

# **AN *in situ* MARKER-ASSISTED CONSERVATION SCHEME OF 11 ITALIAN AVIAN BREEDS**



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Session: **G3.8**

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## **ABSTRACT**

An *in situ* marker assisted conservation scheme (CoVa project) of 11 local poultry breeds of 4 different species (poultry, duck, helmeted guinea fowl and turkey) is currently running, since 2000, in the Veneto region. Objectives of CoVa project are: to preserve local traditions and rural culture of avian productions, to maintain these sources of genetic biodiversity and to minimize average kinship coefficient within breed. A minimum number of 54 mature animals per breed (20 males and 34 females) is guaranteed within each of the 4 nucleus farms located in 4 different environments. A molecular markers information are used to monitor heterozygosity and genetic similarities among and within breeds and to select sires and dams. Production and reproduction performances are also recorded and a biannual complete substitution of sires and dams is applied. Individual identification is based on wing tagging at hatching. A pre-selection of males and females at about 4 months of age is applied using a threshold index based on family attribution, standard phenotype and production and reproduction performances. The future of this local avian breeds is based on their genetic conservation and development of niche production, possibly including crossbreeding with more productive commercial genotypes.

## **INTRODUCTION**

Conservation of animal genetic resources is essential to enable farmers to adapt for changing environmental conditions and consumer demands. It is in the best interest of societies to ensure that farmers and breeders have access to the widest possible range of animal genetic resources so that they can effectively respond to change (FAO, 2004). Biodiversity is essential for the survival of species and populations and it is assuming greater importance in modern animal science because of an expending global emphasis on a few highly selected breeds (Notter, 1999).

The convention on biological diversity of the United Nations of 1992 (UNEP, 1992) permitted to gain much political and social attention for biodiversity of animal genetic resources. Year after, FAO (1993) formulated global programs for management of genetic resources using genetic markers for breed characterization with the aim to make decisions on conservation of genetic diversity based on estimates of variation within and among populations and breeds. A conservation program of animal genetic resources can be based on maintenance of the breeds within their production system, as *in situ* method, or outside their production system, as *ex situ* method based on cryoconservation of

genetic material or live animals (Gandini and Oldenbroek, 1999). A third conservation method may be applied using both *in situ* and *ex situ* techniques together. Henson (1992) pointed out that there is no single method of preservation which is optimal for all situations. However, *in situ* conservation has a number of advantages, and may be the only option available in some instances. *In situ* conservation is very flexible and allows for the development and utilisation of breeds. However, because of limited facilities and budget constraints, *in situ* conservation may be often restricted to a small population with major risk to reduce genetic variability, in particular for breeds without an herd book or pedigree information available. For avoiding risk to compromise genetic variability of conservation programmes of small population of live animals the application of recent technologies in molecular biology seem to be promising (Toro et al., 1998; Toro et al., 1999). At the beginning, molecular markers were advocated as a powerful tool for paternity exclusion and for the identification of distinct populations that need to be conserved (Avisé, 1994). However, their application in conservation programmes of domestic species is only beginning, and in the scientific literature there is a lack of examples of conservation units where markers are routinely scored and utilized.

Aim of this paper is to present an *in situ* marker assisted conservation scheme (CoVa project) of 11 local poultry breeds of 4 different species (chicken, duck, helmeted guinea fowl and turkey) that it is currently running, since 2000, in the Veneto region (Italy).

## **MATERIAL AND METHODS**

### **Description of species and breeds in conservation**

The conservation and valorization of local poultry breed (CoVa project) was planned for 11 historical regional poultry breeds of 4 different species (chicken, duck, helmeted guinea fowl and turkey) that it is currently running, since 2000, in the Veneto region. This project involves four breeding units located in three different areas (mountain, hill and plain) of the regional territory. The local chicken breeds are the Robusta Maculata (PRM), Robusta Lionata (PRL), Ermellinata di Rovigo (PER), Pépoi (PPP), Padovana Dorata (PPD) and Padovana Camosciata (PPC); the local duck breeds are Germana Veneta (AGV) and Mignon (AMG); the local turkey breeds are Bronzato Comune (TCB) and Ermellino di Rovigo (TER) while the local helmeted guinea fowl breed is Camosciata (FCM). All local poultry breeds are dual purpose breeds with meat and eggs production and they are registered in the list of traditional Italian products recognized by the Ministry of Agricultural and Forestry Policy (MIPAF, 2003). It was hoped that the listing of these breeds and their subsequent description and characterization would assist in the development of several areas of the region through the revaluation of local and typical breeds and would thereby promote conservation of local breeds and preservation of biodiversity (De Marchi et al., 2003). The perspectives of these Veneto local breeds and their farmers are to use these genotypes for typical and alternative products of the region in some local market, for favouring the development of poultry sector through the revaluation of local breeds and for leading to the preservation of animal biodiversity.

Preliminary study on the poultry breeds of the Veneto region showed distinctive meat quality characteristics (e.g. good healthy performances and dark colour) that distinguish them from the more common commercial hybrids or genetic lines (Castellini et al., 1994; Cassandro et al., 2002).

### *Characteristics of the chicken breeds*

The six Veneto chicken breeds involved in CoVa project are the Robusta Maculata (**Figure 1**), Robusta Lionata (**Figure 2**), Ermellinata di Rovigo (**Figure 3**), Pépoi (**Figure 4**), and Padovana Camosciata and Dorata (**Figure 5**). Information about these breeds was previously reported in an Italian report (Veneto Agricultural Agency, 2004). The Pépoi and Padovana are small size chicken breeds, whereas the other three breeds are medium-size with heavier mature weight. Characteristics of the birds are shown in **Table 1**. All breeds showed a strong aptitude to organic and extensive breeding.

A preliminary study estimated the daily gain of these chickens breed during summer 2003 at the Agricultural Secondary School of Castelfranco Veneto (TV). This experiment involved four chicken breeds: Robusta Maculata, Robusta Lionata, Ermellinata di Rovigo and Pépoi. At hatch, chicks were individually weighted and reared in an indoor pen with an open grass paddock; feed and water were supplied *ad libitum*. Body weights were recorded every 10 days for 156 days. As expected, the sex difference in average daily gain was highly significant and highest for males. The daily gains of the Robusta Maculata and Pépoi breeds were significantly different and also differed from those of the Robusta Lionata and Ermellinata di Rovigo.

The Robusta Maculata chicken breed was developed in 1965 at the Rovigo Experiment Station from crosses between Tawny Orpington and White American, and it was selected to be a dual-purpose breed (eggs and meat). Adult animals have white plumage with black spots; the skin and tarsus are yellow. The Robusta Maculata exhibited an average daily gain to 156 days of 13.8 and 18.4 g/d, respectively, for females and males and have a strong sexual dimorphism at maturity. The daily gain of Robusta Maculata was higher than the 9.6 g/d reported for the Ancona breed by Castellini et al. (1994).

The Robusta Lionata chicken was also developed in 1965 at the Rovigo Experiment Station from crosses between Tawny Orpington and White American and is also a dual-purpose breed (eggs and meat). At hatch, chicks are a tawny colour with brown spots. Adult chickens retain this tawny coloration with black and greenish tail feathers. Females have a strong aptitude to brood. At 4 months of age, the Robusta Lionata chicken weights around 1.9 to 2.0 kg, and is similar in adult male and female body weight to the Robusta Maculata. The Robusta Lionata had an average daily gain to 156 days of 10.7 and 16.0 g/d, respectively, for females and males which was similar than the result observed in Ancona females breed (9.6 g/d) by Castellini et al. (1994).

The Ermellinata di Rovigo chicken was developed in 1959 for meat production from crosses between the Sussex and Rhode Island breeds. At 3 months, body weight was 1.7 to 1.8 kg. At hatch chicks are yellow, but adult birds have white plumage with dark pens, helmets and cape. The skin and tarsus are yellow. The Ermellinata di Rovigo had average daily gains that were similar to those of the Robusta Lionata (11.6 and 15.7 g/d, respectively, for male and female).

The Pépoi chicken breed is very small but has high-quality meat. This breed is typically found in north-west Italy and at the present is one of the few small breeds available in the markets. At hatch, chicks have a clear brown color that changes to a gilded colour. The skin and tarsus are yellow. Females have a strong aptitude to brood. The Pépoi breed grows relatively slowly, with average daily gains to 156 days of 5.6 and 8.7 g/d, respectively, for female and male. The daily gains of the Pépoi breed were significantly lower than those of the other breeds evaluated and were less than those reported for the Ancona breed (Castellini et al., 1994) and the Padovana breed (Lunardi et al., 2001).

The Padovana breed is a fancy breed (FAO, 2004), its origin is very old and it was described for the first time in the *Ornitologiae* book of Ulisse Aldrovandi (1600). The origin of this ancient breed is uncertain; it is thought that the Padovana was introduced to Italy from Poland in 1300 by a Padova noble, Giovanni D'ondi dell'Orologio. Before 1899, the Padovana chicken was confused with the Polverara breed. Trevisani (1900) and Pascal (1905) were the first authors to separately describe the Padovana and Polverara breeds. Adult males and females have an average weight of 2.0 kg and 1.8 kg respectively, with an average daily gain of 8-10 g/d and exhibited maximum daily gains at around 3 months of age (Lunardi et al., 2001). The Padovana breed has a skull hernia with a tuft of feathers very pronounced. The Padovana has self-black, white, gold, silver, and buff coloured plumage with laced patterns within the feathers (FAO, 2004).

#### *Characteristics of the duck breeds*

The two breeds of ducks in conservation in the Veneto region are: Germanata Veneta and Mignon. The characteristics of the two breeds are shown in **Table 2**. Both breeds have a strong aptitude to organic and extensive breeding.

The Germanata Veneta duck (**Figure 6**) was derived from the Real German; its coloration and form are unchanged. This breed is very rural and the female can be crossed with the Barberia duck to produce fat liver for pate. The female of Germanata Veneta duck produces 100 to 120 eggs per year, which is lower than that reported for the brown Tsaiya and Pekin ducks (Velez et al., 1996). The Mignon (**Figure 7**) duck breed is a small white duck with yellow legs, beak and skin. It is found in the southern and eastern part of the Veneto region and is very rustic. Egg production by the Mignon breed is not very important (50 to 70 eggs per year), but this light duck is used for meat production.

#### *Characteristics of the turkey breeds and the helmeted guinea fowl*

The turkey breeds of the Veneto region are the Ermellinato di Rovigo and Comune Bronzato. The only breed of helmeted guinea fowl is the Camosciata. Characteristic traits of turkey and helmeted guinea fowl are shown in **Table 3**.

The Ermellinato of Rovigo turkey (**Figure 8**) was derived from a mutation in offspring of crosses of local birds to the American Narraganset breed in 1958 and was then selected for increase performance (Veneto Agricultural Agency, 2004). This breed is of medium size and is early feathering. The Ermellinato di Rovigo is very rustic and well suited to pasture production.

The Comune Bronzato turkey is a small breed. The breast, neck, shoulders, and rump are black with rainbow reflexes (**Figure 9**). Young turkeys have a dark brown tarsus, but the tarsus of adult birds ranges from red to violet. Females of this breed can produce 4 to 5 broods at a time, remaining on the nest for more than 100 days.

The Camosciata helmeted guinea fowl (**Figure 10**) was developed in 1922 (Veneto Agricultural Agency, 2004). The neck and throat skin are blackish, the pens are white with pearl stains, and the tarsus coloration varies from orange to grey. The Camosciata breed is small and at maturity, females are usually larger than the males (**Table 3**).

#### *Design and establishment of the conservation project*

The Department of Animal Science of the University of Padova planned a marker assisted conservation scheme based on maintenance and multiplication of these breeds within their production systems (*in situ* conservation system).

The information for this study (pedigree information, production and reproductive data) were recorded since 2000. The number of birds for each breed and the



number of breeding units involved in the conservation project, at the August 2004, are showed in **Table 4**. The four different breeding units are located in the mountain (Feltre-BL), hill (Montebelluna-TV) and plain (Ceregno-RO and Padova-PD) areas of the Veneto region. These four different environments guarantee more security than an unique herd site, in particular during disease outbreaks.

### **Activities and conservation scheme in Co.Va. Project**

The breeding activities and conservation scheme were developed at the same time, in the same manner and in all breeding units. The reproduction season start in February and birds are hatched from April to June. At the start of the reproduction season, 34 pure females and 20 males in each flock are the size group per each breed. Females are grouped all together whereas males are divided in two different families, based on relationship estimated at the start of the project using markers information, and these two male families are rotated among the unique females group using 2 groups of ten homogeneous males every 5 weeks. A total of 2 reproduction periods, 1 period per male family is planned. Within each period, after 2 weeks, for the successive 3 weeks, until to the end of the reproduction period, eggs are used for conservation scheme. For all other periods, eggs are commercialized or used outside the conservation scheme. The breeding scheme is based on a biannual change of all animals. For each breed within each flock, every year the 50% of males (10 males per year) and females (17 females) are changed as showed in **Figure 11**. Near the end of each year, birds are vaccinated (*salmonella pullorum*) and weighted and a blood sample is taken for DNA analysis. In January, males of each breed are rotated among breeding units. Padovana chicken breed is not rotated among breeding units because of it only reared in Padova flock where it were created three different groups to mimic the population structure of others poultry breeds. The marker assisted conservation scheme was established by Department of Animal Science of the University of Padova to avoid the reduction of heterozygosity in poultry populations. Only males individuals are genotyped extracting genomic DNA by whole blood. AFLP genotyping are carried out using three primer combinations E32/T35, E45/T32 and E45/T33 as described by Targhetta et al. (2003). Data obtained by dominant scoring of AFLP markers were firstly used to calculate heterozygosity (H) supposing population to be at Hardy Weinberg equilibrium (Nei, 1987).

To select news males and females in each breeding unit the following criteria are used: date of birth, family origin, phenotypic standards (e.g. colour plumage and morphological defects) and weight performances (e.g. Padovana chicken breed).

### **Results and Discussion**

The statistical description of reproduction traits analysed are summarized in **Table 5**. The overall percentages of fertilized eggs per hatching (pFE) and percentage of chicks born alive (pBA) ranged from 62 to 76% and from 40 to 54%, respectively. The pFE and pBA per each breed over years are presented in **Tables 6 and 7**. The fertility performances over all years showed to be on the increase for TCB, AMG and FCM; whereas showed to be in reduce for PPP and TER. A possible reason of these results might be due to the reduced number of individuals available for TER and the lowest adaptability and stability indexes evidenced by PPP breed (Cassandro et al., 2004). Other breeds showed a quite stable fertility performances over years.

During the last year, of the all chickens breed, the PER, PPC and PPD breeds showed the best pFE with values greater than 70% whereas, PPP showed the lowest value with 53%. Regarding to pBA the chickens breeds that showed the best performances were PER and PPC with values around 65%. The PPP showed also in this case the lowest performance (35% of pBA). The local chicken breed that sensible reduced its fertility performances was PPP. The two ducks breeds showed similar values of pFE even if AGV showed values around 70% during the 2000 and 2001 years. Similar comments can be done on pBA performances where both local duck breeds showed during the last year values similar and equal to 40%. The FCM helmeted guinea fowl breed showed a 59% of pFE and a 45% of pBA.

A total number of 66, 71, 43 and 35 AFLP markers were identified for chickens, ducks, turkey, helmeted guinea fowl breeds, respectively. In **Table 8** are showed the number of birds genotyped for estimating heterozigosity (H) values. The H values estimated per each breed over the three years are showed in **Table 9**. The arithmetical means of the heterozigosity for all breeds considered were 20.1, 24.5 and 26.1 % for 2001, 2002 and 2003, respectively. An average annual change for all breeds was equal to 3.0%, confirming that marker assisted conservation scheme is working on the right direction. All species showed an increment of H with 3.5, 2.0, 2.0 and 1.0 for chicken, duck, turkey and helmeted guinea fowl, respectively. Only TER showed a reduction of H value over years. The few number of live animals for this breed is the main reason of this result. It is interesting to put in evidence that the two breeds (PPP and TER) that showed the worst results on fertility traits are the two breeds with the lowest H values (21 and 15%, respectively). Therefore, more attention and efficiency on conservation strategies are need to improve these two local breeds, whereas for the all other breeds seem to be useful to follow the strategies already applied.

Moreover, application of AFLP methodology could be very interesting to define a genetic traceability system for poultry breeds and products, which would be a very important step for the conservation of rare breeds and for the valorization of their products. De Marchi et al. (2003) and De Marchi et al. (2004) already proposed and showed for chicken breeds the possibility to define a genetic traceability method for chickens and to identify local Veneto chickens breeds respect to commercial broilers for valorization their genetic peculiarities. Genetic peculiarities of a local breed usually do not change if the population is kept as a pure breed. This problem becomes a matter of concern when crossbreeding is use in order to restore genetic variability. Therefore, the most important problem in considering *in situ* conservation is how to keep genetic variability within the populations while maintaining genetic peculiarities without reducing allelic or genotype frequencies.

## Conclusion

Conservation of the Veneto local avian breeds could have a positive impact on the rural economy in some agricultural areas of the regional territory. The most effective way of conserving local genetic resources is through economical utilization of the animals in the production system. However, it is important to increase their heterozigosity for reducing risk of inbreeding. Results obtained in this study suggest that molecular markers, as AFLP fingerprinting technique, can be a feasible method for the characterization of local poultry populations and to assist conservation scheme when pedigree information are scarce or not available. However, AFLP technique, due to the biallelic nature of these markers, seem to be need of an high number of molecular markers for working well, even if the costs genotyping would increase. An alternative way to restore genetic

variability might be the use of crossbreeding method, however, it is important to keep genetic variability within population while maintaining genetic peculiarities.

### Acknowledgements

This research was funded by Regione Veneto. The authors wish to thank all people involved in the Co.Va. project and in particular the teachers and technicians of the Agricultural Secondary School of Castelfranco Veneto (TV), Feltre (BL), Padova and Ceregnano (Ro) experimental center.

Table 1. General characteristics of Veneto local chicken breeds.

Trait	Breed*				
	PRM	PRL	PER	PPP	PPD-PPC
Age of sexual maturity (months)	5-7	5-7	5-7	5-6	5-7
Adult female body weight (kg)	2.8-3.3	2.8-3.3	2.2-2.6	1.0-1.1	1.5-2.0
Adult male body weight (kg)	4.0-4.5	4.0-4.5	3.0-3.5	1.3-1.5	1.8-2.3
Egg production	150-160	160-170	150-160	160-180	120-130
Egg weight (g)	55-60	55-60	55-60	40-45	50-60
Egg colour	Rose	Rose	Rose	Rose	White
Average daily gain (g/d)	14-18	11-16	12-16	6-9	8-10

\* PRM = Robusta Maculata; PRL = Robusta Lionata; PER = Ermellinata di Rovigo; PPP = Pèpoi; PPD = Padovana.

Table 2. General characteristics of Veneto local duck breeds.

Trait	Germanata Veneta	Mignon
Age of sexual maturity (month)	6-8	7-8
Adult female body weight (kg)	2.7	0.8
Adult male body weight (kg)	3.0	0.8
Egg production	100-120	50-70
Egg weight (g)	70	45-50
Egg colour	White	White

Table 3. Characteristics of Veneto turkey and helmeted guinea fowl breeds

Trait	Ermellinato of Rovigo	Comune Bronzato	Camosciata
Age of sexual maturity (month)	7	7	7-9
Adult female body weight (kg)	4-6	3.0-3.5	1.8-2.0
Adult male body weight (kg)	10-12	6-7	1.8
Egg production	70-100	70-100	100-120
Egg weight (g)	70-80	70-85	45
Egg colour	White – Rose	White – Rose	Reddish

Table 4. Number of birds per breeding unit and breed in COVA project (2004).

Flock	Breeds	Breeding Males	Breeding Females
Feltre (Belluno)			
Chicken			
	- Robusta Maculata	15	41
	- Robusta Lionata	16	44
	- Ermellinata di Rovigo	16	15
	- Pépoi	13	43
Duck			
	- Germanata Veneta	24	39
	- Mignon	26	39
Turkey			
	- Bronzato Comune	16	34
	- Ermellino di Rovigo	16	15
Helmeted guinea fowl			
	- Camosciata	24	35
Montebelluna (Treviso)			
Chicken			
	- Robusta Maculata	31	45
	- Robusta Lionata	27	46
	- Ermellinata di Rovigo	28	40
	- Pépoi	29	47
Duck			
	- Germanata Veneta	30	38
	- Mignon	25	40
Turkey			
	- Bronzato Comune	20	43
	- Ermellino di Rovigo	6	5
Helmeted guinea fowl			
	- Camosciata	29	37
Ceregnano (Rovigo)			
Chicken			
	- Robusta Maculata	28	38
	- Robusta Lionata	28	52
	- Ermellinata di Rovigo	29	49
	- Pépoi	36	43
Turkey			
	- Bronzato Comune	18	35
Padova			
Chicken			
	- Padovana Camosciata	30	44
	- Padovana Dorata	23	37

Table 5. Statistical description of reproduction traits of the 11 poultry breeds.

Trait	2000	2001	2002	2003
Hatchings number	133	181	331	232*
All eggs hatched (EH), n.	7,271	10,927	22,230	18,639
Fertilized eggs on EH, %	68	76	62	63
Chicks born alive on EH, %	51	54	40	44

\* data of Conegliano breeding unit were not available.



Table 6. Trend from 2000 to 2003 of the percentage of fertilized on hatched eggs of the 11 local poultry breeds.

Breeds	2000	2001	2002	2003*
Chicken:				
- Robusta Maculata	67	70	60	60
- Robusta Lionata	56	55	50	60
- Ermellinata di Rovigo	84	87	70	77
- Pépoi	82	83	59	53
- Padovana Dorata	-	82	81	73
- Padovana Camosciata	-	89	79	87
Duck:				
- Germanata Veneta	70	71	56	64
- Mignon	50	86	44	64
Turkey:				
- Bronzato Comune	63	87	80	75
- Ermellino di Rovigo	55	47	67	40
Helmeted guinea fowl:				
- Camosciata	50	45	55	59

\* data of Conegliano breeding unit were not yet available.

Table 7. Trend from 2000 to 2003 of the percentage of chicks born alive on eggs hatched of the 11 local poultry breeds.

Breeds	2000	2001	2002	2003*
Chicken:				
- Robusta Maculata	46	49	34	42
- Robusta Lionata	48	53	33	49
- Ermellinata di Rovigo	69	70	51	65
- Pépoi	61	65	39	35
- Padovana Dorata	-	51	49	42
- Padovana Camosciata	-	66	68	64
Duck:				
- Germanata Veneta	54	50	24	42
- Mignon	37	31	21	40
Turkey:				
- Bronzato Comune	49	46	52	53
- Ermellino di Rovigo	29	20	20	15
Helmeted guinea fowl:				
- Camosciata	42	36	42	45

\* data of Conegliano breeding unit were not yet available.

Table 8. Number of individuals genotyped for estimating heterozygosity per each local poultry breed over years.

Breeds	2001	2002	2003
Chicken:	75	239	145
- Robusta Maculata	19	63	36
- Robusta Limonata	13	27	24
- Ermellinata di Rovigo	20	41	29
- Pépoi	13	63	36
- Padovana Dorata	10	25	10
- Padovana Camosciata	not available	20	10
Duck:	25	50	68
- Germanata Veneta	13	25	30
- Mignon	12	25	38
Turkey:	17	59	34
- Bronzato Comune	13	47	22
- Ermellino di Rovigo	4	12	12
Helmeted guinea fowl:			
- Camosciata	21	36	35

Table 9. Trend of heterozygosity (%) estimated by AFLP markers for the 11 local poultry breeds.

Breeds	2001	2002	2003	average annual change
Chicken:	34	38	41	3.5
- Robusta Maculata	16	24	25	4.5
- Robusta Lionata	20	28	25	2.5
- Ermellinata di Rovigo	20	32	30	5.0
- Pépoi	17	25	21	2.0
- Padovana Dorata	21	18	27	3.0
- Padovana Camosciata	not available	15	26	13.0
Duck:	37	41	40	1.5
- Germanata Veneta	19	29	23	2.0
- Mignon	16	29	20	2.0
Turkey:	32	34	36	2.0
- Bronzato Comune	26	29	32	3.0
- Ermellino di Rovigo	16	15	15	-0.5
Helmeted guinea fowl:				
- Camosciata	30	25	32	1.0
Overall mean	20.1	24.5	26.1	3.0

Figure 1. Robusta Maculata (PRM).



Figure 2. Robusta Lionata (PRL).



Figure 3. Ermellinata di Rovigo (PER)



Figure 4. Pépoi (PPP)



Figure 5. Padovana chicken breeds:

Padovana Camosciata (PPC)



Padovana Dorata (PPD)





Figure 6. Germanata Veneta (AGV).



Figure 7. Mignon (AMG) duck breed

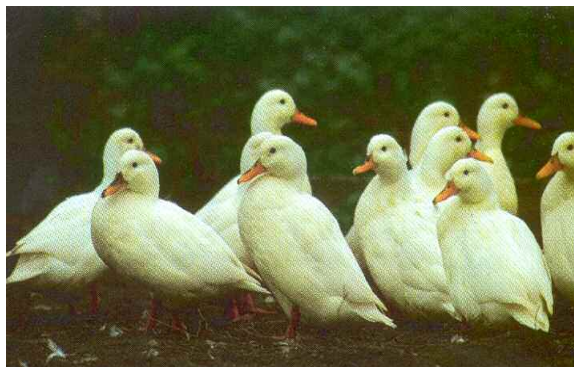


Figure 8. Ermellinato di Rovigo (TER)



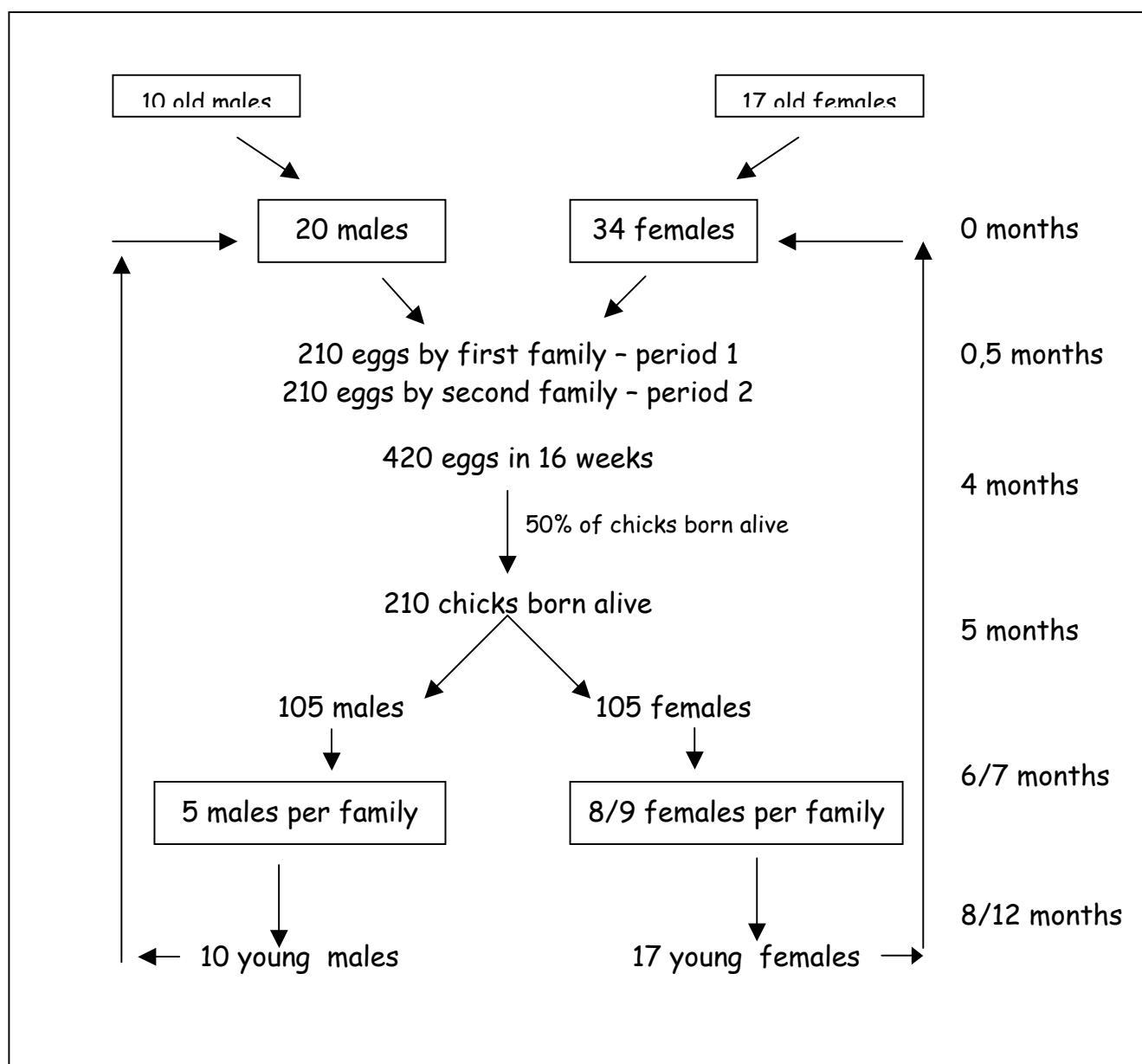
Figure 9. Bronzato Comune (TCB).



Figure 10. Camosciata (FCM) helmeted guinea fowl breed.



Figure 11. Breeding scheme of Co.Va. poultry breeds.



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