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USE OF INDUSTRIAL BY-PRODUCT IN THE SWINE SECTOR IN THE MEDITERRANEAN AREA: EFFECT ON MEAT QUALITY

PhD THESIS

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Abstract

The goal of this thesis work was to include bergamot pulp in pig diets to determine its effects on meat and salami quality.

We conducted two experimental trials, using both ensiled bergamot pulp and dry bergamot pulp.

In the first trial, eighteen pigs were assigned to two experimental treatments and fed a cereal-based concentrate diet (control) or the same diet in which ensiled bergamot pulp replaced 15% dry matter of the diet fed (EBP). The EBP treatment increased α-linolenic acid ($P < 0.05$), docosapentaenoic acid ($P < 0.05$), docosahexaenoic acid ($P < 0.05$) and consequently n-3 PUFA ($P < 0.01$) in meat. In salami, the content of α-linolenic acid, total PUFA and n-3 PUFA increased by feeding the EBP diet ($P < 0.001$). The inclusion of ensiled bergamot pulp in the diet did not alter the oxidative stability in raw and cooked meat and color descriptors. In salami, TBARS values were lower after 5 days of storage ($P < 0.001$) in EBP group.

In the second trial, eighteen pigs were assigned to two dietary treatments and fed a cereal-based concentrate diet (control) or with a concentrate including dried bergamot pulp at the level of 15% dry matter (DBP).

In DBP pork, the proportion of docosapentaenoic acid was significantly increased ($P < 0.05$); it tended to have a greater proportion of n-3 PUFA ($P = 0.09$) and the n-6/n-3 PUFA ratio was lower in DBP treatment ($P = 0.01$). In salami from pigs fed with the DBP, the proportion of α-linolenic acid and the total n-3 PUFA were higher than the control group ($P < 0.001$). In salami, TBARS value was lower after 5 days of storage ($P < 0.001$) in the DBP group.

Finally, dietary supplementation with bergamot pulp to pigs improved nutritional value of meat and meat products.

Riassunto

L’obiettivo di questo lavoro di tesi era quello di includere la polpa di bergamotto nelle diete dei suini per determinarne gli effetti sulla qualità della carne e del salame.

Abbiamo condotto due prove sperimentali, utilizzando sia polpa di bergamotto insilata e sia polpa di bergamotto essiccata.

Nella prima prova, diciotto suini sono stati assegnati a due trattamenti sperimentali e alimentati con un concentrato a base di cereali (controllo) o la stessa dieta in cui la polpa di bergamotto insilata ha sostituito il 15% di sostanza secca fornita (EBP). Il trattamento con EBP ha aumentato nella carne, l'acido α-linolenico ($P < 0,05$), l'acido docosapentaenoico ($P < 0,05$), l'acido docosaesaenoico ($P < 0,05$) e di conseguenza i PUFA n-3 ($P < 0,01$). Nel salame, il contenuto di acido α-linolenico, dei PUFA totali e dei PUFA n-3 è aumentato con la dieta EBP ($P < 0,001$). L'inclusione nella dieta della polpa di bergamotto insilata non ha alterato la stabilità ossidativa della carne cruda e cotta e dei descrittori di colore. Nel salame, i valori di TBARS erano più bassi dopo 5 giorni di conservazione ($P < 0,001$) nel gruppo EBP.

Nella seconda prova, diciotto suini sono stati assegnati a due trattamenti dietetici e nutriti con un concentrato a base di cereali (controllo) o con un concentrato contenente polpa di bergamotto essiccata al livello del 15% di sostanza secca (DBP).

Nel maiale alimentato con DBP, la proporzione di acido docosapentaenoico era significativamente aumentata ($P < 0,05$); tendeva ad avere una proporzione maggiore di PUFA n-3 ($P = 0,09$) e il rapporto PUFA n-6/n-3 era più basso nel trattamento con DBP ($P = 0,01$). Nel salame di suini alimentati con DBP, la proporzione di acido α-linolenico ed il totale di PUFA n-3 erano superiori al gruppo controllo ($P < 0,001$). Nel salame, il valore TBARS era più basso dopo 5 giorni di conservazione ($P < 0,001$) nel gruppo DBP.

Infine, l'integrazione alimentare con polpa di bergamotto nei suini ha migliorato il valore nutritivo della carne e dei prodotti a base di carne.

Keywords

Bergamot pulp, Pork, Meat quality, Oxidative stability, Fatty acid profile

1 – Introduction

1.1. State of the art

The use of by-products from agroindustry as animal feedstuffs is a very old practice. Livestock producers and animal nutritionists are encouraging the use of industrial by-products for several reasons, particularly environmental, economic and social factors. The high prices of conventional feeds have caused the farmers to evaluate utilization of agricultural by-products for animal feeding, particularly to reduce livestock production costs. The alternative feedstuffs, are nowadays available at competitive prices or no cost. On the other hand, feeding animals with agro-industrial by-products could be a valid strategy, in some cases, to enrich diets with bioactive compounds (Biondi *et al*., 2020; Natalello *et al*., 2020). Furthermore, the global intensification of food production has resulted in a significant amount of food wastes, so the use of food residue to livestock is indispensable for reducing the environmental impact.

The definition of agro-industrial by-product is a material that results from a production process, the primary aim of which is not the production of that substance, that can be legally further used directly without any further processing other than normal industrial practice in according to art. 5 of revised waste framework directive (Kasapidou *et al*., 2015).

The growing interest in the use of agro-industrial by-products as zootechnical feed resources is justified by the fluctuation of prices and the supply dynamics of many conventional raw materials (primarily soybeans and maize), which have forced farmers to modify the feeding systems of the animals accordingly. Therefore, the use of local feed resources appears to be able to offer economic advantages through the reduction of conventional feed and environmental costs in terms of reducing the impact that would derive from the need to dispose of many of these biomasses (Vasta *et al*., 2008). Due to the increase in feed costs, fibrous feedstuffs such as citrus pulp, while in the past were considered of marginal quality for monogastrics (Cunha *et al*., 1950), in recent years have been reevaluated for use in swine diets. As outlined by Watanabe *et al*. (2010) the inclusion of a source of fiber for feeding swine could be a strategy to

control fat deposition, e.g. in local breeds that are generally reported to have a higher propensity for fat deposition (Pugliese & Sirtori, 2012). Pigs fall into the category of omnivores. They have a mainly enzymatic digestion, even if microbial fermentation processes of a certain entity take place in their large intestine. In fact, in the omnivores that introduce considerable quantities of cellulose with the diet, the caecum and the colon represent fermentation reservoirs similar to the rumen, omasum and abomasum of ruminants (Bortolami *et al*., 2000).

Fruit of the genus *Citrus* are widespread in the Mediterranean area. The Bergamot (*Citrus Bergamia* Risso) is a hybrid fruit derived from bitter orange and lemon. It is produced almost exclusively in the province of Reggio Calabria (South Italy) to obtain juice and a valuable essential oil. However, a significant amount of waste, which is generally named as bergamot pulp, originates from the processing of bergamot fruit. With the art. 41 – quater of the well-known "decree of doing" (L.D. 69/2013), converted into Law (Law n°98/2013, edited in G.U. n° 184 of 20/08/2013) the citrus pulp (that is the residue of the citrus processing industries), is definitely removed from the waste discipline and submitted to the «citrus by-products». Citrus pulps in general are by-products of the citrus juicing industry that are often included as an energy supplement in ruminant diets (Caparra *et al*., *2007*; Inserra *et al*., 2014; Lanza *et al*., 2015b), especially in citrus-producing regions. Similarly to other citrus pulp (e.g., orange and lemon pulp), bergamot pulp could be used in animal nutrition due to its favorable nutritional composition which make it a valid substitute for cereals in the formulation of concentrates, without particular problems on the production efficiency of animals (Bampidis & Robinson, 2006). Some studies, investigating the chemical composition of bergamot pulp, have reported the presence of some flavonoids such as naringin and neoesperidin (Postorino *et al.*, 2002; Di Donna *et al*., 2009). Flavonoids are substances of particular interest for potential application in the pharmaceutical-nutritional field due to their antioxidant capacities. The use of this citrus by-product in animal feeding may therefore induce some possible antioxidant effects in products of animal origin including meat (Lanza & Bella, 2015a). Moreover, flavonoids show other pharmacological and biological properties: anti-inflammatory, antimicrobical, antiviral activity, effects on capillary fragility, inhibition of platelet aggregation

(antithrombotic effect) antitumor activit (Benavente-Garcìa *et al*., 1997; Delle Monache *et al*., 2013; Navarra *et al*., 2014; Filocamo *et al*., 2015) and it is precisely naringin that is associated with propable protective actions on pre-neoplastic lesions (Kaur & Kaur, 2015).

Recently there has been a growing interest in local pig breeds, such as Apulo-Calabrese, due to high added value and eating quality of their meat, with quality features suitable for the production of Protected Designation of Origin (PDO) salami (Micari *et al*., 2009). The Apulo-Calabrese pig is an Italian autochthonous breed from Calabria region well adaptable to different production systems (Micari *et al*., 2009; Pugliese & Sirtori, 2012).

In the literature, there are some studies on the use of citrus pulp in ruminant nutrition (Bueno *et al*., 2002; Caparra *et. al*., 2007; Inserra *et al*., 2015; Lanza *et al*., 2015b). However, few papers have been reported on the utilization of bergamot pulp in diet for growing ruminants (Scerra *et al*., 2018), and to the best our knowledge, no studies investigated the effects of feeding pigs with bergamot by-products on the quality of meat and typical products deriving from it.

1.2. Meat quality

The term “quality” is commonly defined as a characteristic, a value or a property attributed to an object or a person. It is not possible to define quality objectively and unambiguously since the concept itself can be split into an objective component, linked to the technical aspects of the product that must satisfy the user's needs, and into a subjective one, linked to the aspects that must satisfy their expectations and desires.

According to the official definition of ISO 8402 (1995):

"Quality is the set of properties and characteristics of the product that gives it the ability to satisfy the expressed or implicit needs of customers".

The satisfaction of the quality demand is extremely complex and linked to a multifactorial set of hygienic-sanitary, technological, organoleptic components (Panella *et al*., 1995), very difficult to define univocally and in any case extremely variable in time and space.

In the past, in the purchase of meat, some factors such as color and smell dominated in addition to the economic aspect. In recent years, others have been added to these, such as those related with human health (lean meats, white *vs* red meat preferences, etc.), and the guarantee of origin (possibility of reading information labels or buying branded meats). Crises due to the danger of BSE, foot-and-mouth disease and avian flu have certainly weighed on these last aspects, which have led consumers to ask for more information and traceability. The perception of quality, in fact, especially by consumers in the most economically advanced countries has undergone a progressive change, moving from the concept of “product quality” to that of “production process quality”, as also shown by recent surveys at European level (Rijswijk *et al*., 2008).

In this context, the typicality has not only the purpose of privileging organoleptic sensations to the rediscovery of ancient flavors, but also of evoking production processes that guarantee healthiness and identifiable with a commitment to the territory and the environment.

The factors that plays a major role in determining the quality of meat can be divided into (Sarti, 1992c):

- ENDOGENOUS FACTORS: Age and fatness, conformation, genetics and sex.

- EXOGENOUS FACTORS: nutrition, farm management, climatic factors, stress (farming and transport system) and slaughtering methods (stunning and carcass treatment).

In accordance with regulation 853/2004, at European level, by definition of MEAT we mean all the edible parts of animals including blood. (EC Reg. N° 853/2004).

Meat occupies an important place in human nutrition both for the considerable amount of nutrients it possesses: proteins, lipids, carbohydrates, mineral salts, vitamins and water and because it lends itself well to transformation into products that can be stored for more or less long periods. The study of the qualitative characteristics of meat can be divided into four main aspects:

- hygienic-sanitary quality;
- chemical-physical and nutritional quality;
- sensory quality;
- technological quality.

Hygienic-sanitary quality

The definition of hygienic-sanitary quality refers mainly to the concepts of safety and healthiness.

The “healthy foods” are already defined by the D.L. 155 of 97, such as those “foods suitable for human consumption from a hygienic point of view”.

A safe food is that without biological, chemical or physical agents capable of causing harm to human health.

The increase in intensive farms has led to a greater use of drugs not only in the care of animals, but also to promote their growth, with the consequence that toxic residues, deriving from the biotransformation of these molecules, can be found in the food. Antibiotics, anabolics, cortisones, beta-agonists and/or beta-stimulants can be fraudulently used to increase slaughter yields and reduce feeding costs, with risks for human health.

Another problem may be represented by health risks due to the presence of pathogenic microorganisms, their metabolites and their toxins in quantities exceeding the minimum legal values.

The hygienic-sanitary quality of the meat must therefore be guaranteed throughout the production chain: during the rearing phase, making careful use of pharmaceutical products and respecting the suspension times by using feed that does not cause damage to the animal's health; during slaughter, which must be carried out taking into account the elementary rules of good hygiene practice (i.e. cleaning of equipment, premises and personnel clothing) and avoiding as much as possible the cross-contamination that can occur, for example, with the passage of the staff from “dirty” to “clean” areas.

As a guarantee for the consumer, all animals destined for slaughter and slaughtered meat must undergo a veterinary examination.

“Meat from animals not subjected to ante-mortem and post-mortem inspection is declared unfit for human consumption” (EC N ° 853/2004).

A tool to guarantee the consumer is represented by process certifications regarding the hygiene and safety of meat. The self-control of production processes obliges the manager of the food industry to withdraw products that may present an immediate risk to human health.

Chemical-physical and nutritional quality

The chemical-physical and nutritional quality of meat refers above all to its content in fats, proteins, minerals and vitamins. Meat contains from 50 to 80% water and is made up of muscle tissue (the predominant part), connective tissue and adipose tissue (Piciocchi, 2009).

The connective tissue, organized in the epimysium (membrane that surrounds the muscle mass, from the inside of which the perimysium originates, which incorporates the muscle bundles, blood vessels and major nerves), and in endomysium (dense connective network that departs from the perimysium and surrounds every single muscle fiber. Endomysius, perimysium, epimysium, at the two ends of the muscle, merge into a single structure that forms the tendon, through which the muscle attaches itself to the bones. The percentage of connective tissue varies from muscle to muscle: it is greater, for example, in the forequarters of slaughter animals and in working muscles. Muscle tissue is made up of muscle bundles, within which muscle fibers are distinguished. The muscle fiber is the anatomical-functional unit of the muscle. It is a long and narrow cell, polynucleated and wrapped in a cell membrane called sarcolemma. Numerous fibrils are immersed in the cytoplasm. Each myofibril is surrounded by the sarcoplasmic reticulum which communicates, through tubules, with the sarcolemma, allowing the transmission of the nerve impulse. The myofibrils, observed under the microscope, appear transversely striated: light bands and dark bands alternate regularly forming the contractile units called sarcomeres (Cappelli &Vannucchi, 2007).

The relationship between the three components of the meat (muscle, connective, fat) and therefore the nutritional value of the same, varies according to the breed, age, type of breeding and type of cut. Pork meat is the one with the highest lipid content, followed, in descending order, by that of: adult bovine, turkey, veal, chicken, rabbit and lamb. Regardless of the breed, the lipid content tends to increase with age, although today greater attention by the breeder towards feeding livestock leads to obtaining lean cuts even from animals that are no longer very young (Piciocchi, 2009).

The constituents of meat that exhibit greater variability are water and fat.

The water content can range from 49% (goose) to 77% (lean veal).

The fat content from 0.6 – 0.7% (lean rabbit, guinea fowl breast) to 34% of the goose and 22% of the fat pig (Castello, 2018).

Meat lipids

Apparently lean meat can contain 8 – 9% of lipids (Cappelli &Vannucchi, 2007).

Meat lipids are represented by saturated (40%), monounsaturated (35%) and polyunsaturated (25%) fats, the cholesterol content varies between 45/80 mg per 100 g of edible portion (Piciocchi, 2009).

Fatty acids are the most important and common component in all lipid classes and as such are widely represented in living organisms in which they perform structural, energy and metabolic functions (Agostoni & Bruzzese, 1992).

They can be saturated, no double bond (Palmitic and Stearic) or unsaturated: monounsaturated, one double bond (Oleic) and polyunsaturated, two or three double bonds (Linoleic and Linolenic). The predominant fatty acids in slaughter animals are oleic, palmitic and stearic. Linoleic essential fatty acid and its conjugated isomers (CLA) are also present (Cappelli & Vannucchi, 2007).

The composition of fatty acids in meat is of considerable importance for human health. In recent decades, studies on the presence of fatty acids in the human diet have mainly focused on the role of different fatty acids.

In fact, saturated fatty acids are associated with an increased risk of obesity, hypercholesterolemia and cancer (WHO, 2003).

Polyunsaturated fatty acids (PUFA) lower cholesterol levels, but excessive consumption can lead to immunosuppression and cancer. A low intake of polyunsaturated acids in the diet has shown an increased risk of developing cardiovascular diseases, the main cause of death in industrialized countries (Alfaia, 2006).

Most of the fatty acids in meat are in the form of saturated fatty acids (SFA), in some cases accused of increasing serum cholesterol levels in humans (Groff *et al*., 1996).

Sometimes, the definition of meat quality and its organoleptic characteristics is linked to the ratio of polyunsaturated and saturated fatty acids (PUFA/SFA) and in particular

the ratio between n-6 and n-3 FA is considered responsible for the aroma (Wood & Enser, 2007).

The most common fatty acids are (Cappelli & Vannucchi, 2007):

	ATOMS OF C	STRUCTURE	DENOMINATION OF ACIDS
Linear saturated	12	$CH_3(CH_2)_{10}COOH$	*n*-dodecanoic lauric
	14	$CH_3(CH_2)_{12}COOH$	*n*-tetradecanoic myristic
	16	$CH_3(CH_2)_{14}COOH$	*n*-hexadecanoic palmitic
	18	$CH_3(CH_2)_{16}COOH$	*n*-octadecanoic stearic
	20	$CH_3(CH_2)_{18}COOH$	*n*-eicosanoic arachidic
Monounsaturated	14	$CH_3(CH_2)_3CH=CH(CH_2)_7COOH$	9-tetradecenoic myristoleic
	16	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	*cis*-9-hexadecenoic palmitoleic
	18	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	*cis*-9-octadecenoic oleic
	18	$CH_3(CH_2)_5CH=CH(CH_2)_9COOH$	*cis*-11-octadecenoic vaccenic
	20	$CH_3(CH_2)_9CH=CH(CH_2)_7COOH$	9-eicosenoic gadoleic
Polyunsaturated	18	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	*cis, cis*-9,12-octadecadienoic linoleic
	18	$CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$	*cis, cis, cis*-9,12,15-octadecatrienoic linolenic
	18	$CH_3(CH_2)_4CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_4COOH$	*cis, cis, cis*-6,9,12-octadecatrienoic γ-linolenic
	20	$CH_3(CH_2)_4(CH=CHCH_2)_4CH_2CH_2COOH$	*cis, cis, cis, cis*-5,18,11,14 eicosatetraenoic arachidonic

Proteins

The protein content of meat varies within a very narrow range, from 15% to 23% (Cappelli & Vannucchi, 2007).

Meat contains a high percentage of proteins of high biological value, that is, good quantities and balanced proportions of all the amino acids essential for the formation, growth and maintenance of our body, including some "essential" ones (lysine, tryptophan, amino acids sulphites, etc.) which are generally present in insufficient quantities in proteins of vegetable origin (Wu, 2016).

Depending on the location, we will have myofibrillar, sarcoplasmic and stroma proteins.

Myofibrillar proteins include contractile proteins (Actin and Myosin) and those regulating contraction. These proteins influence the commercial and culinary qualities of meat: water retention (most of the water present in the muscle is located between the protein filaments of myofibrils), emulsifying properties (important in the preparation of sausage dough) and also the softness of the meat.

Sarcoplasmic proteins include: cytoplasmic enzymes that regulate glucose and protein metabolism and mitochondrial and lysosomal enzymes; myoglobin and cytochromes which, with the hemoglobin of the red blood cells, give the red color to the meat (Cappelli & Vannucchi, 2007).

The myoglobin content is maximum in the red fibers, minimum in the white ones. Muscles with more than 40% red fibers are classified as red muscles, those with less than 30% red fibers are white muscles.

Another protein that distinguishes meat and is also important for its nutritional and sensory characteristics is hemoglobin. It is bound to red blood cells and approximately 95% is eliminated after bleeding. The concentration of hemoglobin in the muscle largely depends on the degree of bleeding which in turn can be influenced by the slaughtering techniques.

On the contrary, myoglobin remains in the muscle cells even after slaughter and bleeding.

Finally, the proteins of the stroma, the proteins of the cellular and subcellular membranes and those of the connective tissue, in particular collagen, which gives rise to gelatin after cooking in water, and elastin are part of the proteins.

There are also significant quantities of non-protein nitrogen compounds in meat:

- free amino acids, dipeptides (carnosine, anserine), oligopeptides;
- amines;
- nucleosides, nucleotides, including inosinic acid that forms during maturation;
- pyrimidine and purine bases;
- creatine and creatinine;
- urea and ammonia.

From a nutritional point of view, these substances are sometimes of interest as bioactive and antioxidant components, furthermore their presence contributes to giving the meat its typical flavor; after boiling, they pass, with the water-soluble proteins, into the cooking broth.

Some of these compounds perform important biological functions. Creatine and creatinine are present in almost all vertebrate tissues, but 98% are found in muscles (Kréas = meat in Greek). A typical mammalian skeletal muscle contains 300 – 500 mg/100 g of creatine, combined for 80% with orthophosphoric acid, in the form of dipotassium salt (Cappelli & Vannucchi, 2007).

Vitamins and Minerals

Meat is also an important source of vitamins (B_1, B_2, B_6, niacin and B_{12}) and minerals (zinc, copper, selenium and iron) (Ahmad, 2018).

In particular, vitamin E is a natural antioxidant widely used in the food industry. Numerous researches show that the presence of vitamin E can definitely improve the sensory characteristics of meat and derived products, such as freshness, tenderness, color, and prevents the loss of aroma (Smith *et al*., 1996).

Vitamin E is the main natural active antioxidant in the form of α-tocopherol. It works as a fat-soluble antioxidant of cell membranes (Hajibabaei, 2016). It protects phospholipids and cholesterol by counteracting the formation of free radicals, reduces

the degradation of vitamins of groups A and B, performs an antithrombotic activity and prevents premature aging of tissues.

It has been shown that the intake of vitamin E with the diet increases the amount of α-tocopherol deposited in the muscle and fat tissues (Jensen, 1997) reducing both the oxidative processes and the formation of metmyoglobin (browning of the meat) (Yin, 1993), and improving the organoleptic and nutritional.

Sensory quality

Quality is also defined by sensory parameters, which can be assessed on the raw product, and which therefore mainly influence the consumer's choice to buy the product or not, such as color, aroma, grain, marbling fat, water retention capacity (Lanza & Biondi, 1990; Sarti, 1992c; Panella *et al.*, 1995).

Other parameters, on the other hand, can be assessed at the time of use, i.e. on the cooked product, such as flavor, juiciness, tenderness, decrease in cooking and overall satisfaction (Lanza & Biondi, 1990; Panella *et al*., 1995) and are ascertainable with instrumental laboratory methods or through *panel-tests*.

Color

The first perception that consumers have of all meats is color.

The color of meat is given above all by myoglobin, a red pigment of muscle tissue, prevalent in the muscles compared to hemoglobin in the blood (Lawrie, 1983). The changes in the color of the meat can be attributed to the changes in the chemical state of this pigment, which can vary from purple red (reduced myoglobin), to bright red (oxygenated myoglobin), to brown red (oxidized myoglobin), while serious alterations of the meat, and therefore of the pigment, can give abnormal colors such as brown-greyish or green (Lawrie, 1983). Physical variations of the muscle (low pH, closed and highly reflective myofibrillar network) can give pale flesh (Panella *et al*., 1995).

The color therefore depends mainly on the myoglobin, on the chemical state in which the iron is found inside it, on any alterations of bacterial origin, on the type of muscle fibers that make up the muscle (red, white, intermediate), on the activity carried out by the muscles (more intense color in muscles very stressed through locomotion) and by the presence of other components such as fat (Hendrick *et al*., 1994).

In addition to this, it also depends on its behavior with respect to the absorption and diffusion of light, which in turn are related to the myofibrillar concentration and oxygen penetration (Cattaneo, 1995).

The color is evaluated instrumentally with a reflectometer, generally according to the indications of the CIE, Commission Internationale de l'Éclairage, or according to the Hunter method, investing the surface of the meat with standard illuminants, and

detecting a triad of parameters: L * (luminosity or lightness), a * (red-green index) and b * (yellow-blue index), with the method therefore called CIEL * a * b * or CIELAB, also using the derived quantities: Chroma or Saturation (C), which indicates how much white is mixed with the color, and Hue (H), which indicates the dominant color.

There is also a subjective evaluation of color, not widely used, because it is less satisfactory than the instrumental one, and is also based on numerical scales, for example with values from 1 (pale) to 5 (dark red) (Sañudo *et al*., 1996).

Tenderness

The tenderness of the meat is understood as resistance opposed to chewing (Monsón, 2005).

It is influenced by a number of factors, such as the age of the animal, the duration of the aging process, the marbling, as well as the breed.

Aging is the process during which there is a biochemical transformation of the muscle that acquires the characteristics of tenderness, juiciness and flavor typical of meat (Kim, 2018).

During the maturation process the proteolytic enzymes, which intervene on the structural proteins of the muscle, have little influence on those of the connective tissue (collagen and elastin), which is why increasing the age of the animal, due to the greater presence of connective tissue, the meat is harder. The aging time has a positive effect on tenderness, but is limited by microbial development, with consequent alteration of the meat. The degree of marbling (quantity of intramuscular fat) also influences the tenderness, juiciness, flavor of the meat and limits cold contracture, because it determines a gradual cooling of the carcasses placed to mature in the cold rooms. During cooking, however, the fat retains the right amount of water in the meat, making it more juicy (Piciocchi, 2009).

Tenderness is generally defined as Shear Force measured in kg/cm^2 and is determined with equipment such as bitetenderometer and Instron with Warner Blatzler Shear (Panella *et al*., 1995); it consists of the effort necessary to cross a piece of meat of a certain thickness or to penetrate it to a certain depth, but it can also be measured as the

crushing effort of a sample of meat (Lawrie, 1983). Another method for the assessment of tenderness is the detection of the thermal solubility of collagen (Grau, 1978).
In the panel-tests, tenderness is evaluated as the opposite of the force required to pierce the meat sample with the molars: greater tenderness corresponds to less force used (Campo *et al*., 1999).
Tenderness is related to grain and texture, which are in turn defined by the diameter of the muscle fiber bundles into which the muscle is divided by the connective tissue (Lusetti, 1983).
Grain is evaluated as the cross-sectional aspect of a cut of meat, perpendicular to the muscle fibers. When the cut surface appears soft and velvety the grain is called fine and is indicative of a reduced diameter of the bundles of fibers, while if the cut surface is rough and dry the grain is called coarse, it is attributable to a large diameter of the bundles and is typical of older animals. It should also be noted that different muscles generally have different grains (Lusetti, 1983).
The texture is evaluated instead by dissecting the muscle in the direction of the fibers and stretching it slightly: there is a compact texture in young and well fed animals and lax in very young or old animals, undernourished or poorly fed. Furthermore, the texture also depends on the type of muscle (Lusetti, 1983).
According to Carlucci *et al*., (1999) meat, with regard to texture, can be defined:

- tender, when it is easy to chew,
- fibrous, when you feel the fibers when chewing,
- juicy, when you feel water when chewing,
- cohesive when it is difficult to swallow.

In the panel-tests, the texture is evaluated as the fiber perceived by the taster on the sample after four chewing acts; the residue is also evaluated, defined as the amount of connective tissue perceived by the taster before swallowing (Campo *et al*., 1999).

Aroma

The English term “flavor” is rendered in Italian as “aroma” and generally for meat it is defined as a combination of flavor and smell (Grau, 1978) but, according to some authors, it also includes texture and pH (Lawrie, 1983). The aroma in meat is due to

adipose tissue, predominantly compared to muscle (Lanza & Biondi, 1990), since the former is able to "trap" aromas originating from other chemical compounds and then release them during cooking. Above all, because the volatile substances that are formed during cooking are derived from the oxidation of lipids, as well as from the Maillard reaction between amino acids and carbonyl compounds (Elmore *et al*., 2000).

Juiciness

It is an extremely important sensation to define the liking of a meat: it can be distinguished in an immediate component, given by the sensation of humidity during the first chewing acts, due to the rapid release of liquids from the meat, and a prolonged component, mainly due to the stimulation of salivation by the fat of the meat. This explains why the meat of young animals can initially give a sensation of juiciness, and then appear dry, given their scarcity of intramuscular fat (Lawrie, 1983).

Juiciness is evaluated by the panel-test as a liquid released from the sample after a certain number of chewing acts, usually two (Campo *et al*., 1999), or as a perception of total moisture in the mouth after chewing (Sañudo *et al*., 2000b).

Of course, all the factors that determine water losses, such as defrosting or certain cooking methods, result in a decrease in juiciness, which is closely linked to the water retention capacity (Lawrie, 1983).

Amount of fat

Italian consumers do not like meats that are too fatty, yet a moderate amount of fat, marbling and subcutaneous, gives the meat some positive characteristics, such as greater tenderness, juiciness, aroma and palatability (Jeremiah, 1998; Sañudo *et al*., 2000a; Sañudo *et al*., 2000b).

Technological quality

Technological quality refers to the aptitude of the meat to be preserved and processed. It includes water retention, aptitude for storage in refrigeration conditions and pH, which, despite being a chemical characteristic, following post mortem modifications, plays an important role in the preservation and transformation of meat. The technological characteristics result from objective measurements, by means of instruments, of characters that consumers perceive through their senses.

Among these factors, the following deserve particular importance:

Water holding capacity (WHC)

The water holding capacity (WHC or Water Holding Capacity) represents the ability of the meat to hold water during the application of external forces, such as cutting, grinding or pressure and affects the appearance of raw meat, its behavior during cooking and its juiciness during chewing (Lawrie, 1974).

The water present in muscle tissues is normally found in different stages in quantity and mobility according to the type of protein binding (Cattaneo, 2003):

- constitution or hydration water: localized between protein molecules and chemically bound to proteins, it cannot be mobilized and is not lost with normal drying;
- interface water: localized on the protein surface in mono or multimolecular layers. The WHC is not associated with this;
- free water: makes up most of the muscle water, bound by capillary forces, mainly between the thick and thin filaments. This share is easily lost by evaporation or drying and is more or less detectable by applying a force. It is the most important portion in determining WHC variations.

WHC is one of the qualitative criteria that allow to distinguish meats according to their technological aptitude: those with low WHC, highlighted by a considerable exudation of raw refrigerated meat, are considered undesirable both for direct consumption and for processing. Meat with a high WHC may be unacceptable to the consumer, but it is usable in many processing processes, as it is very important that, especially after cooking, it retains its constitution water (Cattaneo, 2003).

The WHC of the muscle affects not only the performance, but also the sensory characteristics of the product, which certainly does not benefit from a dry consistency. The method commonly used to evaluate the WHC is that, simple but sufficiently exact, of Grau & Hamm (1953), which consists in subjecting the meat, under strictly pre-established conditions, to a given pressure, such as to allow the free water to escape and not the bound one, which remains in the muscle (Grau, 1978).

Other more precise methods use centrifugation with coded parameters (Castellini *et al*., 1998).

WHC is not uniform, varies with individual, race, age, sex, diet, government, manner of slaughter and also varies from muscle to muscle (Lawrie, 1983).

WHC is affected by pH.

pH

The pH is determined at slaughter (pH_0) and after 24 hours (pH_{24}), it is the first indicator of meat quality and allows us to evaluate the potential of the animal muscle to become good meat. This parameter also gives a measure of the aptitude for conservation of this food: in fact, low pH values limit microbial growth and thus prevent possible alterations (Dell'Orto & Sgoifo Rossi, 2000).

In order to have good quality meat, the pH must decrease after slaughter. This is due to the increase in lactic acid in the muscle originating from the post-mortem glycolysis of glycogen. This decrease must be gradual because if it were too rapid, there would be a denaturation of proteins and a lowering of water retention capacity (Lawrie, 1983; Lanza & Biondi, 1990).

The pH is also modified by the storage methods: freezing causes a decrease in pH compared to refrigeration alone (Moore *et al*., 1998).

If the animal is in stressful conditions, especially immediately before slaughter, the muscle reserves of glycogen are reduced, limiting the drop in pH due to glycolysis: thus the pH cannot reach low enough values and the meat is presented "scrambled" or DFD, ie dark, compact and dry (Lawrie, 1983; Sarti, 1992c; Renieri *et al*., 1993; Dell'Orto & Sgoifo Rossi, 2000). Conversely, a too rapid drop in pH can result in pale, soft, exudative or PSE meats (Renieri *et al*., 1993). The defect of PSE meat has a

genetic origin (Bittante *et al*., 2003). The PSE condition is controlled by at least two genes: the first is associated with a high stress-induced speed of glycolysis, while the second, not related to stress syndromes, it is associated with a pHu of very low meats (Church & Wood, 1992).

Each of the post-mortem active enzymatic complexes in the muscles has optimal characteristic pH values, and thus the tenderness, aroma, water retention power and color of the meat are influenced by the pH itself, which thus assumes considerable importance in muscle transformations after slaughter (Panella *et al*., 1995; Dell'Orto & Sgoifo Rossi, 2000).

The rapid acidification brings the muscle pH to values close to isoelectric point of proteins, minimizing their hydrophilicity, it also combines at high temperature, it can denature the myoglobin pigment with color changes and ultimately affect negatively also on the complex of calpaine, the proteases on which meat tenderness mainly depends (Dell'Orto & Sgoifo Rossi, 2000).

PSE meats have an uninviting appearance for the consumer and are unsuitable for processing into typical cured meats (Bittante *et al*., 2003).

Apulo-Calabrese meat is mainly used for the manufacture of typical meat products as salami (Ambrosio *et al*., 2021).

Salami is defined as: cured meat product, consisting of meat obtained from striated musculature belonging to the carcass of pork with added salt and possibly other species of meat animals, minced and mixed with pork fat in proportions variable, and stuffed into natural or artificial casings (G. U., 2005).

Sodium chloride has played a very important role in the processing of these products for millennia because it dehydrates the tissues, inhibits the development of harmful microorganisms and favors the good ones, useful for maturation and seasoning (Asaro *et al*., 2007).

In fact, cured meats can be considered real bioreactors in which, during maturation, numerous enzymatic processes take place, which give them the final organoleptic qualities appreciated by the consumer (Cappelli & Vannucchi 2007).

Finally, what I define as “social quality” is becoming increasingly important and includes bioethical and ethical aspects linked to the production process of a food.
The bioethical aspects are linked to the sensitivity of society towards respect for animal welfare and rights. The ethical aspects refer to the consumption of food in the production of a product of animal origin.

1.3. Aim of the PhD thesis

It is now evident that industrialization has led to the generation of large quantities of food waste and in this regard, in order to try to reduce pollution, industrial ecology and the circular economy are considered the guiding principles of eco-innovation that are focus on a "zero waste" society and economy, where waste can be used as raw materials (Kasapidou *et al*., 2015).

Citrus pulp, now reclassified as a citrus by-product and no longer considered waste, is rich in bioactive compounds, such as unsaturated fatty acids, vitamins and phenolic compounds (Balasundram *et al*., 2006; Ladaniya, 2008).

The goal of this thesis work was to include bergamot pulp in pig diets to determine its effects on meat and salami quality.

2 – Materials and Methods

2.1. Experimental design

The experimental trials were conducted with Apulo-Calabrese pigs, an Italian autochthonous breed, in a farm oriented on heavy pig production. All the procedures were approved (prot. No. 286946) by the Animal Welfare Committee (O.P.B.A) of the University of Catania.

We conducted two experimental trials. In the first trial we used ensiled bergamot pulp and in the second trial we used dry bergamot pulp as a novel food for pigs.

Fresh bergamot pulp was obtained from a juice citrus industry and ensiled for 90 days, while dried bergamot pulp was provided from a pharmaceutical company after the extraction of phenolic compounds. The extraction of these compounds was carried out using the bergamot fruit without its external cuticle and its flavedo. Columns containing polystyrene adsorbent resin were used to absorb polyphenols, which were then eluted and recovered, following further steps.

Apulo-Calabrese barrows were weighed, individually identified and allocated in individual pens. After a period of adaptation to the experimental diet of 10 days, the barrows were finished for 120 days with the dietary treatments offered *ad libitum* and formulated to contain: only concentrate (control group; 9 pigs), concentrate and ensiled bergamot pulp at the level of 15% dry matter (DM) on the diet fed (EBP group, 9 pigs) or with a concentrate including dried bergamot pulp at the level of 15% dry matter (DBP group, 9 pigs).

The ingredients and chemical composition of the experimental diets are reported in Table 1 for the trial with EBP and in Table 2 for the trial with DBP.

The concentrate offered to the pigs of the bergamot pulp group had the same ingredients of the concentrate supplied to the pigs of the control group but, in order to maintain a similar crude protein concentration between treatments, had a higher soybean meal content and a lower percentage of barley and maize (Table 1 and Table 2). The diets with ensiled bergamot pulp or with dried bergamot pulp were a mixture of concentrate with the respective amount of ensiled or dried bergamot pulp (Trial n° 1 or Trial n°2).

All the pigs were offered the concentrate twice daily, at 7:00 am and 4:00 pm. For each pig, offered and refused feed was recorded every day to calculate dry matter intake (DMI). Water was continuously available. Pigs were weighed at the beginning, in the middle and at the end of the experimental trial to determine average daily gain (ADG). Feed conversion ratio (FCR), which measures the amount of feed required for the growth of one kg of live weight of the animal, was also calculated.

Table 1

Ingredients (% on DM basis) and chemical composition of experimental diets (Trial n° 1 with EBP)

	Control Diet	EBP Diet	Ensiled bergamot pulp
Barley	30	23	
Maize	30	23	
Oat	16	12	
Soybean meal	7	10	
Faba bean	14	14	
Ensiled bergamot pulp	-	15	
Vitamin mineral premix[1]	3	3	
Chemical composition			
Dry matter (DM) g/Kg wet weight	885	606	185
Crude protein g/Kg DM	136	132	50.8
Ether extract g/Kg DM	31.9	23.7	13.7
Ash g/Kg DM	39.7	44.8	53.8
NDF g/Kg DM	436	360	246
Total phenolic compounds (g TAe[2]/Kg DM)	1.55	6.58	14.2
Tocopherols, μg/g dry matter			
α-Tocopherol	2.52	68.8	169
γ-Tocopherol	5.32	7.67	11.3
fatty acids (g/100g of total fatty acids)			
C10:0	-	0.04	0.09
C12:0	0.04	0.09	0.16
C14:0	0.13	0.24	0.30
C16:0	14.5	16.3	19.4
C16:1	0.16	0.34	0.58
C18:0	2.52	2.79	2.97
C18:1 n-9	31.3	29.9	25.1
C18:2 n-6	44.4	39.5	34.1
C18:3 n-3	2.13	4.91	8.92
C20:0	0.12	0.15	0.19

[1]The mineral vitamin premix consisted of vitamin A=6750 UI; vitamin D3=1000UI; vitamin E 2 mg; vitamin B12 0,01 mg; vitamin B1 1mg; folic acid 0,2 mg; D-pantotenic acid 5 mg; Co 0,05 mg; Mn 12,5 mg; Zn 15 mg; Mo 0,5mg.

[2]tannic acid equivalent.

Table 2

Ingredients (% on DM basis) and chemical composition of experimental diets (Trial n° 2 with DBP)

	Concetrate Diet	DBP Diet	Dried Bergamot pulp
Barley	30	23	
Maize	30	23	
Oat	16	12	
Soybean meal	7	10	
Faba bean	14	14	
Dried bergamot pulp	-	15	
Vitamin mineral premix[1]	3	3	
Chemical composition			
Dry matter (DM) g/kg wet weight	885	896	940
Crude protein g/kg DM	133	134	136
Ether extract g/kg DM	31.9	31.7	30.7
Ash g/kg DM	39.7	41.4	51.4
NDF g/kg DM	436	449	518
Total phenolic compounds (g TAe[2]/kg DM)	1.55	2.27	6.34
Tocopherols, µg/g dry matter			
α-Tocopherol	2.52	13.74	77.3
γ-Tocopherol	5.33	5.11	3.85
Fatty acids (g/100g of total fatty acid)			
C10:0	-	0.02	0.07
C12:0	0.04	0.06	0.15
C14:0	0.13	0.39	1.85
C16:0	14.5	16.4	27.4
C16:1	0.16	0.51	2.46
C18:0	2.52	2.53	2.56
C18:1 n-9	31.3	27.7	7.73
C18:2 n-6	44.4	43.1	35,6
C18:3 n-3	2.13	4.31	16.6
C20:0	0.12	0.80	0.17

[1]The mineral vitamin premix consisted of vitamina A=6750 UI; vitamin D3=1000UI; vitamin E 2 mg; vitamin B12 0,01 mg; vitamin B1 1mg; folic acid 0,2 mg; D-pantotenic acid 5 mg; Co 0,05 mg; Mn 12,5 mg; Zn 15 mg; Mo 0,5mg.
[2]tannic acid equivalent.

2.2. Slaughter Procedure and Sampling

At the end of the trials, all animals were slaughtered, on the same day, in a commercial abattoir according to the European Union welfare guidelines. Animals were electrically stunned and exsanguinated. Thereafter, the carcass weight was recorded and the muscle *longissimus thoracis et lumborum* (LTL) and the corresponding subcutaneous fat were removed from each carcass. One part of LTL muscle and of subcutaneous fat were immediately refrigerated and transported to the laboratory for the analysis. The remaining parts of the samples were devoted to producing salami from each animal. In a local company, all the salamis were produced with the same technology, ingredients and formulation, on the same day. They used the backfat and meat of each pig, using raw material (% w/w), pork meat (90% w/w), backfat (10% w/w) and 25g/kg of salt. The ingredients were mixed and the paste obtained was stuffed into the casings, subsequently manually tied, hung from steel racks and placed in a fermentation chamber. The ripening was performed as follows: for the first 24 h a temperature of 20 ± 1 ◦C and 75% relative humidity (RH); within five days, the temperature was gradually reduced to 12 °C, while the RH was gradually increased to 80% ± 5%. Ripening was carried out for 30 days. Matured salamis were packed under vacuum and stored frozen at –20 °C for subsequent laboratory analysis.

2.3. Feedstuff analysis and meat proximate analysis

Moisture (method 930.15), ash (method 942.05), crude protein (method 984.13, Kjeldahl method) and crude fat (method 920.39, the solvent used for fat extraction was the petroleum ether) of the experimental diets were determined according to Association of Official Analytical Chemists procedures (A.O.A.C., 1995). Feed samples were analyzed for neutral detergent fiber (NDF; Van Soest *et al*., *1991*). Total phenolic compounds were assessed according to the procedure described by Makkar *et al*. (1993), using aqueous acetone (70% V/V), as extraction solution and Folin-Ciocalteu reagent. The total phenolic compounds were expressed as grams of tannic acid equivalents per kg of dry matter. Lipids, for the analysis of fatty acid, were determined using the method of Gray *et al*. (1967).

Liposoluble vitamins were extracted in the feedstuffs following the methodologies described by Rufino-Moya *et al*. (2020). Briefly, 50 mg of bergamot pulp and 200 mg of concentrates were extracted three times with 3 mL of methanol:acetone:petroleum ether (1:1:1, v:v:v, 0.01% (w/v) of 2,6-di-tert-butyl-4-methylphenol (BHT)) solution. Then, the supernatant (1 mL for the bergamot pulp samples and all the supernatant for the concentrate samples) was evaporated. The dry residues obtained in the extractions were dissolved in 1 mL of methanol HPLC grade, filtered through a 0.22 µm polytetrafluoroethylene (PTFE) filter, and transferred into a 2 mL glass screw-top vial for automatic sampling using 5 µL for ultra-high performance liquid chromatography (UHPLC). Chromatographic conditions were as described later for the meat.

In samples of LTL moisture (method 950.46), ash (method 920.153), crude protein (method 984.13) and crude fat (method 991.36), were assessed according to Association of Official Analytical Chemists procedures (A.O.A.C., 1995), after 24 h thawing at 4 °C.

2.4 Analysis of antioxidant vitamins in meat and salami

In muscle and salami samples, antioxidant vitamins were extracted as described by Bertolín *et al*. (2018). Briefly, 500 mg of lyophilised sample was placed in a 15 mL polypropylene tube. Subsequently, 0.2 g of L-ascorbic acid and 7.5 mL of saponification solution (10% w/v KOH in 50:50 v:v ethanol:distilled water mixture) were added. The tube was vortexed for 30 seconds. The saponification procedure was performed overnight at room temperature in an orbital shaker (250 rpm).

Then, 5 mL of n-hexane:ethyl acetate 9:1 v:v (with 25 µg/mL of BHT) mixture was added. The tubes were vortexed for 30 s, and centrifuged for 5 minutes at 2000 × g at 10 °C. The supernatant was recovered in a glass tube. This procedure was repeated three times. The organic solution was evaporated under nitrogen flow at 40 °C. Then, the residue was dissolved in 1 mL of methanol, the tube leaves at 40 °C for a few minutes, vortexed for 30 seconds and filtered through a 0.2 µm–13 mm PTFE filter into a 2 mL amber vial for UHPLC.

The chromatographic system was a Nexera UHPLC (Shimadzu Corporation, Milan, Italy) equipped with a Zorbax ODS column (250 mm × 4.6 mm, 5 µm; Supelco,

Bellefonte, PA), a photodiode array detector (PDA; SPD-M40, Shimadzu Corporation, Milan, Italy) and a spectrofluorometric detector (RF-20AXS, Shimadzu Corporation, Milan, Italy). The UHPLC system was controlled by the LabSolutions software. The mobile phase was methanol with a flow rate of 1.3 mL min^{-1}. The temperature of the samples and the column were adjusted to 25 °C and 40 °C, respectively. The injection volume was 10 µl.

Tocopherols were detected by fluorescence emission at 295 excitation wavelength and 330 nm emission wavelength, retinol by absorbance at 325 nm. The analytes in the different matrices were identified by comparison of the retention times and spectral analysis with those of the pure standards. Tocopherols and retinol were expressed as µg/g.

2.5 Fatty acid analysis in meat, backfat and salami

Fatty acid composition was analyzed on total lipids extracted according to the procedure of Folch *et al*. (1957).

Briefly, 5 g of homogenized sample were blended with chloroform/methanol (2:1, v/v), mixed with saline solution (0.88% KCl). Two phases are then formed and the chloroform lipid fraction was washed with distilled water/methanol (1:1, v/v) and the aqueous methanol fraction was discarded. After a filtration and evaporation by means of a rotary evaporator, lipid extracts were transferred to test tubes. Methylation was performed in duplicate as follows: 100 mg of lipid, were methylated adding 1 mL of hexane and 0.05 mL of 2 N methanolic KOH (I.U.P.A.C., 1987); the internal standard used was C9:0. The FAME separation was carried out by a Varian gas chromatograph (model CP 3900) equipped with a capillary column CP-Sil 88 (100 m x 0.25 mm i.d., film thickness 0.25 µm). The oven conditions were as follows: 4 min at 140 °C and a subsequent increase to 220 °C at 4 °C/min. The flame ionization detector (FID) temperature was 260 °C; while the injector was set at 220 °C and operated with an injection rate of 120 mL/min. A sample volume of 1 µl was applied. Helium was used as the carrier gas at a flow rate of 0.7 mL/min. Retention time and area of each peak were computed using the Varian Star 3.4.1. software. The identification of fatty acids was achieved by comparison of the retention times of the peaks of each FAME with

those of the Standards (FAME mix 37 components from Supelco Inc., Bellefont, PA). The results were expressed in g/100 g of total fatty acids.

The indexes used to evaluate the risk of atherosclerosis and the potential aggregation of blood platelets, the atherogenic and the thrombogenic indexes respectively, were evaluated (Ulbricht & Southgate, 1991).

2.6 Lipid oxidation and colour measurements

For monitoring the oxidative stability of raw meat, 3 slices of LTL (2 cm thick) were used under aerobic refrigerated storage. The slices were placed in polystyrene trays, covered with vinyl film and stored in the dark in a refrigerated environment (4 °C). Colour stability and lipid oxidation extent measurements were performed after 2 h of blooming (day 0) and after 3 and 7 days of storage, using one slice for each day of storage. For assessing the extent of lipid oxidation in cooked meat, three more slices were used. The slices were vacuum-packaged and cooked by immersion of the bags into a water bath set at 75 °C. After 30 min of cooking, the samples were cooled by immersion of the bags into a water/ice bath. The bags were opened, and 1 slice was used immediately for measuring the extent of lipid oxidation (day 0). The remaining slices were placed into polystyrene trays, overwrapped with vinyl film and stored in the dark at 4 °C. Lipid oxidation of stored cooked meat was measured after 2 and 5 days of storage (Luciano *et al*., 2013).

Similar to meat, three salami slices were used for monitoring the oxidative stability under aerobic refrigerated storage as described above for meat samples. One slice of salami was immediately used for measuring the extent of lipid oxidation (day 0), whereas the remaining slices were placed into polystyrene trays, overwrapped with vinyl film and stored at 4 °C in the dark. Lipid oxidation was measured after 2 and 5 days of storage.

Lipid oxidation was monitored in salami, raw and cooked meat by measuring thiobarbituric acid reactive substances (TBARS) at each day of storage (Siu & Draper, 1978). Shortly, 2.5 g of LTL were homogenized with 12.5 mL of distilled water using an Ultra–Turrax T25 (IKA – Werke GmbH & Co.KG, Staufen, Germany), keeping the vessel containing the samples in a water/ice bath during homogenization. Then, 12.5

mL of 10% (w/v) trichloroacetic acid (TCA) were added to precipitate proteins and samples were thoroughly vortexed. Homogenates were filtered through filter paper (Whatman n°1). After, 4 mL of clear filtrate were transferred to a 15 mL falcon and mixed with 1 mL of 0.06 M aqueous thiobarbituric acid (TBA) and samples were incubated in a water bath at 80 °C for 90 min. The absorbance of the samples was read at a wavelength of 532 nm by means of the use of Shimadzu double beam spectrophotometer (model UV – 1800; Shimadzu Corporation, Milan, Italy). The assay was calibrated using solutions of known concentrations of TEP (1,1,3,3,-tetraethoxypropane) in 5% (w/v) trichloroacetic acid ranging from 5 to 65 nmoles/4 mL. Results were expressed as TBARS values (mg of malonaldehyde (MDA)/kg of meat).

At the end of each storage time (0, 3 and 7 days), the colour was measured in the fresh LTL slices using a Minolta CR300 colour-meter (Minolta Co. Ltd. Osaka, Japan). Hue angle (H*) was calculated as: H* = tan – 1 (b*/a*) x (180/π). Measurements were performed using illuminant A and 10° standard observer. For each muscle slice, average values were calculated from triplicate readings made on nonoverlapping areas of the sample.

2.7 Statistical analysis

Data on animal performance and intramuscular FA composition were analyzed using a one-way ANOVA to evaluate the effect of the dietary treatment. Data of meat colour stability descriptors (L*, a*, b*, C*, H*) and lipid oxidation (TBARS values) in raw, cooked meat and salami were analyzed using a mixed model to study the effect of dietary treatment and of the time of storage, as well as of their interaction as the fixed factors, while individual animal was included as a random factor.

Differences between means were assessed using Tukey's multiple comparison test. Significance was declared at $P \leq 0.05$, whereas trends toward significance were considered when $0.05 < P \leq 0.10$.

Statistical analyses were performed by the statistical software Minitab, (version 16, Minitab Inc, State College, PA).

3 – Results and Discussion

3.1. Result of experimental trial n° 1 with EBP

3.1.1. Animals Performance and chemical composition of meat

As shown in Table 3, no effect of the dietary treatment was found on the final body weight (P = 0.415), carcass weight (P = 0.965), dry matter intake (DMI; P = 0.478), average daily gain (ADG; P = 0.692) and feed conversion ratio (FCR; P = 0.285).

As for meat proximate analyses, there were no significant differences between treatments for crude protein, moisture, ether extract and ash.

The intake of total fatty acids (FA), expressed on a dry matter basis, was higher (P < 0.01) for the pigs from EBP treatment compared to control.

Regarding individual fatty acids, α-linolenic acid intake was higher (P = 0.005) in EBP group then in control group and stearic acid intake tended to increase (P = 0.057) in animal from the EBP group.

Table 3

Pig performances in vivo and chemical composition of *LTL* muscle and salami (g/100g wet weight)
(Trial n° 1 with EBP)

	Dietary treatment[1]		SEM[7]	*P* value
	Control	EBP[4]		
Final BW[2], kg	157	154	2.276	0.415
Carcass weight, kg	133	131	5.780	0.965
Total DMI[3], g/d	3.53	3.35	0.167	0.478
ADG[5], g/d	454	431	8.231	0.692
FCR[6], g DMI[3]/g ADG[5]	7.76	8.18	0.392	0.285
Total FA[8] intake, g/d	41.2	51.5	2.321	0.002
SA[9] intake, g/d	1.0	1.4	0.123	0.057
LA[10] intake, g/d	18.3	20.7	0.362	0.129
ALA[11] intake, g/d	0.9	2.2	0.123	0.005
Chemical composition of LTL muscle				
Moisture	71.5	72.1	0.325	0.300
Crude protein	21.4	22.2	0.202	0.065
Ether extract	2.79	2.29	0.869	0.286
Ash	1.23	1.13	0.031	0.135
Chemical composition of salami				
Dry matter	71.9	72.5	0.468	0.527
Crude protein	29.4	29.4	0.410	0.976
Ether extract	17.0	15.8	0.594	0.307
Ash	6.9	6.8	0.192	0.827

[1]Treatments were: only concentrate (control) or concentrate and ensiled bergamot pulp at the level of 15% dry matter on the diet fed (EBP).
[2]BW=body weight.
[3]DMI=dry matter intake.
[4]EBP=ensiled bergamot pulp.
[5]ADG=averange daily gain.
[6]FCR=feed conversion ratio.
[7]SEM= standard error of means.
[8]FA=fatty acid.
[9]SA=stearic acid.
[10]LA=linoleic acid.
[11]ALA=α-linolenic acid.

3.1.2. Fatty Acid Composition of Intramuscular fat and antioxidant vitamins

Table 4 reports the concentration of vitamins E and A in meat. Vitamin E (VE) was mainly represented by α-tocopherol and its concentration was not affected by supplementing ensiled bergamot pulp in the finishing diet of pigs. Also the concentration of retinol (vitamin A) was not affected by the dietary treatment.

The effects of dietary treatment on the individual FA in intramuscular fat (IMF) are reported in table 4. In the present study, the dietary administration of 15% ensiled bergamot pulp tended to reduce the accumulation of IMF ($P = 0.082$) in meat.

The total of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) was not different between groups. Within the SFA class, the EBP diet specifically tended to reduce the concentration of C12:0 ($P = 0.085$).

The level of polyunsaturated fatty acids (PUFA) tended to increase by feeding the EBP diet ($P = 0.081$). Consequently, the PUFA to SFA ratio increased ($P < 0.05$) by EBP treatment compared to the control treatment. Among the individual PUFA, the EBP diet increased the concentration in muscle of α-linolenic acid (C18:3 cis-9, cis-12, cis-15; $P < 0.05$), docosapentaenoic acid (DPA, C22:5 n-3; $P < 0.05$) and docosahexaenoic acid (DHA, C22:6 n-3; $P < 0.05$).

The dietary treatment affected the sum of n-3 PUFA, with the greatest concentration found in meat from the EBP group ($P < 0.01$). Consequently, the EBP treatment reduced the n-6 to n-3 ratio ($P < 0.01$) compared to the control treatment.

The concentration in meat of the highly peroxidisable (HP)-PUFA, with unsaturation degree ≥ 3, increases ($P < 0.05$) by feeding pigs with the diet containing ensiled bergamot pulp. Considering the above mentioned effect of the dietary treatment on the concentration of vitamin E, HP-PUFA ÷ VE ratio tended to increase ($P = 0.059$) in meat from the pigs fed with the diet containing ensiled bergamot pulp compared to the control treatment.

Finally, the thrombogenic index was lower in EBP meat ($P < 0.01$) compared control meat.

Table 4

Effect of the dietary treatment on the antioxidant vitamins (µg/g muscle) and fatty acid composition of *LTL* (g/100 g of total fatty acids)
(Trial n° 1 with EBP)

	Dietary Treatment			
Item	Control	EBP	SEM	*P* value
Tocopherols and retinol, µg/g muscle				
α-Tocopherol	2.37	2.38	0.060	0.880
γ-Tocopherol	0.09	0.11	0.009	0.180
Retinol	7.26	6.52	0.392	0.367
Intramuscular fat, mg/100 g of muscle	2840	2160	195	0.082
C10:0	0.08	0.12	0.011	0.113
C12:0	0.10	0.07	0.010	0.085
C14:0	1.24	1.19	0.027	0.400
C14:1 *cis-9*	0.03	0.03	0.002	0.582
C15:0	0.04	0.05	0.005	0.864
C16:0	20.4	20.5	0.144	0.927
C 17:0	0.41	0.36	0.014	0.215
C16:1 *cis-9*	3.38	3.58	0.089	0.262
C17:1 *cis-9*	0.22	0.20	0.005	0.200
C18:0	9.49	9.55	0.151	0.841
C18:1 *cis-9*	40.1	37.7	0.912	0.201
C18:1 *trans-11* VA[1]	4.68	4.91	0.091	0.208
C18:2 *cis-9, cis-12* LA[1]	11.9	12.3	0.442	0.650
C18:3 n-3 ALA[1]	0.42	1.47	0.235	0.018
C 20:0	0.28	0.25	0.014	0.377
C 20:1 *cis-9*	0.90	0.88	0.020	0.627
C20:2 n-6	0.50	0.49	0.013	0.800
C20:3 n-3	0.22	0.33	0.053	0.344
C20:4 n-6	2.08	2.51	0.353	0.203
C20:5 n-3 EPA[1]	0.11	0.15	0.025	0.409
C22:5 n-3 DPA[1]	0.24	0.39	0.037	0.036
C22:6 n-3 DHA[1]	0.21	0.30	0.021	0.031
∑ SFA[1]	32.0	31.9	0.270	0.951
∑ MUFA[1]	49.3	47.3	0.798	0.346
∑ PUFA[1]	15.7	18.0	0.644	0.081
∑ n-3	1.20	2.64	0.278	0.004

∑ n-6	14.5	15.3	0.710	0.361
n-6/n-3	12.1	6.87	0.928	0.002
∑ PUFA[1]/∑ SFA[1]	0.49	0.56	0.022	0.039
Thrombogenic index[2]	0.84	0.76	0.016	0.005
Atherogenic Index[3]	0.39	0.38	0.005	0.235
HP-PUFA[4](mg/g muscle)	0.65	0.84	0.043	0.026
HP-PUFA ÷ VE[5]	2.42	2.52	0.026	0.059

[1]VA: vaccenic acid; LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
[2]Thrombogenic index: (C14:0 + C16:0 + C18:0)/(0.5 MUFA + 0.5 PUFA n-6 + 3 PUFA n-3 + PUFA n-3/PUFA n-6).
[3]Atherogenic index: (C12:0 + 4*C14:0 + C16:0)/(MUFA + PUFA n-6 + PUFA n-3).
[4] Highly peroxidizable (HP)-PUFA: calculated as the sum of PUFA with ≥3.
[5] Calculated as the ratio between HP-PUFA and total vitamin E, both expressed as mg/g muscle. Original values obtained were not normally distributed according to the Anderson-Darling test. Therefore, logarithmic transformation was adopted and values in table are presented as LOG10.

3.1.3. Fatty Acid Composition of backfat

Table 5 reports the effect of the dietary treatment on the fatty acid composition of backfat. The inclusion of ensiled bergamot pulp in the diet did not affect the total of SFA, MUFA and PUFA.

The only fatty acid that showed a difference ($P < 0.05$) was α-linolenic acid, with the highest value found in backfat from pigs fed the EBP diet. Consequently, the proportion of n-3 PUFA tended to increase ($P = 0.074$) in backfat of pigs from the EBP group compared to the control group.

Table 5

Effect of the dietary treatment on fatty acid composition of backfat (g/100 g of total fatty acids)
(Trial n° 1 with EBP)

	Dietary Treatment			
Item	Control	EBP	SEM	*P* value
C10:0	0.07	0.07	0.013	0.726
C12:0	0.13	0.11	0.010	0.639
C14:0	1.46	1.46	0.438	0.963
C14:1 *cis-9*	0.03	0.03	0.002	0.183
C15:0	0.07	0.07	0.005	0.620
C16:0	21.0	21.2	0.259	0.672
C 17:0	0.47	0.41	0.020	0.198
C16:1 *cis-9*	2.03	2.01	0.029	0.715
C17:1 *cis-9*	0.26	0.27	0.020	0.791
C18:0	8.89	10.5	0.711	0.285
C18:1 *cis-9*	42.6	40.1	0.777	0.115
C18:1 *trans-11* VA[1]	2.95	2.83	0.045	0.174
C18:2 *cis-9. cis-12* LA[1]	14.2	14.3	0.361	0.877
C18:3 n-3 ALA[1]	0.55	1.29	0.183	0.036
C 20:0	0.26	0.26	0.006	0.750
C 20:1 *cis-9*	1.15	1.19	0.043	0.633
C20:2 n-6	0.65	0.71	0.080	0.688
C20:3 n-3	0.13	0.09	0.015	0.150
C20:4 n-6	0.20	0.20	0.012	0.955
C20:5 n-3 EPA[1]	0.19	0.18	0.005	0.635
C22:5 n-3 DPA[1]	0.20	0.21	0.019	0.753
C22:6 n-3 DHA[1]	0.19	0.22	0.031	0.729
∑ SFA[1]	32.2	34.0	0.827	0.306
∑ MUFA[1]	49.0	46.4	0.825	0.126
∑ PUFA[1]	16.3	17.2	0.386	0.246
∑ n-3	1.26	1.99	0.207	0.074
∑ n-6	15.1	15.3	0.392	0.817
n-6/n-3	13.1	9.02	1.280	0.110
∑ PUFA[1]/∑ SFA[1]	0.51	0.51	0.020	0.919

[1]VA: Vaccenic acid; LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

3.1.4. Fatty Acid Composition of salami and antioxidant vitamins

The fatty acid composition of salami is shown in Table 6. The dietary administration of 15% ensiled bergamot pulp reduced the content of saturated fatty acids in salami ($P < 0.01$).

Instead, the content of PUFA increased by feeding the EBP diet ($P < 0.001$). Among the individual PUFA, the EBP diet in salami increased the concentration of α-linolenic acid ($P < 0.001$).

The dietary treatment affected the sum of n-3 PUFA, with a greater concentration found in salami from the EBP group ($P < 0.001$). Consequently, the EBP treatment reduced the n-6 to n-3 ratio ($P < 0.001$) and increased the PUFA to SFA ratio ($P < 0.001$) compared to the control treatment. Finally, the thrombogenic index and the atherogenic index were lower in the EBP salami ($P < 0.001$ and $P < 0.05$ respectively) compared to the control salami.

As for the concentration of vitamins E and A (table 6), also in salami the levels α-tocopherol and γ-tocopherol were not affected by supplementing ensiled bergamot pulp in the finishing diet of pigs. Also, the concentration of retinol (vitamin A) was not affected by the dietary treatment. As in meat, the concentration of the HP-PUFA increases ($P < 0.001$) by feeding pigs with the diet containing ensiled bergamot pulp in salami, influencing the HP-PUFA ÷ VE ratio that was higher ($P < 0.007$) in salami from the EBP group compared to the control treatment.

Table 6

Effect of the dietary treatment on the antioxidant vitamins (µg/g salami) and fatty acid composition of salami (g/100 g of total fatty acids)
(Trial n° 1 with EBP)

	Dietary Treatment			
Item	Control	EBP	SEM	*P* value
Tocopherols and retinol, µg/g salami				
α-Tocopherol	0.54	0.54	0.083	0.977
γ-Tocopherol	0.05	0.05	0.005	0.472
Retinol	3.38	2.81	0.554	0.628
Total fat, g/100 g of salami	19.7	17.0	0.479	0.333
C12:0	0.12	0.12	0.004	0.187
C14:0	1.58	1.32	0.066	0.024
C16:0	22.1	20.2	0.420	0.018
C16:1 *cis-9*	2.18	2.19	0.071	0.776
C18:0	10.9	9.55	0.341	0.040
C18:1 *cis-9*	44.9	45.1	0.336	0.803
C18:2 *cis-9. cis-12* LA[1]	11.3	12.3	0.270	0.098
C18:3 n-3 ALA[1]	0.50	2.10	0.240	0.001
C 20:1 *cis-9*	1.04	1.10	0.028	0.342
C20:2 n-6	0.51	0.68	0.048	0.088
C20:4 n-6	0.28	0.19	0.052	0.367
C20:5 n-3 EPA[1]	0.20	0.35	0.061	0.216
C22:5 n-3 DPA[1]	0.15	0.25	0.028	0.327
C22:6 n-3 DHA[1]	0.14	0.24	0.048	0.288
∑ SFA[1]	34.7	31.2	0.733	0.009
∑ MUFA[1]	48.1	48.4	0.342	0.790
∑ PUFA[1]	12.9	16.1	0.537	0.001
∑ n-3	0.82	2.94	0.288	0.001
∑ n-6	12.1	13.2	0.288	0.094
n-6/n-3	14.7	4.48	1.410	0.001
∑ PUFA[1]/∑ SFA[1]	0.37	0.52	0.023	0.001
Thrombogenic index[2]	1.06	0.78	0.043	0.001
Atherogenic index[3]	0.47	0.40	0.014	0.045
HP-PUFA[4](mg/g salami)	1.23	3.81	0.456	0.001
HP-PUFA ÷ VE[5]	3.25	3.87	0.124	0.007

[1]LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
[2]Thrombogenic index: (C14:0 + C16:0 + C18:0)/(0.5 MUFA + 0.5 PUFA n-6 + 3 PUFA n-3 + PUFA n-3/PUFA n-6).
[3]Atherogenic index: (C12:0 + 4*C14:0 + C16:0)/(MUFA + PUFA n-6 + PUFA n-3).
[4]Highly peroxidizable (HP)-PUFA: calculated as the sum of PUFA with ≥3.
[5]Calculated as the ratio between HP-PUFA and total vitamin E, both expressed as mg/g muscle. Original values obtained were not normally distributed according to the Anderson-Darling test. Therefore, logarithmic transformation was adopted and values in table are presented as LOG10.

3.1.5. Meat colour and oxidative stability

In fresh meat, lipid oxidation (TBARS values) was not affected by the dietary treatments (table 7). Regarding colour descriptors, the redness (a*) and lightness (L*) descriptors were not affected by the time of storage or by the dietary treatment. Instead, meat saturation (C*), yellowness (b*) and hue angle (H*) values were affected by the time of storage (respectively $P < 0.001$ for b* and H* values and $P < 0.05$ for C* value), with values overall increasing from 0 to 3 days and stabilizing thereafter.
Also, in cooked meat, lipid oxidation was not affected by the dietary treatments (table 7), while it increased for all treatments over 2 days of storage ($P < 0.001$).

Table 7

Effect of the dietary treatment and time of refrigerated storage on meat colour stability and lipid oxidation
(Trial n° 1 with EBP)

	Dietary treatment[1]		Time [2]			SEM	*P* values		
	Control	EBP	0	1	2		Diet	Time	Diet x Time
L* values[3]	41.7	43.8	42.0	43.3	43.0	0.583	0.084	0.650	0.984
a* values[3]	6.0	6.0	6.6	6.2	5.1	0.306	0.986	0.106	0.320
b* values[3]	8.2	8.2	6.5^{x}	9.6^{y}	8.4^{y}	0.316	0.953	0.001	0.069
C* values[3]	10.3	10.3	9.3^{x}	11.5^{y}	10.1^{xy}	0.337	0.985	0.023	0.065
H* values[3]	53.6	54.2	45.4^{x}	57.5^{y}	58.8^{y}	1.64	0.835	0.001	0.965
TBARS raw meat. mg MDA/kg	0.50	0.52	0.46	0.56	0.50	0.019	0.677	0.101	0.635
TBARS cooked meat. mg MDA/kg	3.08	3.04	2.35^{x}	2.83^{x}	4.01^{y}	0.132	0.827	0.001	0.833

[x.y.z]Within row, different superscripts indicate differences between days of storage ($P < 0.05$) tested using the Tukey's adjustment for multiple comparisons.
[1]Treatments were: only concentrate (C); concentrate and ensiled bergamot pulp at the level of 15% dry matter on the diet fed (EBP).
[2]Times 0. 1. 2 = days 0. 3. 7 for raw meat and 0. 2. 5 for cooked meat at 4 °C under aerobic conditions (meat slices).
[3]L*=lightness; a*=redness; b*=yellowness; C*=Chrome; h*=hue angle. measured in degrees.

3.1.6. Salami oxidative stability

The oxidative stability parameters measured in salami are reported in Fig. 1. The TBARS values increased over storage duration ($P < 0.001$) and the EBP diet reduced the extent of lipid oxidation overall measured in salami over time ($P < 0.001$). A significant diet × time interaction was found ($P < 0.001$). Specifically, compared to day 0, while the TBARS values increased already after 2 days in salami from only concentrate-fed animals, in salami from animals fed the EBP diet, lipid oxidation increased after 5 days ($P < 0.01$). Salami from the EBP treatment had lower TBARS values compared to salami from the control treatment after 5 days of storage ($P < 0.001$; 1.54 *vs* 2.96 respectively).

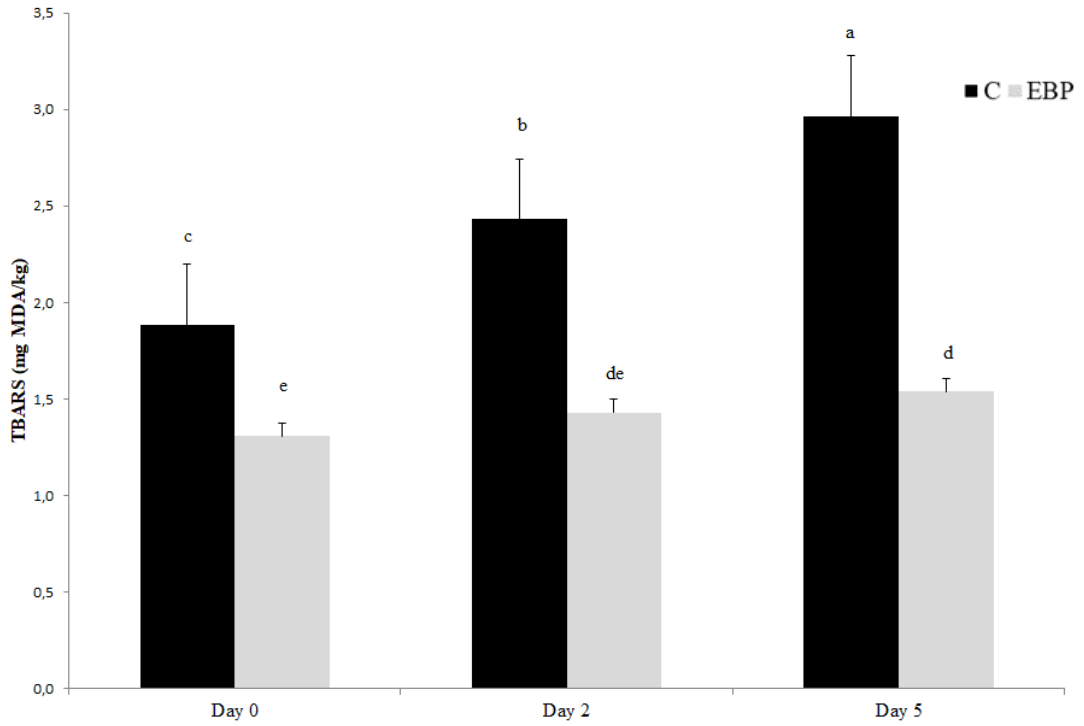

Fig. 1 (Trial n° 1 with EBP)
Effect of the dietary treatment and time of storage on the oxidative stability of salami.
Interactive effect of the dietary treatment (Control and EBP) and time of storage (Days 0, 2 and 5) on the TBARS values measured in salami slices over aerobic storage at 4 °C. Values presented are the estimated least squares means and standard error bars. ^{a,b,c,d,e} Values with different superscripts are significantly different ($P \leq 0.05$).

3.2. Discussion of experimental trial n° 1 with EBP

3.2.1. Fatty acid composition

The dietary treatment did not result in differences in the main performance parameters. As shown in Table 3, the final weight of the animals, as well as carcass weight were comparable between treatments.

In literature, no information has been provided on the effects of supplementing bergamot pulp in the finishing diet of pigs on animal performance and meat quality. However, some data have been provided on the effects of supplementing citrus pulp (from other species than bergamot) in the finishing diet of pigs on animal performance. Crosswhite *et al*. (2013) observed no differences in ADG values in finishing pigs fed a diet with 15% DM of ensiled citrus pulp. Other authors also showed similar results (Strong *et al*., 2015). Cerisuelo *et al*. (2010) observed that the inclusion of up to 10% of ensiled citrus pulp tended to reduce animal growth performance. However, the authors suggested that the lower performance was due to a lower DM feed intake during the first 4 weeks on trial, thereafter differences disappeared.

Apulo-Calabrese is a breed characterized by reduced growth and carcass performance (Aboagye *et al*., 2020), and this justifies the lower ADG values registered in all treatments of our trial in confront of the data reported from the authors mentioned above.

In animal nutrition, in both ruminants and monogastrics, a main objective is to find strategies to increase polyunsaturated fatty acids, especially of n-3 series, and reduce saturated fatty acids in zootechnical products (Scollan *et al*., 2006; Wood *et al*., 2008). In ruminants, after lipid hydrolysis in the rumen, many unsaturated fatty acids are hydrogenated by ruminal micro-organisms, changing dietary fatty acids. Differently from ruminants, in monogastric animals, dietary fatty acids do not undergo substantial changes along the digestive tract and, after absorption, accumulate in animal tissues (Wood *et al*., 2008). Moreover, Aboagye *et al*. (2020) indicated 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 commercial crossbreed pigs, excluding effects of the genetic type over muscle FA synthesis and storage. For these reasons, the results found in the

present study for the fatty acid composition of meat can be mainly explained by the fatty acid profile of the two experimental diets.

To date, no studies investigated the effects of feeding pigs with the solid residue resulting from the industrial processes of bergamot on meat fatty acids composition and also on the use of citrus pulp, which is well documented in ruminants, little is known about its effects on meat quality for pigs.

Cerisuelo *et al*. (2010), in a study where pigs were fed 100 g of ensiled citrus pulp per kg of diet (DM basis), observed no significant differences in total SFA and in total PUFA. However, in our trial, taking into account the fatty acid composition of the C and EBP diets, a greater concentration of α-linolenic acid in the EBP pork meat was expected. This fatty acid was found at a greater amount in meat from the EBP group than in meat from the control group. Also, the level of total PUFA tended to be higher in the meat of animals fed bergamot processing by-products. These results are in agreement with the data of a previous research, where bergamot processing by-products supplementing in the diet increased α-linolenic acid and tended to increase total PUFA in lamb meat (Scerra *et al*., 2018).

Consistent with the higher level of α-linolenic acid in meat from the EBP group, some polyunsaturated fatty acid derived from it through the action of Δ^5 and Δ^6 desaturase enzymes and elongase (Gurr & Harwood, 1996), were higher in meat from animals of this group. In fact, the levels of docosapentaenoic (DPA, C22:5 n-3) acid and docosahexaenoic acid (DHA, C22:6 n-3) were greater in meat from the EBP group than in meat from C group. Durand-Montgé *et al*., (2010) reported that diets rich in linolenic acid, for example diet with linseed oil, may result in an increased level of docosapentaenoic (DPA) in pork.

These findings are of relevance as it is well recognized that long chain n-3 PUFA have a wide range of biological effects, which are believed to be beneficial for human health (Kromhout, 1989; Barlow *et al*., 1990). However, considering that the adequate intake estimated for human is 2.22 g/d for ALA and 0.65 g/d for DHA + EPA (Simopoulos, 2000), the contribution provided by the integration of ensiled bergamot pulp on increased levels of ALA and long chain n-3 PUFA in the meat was small.

These data obviously influenced the total n-3 PUFA content that was higher in meat from the EBP group than in meat from the other group. Instead, the levels of the most important n-6 PUFA such as linoleic and arachidonic acids were comparable between treatments. Consequently, the level of n-6 to n-3 PUFA ratio was significantly lower in meat from pigs fed with the EBP diets than in meat from pigs fed only concentrate. In this trial, consequently to the positive effects of ensiled bergamot pulp on some desirable fatty acids, the thrombogenic index, a lipid nutritional quality index, tended to be lower in meat from pigs fed the EBP diet than in meat from pigs fed the control diet.

Similarly to meat, the concentration of total n-3 PUFA tended to increase in backfat from the EBP fed pigs. In fact, feeding diets with a high concentration of α-linolenic acid to pigs generally results in the accumulation of this fatty acid and its long-chain derivatives in the backfat. Consistent with this, we found a greater concentration of α-linolenic acid in the backfat from the EBP fed animals. However, its long-chain derivatives were found at comparable amounts between the two groups.

As in meat and backfat, the use of ensiled bergamot pulp influenced the salami fatty acid composition (table 6). In all the salami, the major FA was C18:1 *cis-9*, which occurred at comparable levels between treatments. Among the SFA, significant differences were found for C14:0, C16:0 and C18:0, with higher values observed in salami from animals fed the C diet than in salami from animals fed the EBP diet.

Salami produced from pigs fed with ensiled bergamot pulp had a four-fold greater content of C18:3 n-3 compared to the control group. Considering the above described effect of feeding bergamot by-products on the content of α-linolenic acid in meat and in backfat, this result was expected. This result strongly influenced the total n-3 PUFA content which was higher in meat from the EBP group than in meat from the control group.

Similar to meat, also in salami the levels of the most important n-6 PUFA such as linoleic and arachidonic acids were comparable between treatments. Consequently, the level of n-6 to n-3 PUFA ratio was significantly lower in salami from pigs fed with the EBP diet than in salami from pigs fed the control diet. Furthermore, the data reported above influenced the level of total PUFA, which was higher in the meat of animals

from the EBP group. In this trial, the PUFA content was comparable with those reported by Zanardi *et al*., (2002) and Warnants *et al*., (1998) for salami, who described values of approximately 13.5 and 16.3 %, respectively.

Regarding the thrombogenic index and the atherogenic index, in salami the values were lower in the EBP group than in the control group.

3.2.2. Oxidative stability

The deterioration of colour and the development of rancid off-flavours in meat over time of storage is primarily affected by the oxidation of lipids and myoglobin (Faustman *et al*., 2010). The inherent susceptibility of meat to oxidation is the result of a complex balance in muscle between anti-oxidant and pro-oxidant factors.

The trend of lipid oxidation depends strongly on the dietary background of the animals. Different authors reported that a higher deposition of PUFA, especially n-3 fatty acids, is associated with an increased susceptibility of the meat to lipid oxidation (Dunne, *et al*., 2011; Moloney *et al*., 2012), while the increase of antioxidants compounds, derived directly and indirectly from the diet such as vitamin E, is commonly associated with the improvement of the antioxidant capacity (Luciano *et al*., 2017).

The results of the present study showed that, although the concentration of vitamin E (α-Tocopherol and γ-Tocopherol) was higher in the ensiled bergamot pulp than in the concentrate and consequently in EBP treatment than in control treatment, no difference in vitamin E content was observed in meat from both experimental groups. This latter result was not expected. However, despite the data reported on vitamin E in meat and the higher levels of total PUFA and especially HP- PUFA in meat from EBP animals, the inclusion of ensiled bergamot pulp in the diet did not alter the shelf-life in raw meat. Similarly, colour coordinates mostly related to meat browning (a*, C* and H* values) did not differ between treatments. Probably, the high level of vitamin E in the meat of both experimental groups was enough to protect it from oxidation during the 7 days of storage.

Different authors showed a similar trend in fresh pork stored for up to 5 or more days (Inserra *et al*., 2015; Biondi *et al*., 2020). Aerobic storage of fresh meat in darkness may represent a low oxidative challenge for meat to fully express its resistance to oxidative deterioration. Indeed, it has been reported that differences in oxidative stability between meat samples were masked under refrigerated storage of fresh meat, but become evident when meat is subjected to stronger oxidative challenges (Luciano *et al*., 2019). Therefore, in the present study, we also assessed oxidative stability in cooked meat to assess possible diet-related differences of meat oxidative stability under more pro-oxidant conditions. Also under these conditions, no effect of dietary

treatment was observed, although TBARS values increased during the 5 days of refrigerated storage, reaching much higher values than those recorded in fresh meat. Recently some authors (Scerra *et al*. 2018) observed a stronger effect of bergamot pulp supplementation in ruminant diet, showing a lower TBARS value in meat from lambs fed concentrate and fresh bergamot pulp at the level of 20% DM on the diet fed than in meat from lambs fed only concentrate.

In contrast to the results observed in the meat, significant differences were observed between treatments in salami oxidative stability (Fig. 1). Already on the first day of the oxidative stability test, TBARS values in salami from animals of the control group were significantly higher than in salami from animals of the EBP treatment. Furthermore, while TBARS values strongly increased during the 5 days of refrigerated storage in salami from the control group, already exceeding after 2 days of storage the suggested value of TBARS (2 mg MDA/Kg of meat) considered a maximum level for positive sensory perception (Campo *et al*., 2006), in salami from the EBP group TBARS values increased slightly during the 5 days of refrigerated storage, remaining below the threshold value of 2 mg MDA/Kg for the entire monitoring period.

As in raw meat, no difference in vitamin E content was observed in salami between the experimental groups. However, the level of vitamin E in salami was 4 times lower than in fresh meat, which may be related to the consumption of vitamin E due to pro-oxidation challenges linked to salami production process and ageing. Therefore, it may be supposed that this level was probably not enough to protect it from oxidation during storage. Nonetheless, TBARS values in salami from animals of the EBP group were significantly lower, despite the highest level of HP-PUFA, than in salami from animals of the control treatment after 5 days of refrigerated storage. As mentioned above, aerobic storage of fresh meat in darkness may represent a low oxidative challenge for meat to fully express its resistance but can be more evident when the meat is subjected to strong oxidative stress. Some authors (Warnants *et al*., 1998) observed that oxidative stability of the salami decreased with increasing ripening time and PUFA content. The long storage time has probably allowed to show the resistance to oxidative deterioration of the meat from animals of the EBP group.

We could speculate that ensiled bergamot pulp may offer additional antioxidant effects, which could be due to the occurrence of antioxidant compounds other than tocopherols. Bergamot fruit contains a very high amount of flavonoids, especially naringin and neosperidin, and the highest concentrations of these compounds occur in the peel (Tsiokanos *et al*., 2021). Citrus flavonoids are polyphenolic compounds, secondary metabolites of plants that have been found to have different properties, especially based on their antioxidant activity (Kawaii *et al*., 1999). However, some authors (Bieger *et al*., 2008; López-Andrés *et al*., 2013) confirmed the poor bioavailability of these compounds in animal tissue and that it is not yet possible to reach conclusions on the effects of polyphenols. Nevertheless, several authors suggest an indirect antioxidant effect of phenolic compounds, such as an antioxidant activity on the gastrointestinal tract, interrupting lipid oxidation propagation and formation of toxic molecules (Kerem *et al*., 2006) or a possible protective effect in the gastrointestinal tract of phenols towards other more bioavailable antioxidant compounds (Halliwell *et al*., 2005). In our first trial, ensiled bergamot pulp integrated in the EBP diet, showed a higher amount of total phenolic compounds than concentrate, evaluated by the Folin-Ciocalteu assay (14.2 *vs* 1.55 g TAe/kg DM respectively) and these data indicate that animals from the EBP group ingested a higher quantity of reducing compounds than animals from the control group. Furthermore, it should be pointed out that bergamot peel generally contains essential oils, a complex mixtures of plant metabolites able to exert a wide spectrum of biological activities such as antioxidant (Mandalari *et al*., 2006; Cui, *et al*., 2020), in a phytochemical fraction other than phenolic compounds and that these substances could partly contribute to the overall antioxidant capacity.

Therefore, it may be supposed that all these compounds exerted antioxidant protection which was evident in condition of low levels of vitamin E, as observed in the case of salami.

3.3. Result of experimental trial n° 2 with DBP

3.3.1. Animal performance and chemical composition of meat and salami

Data on animal performances are reported in Table 8. No significant differences between dietary treatments were found for final weight ($P = 0.392$), carcass weight ($P = 0.629$), dry matter intake (DMI; $P = 0.468$), average daily gain (ADG; $P = 0.589$) and feed conversion ratio (FCR; $P = 0.291$).

The intake of total fatty acids (FA), expressed on dry matter basis, was higher ($P < 0.01$) for the pigs from the DBP treatment compared to the control.

Regarding individual fatty acids, stearic acid intake ($P = 0.064$) and linoleic acid intake ($P = 0.091$) tended to increase in the DBP group compared to the control group and the DBP group ingested higher concentration of α-linolenic acid ($P = 0.001$).

The chemical composition of the meat samples is presented in Table 9. No significant differences between groups were found for moisture ($P > 0.05$), crude protein ($P > 0.05$), ether extract ($P > 0.05$) and ash ($P > 0.05$).

The chemical composition of the salami is shown in Table 9. Although there were no significant differences ($P > 0.05$) between the experimental groups as regards crude protein, ether extract and ash, a tendency towards significance occurred for dry matter ($P = 0.09$) being greater in the group fed with the DBP.

Table 8

Pig performances in vivo (Trial n° 2 with DBP)

	Dietary treatment[1]		SEM[7]	*P* value
	Control	DBP[4]		
Final BW[2], kg	157	157	2.132	0.392
Carcass weight, kg	133	127	5.280	0.629
Total DMI[3], g/d	3.53	3.54	0.156	0.468
ADG[5], g/d	454	456	8.163	0.589
FCR[6], g DMI[3]/g ADG[5]	7.76	7.76	0.363	0.291
Total FA[8] intake, g/d	41.2	53.7	2.321	0.002
SA[9] intake, g/d	1.0	1.36	0.123	0.064
LA[10] intake, g/d	18.3	22.2	0.362	0.091
ALA[11] intake, g/d	0.9	3.82	0.123	0.001

[1]Treatments were: only concentrate (control) or concentrate and dried bergamot pulp at the level of 15% dry matter on the diet fed (DBP).
[2]BW=body weight.
[3]DMI=dry matter intake.
[4]DBP=dried bergamot pulp.
[5]ADG=averange daily gain.
[6]FCR=feed conversion ratio.
[7]SEM= standard error of means.
[8]FA=fatty acid.
[9]SA=stearic acid.
[10]LA=linoleic acid.
[11]ALA=α-linolenic acid.

Table 9

Chemical composition of *LTL* muscle and salami (g/100g wet weight) (Trial n° 2 with DBP)

	Dietary treatments[1]		SEM[2]	*P* value
	Control	DBP		
Chemical composition of LTL *muscle*				
Moisture	71.5	71.1	0.342	0.536
Crude protein	21.4	21.7	0.250	0.124
Ether extract	2.79	2.97	0.214	0.694
Ash	1.23	1.17	0.031	0.424
Chemical composition of salami				
Dry matter	71.9	74.1	0.648	0.090
Crude protein	29.4	28.7	0.430	0.465
Ether extract	17.0	15.0	0.953	0.300
Ash	6.9	6.6	0.187	0.495

[1]Treatments were: only concentrate (control) or concentrate and bergamot pulp at the level of 15% dry matter on the diet fed (DBP).
[2]SEM= standard error of means.

3.3.2. Fatty acid composition of intramuscular fat and antioxidant vitamins

Table 10 reports the concentration of vitamins E and A in meat. Vitamin E (VE) was mainly represented by α-tocopherol and its concentration was not affected by dietary treatment ($P > 0.05$), while the concentration of retinol (vitamin A) tended to be influenced by the diet, being greater in the group treated with the DBP ($P = 0.064$).

The effect of dietary treatment on the fatty acid composition of *longissimus thoracis et lumborum* is reported in Table 10. No effect was found ($P > 0.05$) for the proportion of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Individual FA were not affected by the dietary treatment ($P > 0.05$), except the proportion of C22:5 n-3 (DPA) which was increased by the DBP inclusion ($P = 0.019$). Furthermore, the LTL muscle from pigs fed with the DPB diet tended to have a greater proportion of n-3 PUFA ($P = 0.09$); as a consequence, the n-6/n-3 PUFA ratio was lower in the DBP treatment ($P = 0.01$).

As regards for the meat concentration of the highly peroxidizable (HP)-PUFA, with unsaturation degree ≥ 3, no significant differences were found between the two groups ($P > 0.05$). Nevertheless, HP-PUFA ÷ VE ratio tended to increase ($P = 0.056$) in meat from the pigs fed with the diet containing dried bergamot pulp compared to the control group.

The thrombogenic index tended to be reduced ($P = 0.074$) by feeding pigs with the DBP diet compared to the control diet.

Table 10

Effect of the dietary treatments on the oxidant vitamins (µg/g muscle) and fatty acid composition of *LTL* muscle (g/100 g of total fatty acids)
(Trial n° 2 with DBP)

	Dietary Treatment			
Item	Control	DBP	SEM	*P* values
Tocopherols and retinol, µg/g muscle				
α-Tocopherol	2.37	2.32	0.066	0.720
γ-Tocopherol	0.09	0.08	0.006	0.517
Retinol	7.26	10.4	0.858	0.064
Intramuscular fat, mg/100g of muscle	2840	3012	214	0.712
C10:0	0.08	0.07	0.01	0.797
C12:0	0.10	0.08	0.009	0.234
C14:0	1.24	1.22	0.044	0.831
C14:1 *cis-9*	0.03	0.03	0.003	1.000
C15:0	0.04	0.04	0.005	0.711
C16:0	20.4	19.9	0.208	0.271
C 17:0	0.41	0.39	0.02	0.741
C16:1 *cis-9*	3.38	3.44	0.092	0.731
C17:1 *cis-9*	0.22	0.23	0.006	0.261
C18:0	9.49	9.60	0.637	0.686
C18:1 *cis-9*	40.1	40.2	0.637	0.938
C18:1 *trans-11* VA[1]	4.68	4.88	0.099	0.337
C18:2 *cis-9, cis-12* LA[1]	11.9	11.6	0.507	0.780
C18:3 n-3 ALA[1]	0.42	1.11	0.235	0.146
C 20:0	0.28	0.23	0.022	0.253
C 20:1 *cis-9*	0.90	0.82	0.047	0.424
C20:2 n-6	0.50	0.46	0.038	0.599
C20:3 n-3	0.22	0.43	0.083	0.212
C20:4 n-6	2.08	2.05	0.174	0.926
C20:5 n-3 EPA[1]	0.11	0.17	0.025	0.243
C22:5 n-3 DPA[1]	0.24	0.45	0.047	0.019
C22:6 n-3 DHA[1]	0.21	0.31	0.041	0.275
∑ SFA[1]	32.0	31.8	0.227	0.309
∑ MUFA[1]	49.3	49.6	0.736	0.846
∑ PUFA[1]	15.7	16.6	0.66	0.519
∑ n-3	1.20	2.47	0.375	0.090
∑ n-6	14.5	14.1	0.667	0.794
n-6/n-3	12.1	7.70	0.916	0.010

∑ PUFA[1]/∑ SFA[1]	0.49	0.53	0.019	0.352
Thrombogenic index[2]	0.84	0.76	0.022	0.074
Atherogenic Index[3]	0.39	0.38	0.006	0.230
HP-PUFA[4](mg/g muscle)	0.65	0.99	0.113	0.135
HP-PUFA ÷ VE [5]	2.42	2.58	0.042	0.056

[1]VA: vaccenic acid; LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.
[2]Thrombogenic index: (C14:0 + C16:0 + C18:0)/(0.5 MUFA + 0.5 PUFA n-6 + 3 PUFA n-3 + PUFA n-3/PUFA n-6).
[3]Atherogenic index: (C12:0 + 4*C14:0 + C16:0)/(MUFA + PUFA n-6 + PUFA n-3).
[4]Highly peroxidizable (HP)-PUFA: calculated as the sum of PUFA with ≥3.
[5]Calculated as the ratio between HP-PUFA and total vitamin E, both expressed as mg/g muscle. Original values obtained were not normally distributed according to the Anderson-Darling test. Therefore, logarithmic transformation was adopted and values in table are presented as LOG10.

3.3.3. Fatty acid composition of backfat

Fatty acid composition of backfat is reported in Table 11. The DBP treatment tended to increase the total PUFA concentration ($P = 0.078$) in backfat compared to the control diet, while no effect was found for the sum of SFA and MUFA ($P > 0.05$). Evaluating the individual fatty acids was noted that backfat from pigs fed the DBP diet had a greater proportion of α-linolenic acid ($P < 0.05$).

Overall, the proportion of n-3 PUFA in fat tended to increase ($P = 0.067$) by feeding pigs the DBP diet compared with the control diet.

Table 11

Effect of the dietary treatments on fatty acid composition of backfat (g/100 g of total fatty acids) (Trial n° 2 with DBP)

	Dietary Treatment			
Item	Control	DBP	SEM	*P* values
C10:0	0.07	0.06	0.01	0.791
C12:0	0.13	0.12	0.005	0.425
C14:0	1.46	1.39	0.03	0.264
C14:1 *cis-9*	0.03	0.03	0.004	1.000
C15:0	0.07	0.08	0.005	0.250
C16:0	21.0	20.6	0.298	0.519
C 17:0	0.47	0.49	0.012	0.343
C16:1 *cis-9*	2.03	2.01	0.039	0.839
C17:1 *cis-9*	0.26	0.39	0.028	0.015
C18:0	8.89	10.2	0.697	0.383
C18:1 *cis-9*	42.6	40.3	0.734	0.133
C18:1 *trans-11* VA[1]	2.95	3.04	0.046	0.363
C18:2 *cis-9, cis-12* LA[1]	14.2	15.0	0.366	0.291
C18:3 n-3 ALA[1]	0.55	1.19	0.165	0.047
C 20:0	0.26	0.26	0.006	0.843
C 20:1 *cis-9*	1.15	1.28	0.05	0.196
C20:2 n-6	0.65	0.67	0.097	0.896
C20:3 n-3	0.13	0.18	0.033	0.452
C20:4 n-6	0.20	0.18	0.014	0.600
C20:5 n-3 EPA[1]	0.19	0.19	0.015	0.895
C22:5 n-3 DPA[1]	0.20	0.25	0.025	0.336
C22:6 n-3 DHA[1]	0.19	0.23	0.041	0.669
∑ SFA[1]	32.2	33.0	0.813	0.643
∑ MUFA[1]	49.0	47.1	0.757	0.226
∑ PUFA[1]	16.3	17.9	0.456	0.078
∑ n-3	1.26	2.04	0.215	0.067
∑ n-6	15.1	15.9	0.424	0.355
n-6/n-3	13.1	9.14	1.25	0.113
∑ PUFA[1]/∑ SFA[1]	0.51	0.47	0.031	0.477

[1]VA: vaccenic acid; LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

3.3.4. Fatty acid composition of salami and antioxidant vitamins

Fatty acid composition of salami is reported in Table 12. Salami from pigs fed the DBP diet had a smaller proportion of total SFA ($P < 0.05$). In fact, by evaluating the individual fatty acids it emerged that both myristic acid and palmitic acid presented a lower proportion tending to significance (C14:0, $P = 0.10$; C16:0, $P = 0.096$), while the stearic acid was significantly lower (C18:0, $P = 0.026$).

No significant effects were found for the proportion of MUFA ($P > 0.05$), while the proportion of polyunsaturated fatty acids (PUFA) in salami from pigs was affected by the dietary treatment ($P < 0.001$), with the highest value found in the salami from pigs fed the DBP diet. Salami from pigs fed the DBP diet had a greater proportion of α-linolenic acid ($P < 0.001$) and tended to have a greater proportion of linoleic acid ($P = 0.06$) compared to salami from pigs fed the control diet. No effect was found ($P > 0.05$) for the proportion of other long-chain n-3 PUFA, such as eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic (DHA).

Regarding the n-6/n-3 ratio and the PUFA/SFA ratio, both were significantly influenced by dietary treatment, ($P < 0.001$ and $P = 0.003$ respectively), presenting a better value in salami from pigs fed with the DBP diet.

The thrombogenic and atherogenic indexes were affected by dietary treatment ($P = 0.001$ and $P < 0.05$ respectively), with the lowest values found in salami from pigs fed the DBP diet compared with the control group.

As for the concentration of vitamins E and A (Table 12), in salami the levels α-tocopherol and γ-tocopherol were not affected by the dietary treatment ($P > 0.05$). Similarly, the concentration of retinol (vitamin A) was not affected by supplementing dried bergamot pulp in the finishing diet of pigs. The concentration of the HP-PUFA increases ($P = 0.001$) by feeding pigs with the diet containing dried bergamot pulp in salami, influencing the HP-PUFA ÷ VE ratio that was higher ($P = 0.023$) in salami from the DBP group compared to the control group.

Table 12

Effect of the dietary treatment on the antioxidant vitamins and fatty acid composition of salami (g/100 g of total fatty acids)
(Trial n° 2 with DBP)

	Dietary Treatment			
Item	Control	DBP	SEM	*P* values
Tocopherols and retinol, µg/g salami				
α-Tocopherol	0.54	0.60	0.412	0.806
γ-Tocopherol	0.05	0.04	0.004	0.335
Retinol	3.38	3.02	0.544	0.756
Total fat, g/100 g of salami	19.7	16.6	48.08	0.109
C12:0	0.12	0.18	0.029	0.381
C14:0	1.58	1.33	0.075	0.101
C16:0	22.1	20.1	0.591	0.096
C16:1 *cis-9*	2.23	2.39	0.127	0.543
C18:0	10.9	9.49	0.335	0.026
C18:1 *cis-9*	44.9	43.5	0.482	0.173
C18:2 *cis-9, cis-12* LA[1]	11.1	12.0	0.257	0.060
C18:3 n-3 ALA[1]	0.50	2.10	0.230	<0.001
C 20:1 *cis-9*	1.04	1.12	0.028	0.184
C20:2 n-6	0.51	0.61	0.047	0.300
C20:4 n-6	0.28	0.30	0.066	0.943
C20:5 n-3 EPA[1]	0.20	0.24	0.055	0.713
C22:5 n-3 DPA[1]	0.20	0.21	0.033	0.967
C22:6 n-3 DHA[1]	0.14	0.15	0.027	0.867
∑ SFA[1]	34.7	31.1	0.874	0.033
∑ MUFA[1]	48.2	47.1	0.524	0.314
∑ PUFA[1]	12.7	15.6	0.492	<0.001
∑ n-3	0.87	2.70	0.270	<0.001
∑ n-6	11.9	12.9	0.288	0.060
n-6/n-3	14.2	4.91	1.400	<0.001
∑ PUFA[1]/∑ SFA[1]	0.37	0.51	0.026	0.003
Thrombogenic index[2]	1.06	0.81	0.043	0.001
Atherogenic Index[3]	0.47	0.41	0.015	0.048
HP-PUFA[4](mg/g salami)	1.23	3.65	0.389	0.001
HP-PUFA ÷ VE [5]	3.25	3.72	0.109	0.023

[1]LA: linoleic acid; ALA: α-linolenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[2]Thrombogenic index: (C14:0 + C16:0 + C18:0)/(0.5 MUFA + 0.5 PUFA n-6 + 3 PUFA n-3 + PUFA n-3/PUFA n-6).
[3]Atherogenic index: (C12:0 + 4*C14:0 + C16:0)/(MUFA + PUFA n-6 + PUFA n-3).
[4]Highly peroxidizable (HP)-PUFA: calculated as the sum of PUFA with ≥3.
[5]Calculated as the ratio between HP-PUFA and total vitamin E, both expressed as mg/g muscle. Original values obtained were not normally distributed according to the Anderson-Darling test. Therefore, logarithmic transformation was adopted and values in table are presented as LOG10.

3.3.5. Meat colour and oxidative stability

The effect of the dietary treatment and time of refrigerated storage on the oxidative stability parameters measured in raw and cooked meat is reported in Table 13. The dietary treatment influenced some colour parameters measured in raw meat. In particular, L* values ($P = 0.029$) and b* values ($P = 0.057$), were higher in the DBP diet. Also the time of storage influenced some colour parameters measured in meat during storage, in particular b* values ($P = 0.001$), H* values ($P = 0.001$) and also tended to influence C* values ($P = 0.073$) with values overall increasing from 0 to 3 days and stabilizing thereafter.

In raw and cooked meat, lipid oxidation (TBARS values) was not affected by the dietary treatment. Whereas, TBARS values increased during the days of storage ($P = 0.001$) only for cooked meat.

Table13

Effect of the dietary treatment and time of refrigerated storage on meat colour stability and lipid oxidation.
(Trial n° 2 with DBP)

	Dietary treatment[1]		Time (T)[3]			SEM	*P* values		
	Control	DBP	0	1	2		Diet	Time	DxT
L* values[2]	41.7	44.3	42.1	43.3	43.6	0.574	0.029	0.524	0.914
a* values[2]	6.0	6.4	6.9	6.2	5.5	0.307	0.462	0.142	0.153
b* values[2]	8.2	9.3	7.1^{x}	10.1^{y}	8.9^{zy}	0.350	0.057	0.001	0.080
C* values[2]	10.3	11.4	10.0	11.9	10.7	0.371	0.121	0.073	0.052
H* values[2]	53.6	55.4	46.4^{x}	59.0^{y}	58.0^{y}	1.58	0.529	0.001	0.894
TBARS raw meat, mg MDA/kg	0.50	0.54	0.49	0.51	0.56	0.020	0.407	0.376	0.307
TBARS cooked meat, mg MDA/kg	3.08	2.77	2.17^{x}	2.75^{y}	3.86^{z}	0.145	0.115	0.001	0.711

[x,y,z]Within row, different superscripts indicate differences between days of storage ($P < 0.05$) tested using the Tukey's adjustment for multiple comparisons.
[1]Treatments were: only concentrate (C); concentrate and dried bergamot pulp at the level of 15% dry matter on the diet fed (DBP).
[2]L*=lightness; a*=redness; b*=yellowness; C*=Chrome; h*=hue angle, measured in degrees.
[3]Times 0, 1, 2 = days 0, 3, 7 for raw meat and 0, 2, 5 for cooked meat at 4 °C under aerobic conditions (meat slices).

3.3.6. Salami oxidative stability

The lipid oxidation of salami is reported in Fig. 2. The dietary treatment influenced significantly ($P < 0.001$) the extent of lipid oxidation in salami; indeed, the TBARS values were lower in the DBP diet. Furthermore, the TBARS values increased during aerobic refrigerated storage ($P < 0.001$) and after 5 days the salami of the DBP group showed lower values compared to the salami of the control group ($P < 0.01$).

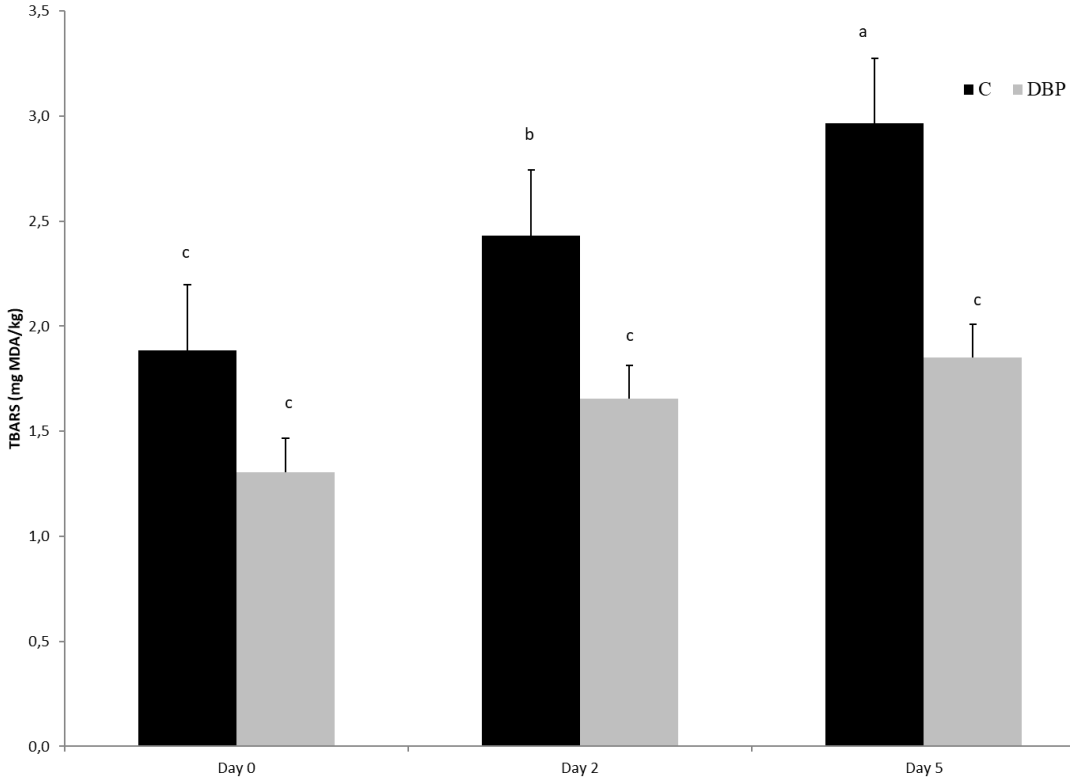

Fig. 2 (Trial n° 2 with DBP)
Effect of the dietary treatment and time of storage on the oxidative stability of salami. Interactive effect of the dietary treatment (Control and DBP) and time of storage (Days 0, 2 and 5) on the TBARS values measured in salami slices over aerobic storage at 4 °C. Values presented are the estimated least squares means and standard error bars. ^{a,b,c} Values with different superscripts are significantly different ($P \leq 0.05$)

3.4. Discussion of experimental trial n° 2 with DBP

3.4.1. Fatty acid composition

To date, the use of by-products in animal nutrition is widely documented. In fact, it is also thanks to the inclusion of by-products in animal feeding that strategies have been pursued to improve environmental and economic sustainability. Ali *et al*., (2017) observed that using by-product, as citrus pulp (dried), in the diets of pigs has the potential to reduce environmental impact of pork production in terms of global warming potential and allows for the use of land for the food crops production intended for human food.

As claimed by Kyriazakis & Emmans (1995), an abundant presence of dietary fibre in pig diets can lead to a reduction of feed intake, due to the specific polysaccharides such as pectins, which absorb water and form a gelatinous compound within the intestinal lumen. Cerisuelo *et al.* (2010), as mentioned previously in the discussion of the first trilal, showed that the inclusion of 5% and 10% of ensiled citrus pulp in the diet for growing pigs resulted in a reduction of animal growth performance. This negative effect may derive from the reduction of DM feed intake during the first 4 weeks of the experimental trial, but the differences subsequently disappeared.

In the current study, no significant differences were observed in terms of DMI between treatments.

Apulo-Calabrese, similarly to the other Italian local pig breeds (Franci & Pugliese, 2007), is characterized by reduced growth and carcass performance (Aboagye *et al*., 2020). Growth is slow and this was probably the main cause that led breeders to prefer other breeds that are earlier and with a better feed conversion index. Actually, this slow growth, if associated with a balanced diet, could have an effect on the fatty acid profile of the meat and therefore affect the nutritional quality of the food itself. However, in our trial, we have evaluated the fatty acid composition of the meat. Aboagye *et al*. (2020) showed that when the Apulo-Calabrese pigs are managed in the same rearing conditions as crossbreeds, their *longissimus thoracis* muscle fatty acid composition was comparable, therefore the fatty profile of the meat seems not influenced by the genetic type. Conversely, the fatty acid composition of meat can be influenced by the fatty acid composition of the experimental diet. Wood & Enser as early as 1997

asserted that in all species, meat fatty acid composition can be modulated by the diet, especially more easily in pigs, monogastric animals, where the linoleic, α-linolenic and long-chain PUFA responds quicky to high dietary concentration.

Despite the fact that meat has been and still is criticized because of its undesirable fatty acid profile, due to the high proportion of SFA rather than PUFA, meat is an essential food for the human diet. Meat is a valuable source of high biological value protein, iron, vitamin B_{12} as well as other B complex vitamins, zinc, selenium and phosphorus. In fact, the elimination of meat from the diet could increase the risk of severe nutritional deficiencies and impair human health and nutritional status (Pereira & Vicente, 2013). Moreover, pork has a favourable balance between polyunsaturated and saturated fatty acids (PUFA/SFA) and our data were in agreement with these authors. It is well known that pork meat in general has an unacceptably high ratio of n-6 and n-3 polyunsaturated fatty acids (Wood *et al*., 2003).

The World Health Organization (WHO, 2003) recommended a reduction in the intake of SFA in favor of the n-3 polyunsaturated fatty acids (n-3 PUFA), for their beneficial effects on human health. Essentially, SFA are known to increase low-density lipoproteins and in turn, the risk of cardiovascular disease. In our trial, despite no significant increase in the concentration of ALA in the DBP pork meat was observed, the proportion of DPA was affected by the dietary treatment. The DPA is a long chain n-3 PUFA that derives from ALA (Dyall, 2015) thanks to the action of desaturase and elongase enzymes that catalyze the reactions; its beneficial effects on human health are well known (Akiba *et al*., 2000; Morin *et al*., 2013; Morin *et al*., 2014; Morin *et* al., 2015; Tsuji *et al*., 2003). Furthermore, the *longissimus thoracis et lumborum* muscle from pigs fed with the DBP diet tended to have a greater proportion of n-3 PUFA, while the proportion of n-6 PUFA was comparable between treatments and, consequently, the n-6/n-3 ratio was the lower in the DBP treatment compared to the control. A similar trend was observed in the first trial, in meat by feeding pigs with a diet containing EBP.

Thrombogenic index (TI) was calculated to assess the potential for platelet aggregation. The TI tended to be reduced when the pigs were fed the DBP diet

compared with the control diet and this can be explained by the fact that bergamot pulp affected some desirable fatty acids such as those belonging to the n-3 family.

As in meat, the concentration of total n-3 PUFA tended to increase in backfat from DBP fed pigs. In fact, feeding diets with a high concentration of ALA to pigs generally results in the accumulation of this fatty acid and its long-chain derivatives in the backfat. Consistent with this, we found a greater concentration of ALA in the backfat from the DBP fed animals. However, its long-chain derivatives were found at comparable amounts between the two groups.

Fatty acid composition determines the firmness/oiliness of adipose tissue and the oxidative stability of muscle, which in turn affects flavour and muscle colour (Wood, 2008).

Apulo-Calabrese breed is among those authorised for the production of the four Protected Designation of Origin-cured meat products: “soppressata”, “salsiccia”, “pancetta” and “capocollo” of Calabria, all certified by the “Consortium for the protection of Calabria PDO cured meats”. In our study, we analyzed the salami and we noticed that the use of dried bergamot pulp influenced, coherently with that observed in the meat and backfat, the fatty acid profile of the salami, enhancing some desirable fatty acids and improving indexes related to a lipid nutritional quality. Specifically, salami produced from pigs fed with the dried bergamot pulp had a greater content of ALA compared to the control group. Considering the above described effect of feeding DBP diet on the content of ALA in meat and in backfat, this result was expected.

3.4.2. Oxidative stability

Lipid oxidation is one of the main factors responsible for the loss of quality of meat and meat products. Following lipid oxidation, a series of unpleasant tastes and odors develop, as well as changes in color and texture. It is a rather complex process, where unsaturated fatty acids are involved and react with molecular oxygen to form peroxides, from which aldehydes, ketones and acids derive, many of which are responsible for the unpleasant rancid smell of oxidized fats (Byrne, 2000).

Tocopherols are fat-soluble antioxidants and have a protective role against lipid oxidation (Luciano *et al*., 2017). Animals are unable to synthesize tocopherol and its concentration in tissues is therefore strictly dependent on the diet. In the present study, although the concentration of vitamin E, especially α-tocopherol, in dried bergamot pulp was much higher than in the concentrate, no difference in vitamin E content was observed in meat of the two experimental groups. While for retinol, defined as a lipophilic scavenger, its concentration tended to be influenced by diet, in fact it was greater in meat of the DBP group. As claimed by Halliwell & Gutteridge (2015), in conditions of oxidative stress there is a shift in the pro-oxidant/antioxidant balance in favor of the former in animal tissues. Despite the beneficial effects, n-3 PUFA are subject to oxidation during the processing and storage phases, inducing a potential alteration of the nutritional composition and product quality (Rosa *et al*., 2012) and as the degree of unsaturation increases, the tendency to oxidation also increases (Decker *et al*., 2010). The results of the present study showed that the highly peroxidizable (HP)-PUFA ÷ VE ratio tended to increase ($P = 0.056$) in meat from the pigs fed with a diet containing dried bergamot pulp compared to the control group. As well as in the first trial with the EBP. It means that other possible compounds came into play, which meant that the use of dried bergamot pulp did not alter the shelf-life in raw meat. Agro-industrial by-products are rich in secondary compounds with antioxidant properties, such as phenolic compounds and essential oils which, when used in animal nutrition, can affect the resistance of meat to oxidative deterioration. The peel of bergamot fruit contains a significant amount of flavonoids, in particular naringin, neoeriocitrin, and neohesperidin (Russo *et al*., 2016), compounds that have been found to have health-related properties, especially based on their antioxidant activity. Moreover, for their

content of polyphenols and other bioactive phytochemicals, several agro-industrial by-products can be considered as functional feedstuffs (Scerra *et al*., 2018). The dried bergamot pulp used in our study showed a considerable residue of phenolic compound as shown in Table 2 (6.34 g TAe/kg DM), despite the fact that it has undergone the extraction of phenolic compounds. This supports the thesis that these compounds may have come into play. Above all, phenolic compounds act against the oxidation of myoglobin by extending the shelf life of the product (Luciano *et al*., 2011). The discolouration of the meat is mainly due to the oxidation of myoglobin and the consequent accumulation of metmyoglobin (Greene, 1969); this process causes the decrease in meat of redness (a*) and saturation (C*) values and the increase in hue angle (H*) during storage time. In the present study, the main descriptor of meat discolouration changed over the time of storage as expected. Indeed, the meat yellowness (b*) and the hue angle (H*) increases during storage time. Dietary treatment significantly affected meat lightness (L*). This finding was consistent with the results from Priolo *et al*., (2000) and Inserra *et al*. (2015), who observed higher meat lightness in lambs and pork, respectively, fed with a diet containing carob pulp and therefore rich in tannins. Conversely, Crosswhite *et al*. (2013), concluded that the inclusion of citrus-pulp did not affect the main colour descriptors.

In the study conducted by Luciano *et al*. (2019), the authors reported that differences in oxidative stability between meat samples were evident with strong oxidative challenges, such as cooking, compared to samples stored in a refrigerated aerobic in darkness environment. In the present study, we also evaluated cooked meat, which is when subjected to strong oxidative stress factors, in order to highlight the influence of dietary treatment. Also under these conditions, lipid oxidation (TBARS values) was not affected by the dietary treatment. Conversely, the dietary treatment influenced significantly the extent of lipid oxidation in salami and the TBARS values were lower in the DBP diet (Fig. 2). The TBARS assay detects the level of malondialdehyde (MDA), which is the major lipid oxidation product (Tsikas, 2017). The limit value that distinguishes the condition of rancidity is indicated in terms of MDA and is a maximum of 2 mg/ kg (Spaziani *et al*., *2011*). In our study, in salami from the DBP group this value has not exceeded this limiting threshold, while in salami from the

control group, the level of MDA was already beyond of 2 on the second day of monitoring. Furthermore, the TBARS values increased during aerobic refrigerated storage and after 5 days the salami of the DBP group showed lower values compared to the salami of the control group.

In salami, the concentration of the HP-PUFA increased by feeding pigs with the diet containing dried bergamot pulp, influencing the HP-PUFA ÷ VE ratio that was higher in salami from the DBP group compared to the control group.

Despite the high levels of HP-PUFA, the TBARS values in the salami of animals fed with the DBP were lower during the storage period, highlighting how salami from animals treated with bergamot responded better to the strong oxidative stresses, such as the grinding and the long storage time. The study of Luciano *et al*. (2017) showed that vitamin E is the greatest contributor in the improvement of the antioxidant capacity of tissues. However, in our study in both raw meat and salami, no difference was found in the content of vitamin E between the two dietary treatments. Phenolic compounds may explain this result as it was reported that dietary administration of hesperidin and naringin exerted a significant effect on the antioxidant capacity of broiler meat (Goliomytis *et al*., 2015). Therefore, it might be speculated that these phenolic compounds may have contributed to delay the lipid oxidation in salami. Also in the first trial we observed, in salami by feeding pigs with a diet containing EBP a TBARS values below the threshold value of 2 mg MDA/Kg for the entire monitoring period. However, after 5 days of refrigerated storage, the TBARS values observed in the first trial, in salami by feeding pigs with EBP were lower than the TBARS values observed in this trial by feeding pigs with a diet containing DBP. The highest amount of antioxidant compounds such as phenols in the bergamot by-prdouct used in the first trial could influence these differences (14.15 *vs* 6.34 g TAe/kg DM respectively in EBP and in DBP).

4 – Conclusion and Future Perspectives

To date, this is the first study that investigated the effect of feeding pigs with the solid residue resulting from the industrial processes of bergamot on meat and meat products on fatty acids composition and oxidative stability. The results showed that the replacement of part of the concentrate with bergamot pulp in the diet increased the content of some of the most desirable fatty acids from a health point of view in meat and salami, primarily linolenic acid.

The inclusion of bergamot pulp in the diet did not alter the oxidative stability in raw and cooked meat. Similarly, colour descriptors mostly related to meat browning did not differ between treatments in fresh meat. Instead, in salami TBARS values were reduced over aerobic storage in bergamot pulp group. Probably, the strong oxidative stresses condition, such as the long storage time, has allowed to show the resistance to oxidative deterioration of the meat from animals of the bergamot pulp groups.

In conclusion, the integration of bergamot pulp at up to 15% in diets for fattening pigs could represent a strategy, in the Mediterranean areas, to naturally improve nutritional value of meat and meat products and to promote the exploitation of this local feed resource.

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