (mean ± SEM)

Table 1.7 – Quantitative characteristics and sperm membrane integrity of native Italian rooster semen

Breed	PA	Donors (n°)	Volume (µL)	Concentration spz × 10 ⁹ /mL	SMI (%)
<i>Siciliana</i>	UniMOL	11	153.8 ± 6.2 ^{ab}	3.35 ± 0.10 ^a	91.9 ± 0.4 ^a
<i>Siciliana</i>	UniPI	11	146.7 ± 8.4 ^{ab}	3.39 ± 0.11 ^a	90.4 ± 0.9 ^{ab}
<i>Livorno bianca</i>		8	195.5 ± 26.3 ^a	3.00 ± 0.24 ^{ab}	89.0 ± 2.1 ^{ab}
<i>Livorno nera</i>		7	187.1 ± 20.8 ^a	3.03 ± 0.13 ^{ab}	90.8 ± 1.4 ^{ab}
<i>Mugellese</i>	UniFI	5	97.8 ± 5.2 ^b	2.09 ± 0.17 ^b	86.1 ± 2.6 ^b
<i>Valdarnese bianca</i>		2	162.5 ± 12.5 ^{ab}	2.63 ± 0.19 ^{ab}	90.4 ± 1.9 ^{ab}

^{a-b}Different superscript letters within the same column indicate a significant difference ($p < 0.05$); values expressed (mean ± SEM)

SMI: sperm membrane integrity

Table 1.8 – Sperm motility and related kinetic parameters outcomes in native Italian rooster

Breed*	PA	TM (%)	PM (%)	VCL (µm/s)	VSL (µm/s)	VAP (µm/s)	LIN (%)	STR (%)
SI	UniMOL	91.2 ± 0.5 ^a	26.7 ± 0.9 ^{ab}	66.8 ± 1.0 ^a	31.3 ± 0.7 ^a	44.2 ± 1.0 ^a	43.4 ± 0.9 ^a	62.3 ± 0.7 ^a
SI	UniPI	85.9 ± 1.0 ^{ab}	32.0 ± 1.3 ^a	67.8 ± 2.3 ^a	28.1 ± 1.3 ^a	43.4 ± 1.8 ^{ab}	37.0 ± 1.2 ^{ab}	56.4 ± 1.3 ^a
LB		83.8 ± 2.2 ^{bc}	28.0 ± 2.8 ^{ab}	65.6 ± 3.6 ^a	26.8 ± 2.4 ^a	40.1 ± 2.7 ^{ab}	35.5 ± 2.5 ^{ab}	56.5 ± 2.6 ^a
LN		83.9 ± 1.9 ^{bc}	26.7 ± 2.3 ^{ab}	66.7 ± 3.5 ^a	29.7 ± 2.5 ^a	40.7 ± 2.5 ^{ab}	41.1 ± 3.3 ^a	60.8 ± 3.3 ^a
MG	UniFI	78.1 ± 3.3 ^c	23.7 ± 4.2 ^b	69.6 ± 5.5 ^a	27.9 ± 2.6 ^a	35.6 ± 4.0 ^b	30.3 ± 2.3 ^b	48.6 ± 2.6 ^b
VB		81.6 ± 6.2 ^{bc}	20.3 ± 4.5 ^b	47.3 ± 4.9 ^b	19.8 ± 1.9 ^b	34.1 ± 5.0 ^b	39.5 ± 2.0 ^a	60.0 ± 1.6 ^a

^{a-c}Different superscript letters within the same column indicate a significant difference ($p < 0.05$); values expressed (mean ± SEM)

*SI: Siciliana, LB: Livorno bianca; LN: Livorno nera; MG: Mugellese; VB: Valdarnese bianca

TM: total motility; PM: progressive motility; VCL: curvilinear velocity; VSL: straight-line velocity; VAP: average path velocity; LIN: linearity; STR: straightness

Table 8.1 – Semen doses stored in the cryobank during period May 2022 – April 2023

Species	Breed	PA	N° of donors	N° of doses
<i>Gallus gallus</i>	<i>Siciliana</i>	UniMOL	11	363
	<i>Mugellese</i>	UniFI	4	6
	<i>Valdarnese bianca</i>		2	8
	<i>Siciliana</i>	UniPI	10	47
	<i>Livorno bianca</i>		7	21
	<i>Livorno nera</i>		6	17
TOTAL			40	462

Table 8.2 – Quality of frozen/thawed semen in Italian chicken breeds

Breed*	PA	SMI (%)	TM (%)	PM (%)	VCL (µm/s)	VSL (µm/s)	VAP (µm/s)
SI	UniMOL	37.2 ± 4.3	30.0 ± 5.9	2.7 ± 0.5	33.4 ± 2.4	12.7 ± 3.3	21.7 ± 3.6
SI	UniPI	40.6 ± 5.9	33.6 ± 4.9	3.6 ± 0.6	32.0 ± 6.7	13.5 ± 2.1	20.5 ± 3.7
LB		42.2 ± 5.8	32.9 ± 4.2	3.2 ± 0.3	30.9 ± 3.8	12.2 ± 2.7	19.1 ± 2.9
LN		33.8 ± 2.1	25.8 ± 2.5	2.3 ± 0.2	26.3 ± 3.0	9.0 ± 1.7	15.2 ± 1.7
MG	UniFI	27.8 ± 2.0	20.6 ± 1.9	1.7 ± 0.2	30.3 ± 2.3	10.5 ± 0.8	17.3 ± 0.6

*SI: Siciliana, LB: Livorno bianca; LN: Livorno nera; MG: Mugellese; values expressed (mean ± SD)

SMI: sperm membrane integrity; TM: total motility; PM: progressive motility; VCL: curvilinear velocity; VSL: straight-line velocity; VAP: average path velocity