

*National Rural Development Program 2014-2022*

*Measure 10.2 – Biodiversity*

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

**REPORT ON THE ACTIVITIES UNDERTAKEN DURING THE THIRD AND FOURTH  
YEAR**

*PA UniPI*

The present report describes the activities carried out from May 1<sup>st</sup>, 2023, to December 31<sup>st</sup>, 2024. The activities are described by Action, according to the original programme.

**Action 1 – Phenotypic characterization of autochthonous breeds and species**

*Task 1.1 - Phenotypic characterization of Livorno, Siciliana, Bianca di Saluzzo, Bionda Piemontese, and Millefiori Piemontese chicken breeds.*

During the period under consideration, Livorno (L) and Siciliana (S) chicken breeds, hatched in April 2021 and raised at the Poultry Breeding facility of the Department of Veterinary Sciences at the University of Pisa, completed their second laying cycle by October-November 2023. All animals were raised on sandy soil in outdoor enclosures with partial roofing, under natural light and temperature conditions.

Monitoring of daily egg production continued until the end of the laying cycle (see figure 1.1.1); the weight of eggs evaluated in the five central months of the reproductive season is shown in figure 1.1.2 and the shape index of these eggs was equal to 74.1 and 68.9 for the Livorno and Siciliana breeds respectively, highlighting also in the second laying a decidedly different shape of the egg in these two breeds: standard oval the first and sharp oval the second. The assessment of food consumption by second-laying hens was completed by June 2023, and the egg FCE was calculated (see table 1.1.1). Replacement subjects born in April 2023 were monitored for growth, mortality, as outlined in the project (figures 1.1.3 and 1.1.4).

The data concerning the Piedmont breeds Bianca di Saluzzo (BS), Bionda Piemontese (BP), and Millefiori di Lonigo (ML) preserved at the Poultry Centre for the Conservation of Local Genetic Resources (Carmagnola, TO - supervised by Prof. Achille Schiavone) have been collected and are made available as part of the technical consultancy contract between UniPI and UniTO starting from 05/03/2022 (refer to the technical report by the UniTO consultant).

*Task 1.2 - Phenotypic characterization of Ancona (A) breed chickens*

Monitoring of daily egg production during the second egg-laying cycle of Ancona hens continued until the end of October 2023 (see figure 1.2.1), while egg weight detection concluded in July (see figure 1.2.2).

### *Task 1.3 - Characterization of laying hens' adaptation capacity to seasonal environmental stimuli*

In 2023, the qualitative characterization of the yolk and albumen of the eggs produced during the second cycle of laying of the L and S breeds was completed in June. The egg components were evaluated (see table 1.3.1), and chemical analyses of the yolk and egg white were conducted (see table 1.3.2).

## **Action 7 - Evaluation and identification of genetic resistance traits in livestock animals against diseases**

### *Task 7.1 - Evaluation of resilience for growth under environmental stimuli*

In September 2023, the growth test for the Ermellinata di Rovigo (ER), Ancona (ANC), Pepoi (PP), and Robusta Lionata (RL) breeds concluded. This test began with hatching at the end of February and was conducted at the UniPI and UniFI facilities. Figure 7.1.1 shows the body weights and growth rates of the Pisa animals during the first 150 days of life; consumption and ICA data are currently being processed. Total mortality rates (1-150 days of age) were 68.4%, 38.1%, 27.3%, and 25.0% for RL, PP, ER, and ANC, respectively, primarily due to colibacillosis, which manifested itself on the 14<sup>th</sup> day of the animals' lives. Figure 7.1.2 displays the average body weights of 120-day-old chickens raised at the two sites.

### *Task 7.2 - Evaluation of resistance/resilience to bacterial and parasitic infections/infestations and resilience of intestinal microbiota*

Table 7.2.1 shows final results of the qualitative and quantitative parasitological analyses to detect coccidia (*Eimeria* spp.), nematodes (roundworms, capillaries, *Heterakis* spp.), protozoa (*Giardia* spp. and *Cryptosporidium* spp.) and *Histomonas meleagridis* in stool samples collected from the growing chickens monitored in task 7.1.

At the age of 120 days, four individual cloacal swabs were taken from the same chickens at both sites to identify pathogens such as *Salmonella* spp., *Clostridium perfringens*, *Campylobacter jejuni*, and commensal bacteria to obtain isolates for evaluating the antibiotic resistance profile and the selection of strains with favourable probiotic characteristics. All samples from the UniPI site tested negative for pathogens. Table 7.2.2 shows the percentage of animals, by breed and location, in the antibiotic susceptibility classes found in the Lactobacilli isolated from them. Isolates PP57E17M and AN03E15M, from Pepoi and Ancona breed respectively, were susceptible to all the antibiotics tested and identified to belong to *Ligilactobacillus salivarius* species. These isolates were the only ones to show anti-Salmonella activity, in particular good inhibitory activity especially against *S. Typhimurium* and *S. Newport*.

At the end of the monitoring period, individual cloacal faecal sampling was conducted to characterize the intestinal microbiota of the chickens, performed by PA UniFI as part of its research program.

## **Action 8 - Collection of Biological Material and Germplasm**

At the end of 2023, the procurement of biological material from Livorno breed chickens from farms in the Tuscan territory was completed.

To conduct task 7.2, five collections of faecal samples were carried out from the four breeds (ER, ANC, PP, and RL) under study at both sites, totalling 16 sampling pools. Cloacal swabs were performed on subjects bred at both the Pisa and Florence sites, totalling 520 samples (four swabs per animal).

## **Action 10 – Information and Dissemination**

### Information Events

- 29 September 2023, Pisa: BRIGHT Night 2023 - The European Night of Researchers.
- 07-08 October 2023, Lucca: Urban Nature 2023 – Event: “Nature takes care”
- 24 November 2023, Pisa: Game of Research (UniPi first edition) – Day to promote research in veterinary sciences, dedicated to non-structured staff. Oral presentation “Italian native poultry breeds: zootechnical and parasitological investigations” (PhD student Marta Raffaelli).

### Technical-informative publications for the Pollitaliani website

- Contributed to the update of the phenotypic characterization data sheet relating to the Siciliana breed, the Ancona breed and the White Livorno breed.
- Contributed to the production of the phenotypic characterization data sheet relating to the Black Livorno breed.

### Scientific publications

- Castillo A., Salvucci S., Mancini S., Serra A., Cappucci A., Schiavone A., Soglia D., Zaniboni L., Buccioni A., Mannelli F., Castellini C., Cartoni Mancinelli A., Cassandro M., Iaffaldano N., Cecchi F., Russo C., Cerolini S., Marzoni Fecia di Cossato M. (2024) Physical and chemical characteristics of eggs from eight Italian chicken breeds. *Italian Journal of Animal Science*, 23(1), 342–347
- Cagnoli G., Di Paolo A., Bertelloni F., Salvucci S., Buccioni A., Marzoni Fecia di Cossato M., Ebani V.V. (2024) Occurrence of antimicrobial-resistant *Enterococcus* spp. in healthy chickens never exposed to antimicrobial agents in Central Italy. In Special Issue “Detection of Bacteria and Antibiotics Surveillance in Livestock”, *Antibiotics*, 13, 417.- Physical and chemical characteristics of eggs from eight Italian chicken breeds. *Italian Journal of Animal Science* (DOI: 10.1080/1828051X.2024.2314149)
- o Di Iorio M., Marelli S.P., Antenucci E., Madeddu M., Zaniboni L., Belcredito S., Rusco G., Schiavone A., Soglia D., Penasa M., Castellini C., Buccioni A., Marzoni M., Maiuro L., Iaffaldano N., Cerolini S. (2024) A comparative study on semen quality and cryopreservation ability in Italian native chicken breeds. *Italian Journal of Animal Science*, 23 (1), 1704-1718
- o Perini F., Cendron F., Castellini C., Iaffaldano N., Marzoni M., Buccioni A., Soglia D., Schiavone A., Cerolini S., Lasagna E., Cassandro M., Penasa M. (2025) Genome-wide analysis of Collo Nudo Italiana and Millefiori Piemontese local chicken breeds: genetic variability and structure analysis in the context of Italian chicken biodiversity. *Italian Journal of Animal Science*, 24 (1), 137-148
- o Cappone E.E., Zambotto V., Mota-Gutierrez J., Soglia D., Daniele G.M., Cianciabella M., Pieroni A., Soukand R., Penasa M., Buccioni A., Marzoni M., Iaffaldano N., Castellini C., Cerolini S., Forte C., Schiavone A. (2025) Native Italian poultry products: the factors influencing consumer perceptions. *Italian Journal of Animal Science* 24 (1), 347-360

#### Abstracts in national conferences

Resci I., Turchi B., Raffaelli M., Salvucci S., Olivieri F., Marzoni M. (2024) Preliminary evaluation of lactobacilli isolated from autochthonous Italian chicken breeds for their use as probiotics: susceptibility to antimicrobials and pH resistance. Proceedings 77° SISVET Conference, Parma 12-14 Giugno 2024, abstract pag 130.

Raffaelli M., Perrucci S. (2024) Eimeria spp. identification in Italian native chicken breeds. Proceedings 33° National Congress of the Italian Society of Parasitology, Padova 18-21 Giugno 2024, abstract pag 267.

#### In course of publication

Raffaelli M., Salvucci S., Jaramillo-Ortiz José, Cagnoli G., Olivieri F., Schiavone A., Buccioni A., Cecchi F., Saia S., Castillo A., Ebani V.V., Perrucci S., Marzoni Fecia di Cossato M. (2025) Poultry resilience to open air environment: evaluation of growth performance and egg production at onset of laying in four Italian native chicken breeds naturally infected by coccidia (*Eimeria* spp.). *Animals* (submitted ID 3462554)

Resci I., Raffaelli M., Pedonese F., Forzan M., Fratini F., Salvucci S., Torracca B., Marzoni M., Turchi B. (2025) Lactobacilli isolated from chickens of Italian native breeds: antimicrobial susceptibility profile, probiotic potential and anti-Salmonella activity. *Food Microbiology* (submitted ID n. FM-D-25-00323)

## FIGURES AND TABLES

**Figura 1.1.1 – Egg-laying curve of white Livorno, black Livorno and Siciliana breed hens at second-laying, during the reproductive period from January to December 2023**



Figura 1.1.2 – Average monthly egg weight from white Livorno and Siciliana breed hens groups at second-laying, recorded from February to June 2023



Table 1.1.1 – Feed consumption and FCE for eggs in the different production periods of white Livorno (LB), black Livorno (LN), and Siciliana (SIC) breed hens at second-laying

BREED	average bird/day feed consumption (g)			
	March	April	May	June
LB	128.5 ± 1.4	148.4 ± 4.8	75.1 ± 5.3	93.4 ± 3.0
LN	182.7 ± 33.0	173.0 ± 0.7	125.9 ± 6.2	152.9 ± 13.0
SIC	121.8 ± 36.5	114.3 ± 17.3	99.8 ± 13.8	100.1 ± 19.9
	average FCE (g)			
	March	April	May	June
LB	3.4 ± 0.2	4.5 ± 0.1	3.5 ± 1.7	3.0 ± 0.4
LN	4.3 ± *	3.7 ± 1.0	3.3 ± 1.2	4.2 ± *
SIC	4.8 ± *	5.5 ± 2.9	7.9 ± 3.1	5.8 ± *

\* referring only to the group in production

Figure 1.1.3 - Live weight trends of male White Livorno (LB), Black Livorno (LN) and Siciliana (Sic)

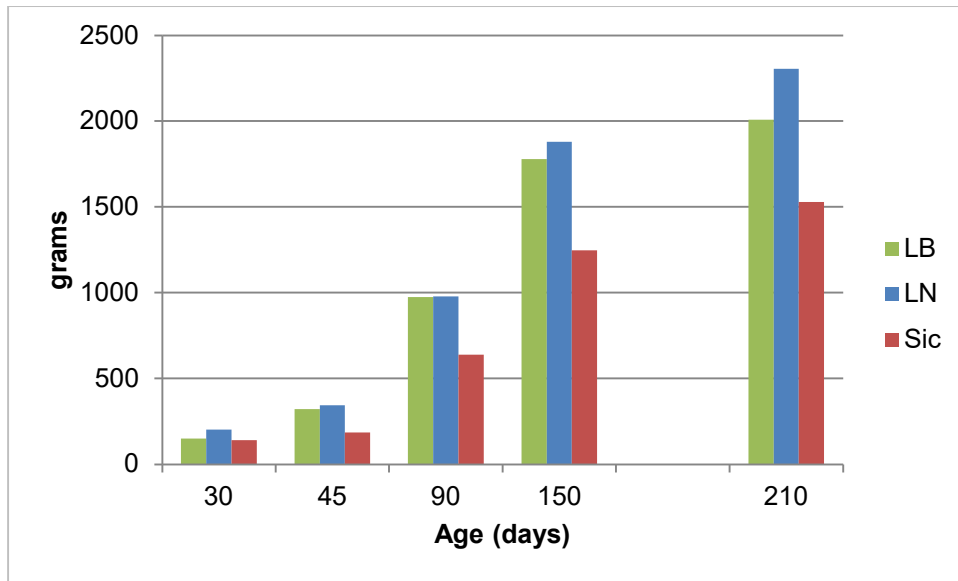
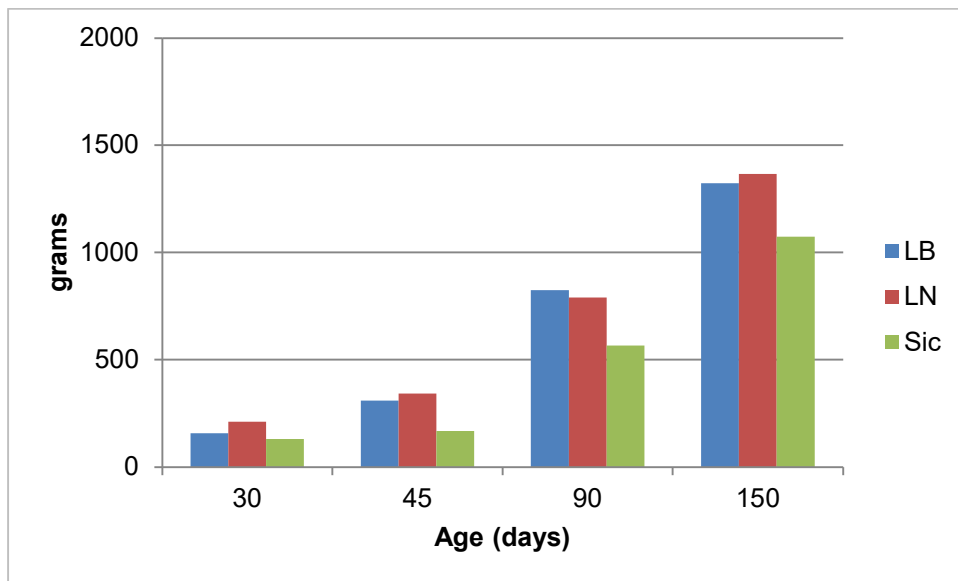
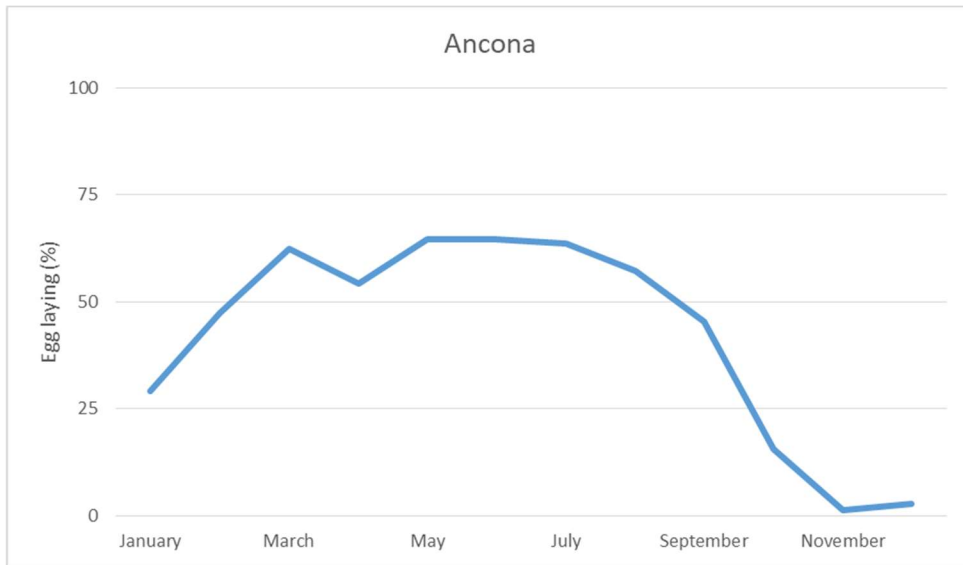


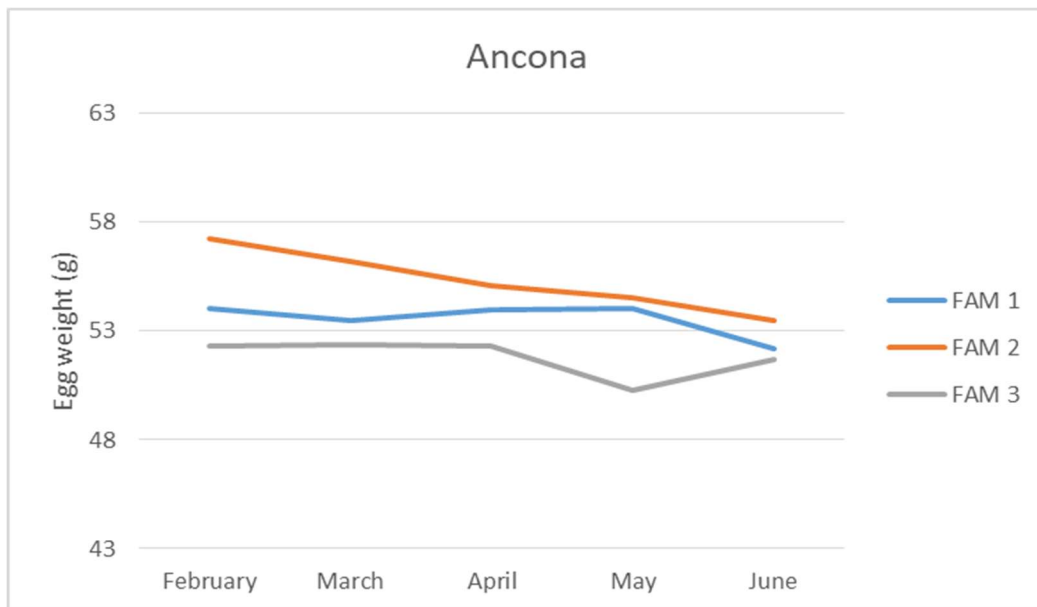
Figure 1.1.4 - Live weight trends of female White Livorno (LB), Black Livorno (LN) and Siciliana (Sic)



**Figure 1.2.1 - Egg-laying curve of Ancona breed hens at second-laying, recorded in the reproductive period from January to December 2023**



**Figure 1.2.2 – Average monthly egg weight from three Ancona breed hens groups in the second-laying, recorded from February to June 2023**



**Table 1.3.1 – Physical characterisation of eggs from white Livorno (LB) and Siciliana (SIC) hens at second-laying**

Breed		Weight (g)	Components weight (g)			Shell thickness (mm)			Components (%)		
		Egg	Yolk	Albumen	Shell	Polo Acuto	Polo Ottuso	Equatore	Yolk	Albumen	Shell
<b>LB</b>	Feb.	60.40 ± 3.26	17.40 ± 1.12	37.42 ± 2.68	6.31 ± 0.50	0.41 ± 0.04	0.40 ± 0.04	0.41 ± 0.03	28.51 ± 2.13	61.16 ± 2.24	10.32 ± 0.68
	March	59.02 ± 3.49	16.73 ± 1.06	36.41 ± 2.68	6.14 ± 0.52	0.41 ± 0.03	0.40 ± 0.02	0.41 ± 0.02	28.26 ± 1.89	61.38 ± 1.94	10.36 ± 0.63
	April	58.99 ± 3.75	17.01 ± 1.41	36.66 ± 2.56	5.94 ± 0.66	0.42 ± 0.04	0.39 ± 0.04	0.40 ± 0.03	28.55 ± 1.82	61.50 ± 2.16	9.95 ± 0.93
	May	58.13 ± 4.86	17.02 ± 1.52	35.49 ± 3.72	5.62 ± 0.51	0.40 ± 0.02	0.39 ± 0.03	0.38 ± 0.02	29.35 ± 2.28	60.96 ± 2.24	9.69 ± 0.62
	June	58.60 ± 2.90	16.89 ± 1.21	35.97 ± 2.35	5.80 ± 0.48	0.41 ± 0.03	0.39 ± 0.03	0.39 ± 0.02	28.81 ± 1.84	61.30 ± 1.95	9.98 ± 0.75
<b>SIC</b>	Feb.	49.49 ± 1.75	15.53 ± 0.93	28.49 ± 1.39	5.50 ± 0.33	0.41 ± 0.02	0.38 ± 0.03	0.41 ± 0.02	31.37 ± 1.60	57.52 ± 1.54	11.11 ± 0.58
	March	50.03 ± 3.58	15.78 ± 1.11	29.23 ± 2.84	5.64 ± 0.82	0.42 ± 0.04	0.40 ± 0.03	0.41 ± 0.05	31.23 ± 2.09	57.65 ± 1.89	11.11 ± 1.32
	April	51.53 ± 3.95	15.75 ± 1.09	30.63 ± 3.21	5.63 ± 0.75	0.43 ± 0.06	0.40 ± 0.05	0.41 ± 0.05	30.47 ± 1.59	59.08 ± 2.76	10.90 ± 1.41
	May	45.36 ± 2.43	15.57 ± 1.25	26.47 ± 2.68	5.39 ± 0.36	0.40 ± 0.03	0.41 ± 0.03	0.40 ± 0.02	33.57 ± 1.44	57.49 ± 4.81	11.30 ± 0.91
	June	50.68 ± 3.90	15.94 ± 1.26	29.68 ± 2.97	5.05 ± 0.85	0.39 ± 0.05	0.37 ± 0.04	0.38 ± 0.05	31.51 ± 2.12	58.53 ± 2.76	9.96 ± 1.49

**Table 1.3.2 – Chemical analysis of the yolk and albumen of eggs from white Livorno (LB) and Siciliana (SIC) hens at second-laying**

Breed	Yolk (%)				Albumen (%)			
	Lipids	Proteins	Dry matter	Sterins	Lipids	Proteins	Dry matter	
<b>LB</b>	February	41.18 ± 0.86	9.04 ± 0.65	54.09 ± 1.21	1.89 ± 0.04	0.70 ± 0.10	13.28 ± 0.77	13.13 ± 0.95
	March	42.24 ± 1.68	8.52 ± 0.80	54.61 ± 1.71	1.95 ± 0.08	0.74 ± 0.09	13.35 ± 0.59	13.11 ± 0.70
	April	43.10 ± 2.09	8.66 ± 0.73	55.82 ± 2.54	2.01 ± 0.13	0.79 ± 0.12	13.38 ± 0.68	13.06 ± 0.76
	May	42.64 ± 1.58	8.46 ± 0.80	54.90 ± 1.74	1.97 ± 0.08	0.77 ± 0.11	12.98 ± 0.70	12.53 ± 0.72
	June	42.07 ± 1.17	8.24 ± 0.64	54.05 ± 1.59	1.95 ± 0.06	0.76 ± 0.10	13.12 ± 0.66	13.72 ± 0.68
<b>SIC</b>	February	42.01 ± 1.53	8.17 ± 0.33	53.74 ± 1.41	1.94 ± 0.07	0.75 ± 0.11	12.40 ± 0.37	12.04 ± 0.48
	March	44.97 ± 2.03	8.44 ± 0.53	57.02 ± 2.73	2.11 ± 0.13	0.67 ± 0.05	12.31 ± 0.54	11.88 ± 0.56
	April	43.72 ± 2.63	8.69 ± 0.56	56.58 ± 3.61	2.06 ± 0.18	0.68 ± 0.08	12.36 ± 0.63	11.93 ± 0.69
	May	43.00 ± 1.62	8.17 ± 0.54	55.76 ± 2.57	2.00 ± 0.10	0.65 ± 0.06	12.03 ± 0.84	11.56 ± 0.99
	June	42.61 ± 1.76	8.19 ± 0.49	54.78 ± 2.25	1.98 ± 0.10	0.71 ± 0.08	11.94 ± 0.48	11.47 ± 0.52

**Figure 7.1.1a - Trend of average body weight in the four breeds (ER, PP, RL and ANC) bred at the Pisa location**

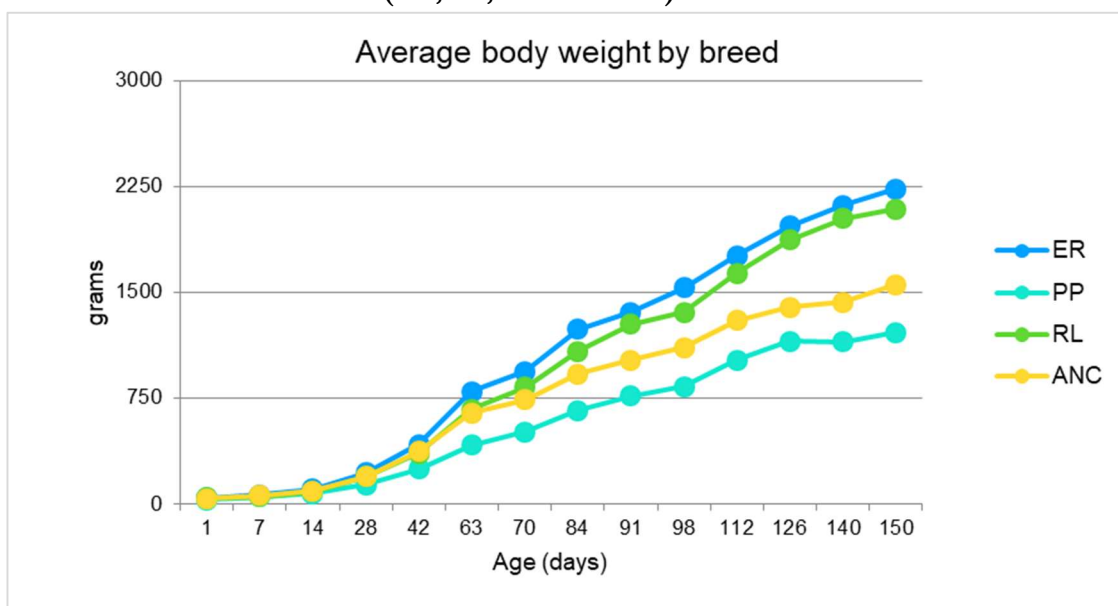


Figure 7.1.1b - Trend of average weight gain in the four breeds (ER, PP, RL and ANC) bred at the Pisa location

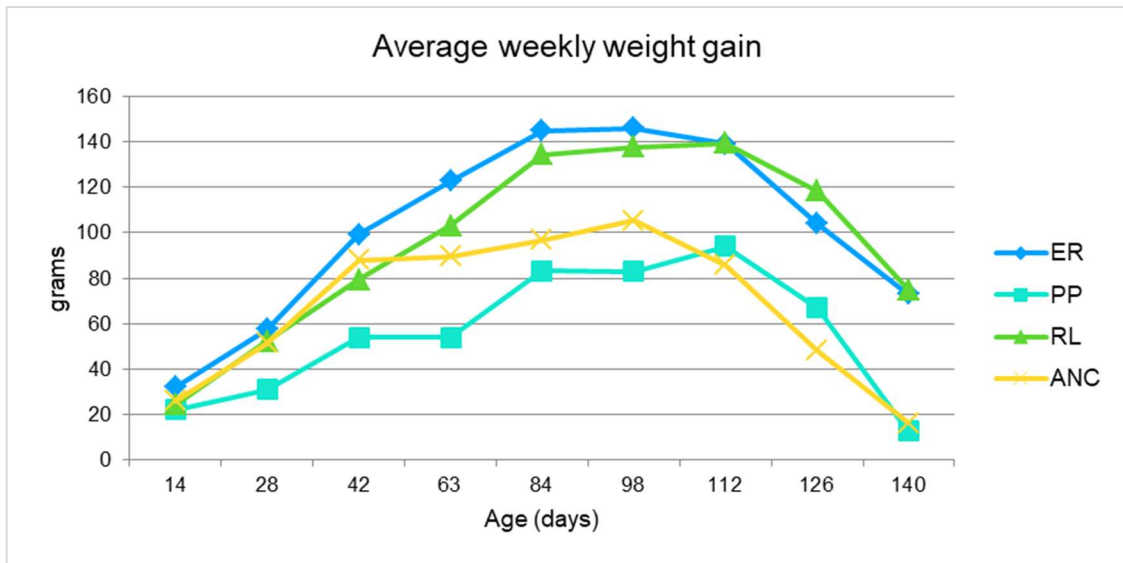
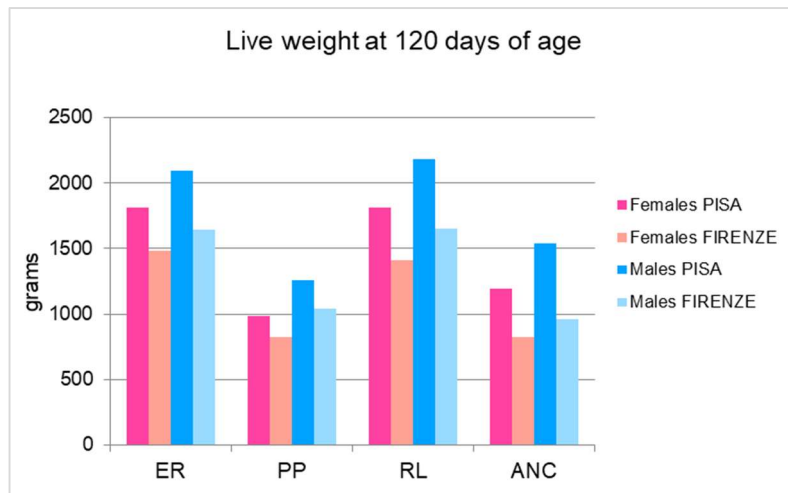


Figure 7.1.2 – Males and females average body weight at 120 days in ER, PP, RL and ANC breeds reared at the two locations



**Table 7.2.1 - Results of qualitative and quantitative parasitological analysis in the four breeds and in the two locations (Pisa and Florence): year 2023**

Location	Breed		Age (days)					
			15	30	60	90	120	
UNIFI	ANC	<i>Eimeria</i> spp.	Neg	11012.5 OPG	2775 OPG	1350.0 OPG	150.0 OPG	
			ER	Neg	Neg	11900 OPG	1187.5 OPG	125.0 OPG
			PP	Neg	Neg	136550 OPG	2650.0 OPG	225.0 OPG
			RL	Neg	Neg	15750 OPG	2412.5 OPG	175.0 OPG
	ANC	<i>Capillaria</i> spp.	Neg	Neg	Neg	Neg	327.3 EPG	
			ER	Neg	Neg	Neg	202.3 EPG	
			PP	Neg	Neg	Neg	387.5 EPG	
			RL	Neg	Neg	Neg	187.5 EPG	
UNIFI	ANC	<i>Eimeria</i> spp.	Neg	Neg	187.5 OPG	1612.5 OPG	337.5 OPG	
			ER	Neg	Neg	200.0 OPG	1687.5 OPG	1087.5 OPG
			PP	Neg	Neg	112.5 OPG	3775.0 OPG	1550.0 OPG
			RL	Neg	Neg	50.0 OPG	465.0 OPG	12.5 OPG

OPG: Oocysts per Gram of feces; EPG: Eggs per Gram of feces

**Table 7.2.2 – Evaluation of the four breeds by susceptibility classes of Lactobacilli towards the following antibiotics: ampicillin (AMP), tetracycline (TE), gentamicin (CN), linezolid (LZN), erythromycin (E), streptomycin (S)**

LOCATION	BREED	AMP			TE			CN			LZD			E			S		
		R	MS	S	R	MS	S	R	MS	S	R	MS	S	R	MS	S	R	MS	S
Firenze	Robusta Lionata	0%	0%	100%	59%	0%	41%	12%	0%	88%	0%	0%	100%	18%	0%	82%	88%	12%	0%
Pisa	Robusta Lionata	0%	0%	100%	0%	0%	100%	33%	0%	67%	0%	0%	100%	0%	0%	100%	67%	33%	0%
Firenze	Ermellinata di Rovigo	0%	0%	100%	20%	5%	75%	35%	0%	65%	0%	0%	100%	10%	0%	90%	95%	5%	0%
Pisa	Ermellinata di Rovigo	0%	0%	100%	8%	0%	92%	0%	0%	100%	0%	0%	100%	0%	0%	100%	58%	42%	0%
Firenze	Pepoi	0%	0%	100%	12%	0%	88%	0%	0%	100%	0%	0%	100%	12%	0%	88%	88%	12%	0%
Pisa	Pepoi	0%	0%	100%	23%	15%	62%	0%	0%	100%	0%	0%	100%	0%	0%	100%	46%	46%	8%
Firenze	Ancona	0%	0%	100%	0%	0%	95%	0%	0%	100%	0%	0%	100%	0%	0%	100%	90%	0%	5%
Pisa	Ancona	0%	0%	100%	18%	0%	82%	0%	0%	100%	0%	0%	100%	0%	0%	100%	55%	36%	9%
	Robusta Lionata n.20	0%	0%	100%	50%	0%	50%	15%	0%	85%	0%	0%	100%	15%	0%	85%	85%	15%	0%
	Ermellinata di Rovigo n.32	0%	0%	100%	16%	3%	81%	22%	0%	78%	0%	0%	100%	6%	0%	94%	81%	19%	0%
	Pepoi n.30	0%	0%	100%	17%	7%	77%	0%	0%	100%	0%	0%	100%	7%	0%	93%	70%	27%	3%
	Ancona n.31	0%	0%	100%	6%	0%	90%	0%	0%	100%	0%	0%	100%	0%	0%	100%	77%	13%	6%
Firenze		0%	0%	100%	22%	1%	76%	12%	0%	88%	0%	0%	100%	9%	0%	91%	91%	7%	1%
Pisa		0%	0%	100%	15%	5%	79%	3%	0%	97%	0%	0%	100%	0%	0%	100%	54%	41%	5%

R: resistant; MS: medium susceptible; S: sensitive