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TRACEABILITY OF SWINE MEAT: AN EXPERIENCE BY EID+DNA IN "SUINO TIPICO SARDO"

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Abstract - 20 suckling piglets, "Suino Tipico Sardo" branded, at 1-7 days old and at an average weight of 2514±895 g, have been electronically identified (EID) by means of intraperitoneal transponders (HDX 32.5×3.8 mm, 134.2 kHz) and from each of them an auricle sample has been kept for DNA analysis. The readability of the transponder of each piglet has been detected by static and dynamic readings, *in vivo* and *post mortem* and was 100%. In the slaughtering chain, from each piglet, slaughtered at 29-35 days of age and at an average weight of 8902±1944 g, a second auricle sample has been kept for DNA analysis. The matching between the two samples of each piglet has been investigated by a genetic test, deploying n. 6 microsatellites ascribed in the FAO-ISAG panel for swine species. The DNA analysis of each electronically identified piglet confirmed the correspondence between the in vivo and post mortem samples. The pairing of EID+DNA can improve the traceability in the commercial net of the "Suino Tipico Sardo".

Introduction - The roasted meat of the suckling piglet "*porceddu arrustìu*", "*porcheddu a ispidu*", probably represents the most characteristic speciality among the regional dishes of Sardinia (Italy) (**2, 5**). It is a niche product from a scattered productive chain of the swine rearing, if we take into account the limited productive power of the region that is just the 2,56% of the pigs reared in Italy (**8**). For local farmers rearing a few sows in more than 17.700 small farms spread all over the isle's territory (**1**), as a matter of fact, the main problems are due to the introduction in the regional market of imported piglets, sold and passed off as local products (**6**). Without omitting the importance of the commercial fraud, an adequate system of traceability across the productive chain can be a more fitting support to protect this limited regional production, applied to the specific conditions of a scattered chain. In previous works, Pinna et al. **(9, 10, 11)** reported the results of experimental trials, about the deployment of the intraperitoneal electronic identification of animals, by means of injectable transponders. In the present paper, Authors take into account the combination of EID+DNA, *in vivo* in farm and *post mortem* in the abattoir, by pairing the anagraphical datum based on electronic identification (EID), to the genetic profile (DNA) of the suckling piglet produced according to "Suino Tipico Sardo" disciplinary.

Materials and Method - 20 suckling piglets at 1-7 days old and at an average weight of 2514±895 g have been electronically identified (EID) by means of injectable transponders, according to Caja et al. technique (**4**). HDX encapsulated in bioglass transponders 32.5×3.8 mm, 134.2 kHz (TIRIS™) have been used, in agreement with ISO11784 and 11785 Standards. Together with the electronic identification, each piglet has been also identified by means of ear tag. Transponder's and ear tag's codes have been paired directly on a handy reader Gesreader

2S ISO® (Gesimpex Com. S.L., Barcelona, Spain). During the application of the ear tag by means of a pincer an auricle sample has been stored in a tube 2 ml and frozen at -20°C in the laboratory. The anagraphical identity of each piglet has been verified weekly *in vivo* in farm, by controlling transponder's and ear tag's codes. Animals have been clinically checked all over the experimental trial, in order to reveal possible effects due to the transponders' presence in the animal body. The presence of the ear tags and the functioning of the transponders have been checked *ante* and *post mortem* (29 days after the identification) in the slaughterhouse. In particular, in each of the main phases of the slaughtering chain, the effective readability of the transponders has been tested by means of the abovementioned reader [at unloading of animals from the mean of transport to the boxes before slaughter, immediately before entering the slaughtering chain, after the electric stunning (1.1 sec, 100 Volts and 0.9 Å), after jugulating, after scorching of hairs by a gas blowpipe, after washing under high pressured hot/cold water (60°-15°C), and before and after evisceration]. Readability (R%), defined as ability of transponder to be operative in the animal's body and possibility to be detected by handy reader, was calculated by the formula:

R (%) = (number of read transponders / number of piglets with transponder)*100

After evisceration, the carcasses of the piglets underwent veterinary inspection to reveal possible lesions due to the presence of the transponder in the abdomen cavity. Then, the fatness has been evaluated, the carcass has been weighed and the carcass yield has been calculated. Each transponder collected during the disembowelling has been enclosed to the respective carcass that went on the slaughtering chain to be branded with "Suino Tipico Sardo". By this moment, a second auricle sample from each carcass has been stored together with the respective transponder in a tube 2 ml and frozen at -20°C. From *in vivo* and *post mortem* auricle samples of each piglet, the DNA has been extracted according to an usual internal method of our laboratory, and amplified with PCR, deploying 6 microsatellites [SW122; IGF1; SW240; S0068; SW72; S0225] (**12**), among those suggested for swine species of FAO/ISAG panel (**13**). PCR products have been separated and evidenced by capillary electrophoresis by ABI PRISM 3100 Genetic Analyser (Applied Biosystem) and data elaborated by the soft ware of the equipment. The genetic profiles of the 2 DNA samples of each piglet have been compared to underline possible non identity cases.

Results and Discussion - The clinical and wellbeing checking of the electronically identified piglets confirm what already reported in previous works by Pinna et al. (10, 11). Table 1 shows live weights, the average daily gain and the readability of transponders' and ear tags' codes. Data reported agree with Pinna et al. (10, 11). Anyway, while *in vivo* readability of transponders was 100%, at arrival at the slaughterhouse 2 piglets were lacking of eartags, letting record a eartag loss rate of 10%. The reading of the transponder code by handy reader was easy and immediate. While the reading of the code of the eartag constantly took a longer time because of the need to restrain the animal and to remove the dirt accumulated on the eartag itself. Table 2 shows the readability of the transponders across the slaughtering chain, the transponder's collection and the *post mortem* productive performance. The readability of the transponders across the different stages of the slaughtering chain was 100% and didn't cause any delay of the operator's work, until the evisceration. Actually, even if the collection of transponders was 100%, a delay of the operator at disembowelling activity occurred. It was substantially due to operator's care in recovering the transponder from abdomen cavity. 15 transponders (75%) were collected in abdomen cavity and 5 transponders (25%) were enveloped by omentum fat. No lesions on abdomen viscera have been detected at post mortem veterinary inspection. The ear tags had to be removed from piglets right before the scorching of hairs, in order not to get burnt. Therefore, for piglets identified by ear tags the *post mortem* traceability, across the slaughtering chain, stopped after jugulating while for those electronically identified arrives until the evisceration. As *in vivo* relieves, also *post mortem* productive performance of piglets were in agreement with previous works by Pinna et al. (**10, 11**). All of the transponders collected were functioning after the period of freezing at -20°C. The auricle tissue matrix resulted to fit the experimental needs as it was suitable for *in vivo* and *post mortem* sampling. DNA amplification was unsatisfactory for 2 samples, the rest of 38 samples has been amplified by means of the pool of the 6 microsatellites that allowed to distinguish univocally all of the piglets by their genetic profiles. No cases of non identity between the *in vivo* and *post mortem* samples from the same piglet happened. Fig 1 shows anexample of the electropherogram of allels in locus microsatellite [SW240], from 2 piglets *in vivo* and *post mortem* samples.

Conclusions - Results obtain in this experimental trial in farm and in the abattoir, highlight a better reliability and accuracy of the electronic identification than ear tags for traceability needs in suckling piglets "Suino Tipico Sardo. The coupling of electronic identification (EID) and genetic test by means of microsatellites (DNA) commonly available for swine species appears a concrete tool for practical deployment, into a perspective. It is predictable, in short/middle time, the availability of cheaper and cheaper electronic identifiers and set for biomolecular analysis that can allow the spreading of the tested system EID+DNA for swine meat traceability.

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Table 1 - Live weight, averaged daily gain (M±SD), transponders' readability and eartags' loss

	Live weight (g)	T readability	Eartags loss
Number of animals	20	20	20
Transponder application (1 d)	2514 ± 895	100%	0%
Transponder recovery (29 d)	8902 ± 1944	100%	10%
Averaged daily gain (1-29 d)	211 ± 72		

Table 2 - Transponders readability, collection and *post mortem* productive performance

	Readability of transponders	Transponder collection	Productive performance
Number of animals	20	20	20
Electric stunning (%)	100		
Jugulation (%)	100		
Scorching (%)	100		
Washing (%)	100		
Evisceration (%)	100		
Transponder collection (%)		100	
Transponder free in abdomen cavity (%)		75	
Transponder enveloped in omentum fat (%)		25	
Fatness (score 1÷5)			3±0.25
Carcass averaged weights (g)			7648±1896
Carcass warm yield (%)			86±3

Figure 1 - Electropherogram of allels in locus microsatellite [SW240], from 2 piglets obtained by automatic analysis (*in vivo* and *post mortem* samples),