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PHENOTYPIC APPROACHES IN LIVESTOCK PRODUCTIONS: NEW METHODS AND TECHNOLOGIES FOR SUSTAINABLE SYSTEMS

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I dedicate this work to GOD Almighty for His infinite mercies and grace.

"*In all your ways acknowledge Him and He shall direct your path"*

Proverbs 3: 6.

I would like to thank my supervisor Professor Leonardo Nanni Costa for his tutorship and intellectual leadership. A special thank you goes to Professor Stefania Dall'Olio for her support and encouragement during my studies. I am also grateful to Doctor Martina Zappaterra for all the help and support.

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SUMMARY

During transport, the pigs are exposed to stressful and uncomfortable conditions like loading, unloading, mixing with unfamiliar animals, and lairage that can have adverse effects on welfare and pork quality traits. It is well known that response to the journey is influenced by the genotype. However, not much is known on the effects of transport stress on the welfare and the meat quality traits of local pig breeds such as Apulo-Calabrese.

The present thesis investigated the effect of transport stress on the welfare and meat quality traits of Apulo-Calabrese in comparison with crossbreed [Duroc x (Landrace and Large White)] pigs. For this purpose, all the animals were blood sampled five days before transport and at exsanguination for the analysis of blood stress parameters. Apulo-Calabrese pigs were slaughtered at 135 kg live weight while crossbreed pigs were slaughtered at 155 kg live weight. Meat quality traits such as pH, color, drip loss, cooking loss, Warner–Bratzler shear force, and intramuscular fat (IMF) were assessed on the *longissimus thoracis* (LT) muscle. In addition, water status was assessed from LT samples using transverse relaxation time (T2) weighted signals registered by Time Domain Nuclear Magnetic Resonance (TD-NMR), and fatty acid composition was determined by Folch method. Data were analyzed using mixed models and principal component analysis.

The results are presented in three manuscripts: In chapter 1, the effect of short distance transport on the behavioral occurrences, blood parameters, and meat quality traits was investigated. Although a large number of the measured blood parameters were within the normal physiological range, Apulo-Calabrese pigs showed the highest value of exsanguination lactate when compared with crossbreeds which indicate a more intense physiological response. Meat quality traits were similar for both genetic types. However, significantly higher a* and lower L* coordinates were found in the samples of Apulo-Calabrese which showed meat with a deeper red color than crossbreeds. In chapter 2, results from the TD-NMR showed higher percentage of extra-myofibrillar water in the samples of Apulo-Calabrese which was in agreement with the higher values of cooking loss found in this breed

at 24 and 72 hours postmortem. The IMF content and fatty acid profile between the two genetic types of pigs have been shown in chapter 3. Except for heptadecenoic acid, there were no great differences in the *longissimus thoracis* muscle fatty acid profile between the two genetic types.

The work underlying this thesis provided insight on the effects of pre-slaughter handling and transport on the well-being being and meat quality traits of Apulo-Calabrese pig. The present study supports the hypothesis that fresh meats obtained from crossbreed and Apulo-Calabrese pigs fed the same diet and reared in the same environment have similar quality traits.

INTRODUCTION

Transport

Road transportation of pigs is an essential process in the production chain that occurs during the pigs' life event for purposes such as fattening, breeding, marketing, and slaughter. Transport and its associated handling may impose uncomfortable conditions of stress (feed and water deprivation, contact with stockmen, repeated regrouping, vibrations and changes in acceleration, high stocking densities, unfamiliar sounds and noise, extreme temperatures, and driving methods) which affect various behavioral, physiological and metabolic changes in the animal (Dalla Costa et al., 2007; Gajana, Nkukwana, Marume, & Muchenje, 2013; Rocha, Velarde, Dalmau, Saucier, & Faucitano, 2016; Sutherland, McDonald, & McGlone, 2009; Torrey et al., 2013). As an example, Bergmann (1979) observed grave ultrastructural change in the cardiac and skeletal muscle of pigs immediately after loading and transport stress. Furthermore, stressful conditions during transport may cause significant changes in live weight (Garcia et al., 2015), as well as blood concentrations (Averós, Herranz, Sanchez, Comella, & Gosalvez, 2007) and other bodily parameters such as heart rate (Goumon et al., 2013). If the process of transportation is too stressful, the pig may become fatigued, non-ambulatory, or eventually die (Sutherland et al., 2009). Mortality during transit is a reflection of the level of stress experienced by the pigs and can vary between 0.03% to 0.5% for journeys less than 8 h within the European Union (Temple et al., 2014). The range of variation is dependent on the conditions of transport and genotype (Lambooij, 2000; Warriss, 2000). According to Warriss (2000) stress-susceptible breeds of pigs, which often have better carcass conformation and greater muscular development are much more prone to die during transport.

In the past decade, the welfare of transported animals has become an issue of great importance amongst consumers, stakeholders, and animal welfare professionals resulting in legislation being passed in the European Union (Council Regulation 1/2005/EC). A recent survey in Europe showed that consumers were willing to change their place of shopping in order to buy animal-friendly

products (Velarde & Dalmau, 2012). Additionally**,** high standards of animal welfare are essential for productivity, profitability and to meet requirements set by domestic and international markets (IFC, 2014). Extensive literature on the effect of transport in pigs exists (Averós et al., 2007; Broom, 2003; Dalla Costa et al., 2007; Gajana et al., 2013; Weschenfelder et al., 2013). According to these authors, the duration of the journey, vehicle design, climatic conditions, stocking density, and driving methods are factors that can negatively affect the welfare and pork quality traits. Nevertheless, most of the studies have been carried out in commercial pigs and their crossbreeds but scarcely investigated in local breeds.

It is well known that the pig's genetic background may influence the response of the animal to stress (Lambooij, 2000). As an example, pigs with the halothane genotype are more susceptible to stress and are characterized by meat with a higher lean carcass content compared with pigs from the halothane-free population (Fàbrega et al., 2002; Gispert et al., 2000)**.** Additionally, mortality in transit has been reported to be higher in stress susceptible breeds like Pietrain and Belgium Landrace (Warriss, 1998)**.** In a previous study, Li et al. (2008) observed distinct coping strategies in Pietrain pigs and Erhualian local pig breeds based on variations in their behavioral, endocrine, and biochemical response during transport. More recently, specific rearing and transport conditions were found to influence indexes of stress in the French local Basque pig (Lebret et al., 2015)**.** In Italy, little or no evidence exists on the effect of transport stress on the behavioral, physiological, and meat quality traits of native pig breeds although these breeds are a source of raw material used in the production of protected designation of origin (PDO) salami.

At the beginning of the twentieth century, there were twenty-one local pig breeds in Italy. The post-war transformations (Mascheroni, 1927) in the agricultural system, the intensification and industrialization of pig farming, the modification in land use, and the massive utilization of foreign high productive breeds caused the decline and risk of extinction of the local breeds. The Italian pig breeds that showed greater resistance to their replacement with conventional breeds are mainly the

breeds of Central and Southern Italy (Franci & Pugliese, 2007). Among those of the Southern region is the Apulo-Calabrese pig (Franci & Pugliese, 2007).

In the last decade, there has been a renewed interest in Italian autochthonous pig breeds in particular for the Apulo-Calabrese pig, previously known as Nero Calabrese (Micari et al., 2009). This pig is included in the list of endangered breeds by FAO (Bittante, 2011) and registered in the herd book of National Breed held by the Italian National Association of Pig Breeders (*Associazione Nazionale Allevatori Suini*, ANAS).

Apulo-Calabrese is a black-skinned, medium-sized pig with small socks on the forelimbs and large socks on the hind limbs. It has a long head, front lop ears, and strong forelegs and shows no excessively developed muscles but a strong skeleton (Maiorano, 2009). This breed is distinguished by strong maternal instinct in the sow and distinct sexual energy in the boar (Micari et al., 2009). Similar to the other Italian local pig breeds, Apulo-Calabrese is characterized by late sexual maturity, low prolificacy, reduced growth, and carcass performance (Franci & Pugliese, 2007). It is an excellent grazer, noticeable for its remarkable frugality and feeding versatility (Micari et al., 2009).

This breed has a strong resistance to illness, zootechnical adaptability to different climatic conditions as well as the ability to procure food thanks to their strong inclination to grazing, high rusticity, and energetic temperament (Maiorano, 2009).

In the year 2017, the breeding population counted 540 sows and 63 boars reared in 63 farms, of which 31 can be found in the Calabria region (http://anas.it). This breed is well adaptable to different production systems and can be reared outdoor and indoors in a conventional system (Pugliese & Sirtori, 2012). Meat from Apulo Calabrese is excellent for the fresh meat market and suitable for the production of Protected Designation of Origin (PDO) salami (*Salsiccia*, *Soppressata, Capocollo, and Pancetta*), typical of the Calabria region (Micari et al., 2009).

However not much is known about the response of this breed to the pre-slaughter handling procedures.

The pre-slaughter handling procedure and treatment includes all the activities an animal undergoes before sticking such as mixing of unfamiliar animals, loading, transport, unloading, lairage conditions, and driving to the stunner (Rosenvold & Andersen, 2003). Exposure to these activities may induce either psychological or physical stress with implications on welfare and meat quality attributes.

Rearing system

Pre-slaughter treatment and handling involve the exposure to novel stimuli like handling, loading, and unloading, mixing with unfamiliar animals, unusual sounds, and motion of the lorry that elicit a stress response (Lambooij, 2000). The reaction of pigs to the pre-slaughter handling procedures is dependent on their prior experience (Terlouw, 2005) which is influenced by the rearing system (De Jong et al., 2000; De Jonge et al., 1996; Petersen et al., 1995). In a previous study, pigs reared in a barren environment showed a higher behavioral reactivity and were likely to experience more stress during pre-slaughter handling procedures than their counterparts from the enriched environment. Furthermore, pigs from the barren environment showed more manipulative social behavior like biting, noising, and massaging of pen mates than pigs from the enriched environment (De Jong et al., 2000). According to Geverink et. al. (1999) pigs from the intensive system (barren treatment) were easier to handle during the pre-slaughter handling procedure than their counterparts from the extensive system (enriched treatment). In contrast, Warriss et. al. (1983) observed that extensively housed pigs were easier to load at slaughter than intensively housed pigs. The extent to which the animals can cope with the stress of transport is not only of importance to their wellbeing but a decisive factor in the ultimate meat quality. It has been suggested that the free-range housing system may lead to an increased glycogen level before slaughter indicating meat with a higher incidence of pale, soft, and exudate meat defect (Geverink et al., 1999). More recently, Lebret et al. (2015)

indicated that the extensive rearing system influenced the level of stress at slaughter as well as the physicochemical traits of the meat.

In the present study, pigs of both genetic types were reared in the indoor system and fed the same commercial diet to eliminate the effect of the rearing system on the behavioral response, blood parameters, and meat quality traits.

Loading

The day of transport involves the transfer of pigs through alleys and chute into the vehicle. The movement of pigs by unfamiliar stockmen, regrouping and the change from a known to an unknown surrounding may induce physical and psychological stress (Bradshaw et al., 1996; Correa et al., 2013). In addition, the pigs may become fatigued and more difficult to handle (Faucitano, 2001) resulting in the excessive use of driving tools. The increase in heart rate during loading is proportional to the slope of the ramp as well as the physical effort needed to negotiating them (Correa et al., 2013). As consequence, loading is considered to be a critical phase in the transport process.

The design of the loading facility may influence the well-being and meat quality of pigs. Steep loading ramps may affect the ease of handling (Garcia & McGlone, 2015) since for pigs, climbing a loading ramp can be psychologically disturbing (Lambooij, 2000). According to Grandin (1987), the ramp should not exceed 20 degrees for a non-adjustable ramp and 25 degrees for an adjustable ramp. Currently, the guidelines available recommend the use of ramps with slopes below 20° (Garcia & McGlone, 2015).

The stress of being handled through a steep ramp (up to 22° slope) may increase the number of dead on arrival (DOA), downers, and pigs with rectal prolapses (Correa et al., 2013). Moreover, the lack of the appropriate bedding on the ramp during loading may increase the incidence of slipping, falling, bruises, and vocalization (Garcia & McGlone, 2015). Behavioral response during loading is

an indication of the animal's aversion to the situation. According to Aradom et al.(2012) behaviors like rooting, reversal and vocalization indicate higher severity of stress during loading than unloading.

The time taken to load pigs into vehicles may influence meat quality. According to Guàrdia et al. (2004) longer loading time may result in a higher risk of carcass bruises. Again, Ritter et. al (2007, 2008) observed a significant effect of the distance (61 to 90 vs 0 to 30 m) that pigs were moved on the proportion of pigs showing signs of fatigue (open mouth breathing and skin discoloration) during loading and on the incidence of non-ambulatory, injured pigs at the plant.

According to Grandin et al. (1987) the breed of the livestock can affect the way they react to handling. As an example, Yorkshire hogs took more time to load and moved more slowly into a truck than the Pietrain breed (Grandin, 1987). In a previous study, loading at different farms either alone or interacting with distance was shown to decrease welfare, increase mortality, and carcass problems (Gosálvez et al., 2006). In contrast, Averós et. al. (2008) observed no effect from loading pigs from different farms on the risk of mortality during transit.

In the present study, pigs were loaded from the same farm to eliminate the effect of the farm on the loading time.

Transport Duration

The duration of the journey is an aspect of transport that can affect the welfare and meat quality of pigs (Bradshaw et al., 1996; Mota-Rojas et al., 2006; Pérez et al., 2002; Warriss, 1998). According to Werner et al. (2007) long (8 h) as well as short (1 h), transportation increased the mortality rate of the animal both during and after transport and affected the welfare of the animal based on the percentage of pathological findings. In a previous study pigs subjected to short transport (15 min) showed a more intense physiological response and poorer meat quality than pigs subjected to moderately long (3 h) transport (Pérez et al., 2002). According to this author, the effect of transport

time on the welfare and meat quality was more important than the genotype. Hence, the detrimental effect of the short journey maybe because the pigs may not have had enough time to recover from the pre-slaughter stressful procedures.

When transporting pigs to the parking plant in the USA, Sutherland et al. (2009) reported an increase in the percentage of dead and injured pigs as the duration of the journey increased from 0.5 to 4 h but indicated a decrease in risk of mortality for journeys lasting between 5 and 11 hours. Moreover, Mota Rojas et al. (2006) observed that animals transported under acute stress showed a higher degree of bruising and pale carcasses.

In contrast, Weschenfelder et al. (2013) indicated a larger impact of genetics on the animal welfare variables and pork quality traits in a study on the effect of vehicle design on three Pietrain crosses during short distance transportation.

In a previous study, Averós et al. (2008) reported that the risk of mortality in relation to journey duration increased not only with temperature and journey conditions but also when the pigs have not fasted. The results of these studies indicate that interacting factors of genetics and transport conditions may influence the response of the animal to transport stress.

Unloading

During unloading, the animals are exposed to a novel environment and handling that may cause fear (Dalmau et al., 2009). According to Lambooij et al. (1996) pigs should be unloaded as soon as possible upon arrival at the slaughterhouse and this must be done carefully, because ventilation in stationary vehicles is often poor, reducing air quality. Goumon et al. (2013) also recommend the use of appropriate bedding and access to adequate water after unloading.

The slope and the slipperiness of the unloading floor may induce fear and increase stress levels (Gregory,1998). Additionally, the pigs may show reluctance to move (Dalmau et al., 2009) with a consequent increase in the unloading time (Garcia & McGlone, 2015; Weschenfelder et al., 2013).

According to previous studies, pigs should be unloaded by compartment rather than by deck in small groups to reduce the occurrence of slipping, jamming, overlapping, and vocalization (Faucitano & Schaefer, 2008; Rabaste et al., 2007).

Lairage

The main purpose of lairage is to ensure a continuous supply of pigs to the slaughter line. Time in lairage also serves as a recovery period for pigs after previous stressful handling (Warriss, 1987). Furthermore, lairage is an important factor that can cause variation in meat quality (Nanni Costa et al., 2002) as mistakes made at this point are irreversible (Faucitano, 2018). Longer lairage times have been shown to improve meat quality by decreasing the incidence of PSE but increased the presence of DFD meat (Dokmanovic et al., 2017; Dokmanović et al., 2014; Guàrdia et al., 2009; Nanni Costa et al., 2002; Warriss et al., 1998) Furthermore overnight lairage may decrease backfat thickness (BFT) and increase the proportion of pigs with severe skin damage due to fighting (Nanni Costa et al., 2002; Warriss et al., 1998).

Short or no lairage may increase the proportion of meat with PSE (De Smet et al., 1996; Driessen et al., 2020; Fraqueza et al., 1998; Van de Perre et al., 2010) and increase in the levels of lactate, cortisol, and creatine kinase in the blood (Pérez et al., 2002; Saco et al., 2003; Salajpal et al., 2005). Thus, an optimal lairage time between 2-3 hours has been recommended (Milligan et al., 1998; Van Der Wal et al., 1997; Warriss et al., 1998; Zhen et al., 2013)**.** In a previous study, Van der Wal et al. (1999) observed that pigs became calm and stopped fighting after approximately 2 h in lairage. However the impact of lairage time may depend on the lairage conditions, the stress experienced by pigs during transport, and the genotype of the animal (De Smet et al., 1996; Warriss et al., 1998).

In a previous study, De Smet et al. (1996) observed a more pronounced effect of lairage time in stress susceptible pigs (nn pigs) than in stress-resistant pigs. Additionally, Warriss et al (1998) observed that more stress susceptible pigs reacted differently from fatter potentially more resistant animals during lairage. Previous authors have shown that a recovery period in lairage is less

important under low pre-slaughter stress in pig populations without the halothane gene (Aaslyng & Barton Gade, 2001).

Stunning

Transfer to the stunning system and restrainer before slaughter also influences an increase in the stress level of the pigs and can increase the heart rate up to 200–250 beats/min (Álvarez et al., 2009). Poor handling immediately before stunning has been shown to give a higher temperature in muscle and a faster pH fall early post mortem, as well as a higher drip loss and a greater PSE-incidence than careful handling (Barton-Gade, 1984). Furthermore, severe stress one minute before stunning reduced the ultimate pH and increase drip loss of pork (Hambrecht et al., 2005; Rabaste et al., 2007; Van Der Wal et al., 1999). Additionally**,** Intense stress before stunning provoked by rough treatment increased concentrations of cortisol and lactate (Hambrecht et al., 2004). In a previous study, Betram et al. (2002) demonstrated a noticeable effect of stunning on post-mortem phosphorus metabolism, post-mortem pH development, and water holding capacity of meat.

Behavioral Measurements

The effect of transport stress on the welfare of pigs could be assessed through various stress indicators. Among these, behavioral measurement is the most basic and direct method since they include the individual animal (Broom, 2003). Assessment of behavior during transport provides information about how the animals adapt and cope and show where modifications are necessary for improving transport procedures (Lambooij, 2000)**.** Behavior occurrences like turning back, vocalization, reluctance to move, kicking and retreat attempts are often a sign of distress and discomfort and may indirectly be an indicator of animal fear (Broom, 2003; Dalmau et al., 2009)

The variations in behavioral response may not only be attributed to the condition of transport itself, but also to the coping style of different breeds of pigs (Geers et al., 1994). Notwithstanding, Weschenfelder (2013) observed no effect of breed on the behavioral response during loading.

Physiological Measurements

Blood parameters at exsanguination have been largely used to assess the stress of transport in livestock (Mota-Rojas et al., 2009; Probst et al., 2014; Werner & Gallo, 2008). The measurement of plasma constituents in the blood can provide information about the stress status of the animal (Shaw & Tume, 1992). In pigs, the physiological values of these parameters have shown variation between and within populations and can be influenced by genotype, breed, age, and environmental factors (Dikic et al., 2010; Geers et al., 1994; Reed & McGlone, 2000; Warriss et al., 1994).

The pre-slaughter handling procedures can induce stress which influences body homeostasis with a consequent increase in the activity of hormones and enzymes (Averós et al., 2007; Becerril-Herrera et al., 2010; Mota-Rojas et al., 2012; Mota-Rojas et al., 2009; Salajpal et al., 2005; Sommavilla et al., 2017). An animal's response to stress activates the hypothalamic-pituitary-adrenocortical (HPA) axis resulting in the secretion of cortisol into blood (Li et al., 2008). An elevated level of cortisol in the blood is an indication of physiological stress (Warriss, 1998) and includes the exposure of the animal to a new environment, and handling during loading and unloading (Bradshaw et al., 1996). According to (Warriss, 2000) transport stress increase the levels of cortisol, but longer periods in lairage allow them to recover**.** Moreover, Aradom et al. (2012) observed a significant increase in the levels of cortisol during short transport but the rate of elevation decreased with an increase in transport time. In a previous study, Désautés et al. (1999) observed breed differences in the neuroendocrine responses of the HPA axis following exposure to a novel environment.

Pre-slaughter stress can also activate the sympathoadrenal medullary axis, causing the release of catecholamine (Minton, 1994; Warriss, 2000). The rise of catecholamine increases blood glucose levels through the rapid breakdown of glycogen in the liver. (Mota-Rojas et al., 2012; Shaw & Tume, 1992).

Subjecting pigs to vigorous exercise will increase the levels of creatine kinase, lactate, and potassium due to the mobilization of energy reserve into the bloodstream and damage to the muscle

as a result of stress (Knowles, Warriss, & Vogel, 2014; Peinado, Fernandezarias, Zabala, & Palomeque, 1995).

In a previous study, Mota-Rojas et al. (2009) observed significantly higher values of creatine kinase (CK) after transport compared to baseline values. Also, Averós et al. (2007) found that CK increased significantly after unloading compared to before loading and that the level was slightly decreased in the exsanguination blood compared to after unloading**.**

According to Poltarsky et al. (1989) more stress-susceptible pigs generally have higher levels of CK than resistant pigs. In a study on the effect of transport stress on the breed-specific coping style, Li et al. (2008) observed significantly higher levels of CK in Pietrain pigs than in Erhualian pigs. In contrast, Lebret et al. (2015) observed no effect of breed on this measure after transport.

Higher levels of blood lactate may be due to pre-slaughter stress and have been shown to have a detrimental effect on pork quality (Edwards et al., 2010).

Total protein concentration is an index of dehydration and is increased by the loss of water from the blood as a result of long distance transportation (Bradshaw et al., 1996). In addition, levels may also be influenced by nutritional and metabolic factors (Swenson, 1993).

Transportation stress may cause an increase in plasma urea and creatinine concentrations, which indicates an increase in protein and nucleic acids breakdown in the muscles due to muscular activity and renal vasospasm produced by catecholamines (Warriss, 2000).

The activities of AST, ALT increased in the blood after tissue damage, poor muscular tissue, and fatigue and were as a result of an increase in the permeability of muscle membrane induced by loading and transportation stress (Guàrdia et al., 2009).

Mota Rojas et al. (2012) associated higher levels of sodium to transport stress while stunning conditions elevated the levels of potassium. In addition, Salajpal et al. (2005) observed significantly

higher levels of sodium and potassium in pigs when studying crosses of (Swedish Landrace x Large White) and (Duroc x Swedish Landrace) x Pietrain during a short lairage (2 h).

Whenever a physiological measurement is to be interpreted it is of significant importance to obtain basal levels of the measure (Dikic et al., 2010). As an example, plasma cortisol is highly variable, and different factors can influence its concentration such as the time elapsed from stress to sampling, secretion due to diurnal pattern, genetics, and the effect of chronic stress (Désautés et al., 1999; Mormède et al., 2007).

Meat Quality Assessment

Pork quality can be defined as a combination of different properties which includes aspects of importance for consumer acceptance and technological qualities such as pH, color, water holding capacity, and texture which are all affected by the post-mortem pH fall (Van Der Wal et al., 1997). According to this author, the handling of the animals on the day of slaughter may cause variations in pork quality.

It is generally accepted that stressful conditions before slaughter may lead to increased glycogen depletion and a greater decrease in glycogen store resulting in rapid acidification of the meat with possible implications for the development of pale, soft, and exudative (PSE) condition. According to Lambooij (2000), this explanation is simply postulated, because the physiological response to stress from the environment is partly influenced by the genotype (the coping ability of the animal). Different genetic types of pigs under the same production system, subjected to identical pre-slaughter handling may show distinct values of meat quality parameters. Furthermore, the type of animal being handled and exposed to stress is essential in determining the extent to which the meat quality can be compromised (Adzitey, 2011). As an example, animals of a very stress-resistant nature may hardly ever produce meat with PSE irrespective of poor handling (Warriss, 1987). Conversely, pigs that are susceptible to stress, such as those with the halothane genotype may

produce meat with PSE no matter how careful the pre-slaughter handling is, because the trauma of slaughter alone can initiate its development (Briskey, 1964; Channon et al., 2000; Warriss, 1998). On the other hand, an increase in muscle pH beyond normal levels as a result of prolonged stress may produce meat that is DFD.

In addition to the pre-slaughter handling procedures, the production system may influence the quality of pork (Bonneau & Lebret, 2010; Olsson & Pickova, 2005). According to these authors, pigs from an enriched environment subjected to careful handling during the husbandry phase may show better physiological and behavioral response at slaughter. Also, the diet of the animal may influence their growth rate and slaughter age which are important in determining the quality of the meat.

pH

The rate and extent of postmortem pH decline is an important factor in determining the final quality of meat as it affects the degree of protein denaturation, water holding capacity, and color of pork (Bidner et al., 2004; Gregory, 1998). Factors such as stress antemortem, the type of breed, and variation within the breed can influence ultimate pH (Terlouw, 2005).

Several authors have found ultimate pH to be higher in local pig breeds than in selected genetic types (Franci et al., 2005; Tomović et al., 2016; Wojtysiak & Połtowicz, 2014) indicating a slower rate of postmortem glycolysis in this breed.

Stress associated with transport may influence the muscle glycogen content which determines the postmortem pH fall. When an animal is subjected to acute stress, quick glycolysis takes place, and therefore, the meat gets acidified increasing the PSE meat likelihood (Mota-Rojas et al., 2006). Also, Perèz et al. (2002) observed a quicker post-mortem pH fall in pigs after a short distance transported (15 min). According to Klont et al. (1995) the genotype effect on postmortem pH fall was minimal under low pre-slaughter stress conditions. Notwithstanding, Nevrkla et al. (Nevrkla et al., 2017) observed a more favorable pH in the Prestice-Black Pied (PBP) breed than in crossbreeds

of Large White x Landrace sows and Duroc x Pietrain boars (LWLDP). The pigs in this study were transferred for a distance of 40 km although details on the conditions of transport were not provided. Additionly, Maiorano et al. (2013) observed the highest pH value in the meat of native breed Casertana when compared with crossbreeds of (Landrace x Large White) x Duroc pigs when the animals were transported for a short time (35 min).

Color

Meat purchasing decisions are influenced by color more than any other quality trait. Due to this normal colored meat is preferred over meat that too dark or too pale because consumers use discoloration as an indicator of freshness and wholesomeness (Mancini & Hunt, 2005).

According to literature (Franci et al., 2005; Lebret et al., 2015; Maiorano et al., 2013; Tomović et al., 2016) local pig breeds are characterized by meat with a more intense red color than commercial breeds and their crossbreeds.

Genotypes with fewer discoloration result from the positive effect of increased pH (Van Oeckel et al., 2001). This is because the low pH values result in the partial denaturation of myoglobin and other muscle proteins which causes them to precipitate and reflect rather than absorb light (Huff-Lonergan et al., 2002). Additionally, high pH values reduce the amount of myoglobin denatured at a given temperature (Trout, 1989).

The rate of pH fall post-slaughter may be affected by the pre-slaughter handling and stunning conditions and this effect may depend on the genotype: the coping style of the animal (Channon et al., 2000). According to this author, the rate of muscle pH fall post-slaughter was faster in stress susceptible pigs (Nn) than stress-resistant pigs (NN). Also, pork from Nn was paler in color and had a lower solubility of sarcoplasmic and myofibrillar proteins than NN pigs. Additionally, Maiorano et al (2013) observed that meat from the native Casertana pig exhibited a redder color than commercial and crossbreed pigs after a short distance transport.

Aside from genetics and pH, the color of the meat may also be explained by the age of the animal (Franco et al., 2016; Zemva et al., 2015). In a previous study, myoglobin was reported to increase as age progresses and to be higher in the meat obtained from autochthonous pig breeds than in commercial breeds (Sans et al., 2004). Additionally, meat with higher myoglobin content displays a more intense reddish color (Karamucki et al., 2013).

Water holding capacity

The water holding capacity (WHC) is an important quality attribute for both consumers and producers. It determines visual acceptability, thus the consumers' willingness to purchase the product (Warner, 2017). Poor WHC will affect the texture and how the meat is perceived. Besides, the low water-binding capacity of meat may reduce yield with a consequent decrease in economic returns (Bertram, Stødkilde-Jørgensen, et al., 2002).

The handling of the animal before and after slaughter has the potential to greatly influence the rate of postmortem energy metabolism, lactate formation, and pH decline which affects the WHC of the meat (Muchenje & Ndou, 2011; Van Der Wal et al., 1999).

Long-term stress results in the depletion of glycogen in the muscle restricted lactate formation, and higher pH values. Meat with high pH tends to be dark firm and dry with a high WHC (den Hertog-Meischke et al., 1997). Conversely, stress applied immediately before stunning may decrease muscular glycogen reserve and lead to reduced WHC (Van Der Wal et al., 1999). The postmortem glycogen change is associated with the genotype of the animal. In a study on the use of NMR to elucidate the effects of pig breed on the biophysical and biochemical characteristics of meat. Straadt et. al. (2011) indicated an effect of breed on the post-mortem lactate formation which probably explained the variation in the WHC between breeds. Additionally, Schivazappa et al.(2002) observed significantly higher WHC in the Duroc pigs than in Large White and Landrace pigs and associated the result to the higher intramuscular content in the Duroc breed which decreased the water content and thus in water lost during compression for WHC assay.

Previous studies have reported better values in WHC for local breeds than in modern breeds and their crosses (Franci et al., 2005; Nevrkla et al., 2017). During an estimation of the WHC, Franci et al. (2005) observed better results for Cinta Senese (CS) than Large White (LW), and LWxCS crosses. In contrast, Maiorano et al. (2013) observed no effect of the genetic type on the WHC when comparing the meat quality traits of Casertana, Large White, and crossbred pigs.

Drip loss

Dripping is the loss of fluid from pork cuts and water evaporation from the shrinkage of muscle proteins (actin and myosin) in the form of drip (Muchenje & Ndou, 2011). Drip loss not only affects the final weight of the product but also the nutritive value because drip contains about two-thirds of the protein concentration of whole meat. Drip loss increases markedly with decreasing ultimate pH, through stronger myofibrillar shrinkage brought about by reduced electrostatic repulsion between the filament (Muchenje & Ndou, 2011).

Previous authors have reported better results for drip loss value in local pig breed in comparison to crossbreed pigs (Kasprzyk et al., 2015; Nevrkla et al., 2017). In a previous study, Nevrkla (2017) reported lower values of drip loss in Prestice Black-Pied pig (PBP) than in crossbred pigs. Similar findings were also described by Kasprzyk et al (2015) in Pulawska breed when compared with Polish Landrace pigs. According to Betram et al. (2002) the stunning method may have a noticeable effect on the values of drip loss. Moreover, it has been shown that pigs exposed to electrical stunning more generally had higher drip loss compared with pigs exposed to CO2-stunning (Casteels et al., 1995; Channon et al., 2000).

Cooking loss

Cooking loss is the percent weight difference between fresh and cooked meat in relation to the weight of the fresh meat (Honikel, 1998; Moelich et al., 2003; Muchenje & Ndou, 2011; Torley et al., 2000). The denaturation of proteins due to cooking results in structural changes that cause fluid to be expelled (Offer & Knight, 1988).

The amount of fluid loss during cooking is highly dependant on the cooking method, cooking time/temperature, and the endpoint temperature (Aaslyng et al., 2003; Pearce et al., 2011) but can also be influenced by the *postmortem* pH. High WHC and low pH results in a high cooking loss (Aaslyng et al., 2003) which implies that the meat is less juicy and tougher (Martens, Stabursvik, & Martens, 1982). Stress impose on the animals during transport may influence the postmortem pH fall with a consequent effect on the water holding capacity, acidity, structural linkage, and cooking loss (Muchenje & Ndou, 2011). In a previous study, the mixing of pigs from different batches resulted in pork with more cooking loss (Beattie et al., 2002). Also, other authors have shown an effect of the stunning method on the water content of meat (Bertram, Dønstrup, et al., 2002; Casteels et al., 1995; Channon et al., 2000).

Meat from pigs with the Rendement Napole (RN- gene) has been associated with high cooking loss due to the higher glycogen content and lower protein from RN^- carriers (Jonsäll et al., 2001; Lundström et al., 1996). Furthermore, Teixeira & Rodrigues (2013) observed higher cooking losses in commercial pig breeds than in Preto Alentejano. Additionally, Franci et al (2005) observed less cooking loss in the pigs of Cinta Senese when compared with Large White and crossbred pigs.

Shear Force

In general, meat tenderness is considered to be the most important palatability trait (Warner et al., 2010) for which consumers are willing to pay a premium. According to previous authors, consumers' eating satisfaction and intention to repurchase is primarily influenced by variations in tenderness in particular for toughness (Lee et al., 2012).

Among the many factors influencing pork shear force, genetics is one of the most important determinants that explain about 30% - 40% (heritability) of the variation in shear force (Miar et al., 2014). Tenderness is also positively correlated with intramuscular fat ($r = 0.32$) (Devol et al., 1988).

Several authors have reported meat from modern breeds seems to be more tender than traditional ones (Teixeira & Rodrigues, 2013; Wood et al., 2004). In a previous study, Franci et al (2005) reported that raw meat was tougher in Cinta Senese (CS) than in Large White and their crosses. The higher shear force in the meat of CS was associated to their higher age with an increase in the proportion and maturity of collagen (Mccormick, 1999).

Intramuscular fat

Intramuscular fat (IMF) is an important factor that influences consumer acceptance of fresh pork in terms of texture and sensory properties (Rosenvold & Andersen, 2003). It is generally accepted that higher levels of IMF enhance the eating quality of pork (Bidner et al., 2004; Fernandez et al., 1999; Schwab et al., 2009; Wood et al., 2004) although this can be counterbalanced by possible rejection by consumers due to visible fat. According to Fernandez et al. (1999), optimal levels of IMF associated with an improvement in consumer perception of texture and taste is in the range of 2 – 3.5%. The positive correlation between IMF and eating quality has been associated with the breed of the animal (Wood et al., 2004).

In a study on the effect of breed on the muscle IMF content, Tyra et al. (2013) found that Polish Large White, Pietrain, and Polish Landrace pigs were characterized by lower IMF levels than Duroc and Puławska breeds. According to Pugliese & Sirtori (2012) the variation between local breeds and commercial breeds is better distinguished by evaluating intramuscular fat content. It is generally accepted that local pig breeds have a higher propensity for fat deposition than commercial breeds and their crosses and in some muscles, IMF levels have been reported to show variations from 3 to 10 % depending on the breed (Pugliese & Sirtori, 2012).

In a previous study, Nervkla et al. (Nevrkla et al., 2017) observed higher levels of IMF in the meat of the native PBP pigs than in LWLDP pigs. Similarly, Kasprzyk et al. (2015) showed an advantage of Pulawska pig in IMF levels when compared with Polish Landrace pigs.

Compared to modern breeds, local pig breeds have a higher potential to accumulate fat, which mainly contains more monounsaturated fatty acids and saturated fatty acids (Franci et al., 2005; Pugliese & Sirtori, 2012; Teixeira & Rodrigues, 2013). Aside from genetics, the fatty acid composition is affected by the dietary fatty acid intake, production system, and age of the animal (Wood et al., 2008).

SCOPE OF THESIS

The thesis aimed to fill the gap in knowledge on the effect of transportation on the behavioral response, blood parameters, and meat quality traits of Apulo-Calabrese with regards to crossbreed pigs.

Apulo-Calabrese pig is a local endangered breed from Calabria in the southern part of Italy. In recent decades, renewed interest in the conservation of endangered germplasm and the desire by consumers to rediscover typical products with almost forgotten flavors has led to the re-evaluation of many indigenous breeds such as Apulo-Calabrese from which products of the area are obtained.

However, many aspects concerning the Apulo-Calabrese pig have not been investigated. In particular, on the response of this breed to pre-slaughter handling and transport stress despite the increasing interest in the welfare and safety of animals during transport.

For this purpose, Apulo-Calabrese pigs and crossbred pigs were reared indoors and subjected to the same transport conditions in order to identify peculiar differences between the two genetic types.

The main subjects developed were:

To assess the physiological response and meat quality traits of Apulo-Calabrese after the short-distance transportation in comparison with crossbreed pigs.

To assess the water status in the meat of the two genetic types using transverse relaxation time (T2) weighted signals registered by Time Domain Nuclear Magnetic Resonance (TD-NMR).

To compare the fatty acid profile of Apulo-Calabrese with crossbreeds when fed a commercial diet.

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CHAPTER 1

Apulo-Calabrese and crossbreed pigs show different physiological response and meat quality traits after short distance transport.

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Summary

Despite the increasing interest in the welfare of animals during transport, very little is known on the response of local pig breeds to the transport procedures. This study aims to compare the effect of short journey on behavior, blood parameters, and meat quality traits in 51 Apulo-Calabrese and 52 crossbreeds [Duroc x (Landrace x Large White)] pigs. All the animals were blood sampled five days before delivery (basal condition) and at exsanguination for the analysis of creatine kinase, cortisol, glucose, lactate, albumin, albumin/globulin, total protein, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase, alkaline phosphate, sodium, and potassium. *Post mortem* pH, color, drip loss, cooking loss, and Warner-Bratzler shear force were measured at different times in *longissimus thoracis* samples. Univariate and multivariate analyses showed that glucose, albumin/globulin, urea, and AST at exsanguination were influenced by the genetic type. Apulo-Calabrese showed the highest increase in blood values of lactate, creatinine, sodium, and potassium after the short distance transport. Behavioral occurrences were similar in both genetic types during unloading and lairage. Small differences were observed for meat quality although significantly higher a* and lower L* were found in Apulo-Calabrese pigs, showing meat with a deeper red color than crossbreeds.

Introduction

Stress associated with transport has been documented in pigs by a large number of studies (Brandt & Aaslyng, 2015; Faucitano & Goumon, 2017; Terlouw et al., 2008). According to literature, transport stress can cause changes in the behavior and normal physiological function affecting negatively the welfare of the animals and meat quality attributes (Brown, Knowles, Edwards, & Warriss, 1999; Warriss, 1998). It is well known that response to the journey can be influenced by the genotype (Gispert et al., 2000), and factors such as temperature and humidity (Lambooy, 1988; Sutherland, McDonald, & McGlone, 2009), truck conditions (Dalla Costa et al., 2007; Torrey et al., 2013), transport, and/or lairage durations (Gajana, Nkukwana, Marume, & Muchenje, 2013) and handling of the animals (Rocha, Velarde, Dalmau, Saucier, & Faucitano, 2016). Blood parameters at exsanguination have largely been used to assess the stress of transport in livestock (Daniel Mota-Rojas et al., 2009; Probst et al., 2014; Werner & Gallo, 2008). In pigs, extensive studies on blood parameters after transport have been carried out in conventional commercial breeds and their crossbreeds, whilst little information exists on local pig breeds such as Erhualian (Li et al., 2008) and Basque (Lebret, Ecolan, Bonhomme, Méteau, & Prunier, 2015). Among the indigenous Italian pig breeds is Apulo-Calabrese which is included in the list of endangered breeds by the United Nations' Food and Agriculture Organization (Bittante, 2011) and registered in the herd book held by the Italian National Association of Pig Breeders (Associazione Nazionale Allevatori Suini, ANAS). In the year 2017, the breeding population counted 540 sows and 63 boars reared in 63 farms, 31 of which can be found in the Calabria region (ANAS, 2018). Apulo-Calabrese is a black-skinned, medium-sized pig with small socks on the forelimbs and large socks on the hind limbs (Micari, Racinaro, Sarullo, Carpino, & Marzullo, 2009). It is well adaptable to different production systems and can be reared outdoor or indoor in a conventional system (Pugliese & Sirtori, 2012). Meat from Apulo-Calabrese is processed into four Protected Designation of Origin (PDO) salami, typical of the Calabrian region (Micari et al., 2009). In Italy there is an increasing interest in the welfare of local breeds due to the growing consumer preference for PDO animal friendly products,

however existing research does not provide information on the response of this breed to the transport procedures. The aim of this study was therefore to investigate the effect of short distance transportation on behavioural response, blood parameters, and meat quality traits of Apulo-Calabrese with respect to crossbreeds.

Materials and Methods

Animals

Blood collection at the farm was carried out by a veterinarian in conjunction with routine sampling for sanitary controls. Transportation and slaughter of all pigs were carried out in compliance with EC regulation No 1/2005 and EC regulation No 1099/2013, respectively. Fifty-one Apulo-Calabrese pigs registered in the herd book of ANAS and 52 crossbreeds [Duroc x (Landrace x Large White)] were used. Apulo-Calabrese pigs were born in this farm from the mating of 13 sows by seven boars of Apulo-Calabrese whilst crossbreeds were bought at about 30 kg live weight from another piggery in the same region. All the pigs were fattened in the same finish facility in separate pens (7–10 pigs per pen) according to their genetic type and were fed the same commercial diet (14,644 KJ DE/kg, 155 g crude protein/kg, 22 g crude fat/kg, 80 g lysine/kg, 58 g ash/kg) in a liquid feeding system with dry feed and water mixed in a 1:4 ratio. All the pigs were identified by a numbered plastic ear tags. Apulo-Calabrese pigs were slaughtered when they reached 135 kg live weight (364 ± 58 days old) due to their slow growth whilst crossbreeds were slaughtered at approximately 155 kg live weight (300 ± 30 days old).

Pre-Slaughter and Slaughter

Approximately 12 h prior to transport, feed was withdrawn. Loading was carried out at 7 a.m. using a mobile ramp (length 4.5 m, width 0.7 m, with solid side walls of 1.0 m and adjustable height) available at the farm. Pigs were delivered through three consignments to the slaughterhouse. At

each delivery, pigs from the four pens were herded by electric prods and walked the same distance (25 m) to reach the ramp which was positioned in correspondence with the facility door. The lorry was a hydraulic three tier equipped with internal partition and mechanical ventilation on the left side. Pigs were transported for approximately 1 h to a local processing plant (Piano Lago, Cosenza, Italy) on two decks with a space allowance of about 0.50 m^2/100 kg live weight. Unloading was done using the ramp of the lorry and pigs were driven for 10 m to the lairage pens where they rested for 30 min. Outdoor temperature and relative humidity were recorded for each journey by a thermo-hygrometer (mod. HI9065, Hanna, Padua, Italy) during loading and unloading at the entrance of the ramp and at the entrance of the resting pen, respectively (Table 1). During transport and lairage, mixing between pens was avoided. The pigs were stunned by electrical tongs (head only; 220 V, 1.3 A). After stunning, exsanguination blood was collected from each pig (Table 1).

Behavioral Response

The behaviour recordings during loading at the farm and unloading at the abattoir included slipping, falling, reluctance to move, turning back, overlapping, and vocalization as previously describe (Correa et al., 2010; Dalmau, Temple, Llonch, & Velarde, 2009). Lying, sitting, and standing behaviours after 25 min of resting time was directly observed for a period of 2 min.

Blood Sampling and Analysis

The animals were blood sampled five days before delivery as reference for basal blood parameter level (T0) and at exsanguination (T1) for the analysis of creatine kinase (CK), cortisol, glucose, lactate, albumin, albumin/globulin ratio, total protein, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphate (ALP), sodium ($Na^{-)}$, and potassium (K^+). All biochemical parameters except for glucose, were measured in serum obtained from blood collected in serum separator tubes with gel separator and clot activator (Vacutest Kima, Padua,

Italy), let to clot and centrifuged at 1300 x g at 20 °C (Eppendorf 5702R, Eppendorf, Milan, Italy) for 10 min. Plasma for the measurement of glucose was harvested from anticoagulated blood collected in Na2EDTA test tubes containing the glycolysis inhibitor potassium fluoride (Vacutest Kima, Padua, Italy), centrifuged at 1300 x g at 20 °C for 10 min. Parameters were measured by colorimetric assays on automated analyser (Olympus AU 400, Beckman Coulter, Milan, Italy) with the exception of cortisol. Serum cortisol was determined using the Kit Immulite One Cortisol (medical system code LKC01, Siemens Health Care Diagnostic Limited, Glyn Rhonwy, Gwynedd, UK).

Skin Bruises Measurement

Carcasses were horizontally exsanguinated for 5 min and then hung for 10 min before being submerged in a scalding tank for dehairing at 62 °C for 8 min. After dehairing, skin damages were subjectively assessed by a trained observer, using a four-point scale (1 = none to 4 = severe) based on the scale developed by the Danish Meat Research Institute (DMRI) (Barton Gade, Warriss, Brown, & Lambooij, 1996). The DMRI scale was used to score all skin lesions on the front (head included), middle and hind quarters of each carcass. Moreover, a skin damage score for the whole carcass was calculated using the highest score assigned to each quarter (Barton Gade et al., 1996). At about 40 min post mortem, carcasses were split, weighed, and transferred to the chilling room.

Meat Quality Measurements

Measurements of pH on the *longissimus thoracis* muscle (LT) at the level of six/seven thoracic vertebra were recorded at 1, 3, and 6 h post mortem on the left side, with a pH-meter (mod. HI8424, Hanna, Padua, Italy) equipped with Crison electrode (Crison Instruments, SA, Barcelona, Spain) and an automatic temperature compensation probe. At 6 h postmortem the left side was sectioned in the primal cuts and a sample of LT muscle between 6 and 10 thoracic vertebra (10 cm thickness) was collected. After 30 min of blooming, L*, a*, and b* coordinates were determined using Minolta chroma-meter (CR-300, Minolta Camera Co., Ltd., Osaka, Japan) with D65 light source and 0 °C

viewing geometry. Samples were transferred (by air) to a laboratory of the Department of Agricultural and Food Sciences (Bologna, Italy). Measurements of pH were repeated at 24 and 72 h post mortem and colour measurements were repeated at 24, 72, and 144 h after slaughter. At the same time interval, cooking loss was determined on a slice of the LT muscle according to Honikel (Honikel, 1998) and Warner-Bratzler shear force (WBSF) was measured after cooking using Instron apparatus (mod. 1140, Instron, Norwood, MA, USA). Drip loss was determined at 24 h post mortem (Honikel, 1998).

Genotyping

To determine the genotype of the Halothane (ryanodine receptor 1, RYR1) and Rendement Napole (protein kinase AMP-activated non-catalytic subunit gamma 3, PRKAG3), major genes that influence the reactivity of pigs to stress and pork quality, genomic DNA was isolated from blood collected in tubes containing EDTA as an anticoagulant. Genotyping of the c.599G > A single nucleotide polymorphism (SNP) PRKAG3 gene and the g.1843C > T SNP of the RYR1 gene were done by PCR-RFLP analyses (J Fujii et al., 1991; Milan et al., 2000).

Statistical Analysis

Loading and unloading duration of the two genetic types were compared using a T-test. The incidence of pigs showing the different behaviors at loading, unloading and lairage was calculated and the data were processed by Fisher Exact Test procedure of SAS v. 9.3 (SAS Institute, Cary, NC, USA). Data from blood parameters were transformed to meet assumptions of homogeneity of variance and normality of residuals. Concentrations of CK, lactate, albumin, albumin/globulin ratio, creatinine, and AST were log10 transformed. A square root transformation was used to normalize cortisol and ALT results, while an inverse transformation was used to normalize glucose and K^+ results. All transformed estimates were back-transformed for presentation to their original scale. Blood parameters were analysed using the mixed model (PROC MIXED of SAS) including the

genetic type (two levels), sampling time (two levels: T0 and T1) and their interaction as fixed effects and subject within the day of slaughter as random effect. Sex as a fixed effect and hot carcass weight as a covariate were initially included in the model but they never reached statistical significance ($p > 0.05$) and were removed. Differences between means were tested by the Tukey-Kramer test ($p < 0.05$). Data of pH, color, cooking loss and shear force were analyzed using PROC MIXED of SAS for repeated measures. The same model for blood parameters was used replacing sampling time factor with measuring time (five levels for pH, four levels for color, three levels for cooking loss, and shear force). Sex and hot carcass weight did not reach the significant level ($p > 0.05$) and were removed from the model. The data of drip loss was analyzed using the same model without measuring time. In order to highlight possible differences between the two genetic types in blood parameters responses to short distance transport, the variation between blood parameters at exsanguination (T1) and basal blood parameters (T0) has been used to perform an unsupervised multivariate principal component analysis (PCA). All the new variables resulting from the difference between T1 and T0 blood parameters were normally distributed except for cortisol difference, which was root squared transformed to meet normal distribution criteria. Furthermore, a PCA has also been used to test the presence of differences in meat quality traits between the two studied genetic types. Unsupervised PCAs have been performed using ropls package in the R environment version 3.4.4 (R Core Team, 2018). The data were mean centred and unit- variance scaled. The results of multivariate models were plotted on both scores and loadings plot. The combined use of univariate and multivariate analyses was employed to test if the results obtained with the multivariate analysis (PCA) were in agreement with what could be observed with the mixed model.

PROC GLIMMIX was used to analyse the effects of genetic type on skin damage scores recorded on each quarter separately as well as on the whole carcass. Because these data approximated a Poisson distribution, the GLIMMIX procedure's POISSON option was used. The differences in least squares means (L.S.M.) were evaluated using Tukey–Kramer's test.

Results and Discussion

Both genetic types did not carry the recessive allele (c.1843T) of the RYR1 gene (Fujii et al., 1991) and the dominant allele (c.599A or p.200Q) of the PRKAG3 gene (Milan et al., 2000) that influence performance and meat quality traits (Cherel et al., 2010; Otto et al., 2007). Few research studies have been focused on the effect of transport on local breeds (Lebret et al., 2015; Li et al., 2008) although a great deal of literature exists on conventional commercial pigs and their crossbreeds (Averós, Herranz, Sanchez, Comella, & Gosalvez, 2007; Brown et al., 1999; Chai et al., 2010). The present study reports for the first time the effects of short distance transport on blood parameters and meat quality traits of Apulo-Calabrese. The results obtained showed different physiological response and meat quality attributes in both genetic types after the transport procedure.

Behavioral Recordings and Carcass Bruises

Behavioral occurrences on both genetic types were collected at loading, unloading and lairage. The two genetic types showed no differences in the behavioral occurrences during unloading and lairage, while at loading Apulo-Calabrese pigs showed significantly lower percentages ($p < 0.05$) of reluctance to move and vocalization (Table S1). During lairage, the posture was recorded after 25 min close to the end of the resting time (30 min) as planned routinely by the abattoir. For the duration of time spent in lairage no pigs were observed sitting, 94% of the pigs were lying down and only 6% of the pigs were observed standing. The genetic type did not show significant effect ($p > 0.05$) on skin damage score(whole carcass: 1.14 ± 0.34 and 1.12 ± 0.32 for Apulo-Calabrese and crossbreeds, respectively).

Blood Parameters

Table 2 shows the effects of sampling time, genetic type and their interaction on blood parameters of Apulo-Calabrese and crossbreeds. Sampling time statistically influenced ($p < 0.05$) all blood parameters, except ALP and sodium whilst the effect of genetic type was significant ($p < 0.05$) for glucose, albumin/globulin ratio, urea, creatinine, AST and potassium. Of particular interest was the interaction between the genetic type and the sampling time since it lays emphasis on the possibility that variation of plasma components between basal and exsanguination is influenced by the genetic type. This interaction was statistically significant ($p < 0.05$) for lactate, albumin/globulin, urea, creatinine, AST, ALT, ALP, sodium, and potassium. At exsanguination, significantly higher levels of lactate ($p < 0.05$) were found in both genetic types. Higher levels of lactate in the blood have been associated with physical stress (Pérez et al., 2002). The highest value of lactate in this study was found in Apulo-Calabrese which showed a lower concentration of basal lactate and were driven with less difficulty (minor duration) during loading, as shown in Table 1. According to Broom et al. (2003), different breeds cope differently to the handling and transport procedures which could explain the higher levels of lactate found in Apulo-Calabrese in this experiment. Other welfare indicators of stress such as CK and cortisol did not differ between the two genetic types, which is in agreement with the results found by Lebret et al. (2015) in the French local Basque and Large White pigs. The similar levels of basal cortisol found in Apulo-Calabrese and crossbreeds in this experiment contrasted with the result by Li et al. (2008) who found higher levels of plasma cortisol in Erhualian with respect to Pietrain. Plasma glucose did not differ between both genetic types at exsanguination. However, slightly lower levels of glucose were found in Apulo-Calabrese compared with crossbreeds. Significantly lower levels ($p < 0.05$) of albumin/globulin were found at exsanguination in Apulo-Calabrese than the crossbreeds, the values obtained were however within the normal physiological range fo**r** pigs (Swenson, 1993). With the exception of some globulins, plasma proteins are produced in the liver and are indicators of colloid osmotic pressure of the blood.

Lower values of the concentrations may be due to a lack of dietary protein or hepatic damage (Swenson, 1993), whilst higher values have been associated with dehydration due to the length of the journey (Brown et al., 1999). Higher levels of urea and creatinine in the blood have been associated with food deprivation stress and an increase in physical activity as a result of the transport procedures (Bórnez, Linares, & Vergara, 2009). In this experiment, significantly lower levels of creatinine ($p < 0.05$) were found in Apulo Calabrese at the basal condition when compared with the crossbreeds whereas serum urea did not differ between the two genetic types. At exsanguination Apulo-Calabrese showed significantly higher levels of urea and lower levels of creatinine when compared with crossbreeds. The values of urea and creatinine obtained in this study were within the normal physiological range for pigs and were in agreement with the results found by Dikic et al. (2010) in local Turopolje breed and their crossbreeds [Turopolje x (CHypor x Swedish Landrace)]. AST, ALT, and ALP are chemical indicators of tissue function: elevated levels of these enzymes occur when liver and pancreas are damaged. Significantly higher levels ($p < 0.05$) of AST were found in Apulo-Calabrese at exsanguination when compared with crossbreeds. It is interesting to note that the concentration of AST increased slightly in Apulo-Calabrese from T0 to T1, unlike in the crossbreeds which demonstrated a remarkable increase at slaughter compared with the values obtained at the basal level. According to Pugliese and Sirtori (2012) local breeds are reared mostly in an extensive system where pigs forage for food in their surroundings. The elevated levels of AST found in Apulo-Calabrese at the basal condition could be a marker of an overworking hepatic metabolism due to their feeding with formula rations given to conventional fast-growing breeds. Nevertheless, the value obtained was within the range of values found in healthy pigs (Odink, Smeets, Visser, Sandman, & Snijders, 1990). Despite the similar levels of ALT found in both genetic types of pigs at exsanguination, there was an increase in this parameter within both genetic types from T0 to T1. The levels of sodium and potassium found in Apulo-Calabrese at exsanguination were higher when compared with crossbreeds and with values obtained at the basal condition. According to Mota-Rojas et al. (Mota-Rojas et al., 2012) transport and slaughter can

cause an increase in the concentrations of sodium and potassium, respectively. The values reported in this study were, however, within the normal physiological range for pigs (Swenson, 1993). The results of the PCA performed on the changes in blood concentrations between T1 and T0 are reported in Figure 1, where the score and the loadings plots are shown. Two samples were not included in this analysis since they appeared to be outliers in orthogonal distance plot. Multivariate analysis generated three Principal Components (PCs: PC1, PC2, and PC3) explained 30%, 14%, and 10% of the total variance, respectively. The two components that explained the most differences between the two genetic types were PC2 and PC3, since plotting these two components samples displayed to be clustered (Figure 1a). The score plot (Figure 1a) presents the graphical projection of the samples into a two-dimensional space with PC2 (t2 in Figure 1a) values as the x axis and PC3 (t3 in Figure 1a) as the y axis. The red and blue ellipses represent the Mahalanobis distances for the crossbreed and Apulo-Calabrese pigs, respectively, while the black ellipse showed the average area of Mahalanobis distances for the complete population. Figure 1b graphically displays PCA loadings, numerically presented in Table S2. In Figure 1b the variables weighting the most in each PC are displayed: variables which have little contribution to a direction (PC) have almost zero weight (like urea for PC3), while the ones that contribute the most in the definition of the PCs show higher or lower weights (like glucose for PC3). Therefore, the blood parameters that contribute the most in the explanation of the differences among crossbreed and Apulo-Calabrese pigs are grouped together at the opposite quartiles. The results obtained from the PCA were consistent with those observed in the mixed model. Interestingly, the variables that weighted the most in PC2 were urea (0.431), AST (-0.406), alb/glob (-0.350), lactate (0.331), and ALP (-0.327) (Table S2), which were the blood parameters that were influenced by the time x genetic type interaction in univariate results. PC3 resulted to be mainly related to glucose (-0.587), which was influenced by the genetic type in the univariate analysis.

Meat Quality Traits

The significance of factors of variations on meat quality parameters of the LT muscle is reported in Table S3. The interaction between measuring time and the genetic type was significant for all color coordinates and shear force.

Post mortem pH decline (Figure 2a) was similar between genetic types although a significant ($p < 0.05$) decrease was reported in the first 6 h after slaughter, which stabilized during subsequent measurements. A similar pH trend was observed by Shen et al. (2014) when comparing local Chinese breeds and crossbreeds of pigs.

L* (lightness) measured at 6, 24, 72, and 144 h after slaughter increased progressively as post mortem time increased (Figure 2b). The L* values measured in the meat of Apulo-Calabrese were significantly lower ($p < 0.05$) than those recorded in the meat of crossbreeds in all measuring times, with the exception of those recorded at 144 h after slaughter. The L* coordinate in the meat of crossbreeds stabilized after 24 h whilst that of Apulo-Calabrese maintained an almost constant increase. According to Scheffler and Gerrard (2007) post mortem pH can affect muscle color Despite the similar levels of pH in this experiment significantly higher ($p < 0.05$) values of a* (Figure 2c) were found in Apulo-Calabrese at each detection time. This indicates that the meat from this breed is distinguished by a deeper red color like other local European pig breeds (Franci & Pugliese, 2007; Lebret et al., 2015; Peinado, Poto, Gil, & López, 2004). The trend of the a* coordinate in Apulo-Calabrese pigs showed limited variations and the only value that was significantly different from the others was that measured at 24 h post mortem. The b* value did not differ between the two genetic types as shown in Figure 2d. For both breeds the highest b* values were recorded at 72 h postmortem while the value decreased significantly at 144 h postmortem.

Higher values of cooking losses were reported for both genetic types at 24 h post mortem (Figure 3a) compared to lower values recorded at 72 and 144 h after slaughter. Apulo-Calabrese pigs showed slightly higher values at 24 h post mortem compared to crossbreeds, but these differences did not reach statistical significance. Apulo-Calabrese showed lower values of cooking loss when

compared with the values of other local breeds, like Cinta Senese (Franci et al., 2005) and Nero Siciliano (Pugliese et al., 2003). There was no effect of genetic type on drip loss (4.19 ± 0.2 and 4.78 ± 0.2 for Apulo-Calabrese and crossbreeds respectively).

Warner-Bratzler shear force measured after drip and cooking loss (Figure 3b) decreased as post mortem time increased and showed a similar trend in both genetic types. Nevertheless, higher values were reported for Apulo-Calabrese at 24 and 144 h after slaughter, suggesting the need to subject the meat of Apulo-Calabrese to ageing if it is intended for fresh consumption.

Figure 4 reports the results of the PCA performed on meat quality traits. Multivariate analysis generated four PCs: PC1, PC2, PC3, and PC4 explained 25%, 14%, 9%, and 8% of the total variance, respectively. The component that explained the most differences between the two genetic types was PC2, since samples displayed to be clustered for PC2 (Figure 4a). Figure 4b graphically displays PCA loadings, numerically presented in Table S4.

The variables that weighted most in PC2 were colour coordinates a* at 24 h (−0.409), a* at 6 h (−0.394), a* at 72 h (−0.368), a* at 144 h (−0.363), L* at 72 h (−0.257), and pH measured at 24 h (0.276). The high weights observed for a* colour coordinate are in agreement with the significant differences obtained from univariate analysis reported in Figure 2c, where redness- greenness value a* was highly divergent at all the measuring times between the two pig genetic types. Together with a*, other variables that contribute in differentiating the two genetic types were L* and pH, as can be noticed both by PCA loadings in Figure 4b and Table S4 and by mixed model results in Table S3. Interestingly, despite the different statistical assumptions of mixed and multivariate analysis, the results obtained are quite concordant, highlighting that colour coordinates represents the meat quality attributes discriminating the most the two genetic types. Anyway, PCA results suggested that, when considering together all the meat quality variables and taking into account their correlated nature, also pH measured at 24 h has a consistent weight in differentiating Apulo-Calabrese from crossbreed pigs. This result may also be noticed in Table S3, from the mixed model results. Despite the genetic type had not a significant effect on longissimus thoracis pH, the

estimated L.S.M. for pH at 24 h were the most divergent between the two genetic types (5.57 for Apulo-Calabrese and 5.45 for crossbreeds) when compared with the pH measured at the other times. This result suggests that using a combined statistical approach may allow to highlight the main differences that would have not been appreciable with the use of univariate statistics alone.

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Table 1. Outdoor temperature (Temp), relative humidity (RH), and duration of loading, transport, and unloading were recorded in pigs in the three deliveries.

Delivery	Genetic type	Number of pigs	Loading			Transport	Unloading		
			Duration[1] (min)	Temp (°C)	RH (%)	Duration (min)	Duration[2] (min)	Temp (°C)	RH (%)
1	Apulo-Calabrese	19	8	10.3	79	63	2	11.2	88.4
	Crossbreed	20	16				9		
2	Apulo-Calabrese	17	10	18	72.3	67	7	19.5	65.8
	Crossbreed	17	16				3		
3	Apulo-Calabrese	15	6	21.5	59.6	60	5	19.7	65.5
	Crossbreed	15	6				2		

[1]. From the opening of the farm gate until the last pig entered the lorry. [2]. From the opening of the gate of the lorry until the last pig entered the lairage pen

Table 2. Effects of sampling time (T), genetic type (GT) and their interaction (T x GT) on least square mean (L.S.M) and standard error of means (S.E.M) of blood parameters of Apulo-Calabrese and crossbreed pigs on farm (baseline) and at exsanguination

Blood parameters	**Baseline**		**Exsanguination**					
	Apulo-Calabrese	**Crossbreed**	**Apulo-Calabrese**	**Crossbreed**		**P-values**		
	L.S.M				**S.E.M**	**T**	**GT**	**T x GT**
Creatine Kinase, CK (U/L)	954.99	831.76	2089.30	2187.76	0.05	<.0001	0.7529	0.2130
Cortisol (mg/dL)	16.72	17.38	56.31	56.73	0.26	<.0001	0.8730	0.8690
Glucose (mg/dL)	71.23	78.13	100.57	114.59	0.00	<.0001	0.0004	0.9497
Lactate (mg/dL)	24.23^{c}	33.85^{b}	181.01^{a}	143.81^{a}	0.03	<.0001	0.4549	<.0001
Albumin (g/dL)	3.47	3.55	3.63	3.80	0.00	<.0001	0.1747	0.2862
Albumin/globulin, Alb/glob	0.93^{a}	0.99^{a}	0.82^{b}	0.95^{a}	0.01	<.0001	0.0031	<.0001
Total protein (g/dL)	7.30	7.20	8.15	7.84	0.10	<.0001	0.0686	0.0674
Urea (mg/dL)	37.71^{a}	36.91^{a}	37.55^{a}	33.50^{b}	0.96	0.0103	0.0404	0.0185
Creatinine (mg/dL)	1.65^{c}	1.89^{b}	2.08^{a}	2.25^{a}	0.00	<.0001	<.0001	<.0001
Aspartate aminotransferase, AST (U/L)	70.80^{a}	33.25^{c}	77.73^{a}	54.29^{b}	0.03	<.0001	<.0001	<.0001
Alanine aminotranferase, ALT (U/L)	54.83^{a}	52.51^{b}	58.52^{ab}	59.41^{ab}	0.11	<.0001	0.7309	0.0357
Alkaline phosphatase, ALP (U/L)	145.14^{a}	118.12^{b}	134.57^{ab}	119.03^{b}	5.69	0.1105	0.0527	0.0124
Sodium, Na (mEq/L)	139.03^{b}	142.00^{b}	147.97^{a}	145.77^{a}	1.18	0.7937	0.7937	0.0017
Potassium, K (mEq/L)	5.34^{c}	5.74^{b}	6.93^{a}	6.20^{ab}	0.00	<.0001	0.0017	0.0004

Means on the same row with different superscript letters (a, b, c,) indicate significant effects ($P < 0.05$) of the interaction between Sampling time (T) and genetic type (GT).

Figures

Figure 1. Results of Principal Components Analysis (PCA) on the variations of blood parameters between T1 and T0: (a) score plots for principal component 2 (t2) and principal component 3 (t3) of Apulo-Calabrese (blue) and crossbreeds (red) samples; (b) loadings plot with the weights of variables included in principal component 2 (p2) and principal component 3 (p3).

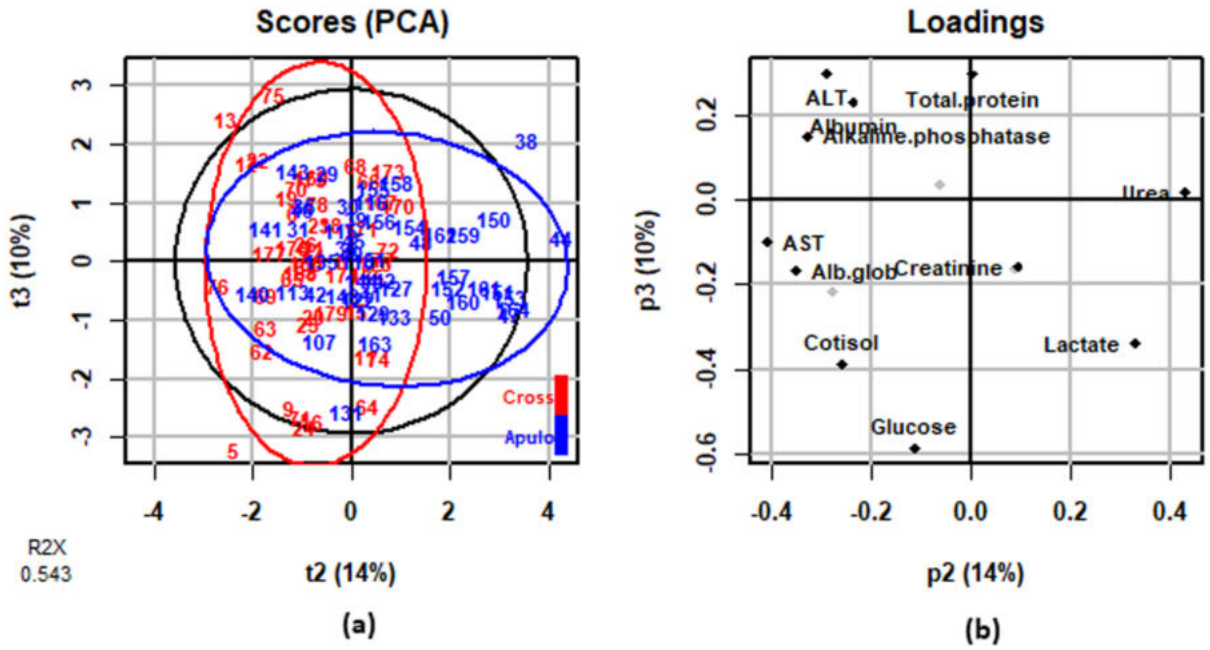


Figure 2. Changes in pH **(a)** and color coordinates (L* in b, a* in c and b* in d) in *longissimus thoracis* in Apulo-Calabrese and crossbreed pigs over time. Different letters in the graphs (a, b, c, d) indicate significant effects ($P < 0.05$).

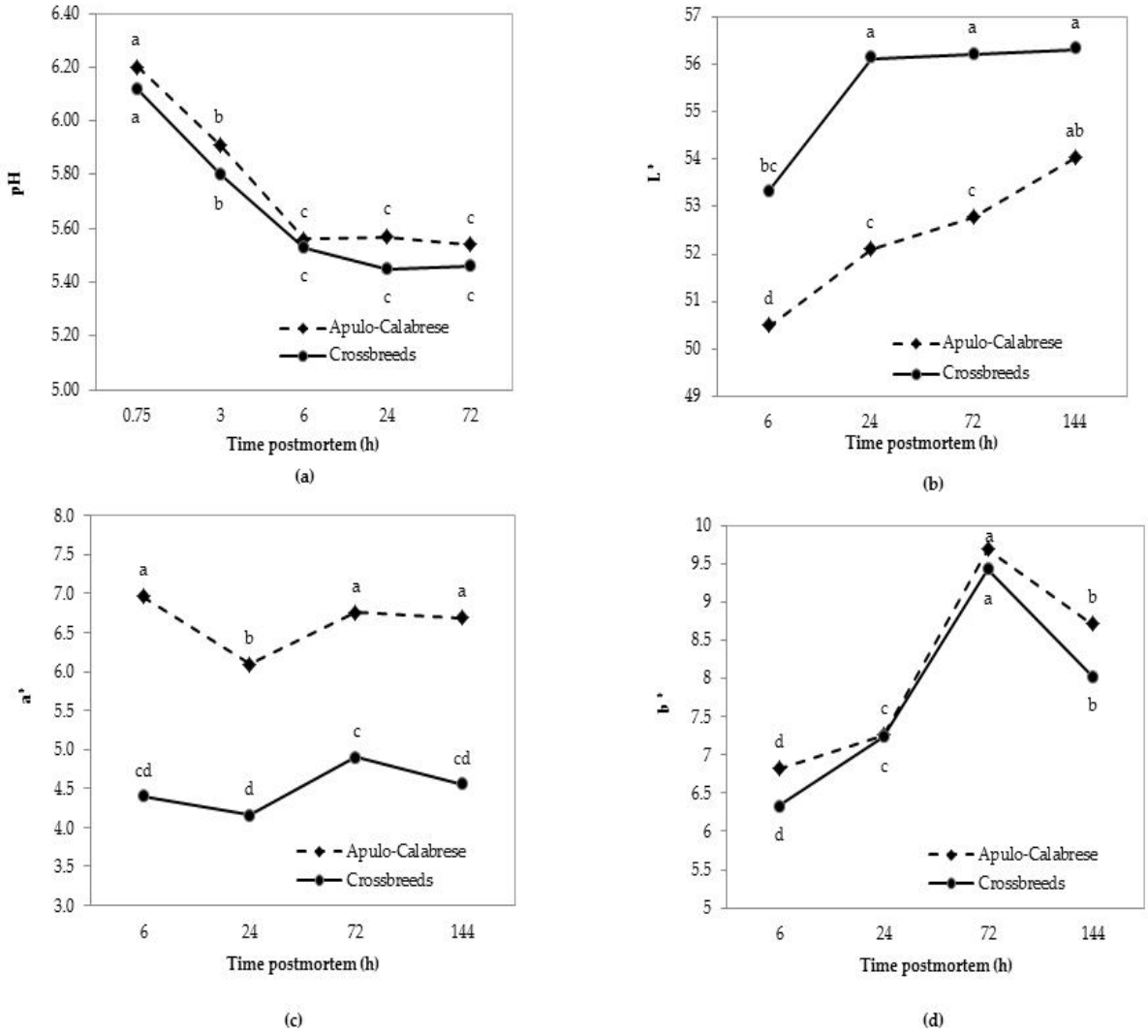


Figure 3. Changes in cooking loss (a) and shear force (b) in the *longissimus thoracis* in Apulo-Calabrese and crossbreed pigs over time. Different letters in the graphs (a, b, c) indicate significant effects ($P < 0.05$).

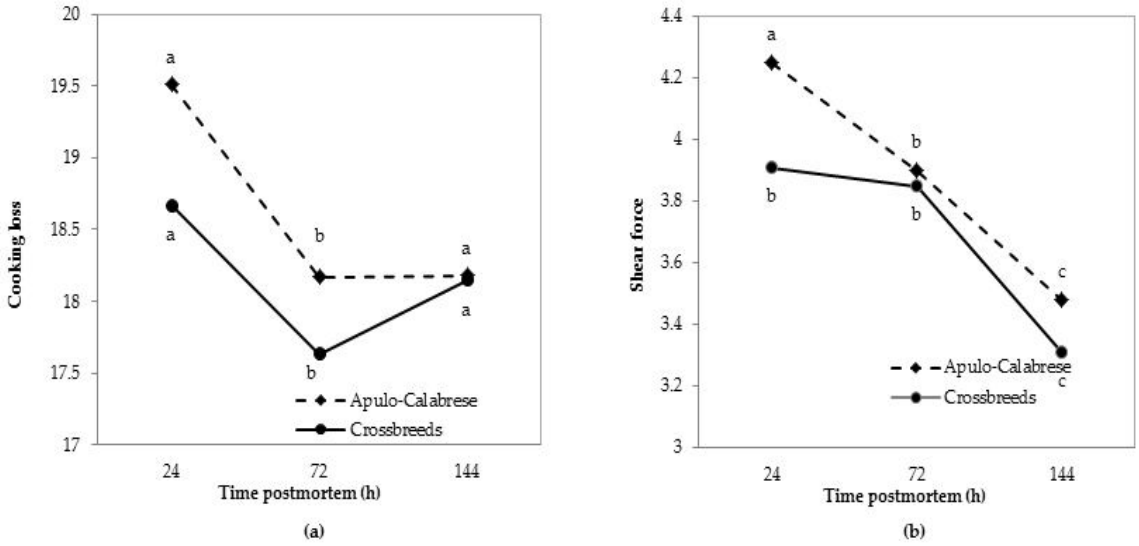
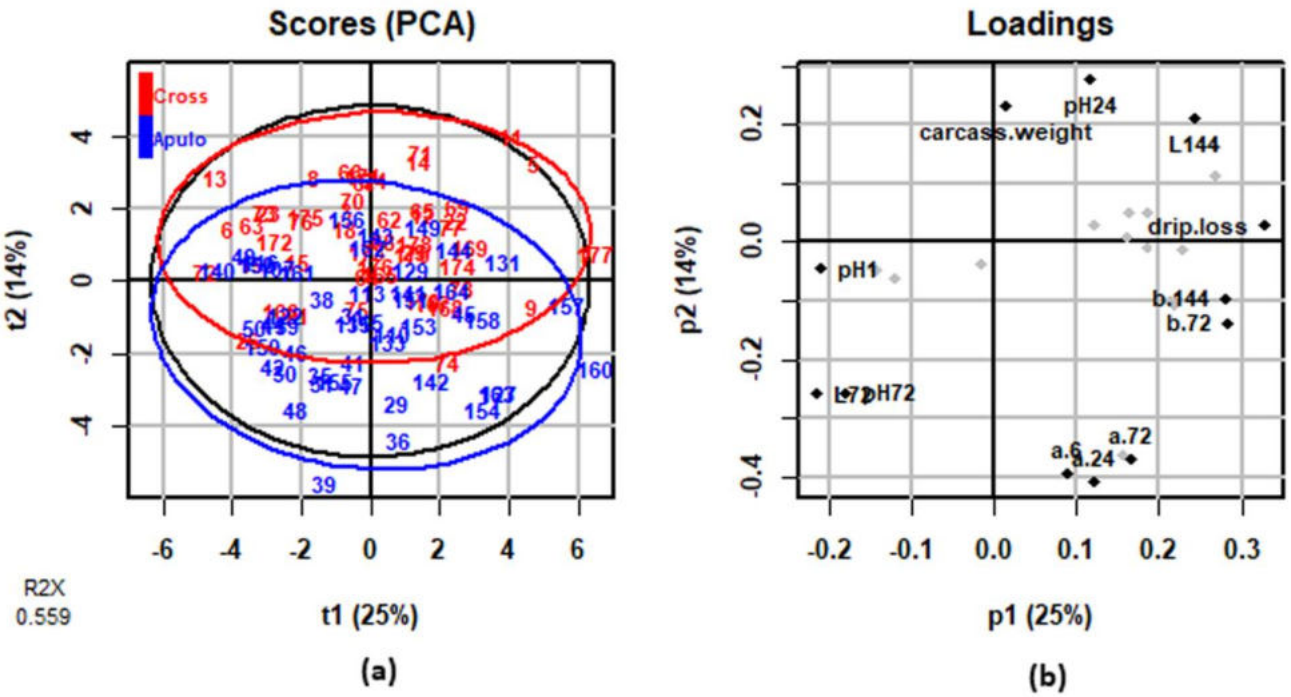


Figure 4. Results of Principal Components Analysis (PCA) on the meat quality traits: (a) score plots for principal component 1 (t1) and principal component 2 (t2) of Apulo-Calabrese (blue) and crossbreeds (red) samples; (b) loadings plot with the weights of variables included in principal component 1 (p1) and principal component 2 (p2).



Supplementary Table S1. The percentage of Apulo-Calabrese and Crossbreed pigs displaying slipping, falling, reluctance to move, overlapping and vocalization behaviors during loading and unloading and lying, sitting and standing behaviors in lairage have been presented below.

Check point	Measurement	Apulo-Calabrese (%)	Crossbreed (%)	P (Fisher Exact Test)
Loading	Slipping	2	8	0.363
	Falling	2	2	0.980
	Reluctance to move	10	34	0.004
	Turning back	6	6	0.980
	Overlapping	10	16	0.555
	Vocalization	12	38	0.003
Unloading	Slipping	2	6	0.618
	Falling	0	0	---
	Reluctance to move	2	10	0.205
	Turning back	2	8	0.618
	Overlapping	0	6	0.243
	Vocalization	0	10	0.057
Lairage	Lying	94	94	0.984
	Sitting	0	0	--
	Standing	6	6	0.984

Supplementary Table S2. Loadings of the PCA were performed on blood parameters.

	PCA loadings		
Blood parameters	PC1	PC2	PC3
Creatine Kinase, CK (U/L)	0.005	-0.274	-0.218
Cortisol (mg/dL)	0.125	-0.256	-0.389
Glucose (mg/dL)	0.134	-0.109	-0.587
Lactate (mg/dL)	0.261	0.331	-0.340
Albumin (g/dL)	0.397	-0.236	0.229
Albumin/globulin, Alb/glob	-0.055	-0.350	-0.168
Total protein (g/dL)	0.430	0.006	0.295
Urea (mg/dL)	0.145	0.431	0.017
Creatinine (mg/dL)	0.389	0.094	-0.157
Aspartate aminotransferase, AST (U/L)	-0.050	-0.406	-0.099
Alanine aminotransferase, ALT (U/L)	0.272	-0.286	0.296
Alkaline phosphatase, ALP (U/L)	0.048	-0.327	0.151
Sodium, Na (mEq/L)	0.412	-0.061	0.036
Potassium, K (mEq/L)	0.363	0.089	-0.162

Table S3. Effects of measuring time (T,) genetic type (GT) and their interaction (T x GT) on least square mean (L.S.M.) and standard error of means (S.E.M) of meat quality traits of Apulo-Calabrese and crossbreed pigs

Meat quality traits	Apulo-Calabrese						Crossbreed						S.E.M.	T	GT	T x GT
	L.S.M. at different time *postmortem* (h)															
	0.75	3	6	24	72		0.75	3	6	24	72	144				
pH	6.20	5.91	5.56	5.57	5.54	0.5221	6.12	5.80	5.53	5.45	5.46		0.0256	<.0001	0.0583	0.5221
L* (brightness)			50.5	52.1	52.8	0.0095			53.3	56.1	56.2	56.3	0.6064	<.0001	<.0001	0.0095
a* (redness)			7.00	6.10	6.80	0.0359			4.4	4.20	4.90	4.60	0.2663	<.0001	<.0001	0.0359
b* (yellowness)			6.82	7.26	9.69	0.0316			6.33	7.24	9.43	8.03	0.2269	<.0001	0.2403	0.0316
Drip loss (%)				4.16		--				4.78			0.2296	--	0.3568	--
Cooking loss (%)				19.51	18.17	0.1896				18.67	17.63	18.15	0.5186	0.0286	0.2089	0.1896
Shear force (N)				4.25	3.90	<.0001				3.91	3.85	3.31	0.0702	<.0001	0.0645	<.0001

Table S4: Loadings of the PCA performed on meat quality traits.

	PCA Loadings			
	PC1	PC2	PC3	PC4
Carcass weight	0.016	0.231	0.110	0.295
pH 1 h	-0.209	-0.045	0.057	0.196
pH 3 h	0.186	0.050	-0.154	-0.292
pH 6 h	0.228	-0.012	-0.102	-0.364
pH 24 h	0.118	0.276	0.278	-0.140
pH 72 h	-0.179	-0.256	-0.227	0.061
L* 6 h	0.270	0.113	-0.159	-0.133
L* 24 h	0.269	0.164	0.174	-0.157
L* 72 h	-0.214	-0.257	0.054	-0.114
L* 144 h	0.244	0.210	-0.181	0.091
a* 6 h	0.090	-0.394	0.007	-0.074
a*24 h	0.122	-0.409	0.104	-0.043
a* 72 h	0.165	-0.368	0.103	0.159
a* 144 h	0.156	-0.363	0.077	0.111
b* 6 h	0.219	-0.107	-0.257	-0.148
b* 24 h	0.214	-0.098	0.393	-0.171
b* 72 h	0.284	-0.137	0.080	0.183
b* 144 h	0.281	-0.095	-0.023	0.084
Cooking loss 24 h	0.185	-0.011	-0.157	0.344
Cooking loss 72 h	0.162	0.007	-0.323	0.085
Cooking loss 144 h	0.122	0.030	0.137	0.031
Shear force 24 h	-0.014	-0.037	-0.110	-0.340
Shear force 72 h	-0.142	-0.049	-0.312	-0.143
Shear force 144 h	-0.119	-0.063	-0.267	-0.263
Drip loss	0.329	0.029	0.015	0.011

CHAPTER 2

Water status in meat from pig breeds strongly differing in growth performances

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Summary

This research compared the distribution and mobility of water in the longissimus thoracis muscle of 51 Apulo-Calabrese and 52 crossbred pigs differing in growth performances. The Apulo-Calabrese and crossbreed pigs were fed the same diet and slaughtered at 135 and 155 kg live weight, respectively. Besides meat quality measurement, water status was assessed from transverse relaxation time (T2) weighted signals registered by Time Domain Nuclear Magnetic Resonance (TD-NMR). A mixed model indicated that Apulo-Calabrese pigs had higher a* (P-value < 0.0001), chroma (P-value < 0.0001) and total intensity (P-value=0.011) values. A Principal Component Analysis showed that the samples from Apulo-Calabrese had higher scores along Principal Component (PC) 2 (P-value=4.07×10−5) and lower scores along PC3 (P-value=1.50×10−7). However PC2 and PC3 explained a low fraction of the total variance, suggesting that few differences characterize meat quality traits of the two genetic types.

Introduction

Meat contains approximately 75% of water (Tejerina, García-Torres, & Cava, 2012). The capacity of meat to hold water determines consumer satisfaction and the acceptability of the product (Van Oeckel, Warnants, & Boucqué, 1999), as it affects the final quality (Bertram et al., 2003) in terms of texture, juiciness, appearance and other sensory properties. In addition, high fluid loss affects the weight of the meat with a consequent decrease in yield, reducing the profit margins of farmers (Pearce, Rosenvold, Andersen, & Hopkins, 2011). Breeding and slaughtering parameters are important determinants of the water content and the water holding capacity (WHC) of meat. As an example, slaughter weight and age have been reported to affect WHC (Čandek-Potokar, Žlender, Lefaucheur, & Bonneau, 1998), such that meat from pigs slaughtered at a lower age and weight were characterized by higher cooking losses (Bertram, Straadt, Jensen, & Dall Aaslyng, 2007), when compared with older pigs. In addition, the breed is among the main factors that influencing the WHC of meat. Schivazappa et al. (2002) observed significantly higher water holding capacity in Duroc pigs than in Large White or Landrace. In Italy, pork products are mainly produced with meat from commercial breeds like Landrace, Large white, Duroc and their crossbreeds. Recently there has been a growing interest for local breeds, such as Apulo-Calabrese, due to the conservation of endangered germplasm and the suitability of their meat for the production of four Protected Designation of Origin (PDO) salamis: salsiccia, soppressata, capocollo and pancetta. The Apulo-Calabrese pig is an Italian autochthonous breed from Calabria region. It is black-skinned, medium-sized with small socks on the forelimbs and large socks on the hind limbs (Micari, Racinaro, Sarullo, Carpino, & Marzullo, 2009). Similarly to the other Italian local pig breeds, Apulo-Calabrese is characterized by late sexual maturity, low prolificacy, reduced growth and carcass performance (Franci & Pugliese, 2007). This breed is well adaptable to different production systems and can be reared outdoor and indoor in a conventional system (Micari et al., 2009; Pugliese & Sirtori, 2012). Despite the growing commercial importance of this breed, not much is known on the water holding capacity of its meat. A current preliminary study showed differences in cooking

losses between Apulo-Calabrese and crossbreed pigs (Aboagye et al., 2018). The present study has been set up to evaluate the water status in the meat of Apulo-Calabrese, compared to the meat of crossbreeds differing in growth performance. These observations have been mainly based on proton transverse relaxation time (T2) weighted signals, registered by Time Domain Nuclear Magnetic Resonance (TD-NMR). Studies conducted on meat of pig (Bertram, Dønstrup, Karlsson, & Andersen, 2002) and of several other farmed animals, including turkeys (Bianchi et al., 2004; Soglia et al., 2018), have shown that from T2-weighted signals it is possible to observe separately the protons located inside and outside the myofibrils of meat. Also, the T2 of both populations has been reported to be strictly related to WHC and other key meat-quality traits. Bertram et al. (Bertram, Meyer, Wu, Zhou, & Andersen, 2008) found that proton T2 was strongly correlated with salt-induced swelling in pork. Similar correlation was also found when myofibrils were extracted from meat. Therefore, different water compartmentalization and quality traits may characterize the meat obtained from Apulo-Calabrese and crossbreed pigs. To test this hypothesis, the present study combines meat quality measures with the meat water status assessed using transverse relaxation time (T2) weighted signals registered by Time Domain Nuclear Magnetic Resonance (TD-NMR) and compares the obtained data between Apulo-Calabrese and crossbreed pigs.

Material and Methods

Animals and Slaughter Procedure

All procedures performed in this study were following the Italian legislation, D.Lgs 4 Marzo 2014 n. 26 art. 2 punto F, and did not require further specific authorization. Moreover, all farming procedures followed the Council Directive 98/58/EC concerning the protection of animals kept for farming purposes, and Council Directive 2008/120/EC laying down minimum standards for the protection of pigs. Animal transport was performed according to Council Regulation (EC) No 1/ 2005 on the protection of animals during transport and related operations. Slaughter was performed following the Council Regulation (EC) n. 1099/2009 on the protection of animals at the time of

killing and under the control of the Veterinary Service from the Italian Ministry of Health, as indicated in the Regulation (EU) 2017/625 of the European Parliament. The animals used were 51 Apulo-Calabrese from the crossing of 13 sows by 7 boars of Apulo-Calabrese and 52 crossbreed pigs [Duroc × (Landrace × Large White)]. The pigs were reared at the same farm in separated pens based on their genetic type and fed the same commercial diet (14,644 kJ DE/kg, 155 g crude protein/kg, 22 g crude fat/kg, 80 g lysine/kg, 58 g ash/kg). The pigs were slaughtered in three different days at the same plant. Apulo-Calabrese pigs were slaughtered at a live weight of 135 kg at an age of 364 ± 58 days while crossbreeds were slaughtered at 155 kg live weight at an age of 300 ± 30 days old. After 40 min post mortem, the carcasses were weighed and samples were obtained from the *longissimus thoracis* muscle at the 6th and 7th thoracic vertebra for the further analysis.

Meat Quality Measurements

At 24 and 72 h post mortem, pH values were measured using a pH meter (Hanna, mod. H18424) equipped with a Crison electrode (Hach Lange, Spain) an automatic temperature compensation probe. Meat color coordinates (L*, a*, and b*) were determined using Minolta chromameter (CR-300, Minolta camera co., Japan) at 24, 72, and 144 h post mortem. Colorimetric data were reported as the average hue, chroma and CIE values (L*, a* and b*) (Commission Internationale de L'Eclairage, 1978). At the same time interval, the cooking loss was determined following Honikel recommendations (Honikel, 1987) on meat samples of approximately 100 g of weight and 3 cm in height and width. Briefly, samples were placed in a thin-walled polyethylene bag, air layers were removed in order to allow optimum heat flow. The bags were placed in a water bath at 75 °C and cooked until the internal temperature of meat reached 70 °C. The packs were then placed for 40 min in running tap water (about 15 °C), meat samples were then taken from the bags, mopped dry, and weighed. The heating loss was expressed as percent heating loss. Warner-Bratzler shear force was determined after cooking using an Instron apparatus (mod. 1140, Instron, Norwood, MA, USA).

Furthermore, longissimus thoracis muscle samples were used to quantify intramuscular fat content using Folch method (Folch et al., 1957). Intramuscular fat quantitation was performed in duplicate and intramuscular fat amount was expressed as g on 100 g of muscle. TD-NMR measurements T2-weighted signals were registered on one sample per animal at each time-point. From each sample, meat cylinders of about 400 mg weight and 10mm deep were excised with a core borer. This ensured that the height of the samples was not going to exceed the active region of the radiofrequency coil of the spectrometer. The T2 weighted signals were registered with the CPMG pulse sequence (Meiboom & Gill, 1958) using a Bruker Minispec PC/20 spectrometer operating at 20 MHz. Each measurement comprised 30,000 echoes (points), with a 2τ spacing between consecutive 180° pulses of 0.080 ms and a recycle delay of 3.5 s. The specified interpulse spacing was chosen to avoid sample and radio frequency coil overheat and allowed the observation of the protons with T2 higher than a few milliseconds. The number of scans was fixed to 16, granting an S/N ratio in the range 900–1400. To obtain an overview of the protons T2 distribution, the CPMG decays were analyzed with the UPEN (Borgia, Brown, & Fantazzini, 1998) software, which inverts the CPMG signal using a semi-continuous distribution of exponential curves, according to Eq. (1): $I(2\tau n) = \sum_{i=1}^{M} I_0(T_{2,i}) \exp(-2\tau n/T_{2,i})$ where 2τ is the CPMG above mentioned interpulse spacing, n is the index of each CPMG echo, and I0 (T2,i) provides a distribution of signal intensities for each T2 component extrapolated at t=0 (the relaxogram), sampled logarithmically. Default values for all UPEN parameters were used throughout this work. The penalty function avoiding the overfitting of the UPEN algorithm caused a partial overlap of the protons populations in the relaxograms from several samples. To observe them separately, fittings to the sum of an increasing number of exponential curves were performed. An F-test showed that the optimum ratio between fitting ability and complexity of the model was reached for most samples with three exponentials. To make the T2-weighted curves' intensities comparable among the time-points, each of the curves was registered with the same acquisition parameters (namely, receiver's gain of 65,

echo time of 0.1 ms, 32K registered points, relaxation delay equal to 6 s) and its amplitude was adjusted for the sample's weight. Finally, the total average signal from the samples characterized by the highest signal amplitude (namely, crossbreed at 72 h) was set to 100 arbitrary units and the others were normalized accordingly. We then referred to this parameter as total amplitude.

Statistics

Data from meat quality measurements and TD-NMR were tested for normality using the Shapiro test of stats package in R (R Development Core Team, 2007). Variables that were not normally distributed were transformed using the Box Cox transformation option in the car package. To highlight significant differences between the two genetic types, data were analyzed using the Mixed model procedure of SAS (SAS Institute, Inc., Cary, NC, USA). The model included the fixed effects of genetic type, measuring time, sex and all the interactions among these variables and subject within the day of slaughter as a random effect. Sex as a fixed effect was not statistically significant and hence it was removed from the model. In order to have an overview of the overall trends underlying the parameters evaluated, an unsupervised multivariate Principal Component Analysis (PCA) model was calculated using the ropls R package. First, the projection of the samples in the Principal Components (PC) space (scores) was calculated. For this purpose, samples were indicated as outliers and not included in the analysis based on orthogonal distance. In particular, samples with a high value for at least one of the distances within and orthogonal to the projection plane (Hubert, Rousseeuw, & Vanden Branden, 2005) were considered as outliers and not further considered for the PCA analysis. Then, to test if the samples clustered according to the genetic type, a Student's t-test was performed on the PC scores. Finally, the importance of the meat quality traits over the PCs (loadings) were calculated, to find those mostly determining the clustering of the samples. The PC explaining most of the differences between the two genetic types were extracted, and a Pearson correlation was performed to highlight the variables that weighted the most in differentiating meat samples from the two pig genetic types. Pearson correlation was

performed using the psych package in R environment and P-values were Bonferroni-adjusted. An adjusted P-value < 0.01 was considered as statistically significant, and the variables showing significant correlations with PC scores were then further discussed.

Results

Interpretation of the TD-NMR Data

Typical relaxograms from T2-weighted signals obtained by TD-NMR are represented in Fig. 1. The fitting towards the sum of an increasing number of exponential curves revealed that three protons populations could be observed separately in both groups of samples. By following Bertram et al. (2008) the proton pool with the shortest relaxation time (T2 < 20 ms), accounting for roughly 4% of the relaxogram signals, was assigned to the water strongly associated to the macromolecular constituents of meat (bound water). The population accounting for the main percentage of the signal, with a T2 in the 20–60 ms range, was considered as water entrapped in the contractile protein reticulum of myofibrils. Finally, the population with a relaxation time longer than 60 ms was assigned to extra-myofibrillar water or water physically located outside of the proteins network.

Mixed model results

The complete set of the means and standard deviations (SD) for the measured data is reported in Table S1. Table 1 shows the effect of the genetic type, sampling time and their interactions on the T2 and amplitude of the signal from protons populations, as studied by TD-NMR. The effect of genetic type was significant for the amplitude of the signal from extra-myofibrillar water, T2 of intra-myofibrillar water and total signal. In each respect, Apulo-Calabrese pigs showed higher values than crossbreeds. Table 2 shows the effect of the genetic type, sampling time and their interactions on meat quality traits. The samples of Apulo-Calabrese showed significantly higher values for redness (a*), significantly lower values for hue and L* color coordinates and a trend towards significance for pH and shear force, both higher in samplesfrom Apulo-Calabrese pigs. These results have already been discussed in a previous study (Aboagye et al., 2018) and are briefly

described to better differentiate the water status between the two genetic types and to perform the multivariate analysis.

Overview of the Trends

Data obtained from TD-NMR and meat quality analyses, centered and scaled to unit variance, were employed as a base for a PCA model, to obtain an overview of the influence of the pig genetic types on them (Fig. 2). Three samples were not included in this analysis since potential outliers. Three principal components were retained by the model, explaining 22.0%, 15.7% and 12.8% of the total variance, respectively. While the samples scores along PC1 did not show any statistical difference between the two genetic types (P-value=0.40), the samples from Apulo-Calabrese appeared at significantly higher scores along PC2 (P-value=4.07×10−5) and lower scores along PC3 (Pvalue =.50×10−7). The score plot of the PC2 and PC3 is represented in Fig. 2A, while Fig. 2B and C graphically displays the corresponding loadings, numerically presented in the supplemental material as Table S2. The significance of the correlations between the scores of PC2 and PC3 and the measured variables is reported in Table S3. The variables that had the greatest influence on PC2 were a* color coordinate and chroma measured at each time, higher in Apulo-Calabrese samples, and hue measured at each time, higher in crossbreed samples. Besides, T2 of structural protons had high importance over PC2, with higher values in crossbreed samples, but only at 24 h post mortem. On the other hand, the characteristics of meat that showed the greatest weight over PC3 and were higher in crossbreed samples were L* color coordinate, hue and T2 of extra-myofibrillar water at each measuring time of pH at 24 and 72 h were higher in samples of Apulo-Calabrese.

Discussion

Previous Authors have reported differences in the mobility and distribution of water among different genetic types of pigs (Renou, Monin, & Sellier, 1985; Straadt, Aaslyng, & Bertram, 2011). However not much is known on the water distribution in local breeds of pigs. T2 of any protons population that can be observed by CPMG pulses sequence in meat is a weighted average between

the one of pure water and the one of biopolymers, as determined by the chemical exchange between the two (Hills, 1994; Laghi, Venturi, Dellarosa, & Petracci, 2017). While water has a T2 of around 2.5 s and contributes approximately to the 99% of the total signal (Laghi et al., 2005), biopolymers are characterized by a T2 in the range of microseconds. The T2 of any protons pool can be therefore determined by the ratio between protons of water and exchangeable protons of biopolymers. In turn, such ratio can be influenced by the migration of water among compartments or by the pH, which acts on the number of protons of the biopolymers that can exchange with water at a rate comparable to the acquisition rate the CPMG experiment. The T2 of a proton population can also change in connection to a change in the T2 of water or biopolymers. In this context, it is of importance to mention that water has been considered to have a high T2 when it interacts limitedly with the microstructures of meat (Bertram et al., 2002). The mixed model highlighted that samples from Apulo-Calabrese pigs had a higher fraction of extra-myofibrillar water. Bertram et al. (Bertram, Whittaker, Andersen, & Karlsson, 2004) showed that a conspicuous amount of the water located outside the myofibrils is given by the migration of the mobile water towards the surface of the meat, contributing to its loss during storage or cooking. In agreement with this finding, water loss upon cooking (reported in Table S1) appeared higher for Apulo-Calabrese samples at 24 h and 72 h post mortem and was comparable to the one of crossbreed samples only at 144 h. The greater tendency of water to migrate from intra- towards extra- myofibrillar spaces and then to be lost by Apulo-Calabrese meat structures upon cooking can be rationalized considering the T2 values of intra-myofibrillar protons. These values were, in fact, higher in Apulo-Calabrese samples, suggesting that water interacted less with meat microstructures and was, therefore, less tightly retained by them. This interaction seemed to be lower also for structural water and extramyofibrillar water, although not statistically significant (P-value > 0.05). The pH of meat from Apulo-Calabrese pigs was higher than the one of crossbreeds, although showing only a trend towards significance for the genetic type effect in the univariate analysis. High pH and low water retention ability may be correlated, as described by Renou et al. (1985). However, such correlation cannot be ascribed in the present work

to the number of exchangeable protons of biopolymers regulated by pH. The higher pH of Apulo-Calabrese samples is far from the isoelectric point, reasonably in the 5.0–5.3 range (Puolanne & Halonen, 2010). This would have led to a lower number of chemically exchanging protons (Laghi et al., 2005) and, consequently, to a higher T2, the opposite of what was found. Higher amounts of extra-myofibrillar water have been associated with toughness in meat (Fjelkner-Modig & Tornberg, 1986; Pearce et al., 2011) which was consistent with the tendency to lower tenderness in the meat of Apulo-Calabrese when compared with crossbreeds. The T2 of each proton population higher in Apulo-Calabrese samples can also explain the higher total NMR signal measured in the samples from this breed. Indeed, as detailed in the material and methods section, the NMR sequence could not register the signal from protons with very short T2 which was more abundant in the samples from crossbreeds. In agreement with the results of the mixed model on meat quality traits, the variables that contributed the most in discriminating the samples of the two genetic types were colorimetric measures. In particular, the variables clustered into two linear combinations: in PC2 the a* redness parameter showed positive and consistent loadings at all measuring times, therefore characterizing Apulo-Calabrese samples, while L* lightness measure entered with a positive and consistent weight in PC3, describing the crossbreed cluster. At each detection time, the redness a* value was significantly higher in Apulo-Calabrese pigs, indicating that this indigenous pig breed has deeper red-colored meats, like other local European pig breeds (Franci & Pugliese, 2007; Lebret, Ecolan, Bonhomme, Méteau, & Prunier, 2015), when compared to highly selected pig breeds. Accordingly, lightness and hue were significantly lower in Apulo-Calabrese pigs compared to those recorded in the meat of crossbreeds, characterizing with positive PC3 scores in the samples of crossbreed. Interestingly, despite the mixed model highlighted only a trend towards significance for the genetic type effect on pH, this variable was however among the meat parameters evidenced by PCA unsupervised model in PC3 (Fig. 2C), suggesting that the effect of pH is made clearer when it is considered together with the other variables. It is well known that the post mortem pH decline has a significant effect on the water holding capacity and color of meat. According to Bidner et al.

(2004) meat with higher pH values has a darker color and a lower drip loss, while meat with lower pH is associated with higher lightness values. Indeed, low pH and in particular rapid pH declines are known to alter the color intensity of the meat through a partial denaturation of the pigment-protein myoglobin and the sarcoplasmic proteins in muscle and meat. This chemical process would, in fact, decrease meat color intensity, since the denaturation of these proteins would reduce their solubility, causing them to precipitate and to reflect rather than absorb light (Huff-Lonergan et al., 2002). These results reported in the literature are therefore in agreement with the linear combination of variables weighting the most in PC3 and PC2 since the strong discriminating power in PC3 of the L* variable likely increased also the pH importance in samples differentiation. On the other hand, chroma and b*, which were not significant in the mixed model results, showed high PC2 loadings. Chroma is directly derived from a* and b* color coordinates, since it is calculated as the square root of (a*2+b*2). Consequently, given the strong discriminating power of a* and the mathematical relations occurring between chroma and a* and between chroma and b*, the latter may have entered with consistent weights in PC2 as a direct consequence of the a* importance in this PC. Therefore, the main differences observed between the meat of Apulo-Calabrese and crossbreed pigs may be ascribed to L* and a* color coordinates, which may be at least in part a direct consequence of the different pH decline in these two genetic types. Together with pH decline, the redder meat color in Apulo-Calabrese pigs may also be explained by a higher myoglobin content. Indeed, meat with higher myoglobin content displays a redder color (Karamucki, Jakubowska, Rybarczyk, & Gardzielewska, 2013), and myoglobin was reported to increase as age progresses and to be higher in the meat obtained from autochthonous pig breeds (Sans, Andrade, Ventanas, & Ruiz, 2004). Furthermore, in agreement with univariate model results, the total signal amplitude at 24 and 144 h post mortem showed positive loadings in PC2, thus characterizing the meat of Apulo-Calabrese pigs. Besides, structural water measures and T2 of the extra-myofibrillar water entered with a limited weight in PC2 and PC3, despite these variables did not show significant differences in Table 1 between the pig genetic types. Interestingly, despite the different statistical assumptions of the

performed univariate and multivariate analyses, the results obtained are quite concordant, highlighting that color co-ordinates represent the meat quality attributes discriminating the most the two genetic types. This discriminating power may be of such strength that the differences obtained for TD-NMR measurements lose their weight, except for total signal amplitude. Again, PCA results suggested that, when considering together all the meat quality variables and taking into account their correlated nature, also pH measured at 24 h has a consistent weight in differentiating Apulo-Calabrese from crossbreed pigs. Unexpectedly, despite the common belief that Apulo-Calabrese pig breed has consistently higher potential for intramuscular fat deposition compared to commercial breeds selected for fast growth rates, no significant differences were observed for intramuscular fat depots between the two genetic types, neither in the mixed model results (reported in Table 2), nor in the weight of intramuscular fat in PC2 and PC3 (Table S2). This result, together with the findings reported in this work, suggests that despite the genetic differences occurring between highly-selected commercial pigs and Apulo-Calabrese local pig breed, crossbreed and Apulo-Calabrese pigs fed the same diet and reared in the same environment produce meat with comparable quality traits.

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Table. 1. Effects of genetic type (GT), sampling time (T) and their interaction (GT x T) on least square mean (LSM) and standard error of means (SEM) on intensity and T2 weighted signals of Apulo-Calabrese and crossbreed.

TD-NMR T_2 weighted signals	**Breed**	**Measuring time (LSM)**			SEM	**P values of factors of variation**		
		24	72	144		GT	T	GT x T
Intensity of structural water	Apulo Calabrese	2.03	1.74	2.76	0.22	0.9098	0.8222	0.4308
	Crossbreeds	1.98	1.97	1.44	0.35			
Intensity of intra-myofibrillar water	Apulo-Calabrese	86.49	85.49	82.76	0.87	0.9338	<.0001	0.3570
	Crossbreeds	85.78	85.13	83.57	0.96			
Intensity of extra-myofibrillar	Apulo-Calabrese	7.55	7.69	7.46	0.23	0.0044	0.2457	0.2486
	Crossbreeds	6.90	6.27	6.92	0.40			
T2-structural water	Apulo-Calabrese	8.58	8.51	8.85	0.58	<.0001	0.4770	0.5248
	Crossbreeds	7.86	8.26	8.70	0.85			
T2-intra-myofibrillar water	Apulo-Calabrese	46.06	43.32	43.41	0.34	0.0002	0.0001	0.2988
	Crossbreeds	44.52	42.24	41.10	0.56			
T2-extra-myofibrillar water	Apulo-Calabrese	271.26	256.12	269.41	6.16	0.3358	0.1183	0.1688
	Crossbreeds	262.10	258.92	255.26	7.80			
Total intensity	Apulo-Calabrese	96.06	94.90	92.99	0.69	0.0109	<.0001	0.9766
	Crossbreeds	94.63	93.37	91.91	0.75			

Table. 2. Effects of genetic type (GT), measuring time (T) and their interaction (GT x T) on least square mean (L.S.M.) and standard error (S.E.M) of meat quality traits in Apulo-Calabrese and crossbreed pigs.

Meat quality traits	Apulo-Calabrese			Crossbreeds			SEM	P values of the effects		
	Measuring time (LSM)							GT	T	GT xT
	24	**72**	**144**	**24**	**72**	**144**				
pH	5.57	5.54		5.45	5.46		0.0256	0.0583	<.0001	0.5221
L* (brightness)	52.1	52.8	54.0	56.1	56.2	56.3	0.6064	<.0001	<.0001	0.0095
a* (redness)	6.10	6.80	6.70	4.20	4.90	4.60	0.2663	<.0001	<.0001	0.0359
b* (yellowness)	7.26	9.69	8.72	7.24	9.43	8.03	0.2269	0.2403	<.0001	0.0316
Cooking loss	19.51	18.17	18.18	18.67	17.63	18.15	0.5186	0.2089	0.0286	0.1896
Shear force	4.25	3.90	3.48	3.91	3.85	3.31	0.0702	0.0645	<.0001	<.0001
Drip loss	4.19			4.78			0.2296	0.3568		

Figures

Figure. 1. Two typical transverse relaxation time spectra (T2) obtained on meat at 24 h after slaughtering from crossbred (black line) and Apulo-Calabrese (gray line) samples. To allow for a direct comparison among them, the intensities are scaled so that the total area equals 100.

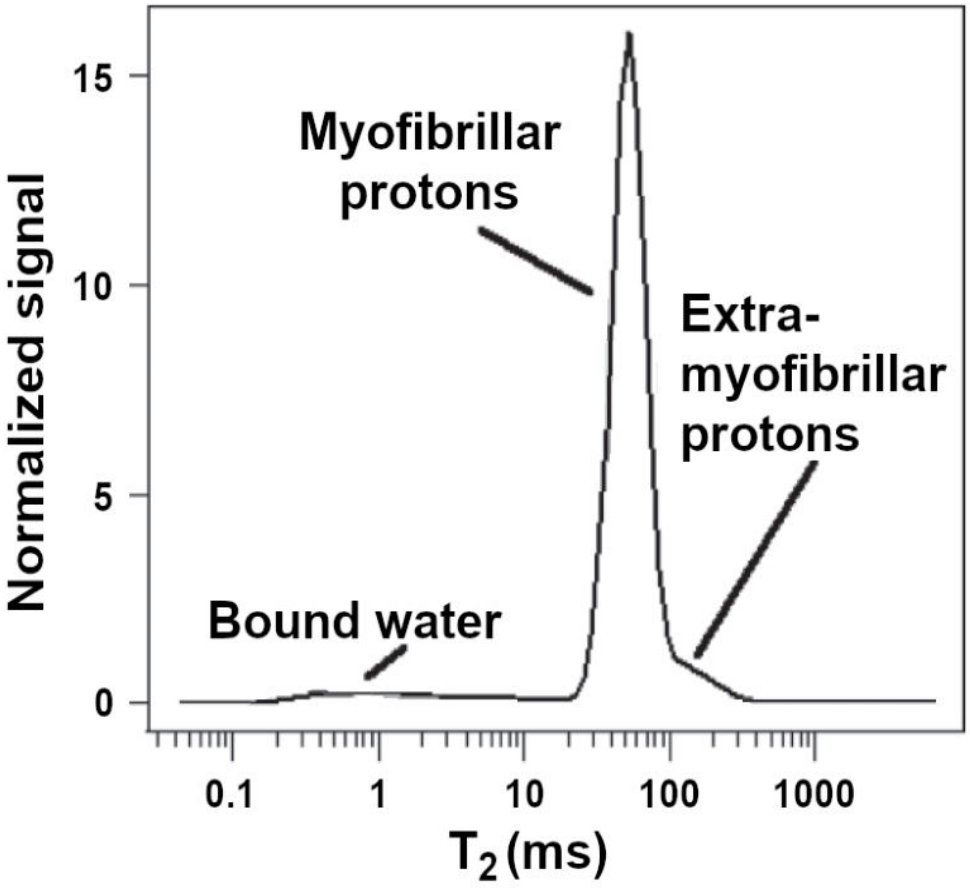
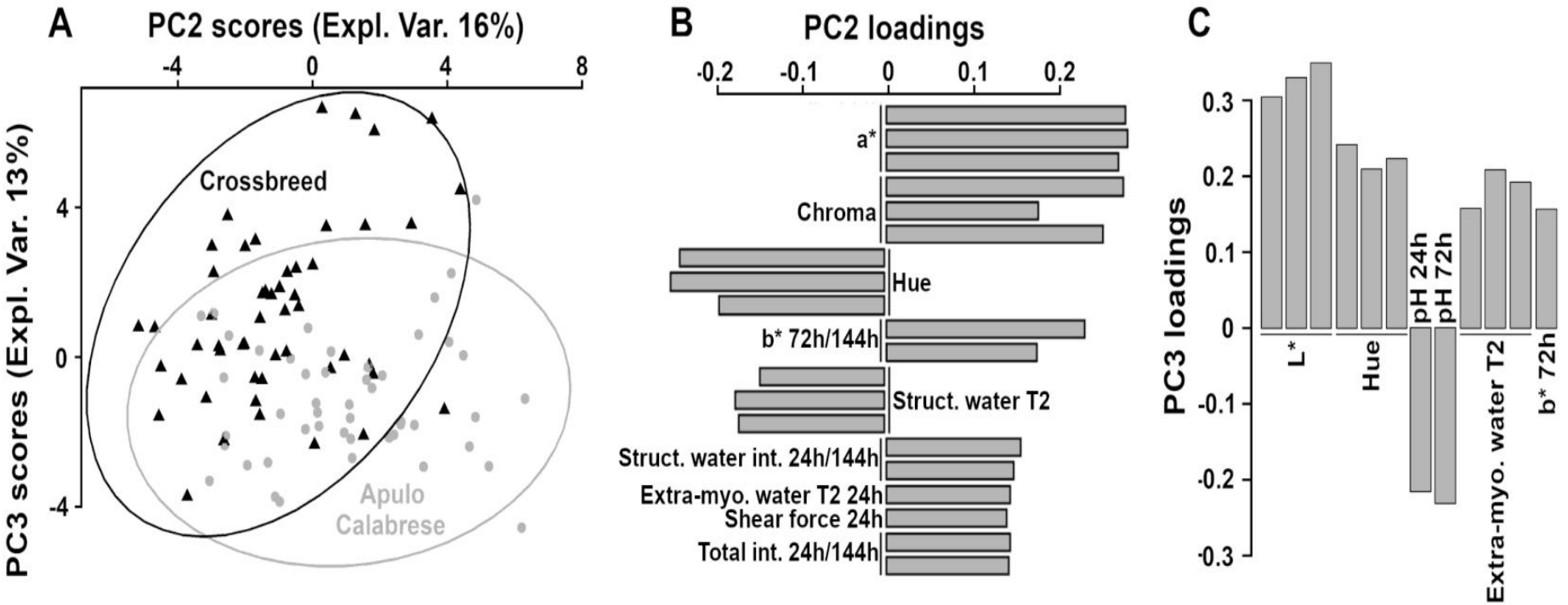


Figure. 2. Principal Components Analysis (PCA) model on the measured traits listed in Table S1. (A) Scoreplot for principal component 2 and 3, where crossbred (black) and Apulo-Calabrese (gray) samples are highlighted. The ellipses represent their Mahalanobis distances. (B and C) Weights of the measured traits along PC2 and PC3. For readability, only the traits significantly correlated with the PCs are reported.



Supplementary Table S1. Principal component loadings for the variables included in Principal Component Analysis.

Variables	PC1	PC2	PC3
Intensity of structural water 24 h	0.188	0.158	0.102
Intensity of intra-myofibrillar water 24 h	0.157	0.033	-0.136
Intensity of extra-myofibrillar water 24 h	-0.093	0.086	0.125
T2 structural water 24 h	-0.236	-0.176	-0.122
T2 intra-myofibrillar water 24 h	-0.092	-0.039	-0.067
T2 extra-myofibrillar water 24 h	0.070	0.146	0.206
Total intensity 24 h	0.197	0.146	-0.042
pH 24 h	0.093	0.129	-0.214
L* 24 h	-0.107	-0.003	0.326
a* 24 h	-0.138	0.283	-0.102
b* 24 h	-0.222	0.045	0.131
Chroma 24 h	-0.216	0.179	0.035
Hue 24 h	-0.020	-0.266	0.207
Shear Force 24 h	0.195	0.142	-0.019
Cooking loss 24 h	-0.107	0.049	0.065
Intramuscular fat	-0.008	0.049	-0.111
Intensity of structural water 72 h	0.131	0.098	0.138
Intensity of intra-myofibrillar water 72 h	0.186	0.033	-0.091
Intensity of extra-myofibrillar water 72 h	-0.137	0.017	-0.083
T2 structural water 72 h	-0.227	-0.147	-0.121
T2 intra-myofibrillar water 72 h	-0.122	-0.094	-0.111
T2 extra-myofibrillar water 72 h	0.025	0.090	0.190
Total intensity 72 h	0.188	0.078	-0.084
pH 72 h	0.118	0.107	-0.229
L* 72 h	-0.053	0.020	0.345
a* 72 h	-0.159	0.280	-0.105
b* 72 h	-0.172	0.233	0.155
Chroma 72 h	-0.179	0.278	0.040
Hue 72 h	0.117	-0.241	0.221
Shear Force 72 h	0.227	0.091	-0.007
Cooking loss 72 h	0.000	0.075	0.104
Intensity of structural water 144 h	0.089	0.150	0.076
Intensity of intra-myofibrillar water 144 h	0.178	0.119	0.098

Intensity of extra-myofibrillar water 144 h	-0.164	-0.029	-0.056
T2 structural water 144 h	-0.230	-0.172	-0.130
T2 intra-myofibrillar water 144 h	-0.120	-0.021	-0.071
T2 extra-myofibrillar water 144 h	-0.048	0.065	0.156
Total intensity 144 h	0.153	0.144	0.104
L* 144 h	-0.057	0.041	0.301
a* 144 h	-0.129	0.272	-0.145
b* 144 h	-0.180	0.177	0.151
Chroma 144 h	-0.186	0.254	0.021
Hue 144 h	0.055	-0.195	0.239
Shear Force 144 h	0.200	0.118	0.038
Cooking loss 144 h	-0.084	-0.001	0.105

CHAPTER 3

Fatty acid composition of the intramuscular fat in the *longissimus thoracis* muscle of Apulo-Calabrese and crossbreed pigs

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Summary

This study aimed to investigate the fatty acid profile of the longissimus thoracis muscle in two genetic types of pigs. Fifty one pigs of the Italian local breed Apulo-Calabrese and 52 crossbreed [Duroc x (Landrace x Large White)] pigs were fed the same commercial diet and reared in the same indoor system. Fatty acid composition was assessed through Folch method and the obtained data were analyzed with a mixed model to identify possible differences between the two genetic types.The Apulo-Calabrese pigs showed significantly higher contents of heptadecenoic acid ($P< 0.0001$), myristic ($P= 0.03$), arachidic ($P= 0.04$), myristoleic ($P= 0.004$), palmitoleic ($P= 0.01$)and gondoic ($P= 0.01$) acids. On the other hand, crossbreed samples presented higher contents of docosahexaenoic ($P= 0.01$) andarachidonic acid ($P= 0.01$).Except for heptadecenoic acid, there were no great differences in longissimus thoracis muscle fatty acid profile between the two genetic types, suggesting that when Apulo-Calabrese pigs are managed in the same rearing conditions as crossbreeds their longissimus thoracis muscle fatty acid composition is similar.

Introduction

Fatty acid (FA) composition has profound effects on the organoleptic properties and nutritional value of meat with regards to human health. However, the technological requirement by the food industry and dietary demands by consumers do not completely match. In particular, pork processing industry requires meat with a limited amount of polyunsaturated FA (PUFA) as they are more likely to incur in lipolytic and oxidative processes, causing rancidity, abnormal flavors, fat softness and altered organoleptic properties (Wood et al., 2008). On the other hand, high proportions of monounsaturated FA (MUFA) and omega 3 (*n*-3) PUFA have been reported to have beneficial effects on consumers' health (Briggs, Petersen, & Kris-Etherton, 2017). Recently, there has been an increasing consumer interest in niche products derived from local pig breeds (Pugliese & Sirtori, 2012) due to their high added value and eating quality. Among the Italian local pig breeds listed in the national herd book is Apulo-Calabrese, from the Calabria region. This breed is characterized by reduced growth and carcass performance, but its meat has quality features suitable for the production of Protected Designation of Origin (PDO) salami (Micari et al., 2009) which is well appreciated not only in Italy but also abroad. This breed is well adaptable to different production systems (Micari et al., 2009; Pugliese & Sirtori, 2012) but is often reared outdoor, which has a negative impact on the environment (Acciaioli, Grifoni, Fontana, Esposito, & Franci, 2012). FA composition of pork is affected by the animal genetics (Wood et al., 2008) and local breeds are generally reported to have a higher propensity for fat deposition. However, most literature in the scientific domain compares the fatty acid profiles of pigs belonging to local breeds reared outdoors, with highly selected pigs reared under the intensive system. This environmental variability makes it difficult to verify the real difference between the two genetic types. The aim of this study was to compare the FA profile of Apulo-Calabrese and crossbreed pigs reared indoors and fed the same commercial diet, in order to identify the effects of the genetic type over muscle FA synthesis and storage.

Materials and Methods

All procedures performed in this study were in line with the Italian and European legislation concerning the protection of animals kept for farming purposes, their transport and their slaughter procedures, and therefore did not require further specific authorization. Slaughter was performed under the control of the Veterinary Service from the Italian Ministry of Health.

Animals

For this study, 51 Apulo-Calabrese pigs (45 gilts and 5 barrows) registered in the herd book of National Pig Breeder Association (ANAS) and 52 [Duroc x (Landrace x Large White)] crossbreed pigs (24 gilts and 26 barrows) were used. All the animals were free from the deleterious alleles of the *Protein Kinase AMP-Activated Non-Catalytic Subunit Gamma 3* (*PRKAG3*) and the *Ryanodine Receptor 1* (*RYR1*) genes (Aboagye et al., 2018). The pigs were reared in the indoor system on the same farm and were fed the same commercial diet (Supplementary Table 1) in a liquid feeding system with dry feed and water mixed in a 1:4 ratio. At the end of the trial, the pigs were transported to a local processing plant and slaughtered after being electrically stunned by tongs (head only; 220 V, 1.3 A). Apulo-Calabrese pigs were slaughtered at 135 kg live weight (364 ± 58 days of age) due to their slow growth whilst crossbreeds were slaughtered at approximately 155 kg live weight (300 ± 30 days of age). These weights were chosen base on the commercial weights of both genetic types. Pigs of the two genetic types were slaughtered in three days. At 40 min postmortem carcasses were eviscerated, weighted and chilled for 3 h at 2 °C. After the chilling, samples of the *longissimus thoracis* (LT) muscle at the level of 6th/7th thoracic vertebrae were collected and stored at -18 °C until further analysis.

Fatty acid analyses

FA composition was measured from *longissimus thoracis* samples and lipids were extracted according to Folch et al. (Folch, 1957). A cold transmethylation was performed on fat according to Christopherson and Glass (Christopherson & Glass, 1969), with some modifications, to convert

fatty acids to the corresponding methyl esters (FAME). About 50 mg of fat was weighed in a conical vial and dissolved in 1 mL of n-hexane, added to 1 mL of the internal standard solution (2.1 mg of tridecanoic acid methyl ester dissolved in n-hexane) and 100 µL of KOH in methanol (c = 2 mol/L). The mixture was vigorously shaken for 30 sec and centrifuged at 252 x g for 3 min. 1 mL of the supernatant was diluted with 2 mL of n-hexane. 1 µL of the organic solution was analyzed by capillary gas chromatography (CGC) employing a RTX-2330 fused silica capillary column coated with 90% bis-cyanopropyl/10% phenyl cyanopropyl polysiloxane (30 m × 0.25 mm i.d., 0.2 µm f.t.) from Restek (Bellefonte, PA, USA) that was fitted on a Clarus 500 gas chromatograph from Perkin Elmer (Shelton, CT, USA). The injector and detector temperatures were set at 240°C. Helium was used as carrier gas at the flow of 1.25 mL/min. The oven temperature was held at 120°C for 1 min, increased from 120°C to 240°C at 4.0°C/min and finally held at 240°C for 10 min. The split ratio was set at 1:40. Peak identification was accomplished by comparing the retention times with those of two FAME standard mixtures: GLC-463 from Nu-Check (Elysian, MN, USA) and FAME 189-19 from Sigma (St. Louis, MO, USA). From each carcass sample were carried out three lipid extractions and the FAMEs composition was measured in 2 replicates for each lipid extract. FA were expressed as percentage on the total FA. Furthermore, as proposed by Boschetti et al.(2016), in addition to the omega 6 to omega 3 PUFA ratio (*n*-6/*n*-3), the following FA ratios were estimated: the palmitoleic isomers to palmitic acid (C16:1/C16:0), the oleic to stearic acids (C18:1/C18:0), the dihomo-γ-linolenic to linoleic acid (C20:3 *n*-6/C18:2 *n*-6), the docosapentaenoic to adrenic acid (C22:5 *n*-3/C22:4 *n*-6) and the arachidonic to linoleic acid ratios (C20:4 *n*-6/C18:2 *n*-6).

Statistical analysis

Data were analyzed using the mixed model procedure of SAS (SAS version 9.3. Cary, NC, USA), including the fixed effect of slaughter day, sex and genetic type, and the random effect of the subject within the day of slaughter. Means were compared using the Turkey-Kramer test,

comparisons showing a $P< 0.05$ were considered significant and a trend towards significance was considered for those showing a $P< 0.10$.

Results and Discussion

The levels of IMF noticed for Apulo-Calabrese and crossbreeds were within the range of values that meet consumers' acceptance (Fernandez, Monin, Talmant, Mourot, & Lebret, 1999). The effect of the genetic type on the fat content and FA profile in the LT muscle is shown in Table 1.

Local pig breeds have been reported to have a higher propensity for fat deposition, anyway in the present study the two genetic types showed only a trend towards significance ($P= 0.08$) for the IMF percentage in LT (Table 1). Concerning LT FA composition in both genetic types, the most predominant FA were oleic (C18:1 *n*-9), palmitic (C16:0), stearic (C18:0) and linoleic acids (C18:2*n-6*). The most significant variation between the two genetic types was noticed for heptadecenoic acid, which is a minor constituent in monogastric animals' fat and its content in pork was already reported in Lo Fiego et al.(2010). No significant differences were found in the contents of total SFA and MUFA between the two genetic types. However, significantly higher contents of myristic (C14:0) and arachidic (C20:0) acids were found in Apulo-Calabrese. Similar findings were reported by Tomovic et al. (2016) for C14:0 when White Mangalica pigs were compared with crossbreeds and Large White pigs but showed the opposite trend for C20:0. According to literature, autochthonous breeds are a rich source of MUFA, which was confirmed by the significantly higher contents of myristoleic (C14:1), palmitoleic (C16:1 *n*-7), heptadecenoic (C17:1), gondoic (C20:1) acids and palmitoleic isomers to palmitic acid ratio (C16:1/C16:0) in the meat of Apulo-Calabrese compared with crossbred pigs (Table 1). The samples of Apulo-Calabrese pigs showed significantly lower contents of docosahexaenoic (C22:6 *n-3*) and arachidonic (C20:4 *n*-6) acids, and a tendency to lower contents of eicosatrienoic (20:3 *n*-3), PUFA, *n*-3 PUFA, *n*-6 PUFA and the PUFA/SFA ratio (Table 1). Similarly, Nevrkla et al. (2017) observed significantly lower contents of eicosatrienoic, arachidonic and docosahexaenoic acids in Prestice Black-Pied pigs than in hybrid

pigs. The difference in PUFA content between crossbreed and Italian local pigs may be due to the different growth performance and adipogenic potential characterising the different genetic types considered. On the whole, these results are in agreement with the fact that lower is the amount of fat stored, the higher is the proportion of PUFA on the total FA (Wood et al., 2008). The reason for this is that PUFA are essential components of cell membranes, and while the storage of energy through SFA may change among individuals and over time, the amount of PUFA remains stable due to their important roles in membranes flexibility. Interestingly, despite the consideration of Apulo-Calabrese as a breed with a high adipogenic potential, the present study did not find great differences between the crossbreed samples and those belonging to this local breed, in agreement with the results on meat quality traits reported in our previous study.

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Table 1. Fatty acid composition of the *longissimus thoracis* muscle of Apulo-Calabrese and crossbreed pigs with the significance for the genetic type effect.

Traits	Apulo-Calabrese	Crossbreeds	*P*-value
Intramuscular fat (IMF; g/100 g of muscle)	2.09 ± 0.14	1.68 ± 0.20	0.08
Fatty acids (FA; % on total FA)			
C10:0 (Capric acid)	0.10 ± 0.00	0.10 ± 0.00	ns
C12:0 (Lauric acid)	0.11 ± 0.00	0.11 ± 0.01	ns
C14:0 (Myristic acid)	1.40 ± 0.02	1.27 ± 0.03	0.03
C14:1 (Myristoleic acid)	0.02 ± 0.00	0.01 ± 0.00	0.004
C15:0 (Pentadecylic acid)	0.05 ± 0.00	0.04 ± 0.00	0.09
C16:0 (Palmitic acid)	24.15 ± 0.25	23.27 ± 0.44	ns
C16:1 *n*-9 (Cis-7 Hexadecenoic acid)	0.36 ± 0.02	0.34 ± 0.03	ns
C16:1 *n*-7 (Palmitoleic acid)	3.14 ± 0.07	2.78 ± 0.12	0.01
C17:0 (Margaric acid)	0.21 ± 0.00	0.18 ± 0.01	ns
C17:1 (Heptadecenoic acid)	0.20 ± 0.00	0.15 ± 0.00	<.0001
C18:0 (Stearic acid)	12.62 ± 0.24	12.81 ± 0.41	ns
C18:1 isomer (Octadecenoic acid isomer)	0.24 ± 0.29	0.53 ± 0.49	ns
C18:1 *n*-9 (Oleic acid)	38.24 ± 0.93	38.23 ± 1.59	ns
C18:1 *cis*-11 (Vaccenic acid)	5.74 ± 0.59	4.49 ± 1.00	ns
C18:2 *n*-6 (Linoleic acid)	8.72 ± 0.43	9.79 ± 0.74	ns
C18:3 *n*-6 (γ-linolenic acid)	0.07± 0.00	0.05± 0.01	ns
C18:3 *n*-3 (α-linolenic acid)	0.26 ± 0.01	0.28 ± 0.02	ns
C20:0 (Arachidic acid)	0.26 ± 0.01	0.15± 0.00	0.04
C20:1 (Gadoleic acid)	0.76 ± 0.00	0.69 ± 0.01	0.01
C20:2 *n*-6 (Eicosadienoic acid)	0.40 ± 0.014	0.43 ± 0.025	ns
C20:3 *n*-6 (Dihomo-γ-linolenic acid)	0.27 ± 0.02	0.29 ± 0.03	ns
C20:4 *n*-6 (Arachidonic acid)	1.76 ±0.18	2.76 ± 0.30	0.01
C20:3 *n*-3 (Eicosatrienoic acid)	0.12 ± 0.00	0.16 ± 0.02	0.06
C22:2 *n*-6 (Docosadienoic acid)	0.11 ± 0.00	0.12 ± 0.01	ns
C22:5 *n*-3/C22:4 *n*-6 (ratio of docosapentaenoic acid on adrenic acid)	0.32 ± 0.02	0.43 ± 0.04	0.01
C20:5 *n*-3 (Eicosapentaenoic acid)	0.09 ± 0.01	0.43 ± 0.04	ns
C24:0 (Lignoceric acid)	0.01 ± 0.00	0.01 ± 0.01	ns
C22:5 *n*-3 (Docosapentaenoic acid)	0.26 ± 0.02	0.30± 0.04	ns
C22:6 *n*-3 (Docosahexaenoic acid)	0.03 ± 0.00	0.08 ± 0.02	0.01
SFA (Saturated fatty acids)	38.82 ± 0.28	37.96 ± 0.49	ns
MUFA (Monounsaturated fatty acids)	48.76 ± 0.64	47.26 ± 1.09	ns
PUFA (Polyunsaturated fatty acids)	12.42 ± 0.67	14.77 ± 1.15	0.09
UFA (Unsaturated fatty acids)	61.18 ± 0.28	62.04 ± 0.49	ns

n-3 PUFA (omega 3 Polyunsaturated fatty acids)	0.77 ± 0.04	0.90 ± 0.07	0.08
n-6 PUFA (omega 6 Polyunsaturated fatty acids)	11.65 ± 0.64	13.88 ± 1.10	0.09
n-6/*n*-3 (ratio of omega 6 on omega 3 polyunsaturated fatty acids)	15.49 ± 0.47	15.97 ± 0.80	ns
C16:1/C16:0 (ratio of palmitoleic isomers and palmitic acid)	0.14 ± 0.00	0.13 ± 0.00	0.04
C18:1/C18:0 (ratio of oleic acid and stearic acid)	3.54 ± 0.07	3.38 ± 0.11	ns
C20:3 *n*-6/C18:2 *n*-6 (ratio of dihomo-γ-linolenic acid and linoleic acid)	0.03 ± 0.00	0.03 ± 0.00	ns
C20:4 *n*-6/C18:2 *n*-6 (ratio of arachidonic acid and linoleic acid)	0.20 ± 0.00	0.26 ± 0.00	0.03
MUFA/PUFA (ratio of monounsaturated fatty acids on polyunsaturated fatty acids)	4.57 ± 0.21	3.95 ± 0.36	ns
MUFA/SFA (ratio of monounsaturated fatty acids on saturated fatty acids)	1.26 ± 0.02	1.25 ± 0.03	ns
PUFA/SFA (ratio of polyunsaturated fatty acids on saturated fatty acids)	0.33 ± 0.02	0.40 ± 0.03	0.07

ns: not significant.

Table S1. The composition of the finishing diet fed to Apulo-Calabrese and Crossbreed pigs.

Components	Raw diet
Digestible Energy (KJ/kg)	14,644
Crude protein (g/kg)	155.00
Crude fat (g/kg)	22.00
Lysine (g/kg)	80.00
Ashes (g/kg)	58.00
Fatty acids (mg/100 g of feed)	
C14:0	0.12
C16:0	13.73
C18:0	2.93
C18:1 *n*-9	21.77
C18:1 *cis*-11	0.85
C18:2 *n*-6	51.06
C18:3 *n*-3	2.73
C20:0	0.34
C20:1	0.41

CONCLUSION

The overall aim of the thesis was to fill the gap in the knowledge on the response of Apulo-Calabrese (a local Italian pig breed) to the transport procedure. The main topic of the research was to investigate the effect of short distance transportation on the behavioral response, blood parameters and meat quality traits of Apulo-Calabrese with regards to crossbreed pigs. The results obtained in this study broaden the knowledge on the Apulo-Calabrese pig, which showed higher levels of lactate, urea and AST after transport indicating a more intense physiological response when compared with crossbreed pigs. With regards to meat quality, similar trends for pH, drip loss and cooking loss were found for both genetic types. The higher a* coordinate found in Apulo-Calabrese pig indicates that meat from this breed has a deeper red color and can be used for the production of typical cured meat which on the basis of the gathered evidence could be produced without the use of additives intended to improve colour.

In the study on the distribution and mobility of water in the meat of Apulo-Calabrese and crossbreed pigs using transverse relaxation time (T2) weighted signals registered by Time Domain Nuclear Magnetic Resonance (TD-NMR). A significantly higher amount of extra-myofibrillar water, higher intra-myofibrillar T2, and total signal amplitude were found in the meat of Apulo-Calabrese samples compared with crossbreed pigs which suggest that water interacted less with meat microstructures in this breed and was, therefore, less tightly retained by them.

The results from the final study supported the hypothesis that when Apulo-Calabrese pigs are reared in the indoor system and fed the same commercial diet as crossbreeds, their longissimus thoracis muscle fatty acid composition is similar to those observed in crossbreed pigs. However, further research is needed to better differentiate and to improve the knowledge of the biological mechanisms underlying the fatty acid profile of local and commercial pig breeds.