National Rural Development Programme 2014-2022

Measure 10.2 – Biodiversity

Project: TuBAvI-2 (2021-2024)

REPORT ON THE ACTIVITIES PERFORMED DURING THE THIRD YEAR

PA UniMOL

The present report describes the activities performed from May 1st, 2023, to March 31st, 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 characterization of a Siciliana breed conservation nucleus

As part of this task, 28 Siciliana (SI) breeders were raised at the Molise farm, which had been previously agreed. The birds were organized into 2 families and raised outdoors with access to night shelters, following standard poultry breeding guidelines.
Two incubations of SI breed were carried out to verify the level of fertility and hatchability; the results recorded are shown in table 1.1.

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

Within the activities for the turnover of the SI breed conservation nucleus planned for 2023, 3 families were formed based on the genetic analysis performed on a group of 44 individuals (30 females and 14 males). The formation of the families took place considering the genetic distances between the females, the males were identified based on molecular kinships. Each family consisted of 1-2 males and a group of 8-12 females. All subjects were raised at a social farm located in Foggia – Apulia region (Cooperativa Emmaus), which is committed to the protection of animal biodiversity within a socially beneficial community. The project has created a small rural breeding farm of SI subjects. The social farm represents a rural production model that is perfectly suited to local breeds, thus contributing to their conservation and promotion.

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

During 2023, 20 RO-breed and 15 ER-breed turkeys were reared at the agreement farm located in the municipality of San Giovanni Rotondo (FG). The live weight of male and female RO-breed individuals was recorded monthly. The mean weights, expressed as mean ± standard deviation from the first day of life to 180 days, are presented in Table 1.2. Mortality recorded up to 6 months was 40%. Oviposition in the two breeds started in January and peaked in the second half of March in the RO breed and at the end of March in the ER breed. A gradual decrease in oviposition rate was observed from the third week of June. The average number of eggs produced by each turkey hen was 65 for the ER breed and 70 for the RO breed. Adult individuals of both breeds (n = 6, RO; n = 5, ER) were subjected to morphometric measurements according to the FAO 2012 guidelines. The mean values of the quantitative traits recorded are presented in Table 1.3.

Two incubations of eggs were performed for each breed to assess the level of fertility and hatchability, and the recorded results are presented in Table 1.4.

Task 1.5 - Phenotypic characterization of Collo nudo italiana chicken breed

In the project proposal submitted, it was planned to maintain the Bianca di Saluzzo breed. Later, in agreement with the other PAs, this breed was replaced by the Collo Nudo italiana breed, as there was no conservation nucleus of this breed yet. In October 2023, the request for replacement of the breed was approved by the Ministry, and activities were started to breed a group of Collo nudo italiana chickens. All individuals (n = 40) were raised outdoor at the Apulian farm. Activities are underway to evaluate oviposition and reproductive performance of the following zootechnical parameters: daily oviposition, egg weight, fertility, and hatching. Morphometric measurements were carried out on the available adults and the data obtained are presented in Table 1.5.
A preliminary artificial incubation test was carried out on 26 eggs in 2024, with a recorded fertility rate of 84.6% and a hatching rate of 76.9%.

Task 1.6 - Phenotypic characterisation for the assessment of innate immunity

In the present task, the immune resilience of two turkey breeds (RO and ER) was monitored, considering the following markers: complement component 3 (C3), IgY, and lysozyme C (LYZ). Blood samples were collected from 5 males and 5 females of each breed. After coagulation (2 hours at room temperature), the samples were centrifuged at 3000 rpm for 20 minutes, and the resulting serum was stored at -20°C. The quantitative analysis of lysozyme C (LYZ) was conducted using a competitive enzyme immunoassay technique (ELISA kit), while a quantitative sandwich ELISA kit was used for the determination of complement component 3 (C3) and IgY. Optical density was measured at 450 nm using the Tecan-Infinite® M Nano model plate reader.
Table 1.6 presents the results obtained. The GLM procedure was employed to determine the effects of the considered variables (breed, sex, and their interactions) on the evaluated immune markers. A significant effect of breed and sex was observed only for LYZ. Significantly higher values of LYZ (ng/mL) were found in ER males, whereas in the RO breed, the highest values of this marker were observed in males compared to females ($p<0.05$). Although higher levels of C3 (µg/mL) were observed in males of both breeds compared to females, no significant differences were noted. IgY concentrations did not show significant differences between breeds and sexes, but the RO breed exhibited higher concentrations (mg/mL) than the ER breed. Understanding the parameters analyzed provides a preliminary insight into the immune status of these breeds, offering a useful framework for assessing their health and responsiveness to pathogens.

Task 1.7 - Phenotypic characterisation of fresh semen

The assessment of fresh semen quality was carried out for the following turkey breeds: RO and ER. The following quanti-qualitative parameters were evaluated: sperm volume and concentration, cellular membrane integrity (by flow cytometry) total sperm motility, progressive motility, and kinetic parameters (by CASA system with SCA software).
The results obtained from the assessment of fresh semen quality of the native turkey breeds considered are reported in Table 1.7.
Lipid profile analysis was performed on the spermatozoa of SI breed roosters, using the Folch's method to extract total lipids with a commercial kit (Fatty Acid Extraction Kit, Low Standard - Sigma Aldrich). The total lipids were separated into the neutral lipid classes by thin layer chromatography.

Total phospholipid, triglyceride, free fatty acid and cholesterol ester classes were subjected to reflux trans-methylation followed by determination of fatty acid composition by gas chromatography. FAMEs were separated on a capillary column (Zebron ZB-FAME 30 m × 0.25 mm × 20 μm). The fatty acids of the samples were compared with the peak retention times of the 37 FAME standard (Supelco). Analysis and organisation of the data are in progress.

Action 8 - Collection of biological material and germplasm

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

During the reference period the Cryobank of the Semen of Italian Poultry Breeds was expanded with semen doses of the turkey breeds indicated in the *task 1.7*. The seminal material was frozen using the procedure described in the cryobank SOP for the *Meleagris gallopavo* species. The number of donors and semen doses stored for each breed is reported in the table 8.1.

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

The activities carried out within this action include:

- realization of breed technical forms for the Romagnolo and Ermellinato di Rovigo turkey breeds, and Collo nudo italiana chicken breed (qualitative characteristics);
- contribution to the update of the project website;
- production of scientific articles published in open-access journals, including a collective project article, and presentations at national and international congresses, as documented on the website pollitaliani.it/publications.

FIGURES AND TABLES

Table 1.1 – Artificial incubation parameters recorded in the Siciliana breed during 2023

Incubation	Incubated n.	Fertile n.	Died n.	Hatched n.	Fertile %	Died %	Hatched % on total	Hatched % on fertile
1	32	28	9	19	87.5	32.1	59.4	67.9
2	46	37	6	31	80.4	16.2	67.4	83.8
Total	*78*	*65*	*15*	*50*	*83.3*	*23.1*	*64.1*	*76.9*

Table 1.2 – Growth performance of Romagnolo turkey's breed

	Sex	
Average weight (g)	**Males**	**Females**
	Mean ± SD	Mean ± SD
1st day	50.33 ± 4.03	50.00 ± 4.00
1st month	324.67 ± 47.70	382.00 ± 52.00
2nd month	952.17 ± 129.70	610.00 ± 117.18
3rd month	1,706.67 ± 232.35	1,188.33 ± 207.85
4th month	2,713.60 ± 425.54	1,473.67 ± 206.34
5th month	3,760.00 ± 926.80	2,136.67 ± 108.52
6th month	4,684.80 ± 1,223.59	2,488.67 ± 212.35

Table 1.3 – Morphological characters measured in Romagnolo (RO) and Ermellinato di Rovigo (ER) turkey breeders

		Males		Females	
Breed	*Character*	Mean ± SD	min - max	Mean ± SD	min - max
RO	*Body weight (g)*	6,430 ± 1,280	5,528 - 7,895	3,560 ± 75	3,490 - 3,640
	Body lenght (cm)	60.80 ± 4.10	56.80 - 65.00	52.90 ± 4.25	50.30 - 57.80
	Chest circumference (cm)	57.60 ± 1.05	56.50 - 58.60	42.73 ± 3.28	40.50 - 46.50
	Shank lenght (cm)	13.60 ± 0.35	13.20 - 13.80	11.80 ± 0.87	11.30 - 12.80
	Shank circumference (cm)	6.60 ± 0.10	6.50 - 6.70	5.53 ± 0.15	5.40 - 5.70
	Wing span (cm)	57.03 ± 1.62	55.30 - 58.50	50.57 ± 4.95	45.70 -55.60
ER	*Body weight (g)*	5,661 ± 915	4,605 - 6,205	3,094 ± 627	2,650 - 3,538
	Body lenght (cm)	58.40 ± 1.21	59.80 - 57.7	51.50 ± 0.14	51.40 - 51.60
	Chest circumference (cm)	57.23 ± 2.48	55.20 - 60.00	43.65 ± 7.00	38.70 - 48.60
	Shank lenght (cm)	13.07 ± 0.59	12.40 - 13.50	11.35 ± 0.64	10.90 - 11.80
	Shank circumference (cm)	6.60 ± 0.20	6.40 - 6.80	5.55 ± 0.07	5.50 – 5.60
	Wing span (cm)	55.50 ± 1.00	54.50 - 56.50	47.75 ± 6.15	43.40 – 52.10

Table 1.4 – Artificial incubation parameters recorded in Romagnolo and Ermellinato di Rovigo turkey breeds

Breed		Incubated n.	Fertile n.	Died n.	Hatched n.	Fertile %	Died %	Hatched % on total	Hatched % on fertile
RO		24	8	1	7	33.3	12.5	29.2	87.5
		23	11	3	8	47.8	27.3	34.8	72.7
	Totale	*47*	*19*	*4*	*15*	*40.4*	*21.1*	*31.9*	*78.9*
ER		37	6	3	3	16.2	50.0	8.1	50.0
		20	10	3	7	50.0	30.0	35.0	70.0
	Totale	*57*	*16*	*6*	*10*	*28.1*	*37.5*	*17.5*	*62.5*

Table 1.5 – Results of morphometric measurements in Collo nudo italiana breed

	Males		Females	
Character	Media ± DS	min - max	Media ± DS	min - max
Body weight (g)	2,370.00 ± 178.19	2,244 – 2,496	1,793.13 ± 236.70	1,428 – 2,176
Body lenght (cm)	38.45± 0.08	38.40 - 38.50	37.87 ± 1.73	35.10 - 40.80
Chest circumference (cm)	36.05 ± 0.49	35.70 - 36.40	34.37 ± 1.79	32.80 - 37.80
Shank lenght (cm)	9.45 ± 1.20	8.60 - 10.30	9.42 ± 0.46	8.80 - 10.30
Shank circumference (cm)	6.15 ± 0.63	5.70 - 6.60	5.27 ± 0.35	4.60 - 5.70
Wing span (cm)	37.60 ± 5.49	33.40 - 41.80	36.87 ± 1.38	34.50 - 38.50

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

		Markers		
Breed	Sex	Lysozyme ng/mL	Complement µg/mL	IgY mg/mL
RO	M	408.2 ± 46.9[b]	430.2 ± 82.3[a]	4.4 ± 1.2[a]
	F	188.4 ± 19.6[c]	295.3 ± 40.2[a]	4.1 ± 1.7[a]
ER	M	694.4 ± 82.1[a]	400.9 ± 29.4[a]	2.4 ± 0.5[a]
	F	391.2 ± 76.8[b]	269.3 ± 62.7[a]	2.8 ± 0.4[a]
Breed effect		$p = 0.003$	$p = 0.737$	$p = 0.156$
Sex effect		$p = 0.002$	$p = 0.053$	$p = 0.920$
*Breed * sex effect*		$p = 0.912$	$p = 0.893$	$p = 0.751$

[a-c]Values within a column reporting different superscript letter differ significantly at $p < 0.05$; values expressed (mean ± SEM)

**Tabella 1.7 – Semen quality outcomes in Romagnolo
and Ermellinato di Rovigo turkey breeds**

Parameter*	Breed	
	RO	**ER**
Volume (mL)	0.210 ± 0.05	0.245 ± 0.05
Concentration (spz × 10⁹/mL)	5.75 ± 0.61[b]	7.03 ± 0.62[a]
SMI (%)	87.27 ± 3.58[a]	83.27 ± 2.78[b]
Total motility (%)	83.32 ± 3.37	81.51 ± 3.12
Progressive motility (%)	25.71 ± 6.56	24.08 ± 3.10
VCL (μm/s)	48.31 ± 3.97	50.49 ± 5.36
VSL (μm/s)	35.84 ± 6.65	36.79 ± 6.96
VAP (μm/s)	24.03 ± 4.05	24.50 ± 6.06
LIN (%)	49.78 ± 7.91	47.95 ± 7.68
STR (%)	67.56 ± 7.37	66.52 ± 11.34

* SMI: sperm membrane integrity; VCL: curvilinear velocity; VSL: straight-line velocity; VAP: average path velocity; LIN: linearity; STR: straightness

**Tabella 8.1 – Semen doses stored in the cryobank
during period May 2023 – March 2024**

Species	Breed	N. of donors	N. of doses
Meleagris gallopavo	*Romagnolo*	5	28
	Ermellinato di Rovigo	3	26
TOTAL		8	54