

***National Rural Development Programme 2014-2022***

***Measure 10.2 – Biodiversity***

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

**REPORT ON THE ACTIVITIES OF THE SECOND YEAR**

***PA UniPG***

This report describes the activities performed from May 1, 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 - Phenotypical characterisation of Ancona and Leghorn breeds*

Currently, at the Experimental Poultry Centre of the University of Perugia, the native Italian breeds Ancona and Leghorn (White, Black, Golden, and Silver) are preserved, since 2021 the Robusta Maculata breed has also been introduced. Each breed is divided into two families, each family is composed of 12-15 females and at least 3 males. For each family: eggs have been incubated, the characteristics of oviposition, fertility, hatching, chick viability and mortality have been registered (Table 1).

*Task 1.2 - Monitoring the performances of Ancona and Leghorn breeds*

In all subjects belonging to the above-described breeds, performance monitoring is carried out during all stages of growth of the animal. In addition, the animals reaching the adult age (34-36 weeks) are subjected to registration of biometric measures (according to FAO guidelines) to monitor the compliance with the breed standards (Table 2, 3, 4 and 5). All animals are individually identified by a CIP that provides information on the animal's age, sex, parentage, weight, and production parameters.

*Task 1.3 - Genetic characterisation of Ancona and Leghorn breeds*

The selection of the breeders selected by UNIPG involves the evaluation of phenotypic and genetic characterisation. This activity was carried out in collaboration with PA UNIMI (collection of biological material for genetic typing) and UNIMOL (collection of semen for the realization of a cryobank). The sampling activity with the UNIMOL PA was completed in March 2022 (below the steps of the activity), while with the UNIMI PA it was completed in October 2022 and described in more detail in task 4.

- September 2021 - October 2021 Training of Ancona and Leghorn rooster;
- November 2021 First collection and evaluation of semen in collaboration with UNIMOL;
- December 2021- February 2022 selection of male donors and maintenance of semen collection;
- March 2022 Second semen collection. Blood sampling of 10 Leghorn chickens (5 males and 5 females) and 10 subjects of Ancona (5 males and 5 females).
- October 2022 Collection of biological material for the genetic typing to the Ancona and Livorno breeds kept at UNIPG.

#### *Task 1.4 Phenotypical characterisation of Robusta Maculata breed*

During 2021, in collaboration with the PA UNIPD, the Robusta Maculata breed was introduced at the Experimental Poultry Centre of UNIPG. The eggs were incubated during January 2022, with subsequent hatching in February 2022. The characteristics of oviposition (fertility, hatching, chick viability and weaning mortality) were monitored as shown in Table 6. In addition, on reaching adult age (34-36 weeks) the weight of 20 subjects of Robusta Maculata female breed and 20 male subjects was monitored (Table 7). Finally, as for the other breeds, biometric measurements were recorded (in reference to FAO guidelines) in order to monitor compliance with the breed standard (Table 8).

#### *Task 2 Characterisation of the adaptation to low-input breeding systems and evaluation of the performances of the native breeds*

Evaluation of the adaptability to extensive farming systems characterized by low-input farming (use of local raw materials, moderate energy and protein intake) and "natural" environmental parameters (temperature, humidity and light) in particular, the ability to adapt to conditions of high temperatures (heat-stress) typical of the summer season has been evaluated.

Task 2.1 The eggs of Leghorn and Ancona breeds have been incubated and hatched at Experimental Poultry Centre of UNIPG. At 14 weeks of age they were transferred to an outdoor breeding system (external density 10 m<sup>2</sup>/animal), also as control group, the same number of animals were transferred to an indoor breeding system (10 animals/m<sup>2</sup>) fed the same diet but without access to the outdoor area. Live weight, ICA and AMG data were collected (Table 9).

Task 2.2 - During the breeding cycle, the weight gain and the performances relate to the production of eggs have been monitored (Tables 10 and 11), in order to characterize the duration of the productive career in the afore mentioned breeding conditions.

To establish the food consumption of the breeds bred in conventional and free-range systems, feed residue was evaluated weekly and also the degree of use of the pasture was evaluated by the inclusion of 4 enclosures (60 x 60 cm) at a distance of 5 and 10 m from the shelter according to the Lantinga et al. method (2004) (Table 12).

### *Task 3 Behavioral Characterisation*

#### Task 3.1 Behavioural characterisation of the Leghorn breed.

The study protocol of the following test was submitted and accepted by the University Committee of Bioethics (prot n.76527 of 3/3/2022). The trial involves the use of 46 hens and 6 males of white Leghorn, the housing occurred the first week of June 2022 at 14 weeks of age.

The animals were divided into two experimental groups:

I Group reared indoors: the animals were housed in 3 boxes without outdoor access with a density of 10 animals/m<sup>2</sup>. In each box 9 females and 1 male were housed for a total of 27 females and 3 males. This experimental group act as the control group.

II Group with outdoor access: the breeding area is characterised by the presence of three outdoor parks each with a shelter for the animals in which the following densities was used: external 10 m<sup>2</sup>/head, internal 10 animals/m<sup>2</sup>, inside each shelter will be placed 2 feeders and 2 drinking troughs. In each pen 9 females and 1 male were housed for a total of 27 females and 3 males.

The trial for the characterization of the Leghorn breed (described in the 1<sup>st</sup> report) started in June 2022 was completed. In particular, the behavioral observations were carried out during the months of June and July and between September and October 2022, the minimum and maximum temperatures of the day were recorded.

The trial produced a total of 12 h of outdoor recording and 12h of indoor recording using the software Noldus XT.

In addition, the study of kinetic and feeding behaviour was carried out by applying individual devices (bibs) to animals (Figure 1).

The outdoor area and indoor box needed a different number of devices as indicated in Figure 2 (Mancinelli Cartoni et al., 2022): UWB anchors positioned at the antipodes (9 in the outdoor area and 4 in the box); 1 UWB tag, 1 UWB Gateway, 1 Computer for collecting raw files and 10 chips placed on the bibs.

The software used produced a raw report for each animal with a punctual recording of the behavior (Figure 3)

The behaviour of the animal was recorded every 2 seconds, detecting its spatial position within the four quadrants of the area and the Cartesian axis (x, y, z), indicating the peck and the distance covered (Figure 4).

#### Task 3.2 Behavioural characterisation of the Ancona breed

The behavioural characterisation of the Ancona breed is in progress, the housing is scheduled for June 2023 and trial will be conducted as described in Task 3.1.

### *Task 4: Genetic characterisation of native chicken breeds through the study of polymorphisms in candidate genes involved in heat stress response.*

*Task 4.1* The sampling of biological material from breeds reared at the experimental section of UNIPG as described in Table 13. An aliquot of each of the 115 samples was sent to UNIMI on 30<sup>th</sup> October 2022 for the study with markers microsatellites that will provide guidelines for the programming of couplings in the UNIPG experimental centre for proper safeguard of breeds, avoiding consanguinity and identifying

the best breeders. In addition, the aforementioned samples together with the dataset of Tubavi I and with the addition of the samples of the breeds Millefiori Piemontese and Collo Nudo Italiana, will be part of the dataset that will be subjected to the study of polymorphisms in genes belonging to the HSP family.

*Task 4.2* DNA extraction is ongoing for new biological samples taken at the experimental section of UNIPG. In the coming months all the samples of the final dataset for the genotyping of HSP will be sent.

#### *Task 5. Verification of consistency of data and information.*

Objective 5 datasets have been prepared and are being compiled and validated.

Task 1 data have been processed and reported in the following report (Tables 1, 2, 3, 4, 5, 6, 7 and 8).

Task 2 data have been processed and reported in the following report (Tables 9, 10, 11 and 12).

Task 3.1 figures are shown in the following report (Figures 1, 2, 3 and 4).

The data related to the Tasks 1.3 and 4 are under study.

#### *Task 6. Data processing, dissemination, and reporting*

Task 6.1 the collected data was processed to produce intermediate reports etc.

Task 6.2 All data collected are disseminated in conferences and meetings of the poultry sector. For this purpose, two abstracts were accepted at the 25th Congress ASPA - Bari 13-16 June 2023 as oral presentation:

- Assessing the motivation of Leghorn hens to access outdoor space and pasture resources.  
Diletta Chiattelli, Marco Birolo, Alice Cartoni Mancinelli, Laura Menchetti, Cesare Castellini
- Preliminary validation of Ultra-Wide Band device as an innovative tool to assess chicken behaviour.  
Alice Cartoni Mancinelli, Diletta Chiattelli, Laura Menchetti, Gianmaria Bernacchia, Cesare Castellini

Task 6.3 all the data collected in task 5 will be processed with the aim of obtaining a manual including all the parameters characterizing the native Italian breeds Ancona and Leghorn.

#### References:

- Mancinelli, A. C., Chiattelli, D., Bernacchia, G., Nicconi, C., Torroni, J., Castellini, C., & Roselli, L. (2022, November). Assessment of Ultra-Wide Band device for monitoring chicken behaviour reared free-range. In *2022 IEEE Workshop on Metrology for Agriculture and Forestry (MetroAgriFor)* (pp. 91-93). IEEE.
- Lantinga, E. A., Neuteboom, J. H., & Meijs, J. A. C. (2004). Sward methods. *Herbage intake handbook*, 2, 23-52.

## FIGURES AND TABLES

**Table 1. Deposition rate recorded in January-July and results of incubation.**

	AN	WL	SL	GL	BL
Deposition rate** (%)	86	90	80	70	57
Average egg weight (g)	54	55	53	53	53
Hatched/Fertile eggs (%)	73	75	75	71	70

\*AN: Ancona, WL: White Leghorn, SL: Silver Leghorn, GL: Golden Leghorn, BL: Black Leghorn

\*\* Maximum value of oviposition

**Table 2. Productive performance of Ancona and Leghorn breeds (Females, age 36 weeks).**

breed	sex	age(weeks)	weight (g)
Ancona	F	36	1617
White Leghorn	F	36	1660
Black Leghorn	F	36	1632
Golden Leghorn	F	36	1685
Silver Leghorn	F	36	1674

**Table 3. Productive performances of Ancona and Leghorn breeds. (Males. age 36 weeks).**

breed	sex	age(weeks)	weight (g)
Ancona	M	36	2025
White Leghorn	M	36	2440
Black Leghorn	M	36	2360
Golden Leghorn	M	36	2503
Silver Leghorn	M	36	2197

**Table 4. Biometric measures of Leghorn breed.**

	Male		Female	
	Average	Min-max	Average	Min-max
Live adult weight (g)	2375	2300-2570	1648,25	1410-1835
Length of body (cm)	44.2	43-46	40.0	36-44
Chest circumference at sternum tip (cm)	35.0	33-37	32.4	30-34
Length of the tarsus (cm)	11.6	11-12	8.8	8-9.5
Diameter of the tarsus (cm)	1.37	1.3-1.45	1.12	1-1.6
Wingspan (cm)	49.3	46-51	42.3	38-45

**Table 5. Biometric measures of Ancona breed.**

	Male		Female	
	Average	Min-max	Average	Min-max
Live adult weight (g)	2025	1920-2200	1616,5	1410-1765
Length of body (cm)	43.3	43-44	39.2	36.5-41
Chest circumference at sternum tip (cm)	33.8	33-34.5	32.3	31-33.5
Length of the tarsus (cm)	10.5	10-11	9.0	8-9.5
Diameter of the tarsus (cm)	1.26	1.18-1.35	1.05	1-1.16
Wingspan (cm)	48.2	47-49	41.6	39-43

**Table 6. Incubation data of the Robusta Maculata breed.**

INCUBATION DATA	
N. eggs incubated	200
% discarded to candling	65
% born	25
% hatched	25

**Table 7. Productive performances of Robusta Maculata breed. (Males and Females, age 36 weeks).**

	Robusta Maculata	
	Male	Female
Weeks	Weight (g)	Weight (g)
36	4000	2800

**Table 8. Biometric measures of Robusta Maculata breed.**

	Male		Female	
	Average	Min-max	Average	Min-max
Live adult weight (g)	4000	4000-4500	2800	2800-3300
Length of body (cm)	44.3	43-45	40.2	38.5-41
Chest circumference at sternum tip (cm)	35.8	34-36.5	33.2	32-33.5
Length of the tarsus (cm)	10.5	10-11	9.05	8-9.5
Diameter of the tarsus (cm)	2.31	1.80-2.45	1.52	1.3-1.6
Wingspan (cm)	48.2	47.5-49	41.9	39-43

**Table 9. Growth parameters of Leghorn and Ancona breeds bred in conventional and free-range system.**

	W. LEGHORN		ANCONA	
	CONVENTIONAL	FREE-RANGE	CONVENTIONAL	FREE-RANGE
Live weight (g) 36 weeks old	1660	1650	1617	1607
ICA	3.1	2.5	3.2	2.4
AMG	9.2	9.1	8.9	9.0

**Table 10. Average egg production of Leghorn and Ancona breeds reared in conventional and free-range system.**

	W. LEGHORN		ANCONA	
	CONVENTIONAL	FREE-RANGE	CONVENTIONAL	FREE-RANGE
Average eggs/week	41.7	47.7	39.2	43.2
Average eggs/week/hen	4.9	5.3	3.8	4.3
Egg weight (g)	61.0	59.1	61.2	59.3

**Table 11. Egg parameters of Leghorn and Ancona breeds reared in conventional and free-range system.**

	W. LEGHORN		ANCONA	
	CONVENTIONAL	FREE RANGE	CONVENTIONAL	FREE RANGE
Shell weight	5.97	6.27	5.84	5.97
Diameter (mm)	43.7	43.3	42.3	42.1
Length (mm)	57.8	57.0	56.7	56.6

**Table 12. Average consumption of Leghorn and Ancona breed reared in conventional and free-range.**

	W. LEGHORN		ANCONA	
	CONVENTIONAL	FREE-RANGE	CONVENTIONAL	FREE-RANGE
Feed-intake g/hen/d	161.2	116.5	158.1	123.7
Grass-intake g/hen/d	-	105	-	100

**Table 13. Sampling at UNIPG experimental centre.**

BREED	SEX
ANCONA	14 females
	5 males
SILVER LEGHORN	17 females
	3 males
GOLDEN LEGHORN	20 females
	4 males
BLACK LEGHORN	21 females
	5 males
WHITE LEGHORN	22 females
	4 males

**Figure 1. Bibs for poultry**

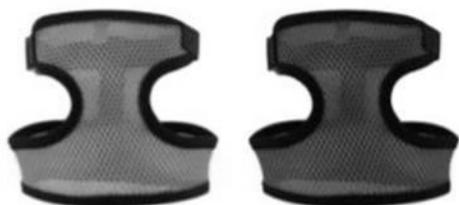




Figure 2. Schematic representation of the devices applied in the outdoor and in the internal box.

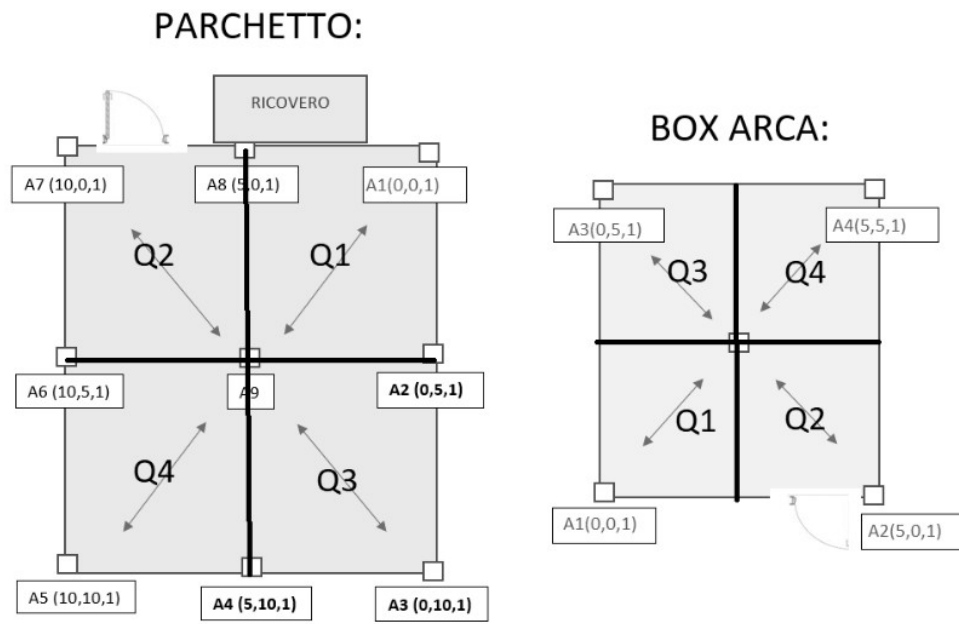


Figure 3. Diagram of the spatial position covered by the animal monitored.

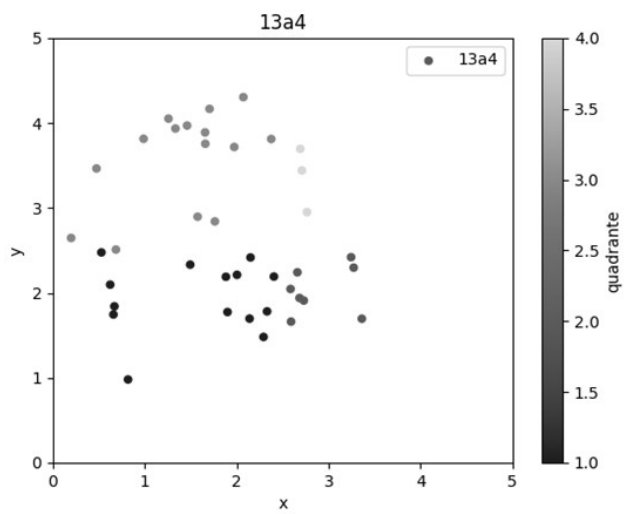


Figure 4. Summary screen of software

ts	x	y	z	time	year	month	day	hour	min	s	check_beccata	distance	quadrante	Q1	Q2	Q3	Q4
88	17.765.064	2.334.008	0.18003267	16.638.300.013.584.800	2022	9	22	9	0	1	1	0.251904	4	0	0	0	1
90	19.679.605	2.312.495	0.19540529	1.663.830.003.361.570	2022	9	22	9	0	3	1	0.192658	4	0	0	0	1
92	19.531.684	22.189.305	0.11527783	1.663.830.005.373.180	2022	9	22	9	0	5	1	0.0	2	0	1	0	0
94	18.550.915	21.362.305	0.1505703	1.663.830.007.315.970	2022	9	22	9	0	7	1	0.0	2	0	1	0	0
96	18.661.147	21.299.515	0.11687712	16.638.300.093.621.900	2022	9	22	9	0	9	1	0.0	2	0	1	0	0
98	19.275.926	20.909.746	0.22100224	16.638.300.113.679.800	2022	9	22	9	0	11	1	0.0	2	0	1	0	0
100	19.936.452	2.231.658	0.03906827	16.638.300.133.603.800	2022	9	22	9	0	13	0	0.0	2	0	1	0	0
102	20.666.513	24.231.689	0.11833142	16.638.300.153.559.900	2022	9	22	9	0	15	1	0.204954	4	0	0	0	1
104	19.176.755	25.944.183	-0.03067288	16.638.300.173.194.600	2022	9	22	9	0	17	1	0.226980	4	0	0	0	1
106	18.592.141	26.909.153	-0.09476725	16.638.300.193.599.100	2022	9	22	9	0	19	1	0.0	4	0	0	0	1
108	18.997.935	26.659.567	0.016837843	16.638.300.213.613.800	2022	9	22	9	0	21	1	0.0	4	0	0	0	1
110	18.599.621	26.702.809	0.030606629	16.638.300.233.607.000	2022	9	22	9	0	23	1	0.0	4	0	0	0	1
112	18.300.484	26.771.917	-0.0097672345	16.638.300.253.686.900	2022	9	22	9	0	25	1	0.0	4	0	0	0	1
114	18.329.134	2.646.595	-0.0017358519	1.663.830.027.383.250	2022	9	22	9	0	27	1	0.0	4	0	0	0	1
116	17.689.579	26.293.218	0.12039436	16.638.300.294.218.800	2022	9	22	9	0	29	1	0.0	4	0	0	0	1
118	17.789.563	26.243.715	0.12196965	16.638.300.313.994.100	2022	9	22	9	0	31	1	0.0	4	0	0	0	1
120	1.769.209	26.222.184	0.0815734	1.663.830.033.398.320	2022	9	22	9	0	33	1	0.0	4	0	0	0	1
122	17.389.742	26.760.566	-0.019462055	16.638.300.353.745.300	2022	9	22	9	0	35	1	0.0	4	0	0	0	1
124	19.013.163	25.233.066	0.19138449	1.663.830.037.366.520	2022	9	22	9	0	37	1	0.222906	4	0	0	0	1
126	19.685.231	24.821.486	0.25431469	16.638.300.393.818.900	2022	9	22	9	0	39	1	0.0	4	0	0	0	1
128	18.698.162	2.590.096	0.13628881	1.663.830.041.357.860	2022	9	22	9	0	41	1	0.0	4	0	0	0	1
130	1.723.592	26.073.689	0.12121255	16.638.300.433.694.600	2022	9	22	9	0	43	1	0.0	4	0	0	0	1
132	16.797.167	26.224.225	0.12242591	1.663.830.045.358.290	2022	9	22	9	0	45	1	0.0	4	0	0	0	1
134	16.907.567	263.539	0.13848743	1.663.830.047.360.390	2022	9	22	9	0	47	1	0.0	4	0	0	0	1
136	1.729.843	2.594.317	0.10821233	16.638.300.493.768.600	2022	9	22	9	0	49	1	0.0	4	0	0	0	1
138	17.718.257	25.724.607	0.020322524	16.638.300.513.597.900	2022	9	22	9	0	51	1	0.0	4	0	0	0	1
140	17.534.941	25.448.465	0.054098893	16.638.300.533.705.400	2022	9	22	9	0	53	1	0.0	4	0	0	0	1
142	17.487.047	25.629.857	0.033351813	16.638.300.553.575.100	2022	9	22	9	0	55	1	0.0	4	0	0	0	1
144	1.751.736	25.307.267	0.025469508	16.638.300.573.747.700	2022	9	22	9	0	57	1	0.0	4	0	0	0	1
146	17.437.391	24.892.557	0.20106219	16.638.300.593.606.900	2022	9	22	9	0	59	1	0.0	4	0	0	0	1
148	17.380.939	25.701.854	0.12961124	1.663.830.061.371.660	2022	9	22	9	1	1	1	0.0	4	0	0	0	1
150	17.966.769	25.917.575	0.10560169	16.638.300.633.714	2022	9	22	9	1	3	1	0.0	4	0	0	0	1
152	17.573.879	27.389.042	0.21607007	16.638.300.653.693.200	2022	9	22	9	1	5	0	0.0	4	0	0	0	1
154	18.214.694	2.870.904	0.2659438	16.638.300.674.045.900	2022	9	22	9	1	7	0	0.0	4	0	0	0	1
156	18.135.203	31.257.207	0.26589623	16.638.300.693.799.700	2022	9	22	9	1	9	0	0.254940	4	0	0	0	1
158	17.583.017	33.518.748	0.31992722	1.663.830.071.369.470	2022	9	22	9	1	11	0	0.232797	4	0	0	0	1
160	19.339.348	34.263.835	0.18733838	16.638.300.733.774.500	2022	9	22	9	1	13	0	0.190783	4	0	0	0	1
162	16.689.994	3.581.341	0.23810863	16.638.300.753.675.900	2022	9	22	9	1	15	0	0.306924	4	0	0	0	1
164	15.021.939	36.540.256	0.26661354	16.638.300.773.677.300	2022	9	22	9	1	17	0	0.181953	4	0	0	0	1
166	13.838.708	37.213.919	0.21372877	1.663.830.079.376.240	2022	9	22	9	1	19	0	0.0	4	0	0	0	1
168	132.883	37.348.874	0.23365928	16.638.300.813.154.600	2022	9	22	9	1	21	0	0.0	4	0	0	0	1
170	13.073.912	37.666.659	0.22290896	1.663.830.083.358.430	2022	9	22	9	1	23	0	0.0	4	0	0	0	1
172	12.964.903	37.727.911	0.21357127	16.638.300.853.678.200	2022	9	22	9	1	25	0	0.0	4	0	0	0	1
174	12.863.605	37.653.489	0.22400855	16.638.300.873.632.600	2022	9	22	9	1	27	0	0.0	4	0	0	0	1
176	12.788.041	3.762.295	0.23080155	1.663.830.089.393.340	2022	9	22	9	1	29	0	0.0	4	0	0	0	1
178	12.754.853	37.984.984	0.19433963	1.663.830.091.357.730	2022	9	22	9	1	31	0	0.0	4	0	0	0	1
180	12.655.591	3.794.064	0.2014011	16.638.300.933.601.600	2022	9	22	9	1	33	0	0.0	4	0	0	0	1
182	12.631.012	38.000.245	0.19580129	16.638.300.953.628.300	2022	9	22	9	1	35	0	0.0	4	0	0	0	1
184	12.522.283	37.935.226	0.20962417	16.638.300.973.594.500	2022	9	22	9	1	37	0	0.0	4	0	0	0	1
186	12.605.946	37.701.161	0.26284167	1.663.830.099.395.720	2022	9	22	9	1	39	0	0.0	4	0	0	0	1
188	12.744.592	37.711.134	0.24987835	16.638.301.013.704.800	2022	9	22	9	1	41	0	0.0	4	0	0	0	1
190	12.721.269	38.345.528	0.18773395	16.638.301.033.731.300	2022	9	22	9	1	43	0	0.0	4	0	0	0	1
192	11.904.817	39.184.134	0.13649262	16.638.301.053.977.700	2022	9	22	9	1	45	0	0.0	3	0	0	1	0