Department of Health,
Animal Science and Food Safety

Università degli Studi di Milano

GRADUATE SCHOOL OF VETERINARY AND ANIMAL SCIENCE

Academic Year: 2018-2019

Improvement in animal health and product quality through dietary manipulation

Chiapparini Sara

(Class XXXII)
Ph.D. Thesis

Tutor:
Prof. Carlo Corino

Coordinator:
Prof. Valeria Grieco

Index

ABSTRACT

The challenge in livestock sector is to maintain a high productivity and food security in a sustainable way, reducing the use of antimicrobials. For these reasons, the use of sustainable dietary interventions seems to be a valuable approach in order to enhance animal performance, health and product quality. So, a nutritional approach, using dietary integration with biotechnological bioactive compounds or sustainable integration are investigated. The aim of this thesis was to: 1) summarize the dietary intervention with seaweed in pig specie 2) evaluate the effects of dietary integration with biotechnological extract in post weaning piglets on growth, immune parameters and oxidative status 3) evaluate the effectiveness of brown seaweed and polyphenols mixture on rabbit does reproductive performances and antioxidant status and 4) evaluate the effect of brown seaweed and polyphenols mixture on growth and meat quality parameters in rabbit.

The present experimental studies highlight that dietary manipulation with natural substances is a useful approach to improve rabbits and piglet's health, productive parameters and product quality in a sustainable way. In particular, the use of dietary biotechnological extract and brown seaweed and plant polyphenols mixture was investigated. This data highlight that natural extracts are effective candidates to improve animal health, reducing the use of antibiotics. This will contribute to the development of a sustainable production system, in order to enhance animal health, product quality and to reduce the environmental impact, as recommended by the One health approach. Considering the heterogeneity of herbs, spices and botanicals, further investigations are required to deepen our knowledge about the mechanism of actions and dosage of seaweeds and polyphenols mixture.

RIASSUNTO

La principale sfida nel settore zootecnico è mantere un'elevata produttivita e la sicurezza degli alimenti in modo sostenibile, riducendo l'utilizzo degli antimicrobici. Diverse strategie alimentari risultano indispensabili per il raggiungimento di questi obiettivi, in quanto sono in grado di migliorare le performance e la salute degli animali e la qualità dei prodotti. In particolare, sono state valutate diverse integrazioni dietetiche con estratti naturali e molecole bioattive. L'obiettivo della tesi è stato: 1) Studiare l'utilizzo di integrazioni dietetiche con alghe nella specie suina. 2) Valutare gli effetti dell'integrazione dietetica nel suinetto in post svezzamento con estratti naturali biotecnologici su performance di crescita, parametri immunitari e lo status antiossidante. 3) Valutare gli effetti dell'integrazione dietetica con alghe brune e polifenoli su parametri riproduttivi e status antiossidante delle coniglie. 4) Valutare gli effetti dell'integrazione dietetica con alghe brune e polifenoli sulle performance di crescita e qualità della carne in conigli in accrescimento.

Gli studi mostrano che l'integrazione dietetica con sostanze naturali riuslta un approccio sostenibile per migliorare la salute, i parametri produttivi e la qualità dei prodotti nella specie suina e cunicola.
In particolare, sono state studiate alcune integrazioni dietetiche con estratti biotecnologici e con una miscela di alghe brune e polifenoli. I risultati hanno dimostrato l'efficacia di queste sostanze nel modulare positivamente la la salute degli animali, con conseguente riduzione di antibiotici.
Tali strategie alimentari possono quindi contribuire allo sviluppo di un sistema produttivo sostenibile migliorando la salute animale e la qualità dei prodotti, riducendo l'impatto ambientale come richiesto dall'approccio *One Health.*
Considerando l'eterogeneità delle sostanze naturali, si rendono necessarie ulteriori indagini per approfondire le conoscenze relative ai diversi meccanismi d'azione e ai corretti dosaggi da applicare nelle diverse specie d'interesse zootrecnico.

CHAPTER 1

FOREWORD

In livestock animal welfare, health, growth performance, sustainability and meat quality are gaining increasing attention not only on the part of the scientific community but also breeders, food producers and consumers.
Disease level is of considerable importance in welfare assessments, and susceptibility to disease is also an important indicator. Management, housing system, stressors, “overcrowding on” farms, environmental conditions must be considered in order to improve health, growth performance and meat quality parameters in different species of pigs, rabbits, cows and poultry.
For years farmers have used antibiotics to improve the growth performance of animals, as growth promoters. Since the ban on the use of synthetic substances, there has been a big increase in animal death and diseases, and thus alternative solutions are needed to enhance animal health. Zinc oxide is an alternative, but there will be in EC ban on the pharmacological dosages of zinc from zinc oxide from 2022.
Probiotics, prebiotics, organic acids, plant extracts, and enzymes therefore present an opportunity to enhance gut health and the productive and reproductive performance of sows, piglets and swine when used as dietary supplements.
Researchers have focused on natural substances that have probiotic, prebiotic, antimicrobial, immuno-modulating and antioxidant actions such as seaweeds, polyphenols aimed at improving animal health, growth performance and meat quality for consumers in animal production.

1.1 ANTIBIOTICS AS GROWTH PROMOTERS IN LIVESTOCK

The benefits of using antimicrobials as antimicrobial growth promoters (AGPs) were first reported in 1950 when two researchers noticed that small subtherapeutic doses of penicillin and tetracycline could enhance weight gain (Stokstad and Jukes, 1950). AGPs are widely added to animal feed to stimulate growth, rapidly increase productivity, and minimize mortality by preventing infections (Van Den Bogaard et al., 2000). In the United States, antimicrobials are also regularly used to treat infections or illnesses in food-producing animals. Food animals are especially susceptible to opportunistic microbes (usually benign or commensal but which can cause disease given the right circumstances), such as bacteria. Thus they are often exposed to antimicrobials, such as antibiotics, to treat and prevent infectious bacterial diseases and/or to promote growth and improve feed efficiency.
Many of these antimicrobials are identical to or closely resemble drugs used in humans (McEwen and Fedorka-Cray, 2002). Typically, the antibiotic is distributed to the animals in the form of livestock feed supplements, as it is more efficient to mass-medicate entire groups as opposed to individual treatments.The use of antibiotics has a drastic effect on the development and occurrence of antibiotic resistance in animals and humans (Devirgiliis, Zinno, & Perozzi., 2013; Barton., 2000). The World Health Organization and the World Organization for Animal Health have encouraged the health, agriculture, and veterinary sectors to reduce the use of AGPs (Aidara-Kane, 2012).

1.2 DEVELOPMENT OF ANTIBIOTIC RESISTANCE

The global situation concerning antibiotic resistance is very alarming. Antibiotics were first studied in the late 1800s and it was in the early 1900s that penicillin was first discovered (Abraham and Chain, 1940). Since the application of penicillin in the 1940s, antibiotics have played an unparalleled role in the prevention, control, and treatment of infectious diseases for humans and animals. Unfortunately, today the increasingly widespread use (especially the misuse) of antibiotics have led to the rapid appearance of antibiotic resistant strains: more and more infections are caused by microorganisms that fail to respond to conventional treatments. In fact, bacteria have a notable genetic plasticity that allows them to respond to a wide array of environmental threats, including the presence of antimicrobials. Bacterial cells derived from a susceptible population are able to develop gene mutations that negatively affect drug activity, causing cell survival in the presence of antimicrobial molecules. The mechanism of antimicrobial resistance (AMR) has been extesnively described by Munita et al., (2016).

Antibiotic usage in intensive livestock systems has been associated with AMR, and the WHO has declared it a risk for both human and animal health (WHO, 2000). The over-use of antibiotics has resulted in the development of AMR in animal microbial populations, with the potential of transferring antibiotic resistance genes from animal to human microbiota (Economou and Gousia, 2015).

The use of antibiotics as growth promoters has thus been prohibited in many countries, with Sweden being the first to ban antibiotics in 1986. Denmark subsequently banned their use in 1998 and was followed by the European Union which introduced a total ban in 2006 (Castanon, 2007). The prohibition on the subtherapeutic use of antibiotics in animal feed resulted in decreased animal production (Cheng et al., 2014) due to higher rates of infections in livestock and has also increased the risk of food-borne infections in consumers (Hao et al., 2014). Ten years later, prophylactic antibiotics are still used at high levels in many countries to sustain animal health and welfare (Aarestrup, 2012; Callens et al., 2012).

AMR is in rapid evolution and greatly affects the efficacy of antimicrobial treatment of patients infected with multi-drug resistant organisms. Therefore, AMR is currently considered to be one of the major public health threats for the near future. Measures are needed worldwide to reduce the use of antimicrobials. In the last few decades, a new approach call "*One Health*" has been adopted. This concept recognizes that the health of people is connected to the health of animals and the environment. Physicians, veterinarians, ecologists, and many others have been working together to monitor public health threats and to learn about how diseases spread among people, animals, and the environment.

It is thus crucial to find a way to improve livestock health and welfare, to decrease antimicrobial use and to produce safe products in a sustainable way. The research and development of innovative strategies is essential for improving animal health by reducing antimicrobial residues and antibiotic-resistant microorganisms and ensuring consumer health. The improvement in livestock health and welfare, through approaches such as the development of novel non-drug approaches is key in order to guarantee the sustainable development of livestock breeding. Several molecules have also been studied to help overcome the problems associated with the ban of antibiotics in livestock production (Cheng et al., 2014).

1.3 ALTERNATIVE SOLUTIONS TO THE USE OF ANTIBIOTICS

Feed and feeding strategies play a key role in affecting animal health and welfare. To overcome the increased mortality and morbidity rate due to the ban of antibiotics, a number of alternatives have been proposed (Seal et al., 2013). These include immunomodulatory agents, probiotics and prebiotics, plant extracts, and enzymes (Cheng et al., 2014). There are also several substances that improve the immune function and consequently the host's resistance to diseases. Since the 1990s, vitamins (A, E, C), nucleotides, essential oils and microelements (selenium, copper and zinc) have been used as immunostimulants. In addition, probiotics, plants and their extracts have been widely studied due to their healthy proprieties (Cheng et al., 2014).

1.4 PROBIOTICS AND PREBIOTICS

In the last few decades, the interest in alternative solutions to the use of antibiotics in livestock nutrition, have assumed great importance. Various strategies are used to reduce the use of synthetic substances with significant impacts on animal health, and reductions in the spread of disease (Griggs and Jacob, 2005).

However, the use of a combination of herbs and probiotics as functional feeds has not been widely studied. Although individual herbs and probiotics are highly effective, their combinations may enhance their performance through synergism (Prakasita et al., 2019). Prevention in the form of an immunization program by vaccination could limit the amount of antibiotics needed.

In the last 15 years, the use of probiotics has greatly increased. The US National Food Ingredient Association defines probiotics (direct-fed microbials) as a source of live naturally-occurring microorganisms, including bacteria, fungi and yeast (Miles and Bootwalla, 1991)

The term "probiotics" has been amended by the FAO/ WHO to "Live microorganisms", which, when administered in adequate amounts, confer a health benefit on the host (Fuller, 1989). Probiotics are also defined as live microorganisms with a health benefit for the host when administered in appropriate and regular quantities. Once ingested, the probiotic microorganisms modulate the balance and activities of the gastrointestinal microbiota, whose role is fundamental for gut homeostasis. Numerous factors, such as dietary and management constraints, affect the structure and activities of gut microbial communities, leading to impaired health and performance in livestock animals. The literature describes the important benefits of yeast and bacterial probiotics on the gastrointestinal microbial ecosystem in ruminants and monogastric animals (equines, pigs, poultry, fish), as well as their implications in terms of animal nutrition and health. (Chaucheyras-Durand and Durand, 2010)

Several lactic acid bacteria (LAB) strains belonging to the genera *Lactobacillus*, *Bifidobacterium*, and *Enterococcus*, are considered beneficial to the host and have thus been used as probiotics and included in several functional foods (Castro et al., 2015). Probiotics enhance intestinal health by stimulating the development of a healthy microbiota (predominated by beneficial bacteria), preventing enteric pathogens from colonizing the intestine, increasing digestive capacity, lowering the pH, and improving mucosal immunity. It is important for the introduced microbes not to disturb the indigenous population, which has already adapted to the environment of the gastrointestinal tract to work both for and with the host (Tannock, 2007).

Prebiotics are non-digestible food ingredients which, when consumed in sufficient amounts, selectively stimulate the growth and/or activity of one or a limited number of microbes in the gut. The impacts of orally-administered probiotics (in this case, referred to as symbiotics) and the intrinsic beneficial bacteria of the gastrointestinal tract can be enhanced by the use of prebiotics (Gibson et al., 2004)The most common prebiotics used to yield health benefits are carbohydrate substrates, such as oligosaccharides or dietary fiber with a low digestibility, which enhance the beneficial growth organisms in the gut, or function as competitive attachment sites of pathogenic bacteria. Fructooligosaccharides (FOSs) and mannanoligosaccharides (MOSs) are two of the most studied prebiotics. Some cereal crops and onions contain FOSs, whereas MOSs are obtained from the cell walls of yeast (*Saccharomyces cerevisiae*). Several studies on the use of prebiotics in animal production have highlighted the potential benefits of FOSs and MOSs (Waldroup et al., 1993).

1.5 ORGANIC ACIDS

For several decades organic acids have been studied to reduce bacteria in livestock production as key to ensuring feed preservation and safety. The majority can be added to the drinking water or in feed. The use of organic acids in animal nutrition may help to reduce the intestinal colonization of *Salmonella* (Van Immerseel et al., 2006). The supplementation effects of organic acids in the water or feed on campylobacter colonization are reported in a study by Chaveerach et al., 2004. They observed certain blends, such as formic, and acetic, and found that propionic acids, in ratios of 1:2:3 and 1:2:5, respectively, were more effective at inhibiting the growth of *Campylobacter* than the commercial products that they tested. At the end of the experiment they concluded that adding organic acids to drinking water reduced the transmission of bacteria in the flock.

The use of acetic acid from vinegar in an *in vitro* study inhibited the growth of several *E. Coli* and *S. typhimurium* bacteria, with positive effects and a reduction in bacteria (Entani et al., 1998).

Among a variety of candidates for the replacement of antibiotic growth promoters, organic acids are promising alternatives (Mroz, 2005).

Organic acids are known to:

-reduce the buffering capacity of diets,

-control harmful microorganisms in digestive and respiratory organs by reducing pH levels in the stomach and gut,

-promote the availability of nutrients in the diet and their absorption and digestion,

-improve immune responses in poultry (Yesilbag and Colpan, 2006; Park et al., 2009; Abudabos et al., 2014), which can make a great contribution to the profitability in poultry production and also provide people with healthy and nutritious poultry products.

However, an important limitation is that organic acids are rapidly metabolized in the foregut (Khan and Iqbal, 2016).

Matrix-coating or encapsulation techniques are a good solution to protect organic acids for targeted delivery to different gut segments. A supplementation blend of a dietary matrix-coated organic acid maintains the optimum pH in the intestinal tract and improve nutrient digestibility (Upadhaya et al., 2014).

Other possible antibiotic replacers are medium-chain fatty acids (MCFAs), which are a type of acid with a strong antibacterial activity against Gram-positive cocci (Bergsson et al., 2001) and

Escherichia coli (Skřivanová et al., 2009). Such positive changes (e.g., greater villus height) may result in improved performance of the poultry. In addition, organic acids could improve the antibacterial effects of MCFAs (Zentek et al., 2011).
A combination of organic acids and MCFAs has beneficial effects on intestinal microecology in piglets (Zentek et al., 2013; Kuang et al., 2015) and nutrient digestibility in laying hens (Lee et al., 2015). They make a fundamental contribution to feed hygiene, as they suppress the growth of mould and bacterial pathogens, thus allowing a better use of feed resources. Organic acids are currently also the most cost-effective and eco-efficient performance-enhancing option available to the feed industry. However, due to their antimicrobial activity, organic acids and their salts not only help to preserve feed and silages, they also reduce bacterial content and maintain the nutritional value of the feed to ensure animal performance. In addition, they improve nutrient digestibility - which in turn leads to stable animal health and increased performance. In animal husbandry, reduced feed conversion rates, improved daily gain and a reduced incidence of diarrhoea all contribute to an enhanced economic return, through lower feed costs and reduced time-to-market weight (Freitag, 2007).

1.6 NATURAL EXTRACTS IN ANIMAL NUTRITION

Interest in natural substances such as plants, herbs, polyphenols and spices has increased in multidisciplinary fields including animal nutrition, health/well–being, and food.
World wide, interest in herbal products has grown significantly, in fact cattle, horses, sheep, goats and pigs represent about 31%, 14%, 17%, 17% and 7%, respectively, of the animals treated with herbal remedies, followed by poultry (9.1%), dogs (5.3%) and rabbits(4.3%). The search for alternatives to antibiotic growth promoters and the increased demand for natural substances by consumers have stimulated the study of the effects and their active compounds in animal feed. Public awareness of the potential health risks associated with the use of in–feed antibiotics, growth hormones and various synthetic pharmaceuticals, combining natural approaches to food production, have changed consumer attitudes (Greathead, 2003).
For centuries, herbs and spices have been known to be rich sources of molecules with several biological properties and thus could become a good solution for reducing the use of antibiotics. Plants have become of great importance in animal nutrition due to their high content in bioactive molecules, and they produce a wide range of active principles with a low molecular weight, known as secondary metabolites. Through secondary biochemical pathways, plants synthesize several compounds, often in defence against damage (Reymond et al., 2000). Some of the roles of secondary metabolites are relatively simple: they have a protective role (e.g. antioxidant, free radical–scavenging) and defend the plant against microorganisms such as bacteria, fungi, and viruses. Some of the roles of secondary metabolites are relatively simple: they have a protective role (e.g. antioxidant, free radical–scavenging) and defend the plant against microorganisms such as bacteria, fungi, and viruses. The range of secondary metabolites can be subdivided into distinct groups based on their chemical structure; alkaloids, terpenes, and phenolic compounds.
Many papers have focused on the clarification of the biochemical structures and their positive role as phytochemicals. Phenolic compounds, phenylpropanoid glycosides and extracted from plants, have been reported to have many antioxidant, anti–microbial and anti-inflammatory activities (Manach et al., 2004; Li et al., 2014).

Plants and their extracts are therefore being increasingly used in animal nutrition as appetisers, digestive stimulants, stimulants of physiological functions, colorants, and antioxidants, as well as for the prevention and treatment of certain pathological conditions. In rabbits, many studies have been carried out in order to evaluate the effects of different polyphenols from grape pomace (Sgorlon et al., 2005), green tea (Eid et al., 2010). In fact, dietary strategies improve the productive performance in intensively-reared animals.

1.6.1 ANTIOXIDANT ACTIVITIES

In response to recent claims that synthetic antioxidants have possible toxicological effects and the increased interest of consumers in purchasing natural products, the meat industry has been investigating sources of natural antioxidants (Karre et al., 2013).

Antioxidants such as vitamin C, vitamin E, carotenoids, and flavonoids have been identified in many natural food products. These substances are used to prevent the oxidative deterioration of foods and thus minimize the oxidative damage in humans, enhancing health and protecting the nutrients during storage. Plants contain a high concentration of redox-active antioxidants, such as polyphenols, carotenoids, tocopherols, glutathione, ascorbic acid and enzymes with antioxidant activities. Many natural antioxidants such as rosemary and spice extracts are more active than synthetic antioxidants, and their use in food needs to be explored. In 2010, the European Union (directives 2010/67/EU and 2010/69/EU) authorized the use of rosemary extracts as new food additives for use in foodstuffs, and the applications specified by the directives include meats.

The majority of papers describe *in vitro* investigations of the potential antioxidant mechanisms (Al–Mariri and Safi, 2014), however in farm animals antioxidants can have a direct influence on the product quality. Several studies have reported that dietary plant polyphenols in pigs enhance the oxidative stress responses of the organism (Pastorelli et al., 2012; Rossi et al. 2013).

Other studies have shown that dietary supplementations with plant extracts improve the animal's antioxidant status and consequently the meat and derived product quality. Dietary supplementation with plant extracts containing verbascoside improved the plasma oxidative status in pigs (Pastorelli, et al. 2012; Rossi et al. 2013) and in Lacaune ewes (Casamassima, et al. 2012). This antioxidant status effect is related to the increased serum levels of vitamins A and E (Palazzo et al., 2011).

In sows, Amrik and Bilkey (2004) found an improvement in the productive performance, feed conversion, feed intake, in sows fed with oregano. This natural substance also controls diarrhoea syndrome in piglets and also reduces the mortality rate.

The use of antioxidants in animal feeding reduces lipid oxidation in pork (Corino at al., 1999), rabbit and chicken meat quality. Polyphenols show antioxidant activities compared to other phenolic compounds. Rossi et al. (2009) investigated and compared the antioxidant activity of extracts from Labiatae and Oleaceae cell cultures and a natural Verbenaceae extract on pig whole blood. Another study in piglets showed the positive effects of verbascoside and teupolioside with an improvement in oxidative stability (Corino et al., 2007).

1.6.2 ANTIMICROBIAL ACTIVITIES

Several plant extracts have revealed a wide spectrum of antibacterial activities against microorganisms, including Escherichia, salmonella, staphylococcus (Dorman and Deans, 2000). Various plant extracts have important antimicrobial abilities, and most studies on this topic are *in vitro*, however a few studies have been carried out with live poultry, rabbits and piglets. Natural extracts could be used to control *Clostridium perfringens*, the bacterium that causes nectrotic enteritis in broilers (Mitsch et al., 2004). *Thymus vulgaris, Curcuma longa, Piper nigrum, Origanum vulgare* have been used in various studies. For example, thyme inhibits the growth of S. *typhimurium* and *E.coli* when added in animal nutrition (Karapinar and Aktug, 1987; Helander et al., 1998). Carvacron, another component of the essential oil of oregano has been shown *in vitro* to be a strong inhibitor of *E. coli* (Friedman et al., 2002). Cinnamon oil has also been shown *in vitro* to have an antimicrobial activity against *E. coli* (Friedman et al., 2004) and garlic (*Allium sativum*) has been shown *in vitro* to have antimicrobial properties. In recent years, the growing interest in evaluating plant constituents and extracts with their properties and good agents constitute an interesting research field. In fact, thyme, oregano, and garlic should be of particular interest to producers and researchers.

1.6.3 ANTI-INFLAMMATORY ACTIVITIES

Inflammation usually occurs when viruses or fungi invade the body in particular tissues and/or circulate in the blood (Isailovic et al., 2015). Hundreds of research and review articles have been published regarding the anti-inflammatory activities of plants, involving both *in vitro* and in vivo study results. The Black cumin (*Nigella sativa*) seed oil fraction contains thymo-quinone, which exerts anti-inflammatory effects *in vitro* (Bordoni et al., 2019).

The anti-inflammatory activities of natural substances such as flavones extracted from the *Eucommia ulmoides* leaf have shown that the dietary supplementation of this substance can alleviate the inflammatory response in piglets (Daixiu et al., 2017).

The most established anti-inflammatory effects of mushrooms containing polysaccharides, proteoglucans, terpenoids, phenolic compounds, steroids, and lectins are highlighted by Elsayed in a review. One of the aims of this study has shown that mushrooms have significant anti-inflammatory properties, in fact the biologically active compounds and the important mechanisms of action of this natural source are well described and are exhibited through the downregulation of different types of inflammatory activities (Elsayed et al., 2014).

1.7 REFERENCES

Aarestrup F. 2012. Get pigs off antibiotics. Nature. 486: 465–466.

Abudabos A. M., Al-Mufarrej S. I., Alyemni A. H., Yehia H. M., Garelnabi A. R., Alotybi M. N. 2014. Effect of using organic acids to substitute antimicrobial growth promoters on broilerchickens' performance. Journal of Food, Agriculture and Environment, 12: 447–451.

Abraham E. P., and Chain E. 1940. An enzyme from bacteria able to destroy penicillin. Reviews of Infectious Diseases, 10: 677–678.

Aidara-Kane A. 2012. Containment on antimicrobial resistance due to use of antimicrobial agents in animals intended for food:WHO perspective. Revue Scientifique Technique, 31: 277–287.

Al–Mariri A., and Safi M. 2014. *In vitro* antibacterial activity of several plant extracts and oils against some Gram–negative bacteria. Iranian Journal of Medical Sciences, 39: 36–43.

Barton M. D. 2000. Antibiotic use in animal feed and its impact on human health. Nutrition Research Reviews, 13: 1–19.

Bela A. and Bilkei G. 2004. Influence of farm application of oregano on performances of sows. Canadian Veterinary Journal, 45: 674–677.

Bergsson G., Arnfinnsson J., Steingr´Imsson 'O., Thormar H. 2001. Killing of Gram-positive cocci by fatty acids and monoglycerides. APMIS. 109: 670–678.

Bordoni L., Fedeli D., Nasuti C.,Maggi F., Papa F.,Wabitsch M., De Caterina R., Gabbianelli R. 2019. Antioxidant and anti-Inflammatory properties of *Nigella sativa* oil in human pre-adipocytes. antioxidants (Basel), 8:51.

Callens B., Persoons D., Maes D., Laanen M., Postma M., Boyen F. 2012. Prophylactic and metaphylactic antimicrobial use in Belgian fattening pig herds. Preventive Veterinary Medicine, 106: 53–62.

Casamassima D., Palazzo M., Martemucci G., Vizzarri F., Corino C. 2012. Effects of verbascoside on plasma oxidative status and blood and milk production parameters during the peripartum period in Lacaune ewes. Small Ruminant Research, 105: 1–8.

Castanon J. 2007. History of the use of antibiotic as growth promoters in European poultry feeds. Poult Science, 86: 2466–2471.

Castro J. M., Tornadijo M. E., Fresno J. M., Sandoval H. 2015. Biocheese: a food probiotic carrier. Biomed Research *Int*ernational, ID 723056, 11.

Chaucheyras-Durand F. and Durand H. 2010. Probiotics in animal nutrition and health. Benef Microbes. 1: 3-9.

Chaveerach P., Lipman L. J. A., Van Knapen F. 2004. Antagonistic activities of several bacteria on *in vitro* growth of 10 strains of Campylobacter jejuni/coli. Internationa Journal of Food Microbiology, 70: 3582–3587.

Cheng G., Hao H., Xie S., Wang X., Dai M., Huang L., Yuan Z. 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Frontiers in Microbiology, 5: 69–83.

Corino C., Oriani G., Pantaleo L., Pastorelli G., Salvatori G. 1999. Vitamin E in heavy pigs. Influence of dietary vitamin E supplementation on heavy pig carcass characteristics, meat quality, and vitamin E status. Journal of Animal Science, 77: 1755–1761.

Corino C., Rossi R., Musella M., Cannata S., Pastorelli G. 2007. Growth performance and oxidative status in piglets supplemented with verbascoside and teupolioside. Italian Journal of Animal Science, 6:292–294.

Daixiu Y., Tarique H., Bie T., Yanhong L., Peng J., Yulong Y. 2017. The Evaluation of Antioxidant and Anti-Inflammatory Effects of *Eucommia ulmoides* Flavones Using Diquat-Challenged Piglet Models. Oxidative and Medicine Cellular Longevity, 2017: 8140962

Devirgiliis C., Zinno P., Perozzi G. 2013. Update on antibiotic resistance in foodborne *Lactobacillus* and *Lactobacillus* species. Frontiers in Microbiology, 4: 1–13.

Dorman, H. J. D., and S. G. Deans. 2000. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. Journal of Applied Microbiology, 88: 308–316.

Economou V., and Gousia P. 2015. Agriculture and food animals as a source of antimicrobial-resistant bacteria. Infection and Drug Resistance, 8: 49–61.

Eid Y., Zeweil H., Ahmed M. H., Basyony M., Farok M. 2011. Effect of plant source of omega-3 fatty acids and green tea powder on the performance and meat quality of growing rabbits, Egyptian Journal of Rabbit Science, 20: 115 – 134.

Elsayed E. A., El Enshasy H., Wadaan M. A , Aziz R. 2014. Mushrooms: a potential natural source of anti-inflammatory compounds for medical applications. Mediators of inflammation, 2014: 805841.

Entani E., Asai M., Tsujihata S., Tsukamoto Y., Ohta M. 1998. Antibacterial activity of vinegar against food-borne pathogenic bacteria including *Escherichia coli* O157:H7. Journal of Food Microbiology, 70: 3582–3587.

European Commission. 2005. Ban on antibiotics as growth promoters in animal feed enters into effect. Press Release Database. http://europa.eu/rapid/press-release_IP-05-1687_en.htm.

Faostat F. 2015. Agriculture organization of the United Nations, 2011. FAO. Retrieved am from http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/S (Access, 20).

Friedman M., Henika P. R., Mandrell R. E. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. Journal of Food Protection, 65: 1545–1560.

Friedman M., Buick R., Elliott C. T. 2004. Antibacterial activities of naturally occurring compounds against antibiotic-resistant Bacillus cereus vegetative cells and spores, Escherichia coli, and Staphylococcus aureus. Journal of Food Protection, 67: 1774–1778.

Freitag M. 2007. Organic acids and salts promote performance and health. In: Acidifiers in Animal Nutrition: A guide for feed preservation and acidification to promote animal performance (Ed. C. Lückstädt). Nottingham University Press, UK, pp. 1–11.

Fuller R. Probiotics in man and animals: A review. Journal of Applied Bacteriology, 66: 365–378.

Gibson G. R., Probert H. M, Loo J. V., Rastall R. A., Roberfroid M. B. 2004. Dietary modulation of the human colonic microbiota: Updating the concept of prebiotics. Nutrition Research Reviews, 17: 259–275.

Greathead H. 2003. Plants and plant extracts for improving animal productivity. Proceeding of the Nutrition Society, 62: 279–290.

Griggs J. P., Jacob J. P. 2005. Alternatives to Antibiotics for Organic Poultry Production. The Journal of Applied Poultry Research, 14: 750–756.

Hao H., Cheng G., Iqbal Z., Ai X., Hussain H. I., Huang L., Dai M., Wang Y. 2014. Benefits and risks of antimicrobial use in food-producing animals. Frontiers in Microbiology, 5: 288.

Helander I. M., Alakomi H. L., Latva-Kala K., Mattila-Sandholm T., Pol I., Smid E.J., Gorris L.G.M, Von Wright A. 1998. Characterization of the action of selected essential oil components on gram-negative bacteria. Journal of Agricultural and Food Chemestry, 46: 3590–3595.

Isailovic N., Daigo K., Mantovani A., Selmi C. 2015. Interleukin-17 and innate immunity in infections and chronic inflammation. Journal of Autoimmunity, 60: 1–11.

Karapinar M., and Aktug S. E. 1987. Inhibition of foodborne pathogens by thymol, eugenol, menthol and anethole. International Journal of Food Microbiology, 4: 161–166.

Karre L., Lopez K, Getty K. J. K. 2013. Natural antioxidants in meat and poultry products. Food Science Institute and Department of Animal Sciences & Industry, Kansas State University, Manhattan, KS 66506, USA. Meat Science, 94:220–227.

Khan S. H., and Iqbal J. 2016. Recent advances in the role of organic acids in poultry nutrition. Journal of Applied Animal Research, 44: 359–369.

Kuang Y., Wang Y., Zhang Y., Song Y., Zhang X., Lin Y., Che L., Xu S., Wu D., Xue B., Fang Z. 2015. Effects of dietary combinations of organic acids and medium chain fatty acids as a replacement of zinc oxide on growth, digestibility and immunity of weaned pigs. Animal Feed Science and Technology, 208: 145–157.

Lee S. I., Kim H. S., Kim I. K. 2015. Microencapsulated organic acid blend with MCFAs can be used as analternative to antibiotics for laying hens. Turkish Journal of Veterinary and Animal Sciences, 39: 520–527.

Li A., Li S., Zhang Y. J., Xu X., Chen Y., Li H. 2014. Resources and Biological Activities of Natural Polyphenols. Nutrients 6: 6020–6047.

Manach C., Scalbert A., Morand C, Remesy C, Jimenez L. 2004. Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition, 79: 727–747.

McEwen S. A., and Fedorka-Cray P. J. 2002. Antimicrobial use and resistance in animals. Clinical Infectious Diseases, 34: S93–S106.

Miles R. D. and Bootwalla S. M. 1991. Direct-fed microbials in animal production. In: Direct-fed Microbials in Animal Production. A Review, National Food Ingredient Association, West Des Moines, Iowa, USA, pp. 117–132.

Mitsch P., Zitterl-Eglseer K., Kohler B., Gabler C., Losa R., Zimpernik I. 2004. The effect of two different blends of essential oil components on the proliferation of *Clostridium perfringens* in the intestines of broiler chickens. Poultry Science, 83: 669–675.

Mroz, Z. 2005. Organic acids as potential alternatives to antibiotic growth promoters for pigs. Advances in Pork Production, 16: 169–182.

Munita J. M., Arias C. A. 2016. Mechanisms of Antibiotic Resistance. Microbiology Spectrum, 4: 10.1128.

Palazzo M., Vizzarri F., Cinone M., Corino C., Casamassima D. 2011. Assessment of a natural dietary extract, titrated in phenylpropanoid glycosides, on blood parameters and plasma oxidative status in intensively reared italian hares (Lepus Corsicanus). Animal, 5: 844–850.

Park, K.W., A. R. Rhee, J. S. Um, and I. K. Paik. 2009. Effect of dietary available phosphorus and organic acids on the performance and egg quality of laying hens. Journal of Applied Animal Research, 18: 598–604.

Pastorelli G., Rossi R., Corino C. 2012. Influence of Lippia Citriodora verbascoside on growth performance, antioxidant status and serum immunoglobulines content in piglets. Czech Journal of Animal Science, 57: 312–322.

Reymond P., Weber H, Damond M, Farmer E. E. 2000. Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell, 12: 707–19.

Rossi R., Corino C., Pastorelli G., Durand P. and Prost M. 2009. Assessment of antioxidant activity of natural extracts. Italian Journal of Animal Science, 8: 655–657.

Rossi R., Pastorelli G., Corino C. 2013. Application of KRL test to assess total antioxidant activity in pigs: sensitivity to dietary antioxidants. Research in Veterinary Science, 94: 372–377.

Seal B. S., Lillehoj H. S., Donovan D. M., Gay C. G. 2013. Alternatives to antibiotics: a symposium on the challenges and solutions for animal production. Animal Health Research Reviews, 14: 78–87.

Sgorlon S., Stradaioli G., Stefanon B., Altimer G., Della Loggia R. 2005. Dietary grape polyphenols modulate oxidative stress in ageing rabbits.Italian Journal of Animal Science, 4: 541–543.

Skřivanová E., Molatová Z., Skřivanová V, Marounek M. 2009. Inhibitory activity of rabbit milk and medium-chain fatty acids against enteropathogenic Escherichia coli O128. Veterinary Microbiology, 135: 358–362.

Stokstad E. L. R., and Jukes T. H. Further observations on the“animal protein factor.” Proc Soc Exp Biology and Medicine, 73: 523–528.

Tannock G.W. 2007. What immunologists should know about bacterial communities of the human bowel. Seminars in Immunology, 19: 94–105.

Upadhaya S. D., Lee K. Y, and Kim I. H. 2014. Effect of protected organic acid blends on growth performance, nutrient digestibility and faecal micro flora in growing pigs. Journal of Applied Animal Research, 44: 238–242.

Van Den Bogaard A. E, Ruinsma N. B, Tobberingh E. E. S. 2000. The effect of banning avoparcin on VRE carriage in the Netherlands (Five Abattoirs) and Sweden. Journal of Antimicrobial Chemotherapy, 46:146–148.

Van Immerseel F., Russell J. B., Flythe M. D., Gantois I., Timbermont L., Pasmans F., Haesebrouck F., Ducatelle R. 2006. The use of organic acids to combat *Salmonella* in poultry: a mechanistic explanation of the efficacy. Avian Pathology, 35:182–188.

Waldroup A. L., Skinner J. T., Hierholzer R. E., Waldroup P. W. 1993. An evaluation of fructooligosaccharide in diets for broiler chickens and effects on salmonellae contamination of carcasses. Poultry Science, 72: 643–650.

World Health Organization. (WHO). 2000. Global Principles for the containment of antimicrobial resistance in animals intended for food: report of a WHO consultation with the participation of the Food and Agriculture Organization of the United Nations and the Office International des Epizooties, Geneva, Switzerland 5–9 June 2000. http://www.who.int/iris/handle/10665/68931.

Yesilbag D., and Colpan I. 2006. Effects of organic acid supplemented diets on growth performance, egg production and quality and on serum parameters in laying hens. Revue de Medicine Veterinaire, 157: 280–284.

Zentek J., Buchheit-Renko S.,Ferrara F., Vahjen W, Van Kessel A. G., Pieper R. 2011. Nutritional and physiological role of medium-chain triglycerides and medium-chain fatty acids in piglets. Animal Health Research Reviews, 12: 83–93.

Zentek J., Ferrara F., Pieper R, Tedin L., Meyer W., Vahjen W. 2013. Effects of dietary combinations of organic acids and medium chain fatty acids on the gastrointestinal microbial ecology and bacterial metabolites in the digestive tract of weaning piglets1. Journal of Animal Science, 91: 3200–3210.

CHAPTER 2
AIM

Take in account the importance of functional nutrition in enhancing animal performance and health in livestock, the aim of the present work is to deepen the knowledge about the effects of dietary integration with natural bioactive compounds in pigs and rabbits. Considering that the consumers and institutions need is moving towards a sustainable agrifood sector, the experimental trials regarding the dietary supplementation with biotechnological extract in piglets (second study) and dietary integration with different levels of brown seaweed and plant polyphenols mixture in does and growing rabbit (third and four studies). Moreover, a review about dietary intervention with seaweed in pig specie was performed (first study), to focus the attention on a sustainable and innovative integration in livestock.

CHAPTER 3

REVIEW

SEAWEEDS IN PIG NUTRITION

Pubblished in:

Animals (2019), 9, 1126

3.1 SIMPLE SUMMARY

In pig nutrition, alternative and safe supplements are needed to enhance the pigs' health and welfare. Natural feed components, such as herbs and plant extracts, are of great importance in animal nutrition, and marine macroalgae can be considered as supplements positively influence animal health parameters. Seaweeds possess several bioactive molecules that are studied for their prebiotic, anti-microbial, antioxidant, anti-inflammatory and immunomodulatory effects. Seaweed benefits are related to their content of sulfated polysaccharides, phlorotannins, diterpenes, omega-3 polyunsaturated fatty acids, minerals and vitamins. This paper reviews the following biological functions of seaweeds and seaweed extracts in pig nutrition: prebiotics, anti-microbial, antioxidant, anti-inflammatory and immunomodulatory effects, promoting intestinal well-being and improving digestibility.

3.2 ABSTRACT

Seaweeds are macroalgae, with different sizes, colors and composition. They consist of brown algae, red algae and green algae, which all have a different chemical composition and bioactive molecule content. The polysaccharides, laminarin and fucoidan are commonly present in brown seaweeds, ulvans are found in green seaweeds and, red algae contain a large amount of carrageenans. These bioactive compounds may have several positive effects on health in livestock. In order to reduce the antimicrobials used in livestock, research has recently focused on finding natural and sustainable molecules that boost animal performance and health. The present study thus summarizes research on the dietary integration of seaweeds in swine. In particular the influence on growth performance, nutrients digestibility, prebiotic, antioxidant, anti-inflammatory, and immunomodulatory activities were considered. The review highlights that brown seaweeds seem to be a promising dietary intervention in pigs in order to boost the immune system, antioxidant status and gut health. Data on the use of green seaweeds as a dietary supplementation seems to be lacking at present and merit further investigation.

3.3 INTRODUCTION

Marine-derived bioactive compounds are valuable as food and feed ingredients due to their biological activities (Rajauria et al., 2015). The term "algae" includes photosynthetic organisms that are usually divided in microscopic unicellular organisms, identified as microalgae and multicellular large-size organisms defined as macroalgae or seaweed. Microalgae usually grow in seawater and freshwater environments and can be prokaryotic, similar to cyanobacteria (*Chloroxybacteria*), or eukaryotic, similar to green algae (*Chlorophyta*). Diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), and golden algae (*Chrysophyceae*) are the most abundant but blue-green algae (*Cyanophyceae*) are also defined as microalgae (Guedes et al., 2011). The bioactive molecules of microalgae are used as food and feed supplements (Garcia et al., 2017). Seaweeds are marine organisms and comprise thousands of species, which are classified on the basis of their pigmentation: brown seaweeds (*Phaeophyceae*), red seaweeds (*Rhodophyceae*) and green seaweeds (*Chlorophyceae*). There are around 1800 species of brown seaweeds include, only 1% of which are recognized from freshwater and the size range varies from 20 m to 30 cm long. The brown color of these algae is related to the main content of carotenoid fucoxanthin, which masks β-carotene,

violaxanthin, diatoxanthin, and chlorophyll. The main reserves are laminarin, fucoidans, and alginates, and the cell walls are composed of cellulose and alginic acid (Peng et al., 2011). Like brown seaweeds, red algae (about 6100 species) are marine, but are able to photosynthesize in deeper water. The size ranges from thin films to filamentous and membranous forms of 1 m. The color results from the presence of the pigments, phycoerythrin and phycocyanin which mask α, β carotene, lutein, zeaxanthin and chlorophyll (O'Sullivan et al., 2010; Koizumi et al., 2018). The main reserves are typically floridean starch and floridoside, and the cells wall are made up of long-chain polysaccharide agars, carrageenans and cellulose (Usov, 2011). There are around 2200 species of green seaweeds. They are a similar size to red seaweeds, only 10% are marine and their color is related to the presence of chlorophyll. The reserves are composed of starch, and the cells wall are made up of polysaccharide ulvan (Kidgell et al., 2019). In 2015, world production of algae amounted to 30.4 million tons, of which about 96% from aquaculture and only 1 million tons from harvesting of wild stock (Fao, 2018). Due to their nutritional value and the content of bioactive molecules, seaweeds are often used as food, herbal medicines, dietary supplements, as a source of agar, alginate and carrageenan for several industrial applications, and as a fertilizer (Lee et al., 2011; Yaich et al., 2011).

3.4 CHEMICAL COMPOSITION

Several studies have been carried out in order to identify the nutritional composition and secondary metabolites of various seaweed species. In fact, it has been reported that seaweed contains several metabolites, such as the sulfated form of polysaccharides, omega 3 fatty acids, phlorotannin, diterpenes, vitamins and minerals, thus demonstrating health effects such as antibacterial, antioxidant, anti-inflammatory functions (Ganesan et al., 2019).

The chemical composition of seaweeds was found to vary in relation to their species and genera, harvesting period, and habitat condition (water temperature, light, salinity, nutrients) (Marsham et al., 2007). The chemical composition and mineral content of brown, red and green seaweeds are reported in table 1. As shown, there is a different nutritional composition range for brown, red and green seaweed, although in the same genus, the values are comparable (Wong and Cheung, 2001).

Of the brown seaweeds, common species such as *Ascophyllum, Laminaria, Saccharina, Macrocystis, Fucus*, and *Sargassum* were considered (Murty and Banerjee, 2012).

Brown seaweed shows a highly variable composition but presented a low protein (7.6-12.6 % DM) and fat content (0.8-6 % DM). The *Fucus* species presents the highest protein content (12.9 % DM), followed by *Sargassum* (10 % DM), *Laminaria* (9.4 % DM) and the *Ascophillum nodosum* (7.4 % DM), as observed by Fleurence et al., (1999). The fat content of brown seaweeds is generally lower with an average value of 3.2 % DM, and high values are observed in *Fucus* spp and *Ascophillum nodosum* (Makkar et al., 2016; Lorenzo et al., 2017).

Red seaweeds contain a higher protein content (16.9 % DM) and fat content (8.9 % DM) than brown seaweeds (Cabrita et al., 2016).

The green seaweed *Ulva lactuca* has a protein content (16.2 % DM) compared to red seaweeds and a comparable fat content (1.3 % DM) with brown seaweeds (Burtin, 2003). The fat content of the studied seaweeds varies between 0.8 to 8.9 % which is a similar range reported for other seaweeds species (Marsham et al., 2007). All seaweeds are characterized by a higher ash content (19.3-27.8 % DM) than those observed in edible plants, in fact they are a considerable source of minerals for livestock nutrition (Cabrita al. 2016; Lorenzo et al., 2017).Seaweeds are rich in potassium, sodium and calcium. Although there is a high variability, in general, the sodium and potassium contents in

Ulva spp. are lower than those reported for red and brown seaweeds. A higher content of potassium has been observed in *Palmaria palmita, Macrocystis pyrifera* and *Laminaria* spp.(Cabrita et al., 2016). All seaweeds present higher levels of calcium than phosphorous, and thus may be a possible natural source of calcium in livestock. Seaweeds are also a source of essential trace elements such as iron, manganese, copper, zinc, cobalt, selenium and iodine. In particular, iron is abundant in all the species considered, and the iodine content is higher in brown than in red and green seaweeds (*Laminaria* spp., with a range 833-5100 mg/kg DM), and a higher zinc content has been observed in red and brown than in green seaweed. The bioavailability of minerals is related to the fiber content of seaweeds. In addition, the interactions with several polysaccharides, such as alginates and agar or carrageenan, lead to the formation insoluble complexes with minerals, decreasing their biovailability (Circuncisão et al., 2018). The mineral content in the insoluble indigestible fraction residues was higher in brown than in red seaweeds with a range of 150-260 g/kg (Ruperez and Toledano, 2003). Some studies in vitro and in rats have been performed on the biovailability of minerals (Circuncisão et al., 2018). In an in vitro study of 13 seaweed species, only *Palmaria palmata* and *Ulva lactuca* showed higher Fe bioavailability than spinach, although six species had a higher Fe content. The apparent absorption values of Na and K were significantly higher in rats supplemented with *Laminaria* spp., while Mg absorption was not affected. It has also been reported that *Laminaria* spp. is rich in alginates, which probably hampers the biovailability of Ca. The absorption of inorganic I, which is the predominant form in brown seaweeds, was observed to be moderate (20-70%). Therefore, the low bioavailability may be related to the iodine interaction with other compounds in the seaweed matrix. The vitamin content showed that seaweeds are a source of water-soluble vitamins (B1, B2, B3 and C) and fat-soluble vitamins (E and provitamins carotenoids, with vitamin A activity). Seasonal effects have a great influence on vitamin content. Most of the red seaweeds, such as *Palmaria Palmata* contained a considerable amount of provitamin A and vitamins B1 and B2. The brown seaweeds *Laminaria* spp., *Ascophillum nodosum* and *Fucus* spp. showed a high content of vitamins E and C (Dominguez, 2013).

The amino acid composition of different seaweeds species is reported in table 2. Red seaweeds have a higher quality of protein than brown and green seaweeds (Angell et al., 2016), however there is considerable difference in the amino acidic content among seaweeds, in relation to the different seasons. It has been reported that seaweeds have a low content of methionine and histidine (Galland-Irmouli et al., 1999; Biancarosa et al., 2017). Leucine was the most abundant amino acid, ranging from 2.43 g/kg DM to 6.63 g/Kg DM for *Palmaria palmata* and *Ascophillum nodosum* respectively, followed by lysine (1.42-7.60 g/Kg DM), threonine (1.26-5.17 g/Kg DM) and valine (2.25-5.87 g/Kg DM). Glutamic and aspartic acids are the most common amino acids found in the non-essential fraction which are responsible of flavor and taste of seaweeds (Saini et al. 2013).

The in vitro protein digestibility (IVPD) was mid-range (82-87%) for *Saccharina latissima* and *Palmaria palmata,* and lower (79%) for *Ascophillum nodosum* and *Fucus* spp. The Red seaweed IVPD of red seaweeds had an average value of 85%, while the brown seaweeds had a lower IVPD, with an average value of 79,7%. A significant inverse correlation the IVPD and total phenolic content was also observed (Tibbetts et al., 2016).

Seaweeds also possess several biological activities due to the presence of several bioactive compounds, such as phenolic compounds, carotenoids, tocopherols, polysaccharides, and peptides. Seaweeds are rich in carboxylated and sulfated polysaccharides, such as alginates, ulvans and fucoidans: their composition and content depend on the algae species and of environmental factors

such as the season and temperature. The total polysaccharide content (% DM) is 29-67% in green algae (*Chlorophyta*), 10-59% in red algae (*Rhodophita*) and 10-75% in brown algae (*Phaecophyta*) (Sardari and Norberg Karlsson, 2018). A major component of brown seaweed cell walls is a salt form of alginic acid, alginate with the content ranging from 140-400 g/Kg DM (Øverland et al., 2019). Brown seaweed walls are also rich in fucoidans, which are saccharide units with different degrees of sulphation. Several species contained fucoidan with distinct structural characteristics, and thus the different types of functional components have various biological applications. The fucoidan content varies in relation to seaweed species and season, although the content ranges from 20 to 200 g/Kg DM, with the highest value in *Fucus vesiculosus* (Makkar et al., 2016; Øverland et al., 2019; Tanna and Mishra, 2019). Laminarin, composed of (1,3)-b-D-glucopyranose residues, is the main reserve carbohydrate of brown seaweeds with a content ranging from 0 to 300 g/Kg DM. In Laminaria spp.and *Saccharina latissima* a high content of laminarin has been reported, while, *Ascophyllum nodosum* and, *Fucus* spp. presented a low laminarin content (Makkar et al., 2016; Øverland et al., 2019; Tanna and Mishra, 2019). Laminarin presents prebiotic, immunomodulator and antioxidant activities (Balboa et al., 2013). The cell walls of red seaweeds are mainly composed of sulfated galactan such as carrageenans (content range: 220-770 g/Kg DM) and agars (content range: 210-420 g/Kg DM). Some red seaweed species contain xylan (in *Palmaria palmita* approximately 350 g/Kg DM) and porphyran (average content 480 g/Kg DM). The floridean starch is the main carbohydrate reserve with a content ranging from 250 to 420 g/Kg DM (Makkar et al., 2016; Øverland et al., 2019; Tanna and Mishra, 2019). Ulvan is one of the main sulfated polysaccharides from green seaweed cell walls with anticancer, antioxidant, antihyperlipidemic, and anticoagulant activities (Pangestuti et al., 2017) and a content ranging from 400 to 500 g/Kg DM (Øverland et al., 2019). For details and information on the monosaccharide composition of the different polysaccharides mentioned, some excellent reviews are available (Xu et al., 2017; Cherry et al., 2019). Considering their valuable source of bioactive molecules, seaweeds have been studied as feed supplements in livestock, particularly in pigs in order to boost growth performance and health (Maghin et al., 2014). Seaweeds exhibit prebiotics, anti-microbial, antioxidant, anti-inflammatory and immunostimulant properties and obviously effective absorption of dietary nutrients.

Table 1. Chemical composition of brown, red and green seaweeds (on DM basis)[1].

Seaweeds	BROWN						RED	GREEN
	***Laminaria spp.* ***	***Ascophillum nodosus***	***Sargassum spp.* ‡**	***Fucus spp.* #**	***Saccharina latissima***	***Macrocystis pyrifera***	***Palmaria palmata***	***Ulva lactuca***
Crude protein, %	9.4 (5.3-16.1)	7.4 (4.9-8.7)	10 (8.5-13.6)	12.6 (12.2-12.9)	7.6 (7.1-8.1)	8.3 (8-10)	21.9 (15.1-31.4)	16.2 (7.06-23.1)
Ether extract, %	1.1 (0.8-2.4)	5.3 (3.9-8.6)	0.8 (0.5-1.2)	6.1 (3.7-8.4)	5.5	1.8 (0.5-3.9)	8.9 (4.9-12.9)	1.3 (0.25-1.64)
Crude Fiber %	11.6 (6.6-16.6)	5.5 (5.4-5.5)	18.2 (6.4-38)	10.7 (5.4-16)	23 (6.6-40)	33.4 (5.5-50)	1.5 (1.49-1.50)	9.6 (6.9-12.3)
Ash, %	27.8 (19.6-31.5)	24.8 (21.1-30.9)	27.6 (19.4-35.9)	21.6 (20.7-22.5)	22.5 (13.3-31.7)	25.8 (20-35)	19.3 (9-24.5)	25.7 (21.3-26.2)
Gross energy, MJ/kg	12.7 (12.5-13)	14.1	9.1	15.7 (15.5-16)	11.1	9	16.9	15.2 (14.7-15.7)
Ca, g/kg	10 (8-12.55)	16.4 (9.8-20)	14,7 (3.8-27.2)	9.9 (8.9-12.8)	9.8 (9.6-10)	14.1 (11.6-16.6)	2.6 (1-4.2)	12.6 (6.1-29.2)
P, g/kg	2.2 (1.2-3)	1	1.7 (1-2.2)	1.9 (1.4-2.3)	2.7 (2.2-3.1)	2.9 (2.6-3.2)	4.0 (3-5)	2.1 (1.3-2.7)
K, g/kg	54 (48.6-59.5)	28.5 (20-37.7)	46.2	22.9 (0.4-36.1)	52.5	67.5 (44.8-112.3)	37.1 (27-47.2)	14.4 (1.5-22.1)
Na, g/kg	23.9 (22.5-25.3)	37.5 (25-45.7)	-	24.2 (0.2-45.8)	33	36.9 (17.1-56.7)	7.2 (3.3-11)	13.9 (2.9-20.2)
Mg, g/kg	6.3 (5.5-7.2)	6.8 (1-8.6)	6.4 (4-7.7)	7.5 (7-8.33)	6.3 (5.1-7.4)	39 (16.2-61.8)	2.3	13 (1.9-20.5)
Mn, mg/kg	7.1 (3.1-11)	17.8 (12-25)	88.3 (26.7-214)	104.7 (8.2-177.8)	8.2 (3.9-12.4)	11	71.6 (11-168)	38.7 (10.1-122)
Zn, mg/kg	22.6 (11-31.5)	116.8 (30.3-181)	79.3 (12-214)	118.1 (45.3-275.3)	35.4 (29.2-41.55)	12	65.1 (23.6-143)	29 (16.1-45)
Cu, mg/kg	2.4 (1.2-5.9)	17.8 (4.2-28)	6.0 (2.3-7)	9.3 (2-23.5)	4.5 (1.1-7.9)	2	11.1 (3.8-24)	8.5 (3.3-12)
Fe, mg/kg	107.3 (58-179)	157.8 (122-241)	2678 (307-7291)	351.9 (189-559)	529 (30-1028)	117	202.5 (139-315)	462.2 (105-1481)
I, mg/kg	2991.7 (833-5100)	777	399.5 (216-583)	376 (232-677)	1448.5 (957-1940)	-	278	56.7
Se mg/kg	0.6 (0.29-0.93)	0.5	1.2 (1.1-1.4)	0.8 (0.2-1.2)	1.1 (0.9-1.3)	-	0.1	1.2 (0.4-1.9)
Co mg/kg	0.1 (0.08-0.11)	0.6	0.4 (0.36-0.47)	1.1 (0.8-1.4)	0.4	-	0.03	0.5 (0.3-0.6)
Vitamin E mg/Kg	672 (3-2000)	230 (80-500)	10	164 (100-356)	1.6	928	69.6 (22-152)	12.95 (2.8-3.5)
Vitamin A mg/Kg **	154.6 (22-229)	57 (35-80)	51	17.8 (7-28.6)	0.42	12.39	142.9 (15.2-270)	3.5 (0.1-7)
Vitamin C mg/Kg **	632 (355-910)	860 (81-1650)	560	383 (141-770)	11.5 (3.5-18.8)	-	78.3 (0.7-156)	141.5 (42-241)
Vitamin B_1 mg/Kg **	7.45 (2.4-12.5)	14(1-27)	4	0.2	0.72 (0.5-0.94)	-	18 (0.7-40)	40
Vitamin B_2 mg/Kg **	4.9 (1.4-8.5)	7.5 (5-10)	65	0.35	1.7 (1.4-2.1)	-	16 (4.3-19)	5.1 (5-5.3)
Vitamin B_3 mg/Kg **	314 (158-612)	15	20	-	-	-	45 (10.1-83)	-
References	Marsham et al., 2007 Makkar et al., 2016 Cabrita et al., 2016 Circuncisão et al., 2018 Chandini et al., 2008 Kolb et al., 2004	Makkar et al., 2016 Lorenzo et al., 2017 Circuncisão et al., 2018 Angell et al., 2016 Tibbets et al., 2018 Chandini et al., 2008 Dierick et al., 2009 Marín et al., 2009	Makkar et al., 2016 Cabrita et al., 2016 Angell et al., 2016 Chandini et al., 2008 Marín et al., 2009	Lorenzo et al., 2017 Cabrita et al., 2016 Circuncisão et al., 2018 Dominguez et al., 2013 Tibbets et al., 2016 Chandini et al., 2008 Pomin et al., 2011	Cabrita et al., 2016 Circuncisão et al., 2018 Dominguez et al., 2013 Tibbets et al., 2016 Schiener et al., 2015	Makkar et al., 2016 Sanz-Pintos et al., 2017 Duis et al., 1995	Makkar et al., 2016 Dominguez et al., 2013 Tibbets et al., 2018 Chandini et al., 2008 Hagen Rødde R. et al., 2004	Makkar et al., 2016 Cabrita et al., 2016 Dominguez et al., 2013 Chandini et al., 2008 Park et al., 1997 Ortiz et al., 2006 Wong et al., 2000

[1] data are reported as mean values and ranges. * values from *Laminaria digitata* and *hyperarborea*. ‡ values from *Sargassum Patens, hemifhyllum, henslowianum*. # values from *Fucus vesciculosus, giuryi, serratus, spiralys*. ** as provitamin carotenoids.

Table 2. Aminoacid profile of brown, red and green seaweeds (mg/g DM)[1].

Seaweeds	BROWN						RED	GREEN
	Laminaria digitata	***Ascophillum nodosus***	***Sargassum spp. ‡***	***Fucus spp. #***	***Saccharina latissima***	***Macrocystis pyrifera***	***Palmaria palmata***	***Ulva lactuca***
Lysine	4.41 (4.1-4.8)	4.77 (4.3-5.4)	3.57 (2.8-4.3)	7.60 (6.7-8.2)	4.05 (4.0-4.1)	6.63 (5.2-7.5)	1.42 (1.2-1.65)	2.09 (0.5-1.9)
Histidine	1,82 (1.3-2.4)	1.63 (1.4-1.9)	0.82 (0.6-1)	1.33 (0.4-2)	2.2 (1.2-3.2)	2.33 (2.0-2.9)	0.4 (0.3-0.5)	0.83 (0.1-2.0)
Isoleucine	2.91 (2.6-3.2)	3.76 (3.1-4.3)	2.70 (1.9-3.5)	3.70 (0.9-6)	3.1 (3.0-3.1)	4.47 (3.2-5.6)	1.31 (0.7-1.9)	1.50 (0.4-5.2)
Leucine	4.93 (4.4-5.4)	6.63 (5.3-7.5)	5.11 (4.4-5.8)	6.42 (1.6-10.5)	5.06 (4.2-5.9)	7.53 (5.5-9.2)	2.42 (1.3-3.6)	2.90 (0.7-6.6)
Arginine	3.2 (2.9-3.4)	5.06 (4.2-6.0)	1.6 (1.3-1.9)	3.24 (1.1-4.6)	4.0 (3.9-4.1)	4.87 (3.5-6.1)	1.9 (1.2-2.6)	2.12 (0.5-1.3)
Methionine	1.50 (1.4-1.5)	1.91 (1.3-2.5)	0.69 (0.68-0.7)	0.81 (0.2-1.8)	2 (1.9-2.1)	2.18 (1.6-2.6)	0.91 (0.5-2.3)	1.21 (0.2-1.8)
Phenylalanine	3.24 (2.8-3.6)	4.23 (3.2-5.0)	2.98 (2.2-3.7)	3.56 (0.9-5.2)	3.82	5.42 (4.1-6.2)	1.7 (0.8-2.6)	2.40 (0.2-3.6)
Threonine	3.68 (3.4-3.9)	4.6 (3.6-5.4)	3.09 (1.2-5.1)	3.09 (1.2-5.1)	4.3 (4.2-4.4)	5.17 (3.6-6.7)	1.26 (0.7-2.8)	2.17 (0.5-3.8)
Tryptophan	1.74 (1.72-1.76)	-	-	1.22 (0.5-1.9)	-	-	0.4 (0.2-0.6)	0.51 (0.4-0.6)
Valine	5.38 (4.7-6.0)	4.76 (4.1-5.5)	3.84 (2.9-4.8)	4.65 (1.1-8.0)	4.10 (3.7-4.5)	5.87 (4.3-7.1)	2.25 (1.1-3.4)	2.03 (0.9-4.4)
Tyrosine	1.74 (1.7-1.8)	2.05 (0.9-3.2)	2.35 (1.8-2.9)	2.26 (1.9-2.6)	-	2.80 (2.1-3.5)	1.15 (0.6-1.7)	1.03 (0.5-1.4)
Alanine	6.68 (4.5-8.8)	5.95 (5.4-6.5)	4.23 (3.3-5.1)	1.58 (1.5-1.6)	6.8 (5.0-8.5)	4.81 (4.5-5.0)	2.23 (1.1-3.3)	3.22 (0.7-5.9)
Glutamine	7.3 (4.6-9.9)	14.5 (1.4-11.6)	19.5 (18.9-20.1)	20.1 (19.6-20.3)	10.5 (10.6-10.4)	14.1 (9.7-18.3)	16.6 (11.0-18.7)	13.2 (11.5-14.8)
Asparagine	6 (3.9-8.1)	8.4 (8.3-8.5)	12 (11.5-12.5)	12.8 (10.9-16.7)	9.4 (8.8-9.8)	10.8 (8.3-13.3)	11.3 (9.9-14.3)	10.4 (7.9-12.2)
References	Kolb et al., 2004 Gaillard et al., 2018	Makkar et al., 2016 Lorenzo et al., 2016	Wong et al., 2001	Lorenzo et al., 2017 Catarino et al., 2018	Gaillard et al., 2018 Saravana et al., 2016	Cruz- Suárez et al., 2000 Duis et al., 1995 Cruz- Suárez et al., 2009	Galland-Irmouli et al., 1999 Tibbetts et al., 2016	Makkar et al., 2016 Lorenzo et al., 2017 Ortiz et al., 2006

[1] data are reported as mean values. ‡ value from *Sargassum Patens* and *hemifhyllum*. # values from *Fucus vesciculosus* and *serratus.*

3.5 INFLUENCE ON GROWTH PERFORMANCE

Brown seaweeds have a generally positive effect on growth, as reported in Table 3. Some interactions between seaweeds bioactive molecules and dietary components should be probable, but considering the heterogeneity of seaweeds species, the effects on growth performances have to be firstly analyzed in relation to seaweed supplement, and bioactive molecules content. With dietary integration in sows at the end of gestation and during lactation, an increase in average daily gain (ADG) of suckling piglets has been observed (from +11.8 to +32.3% compared to the control group). Most of the studies we reviewed involve the dietary supplementation of brown seaweeds in weaned piglets. In weaned piglets, improvements of ADG are observed. The ADG of piglets fed brown seaweeds is higher than the ADG of piglets fed a control diet with an increase between +4.6 and +40.8%. Draper et al. (2016) and Ruiz et al. (2018) appear to be the only two authors to report the effects of long-term dietary supplementation with brown seaweed from weaning to slaughter on ADG. In this case, the influence on ADG was limited but statistically significant, and ranged from +1.2 to +3.3%. Bouwhuis et al. (2017a, b) evaluated the effects of brown seaweed supplementation on pig's growth performance after being challenged with *Salmonella Typhimurium*. When the challenge occured in post-weaning, no significant effect was observed; in pigs with a live weight of 30 kg the seaweed supplement led to a significant increase in growth (+ 16%). It is possible that the bioactive compounds of seaweeds are not able to positively modulate the immune system of the

post-weaning piglet which is still immature. Positive effects on growth are related to the improvement of digestibility and overall health conditions of piglets due to the prebiotic effects of seaweed polysaccharides, as described in the following sections. The effects of seaweed dietary supplementation on the improvement in antioxidant status and the decrease in inflammatory condition may contribute to reduce energy and amino acidic expenditure.

Table 3. Effect of seaweed supplement on average daily gain (ADG) in pigs.

Algae Supplement	Dose	Animal	Control	Supplemented	Diff. %	Ref.
A. nodosum	Dried seaweed 2.5-5-10 g/kg	Weaning to 28 d	0.220	0.209	-5.0	Michiels et al., 2012
				0.198	-10.0	
				0.213	-3.18	
A. nodosum	Dried seaweed 10-20 g/Kg	Weaning to 11 d	0.027	0.054	+100	Dierick et al., 2009
				0.040	+48.14	
Brown seaweed	Alginic acid olisaccharides (50-100-200 mg/kg)	Weaning to 14 d	0.216	0.248 (50)	+14.81	Wan et al., 2016
				0.304* (100)	+40.78	
				0.301* (200)	+39.35	
Brown seaweed	Alginates olisaccharides (100 mg/ kg)	Weaning to 21 d	0.441	0.516	+17.01	Wan et al., 2017
Ecklonia cava	FUC = 0.05 – 0.10 – 0.156 g/kg	Weaning to 28 d	0.344	0.347	+0.87	Choi et al., 2017
				0.368*	+6.98	
				0.360*	+4.65	
Laminaria digitata	LAM + FUC (0.314 -0.250 g/kg) – lactose 15 or 25%)	Weaning to 25 d	0.275	0.293 (15% lact.)	+6.55	O'Doherty et al., 2010
			0.287	0.350** (25% lact.)	+21.95	
Laminaria spp.	LAM (1g/day) – sows, 109d until weaning at 20d	20 d lactation Weaning to 26 d Challenge Salmonella Typhimurium at 10 d post weaning	0.340	0.450 **	+32.35	Bouwhuis et al., 2017 a
	LAM (0.3 g/kg) – piglets		0.410	0.370	-16.13	
Laminaria spp.	LAM + FUC (0.18 + 0.34 g/kg)	30.9 kg pigs for 28 d Challenge Salmonella Typhimurium at 10 d	0.620	0.720***	+16.13	Bouwhuis et al., 2017 b
Laminaria spp.	LAM (0.112 g/kg) [y] FUC (0.089 g/kg) [z]	Weaning to 25d	0.281	0.322**	+14.59	Dillon et al., 2010
Laminaria spp.	LAM + FUC (1 g + 0.8 day) – sows LAM + FUC (0.3 + 0.24 g/kg) – piglets	Weaning to 126 d	0.760	0.850 ** (lactation effect)	+11.84	Draper et al., 2016
			0.800	0.810 (weaning effect)	+1.23	
Laminaria spp.	Extract (1-2-4 g/Kg) [x] LAM = 0.11-0.22-0.44 FUC = 0.09-0.18-0.36	Weaning to 21 d	0.249	0.274 *** (1g/Kg)	+10.04	Gahan et al., 2009
				0.313 *** (2 g/Kg)	+25.70	
				0.303 *** (4 g/Kg)	+21.69	
Laminaria spp.	LAM (0.30 g/Kg)	Weaning to 32 d	0.280	0.353 *	+26.07	Heim et al., 2014
Laminaria spp.	LAM+FUC (0.30 + 0.24 g/Kg)	Weaning to 40 d	0.356	0.374	+5.06	McAlpine et al., 2012
Laminaria spp.	LAM (0.3 g/kg)	Weaning to 21 d	0.288	0.319 * LAM 0.3	+10.7	McDonnell et al., 2010
	FUC (0.36 g/kg)			0.302 FUC 0.36	+4.86	
	LAM + FUC (0.3 + 0.36 g/kg)			0.328 LAM + FUC	+13.89	
Laminaria spp.	LAM + FUC (0.30 + 0.24 g/Kg) [k]	Weaning to 21 d	0.235	0.239	+1.70	O'Shea et al., 2014
		21-40 d	0.489	0.523	+6.25	
Laminaria spp.	LAM (0.15-0.30 g/kg) FUC (0.24 g/Kg) LAM + FUC (0.15 + 0.24 and 0.30 + 0.24 g/kg)	Weaning to 35 d	0.340	0.351 FUC 0.24	+3.24	Walsh et al., 2013a
				0.334 LAM 0.15	-1.76	
				0.347 FUC 0.24 LAM 0.15	+2.06	
				0.390 * LAM 300	+14.71	
				0.358 FUC 0.24 LAM 0.3	+5.29	
OceanFeedSwine	Seaweeed extract (5 g/Kg)	21 to 56 d	0.401	0.380	-5.24	Ruiz et al., 2018
		56-160 d	0.798	0.824 *	+3.26	

Means marked whith *, **, *** showed a significant effect of supplement for $p < 0.05$, $p < 0.01$ and $p < 0.001$ respectively;
LAM, laminarin; FUC, fucoidan.
[y] 990 g/kg Laminarin.
[z] 720 g/kg Fucoidan.
[x] 112 g/kg Laminarin, 89 g/kg Fucoidan and 799 g/kg ash.
[k] 455 g/kg Laminarin and 360 g/kg Fucoidan.

3.6 INFLUENCE ON DIGESTIBILITY

Many authors have evaluated the effects of algae supplementation on the digestibility of the diet in pigs, as reported in Table 4. All digestibility trials were conducted in weaned piglets, except for the study by Gardiner et al. (2008) which investigated male pigs 45 kg live weight. The *Ascophyllum nodosum* does not appear to have significant influence on diet digestibility (Gardiner et al., 2008 and Dierick et al., 2009). On the other hand, *Laminaria digitata*, *Laminaria spp*., *Ecklonia cava* and brown seaweed, titrated in alginates, showed positive effects on the digestibility of nitrogen (N), gross energy (GE), fiber (NDF) and ash in various experiments. Significant improvements from +5.1 and +8% in N digestibility are reported. Also for GE, dietary integration with seaweed improved the digestibility with an increase of between +3.3 and +10%. Some authors have also observed that introducing laminarin and fucoidans in the formula increases the digestibility of the fibrous fraction (NDF). The animals fed seaweed showed a higher digestibility of the NDF (+39 to +73%) compared to the control group. Finally, ash digestibility presented values that in the seaweed group were 25.9-82.4% higher than in the control. The improvement in nutrient digestibility is related to the influence of the seaweed constituents, in particular carbohydrates and antioxidants, on microbiota and on the villous architecture with an increase in absorptive capacity and nutrients transporters (Sweeney and O'Doherty, 2016). These effects are also related to the trophic effect on the intestinal mucosal cells of volatile fatty acids production (i.e., butirric acid).

Table 4. Influence of seaweed on digestibility in swine.

Algae Supplement	Dose g/kg	Animal	Effects on digestibility	Treatment *vs*. Control, %	Ref.
A. nodosum	Dried intact (2.5 g/kg)	Male Pigs, 45 kg LW	NS	-	Sweeney and O'Doherty, 2016
A. nodosum	Dried intact (10-20 g/kg)	Weaned piglets, (35 d age)	NS	-	Dierick et al., 2009
Brown seaweed	Alginates olisaccharides (100 mg/kg)	Weaned piglets, 6.2 kg LW	Improved digestibility of N, fat, ash GE	 +6.7% +10.8% +25.9% +4.0%	Wan et al., 2017
Ecklonia cava	Seaweed (0.5-1-1.5 g/kg) [s]	Weaned piglets, 7.8 kg LW	Improved digestibility of GE	+3.3% (1g/kg)	Choi et al., 2017
Laminaria digitata	LAM + FUC (0.314 - 0.250 g/kg)	Weaned piglets, 7.2 kg LW	Improved digestibility of OM, N, NDF GE	 +4.5% +7.3% +73.3% +5.9%	O'Doherty et al., 2010
Laminaria spp.	Extract (1-2-4 g/kg) [x]	Weaned piglets, (24 d age)	NS		Gahan et al., 2009
Laminaria spp.	Seaweed extract LAM (0.112 g/kg) [y] FUC (0.089 g/kg) [z]	Weaned piglets, (24 d age)	Improved digestibility of N GE	 +6.7% +5.2%	Dillon et al.,2010
Laminaria spp.	LAM+FUC (0.30 + 0.24g/kg)	Weaned piglets, (22 d age)	Improved digestibility of DM, N, NDF	 +8.8% +8.9% +57.5%	McAlpine et al., 2012
Laminaria spp.	LAM (0,15-0,30 g/kg) FUC (0,24 g/kg) LAM + FUC (0,15 + 0.24 and 0.30+0.24 g/kg)	Weaned piglets, (24 d age)	Improved digestibility of DM, LAM and LAM+FUC OM, LAM and LAM+FUC N, LAM NDF, LAM and LAM+FUC GE, LAM and LAM+FUC	 +7.0% - +4.5% +5.9% - +3.5% +5.1% 54.5% - 39.7% +7.3% - +4.3%	Walsh et al., 2013a
Laminaria spp.	LAM (0.30 g/kg) FUC (0.24 g/kg LAM + FUC (0.30 + 0.24 g/kg)	Weaned piglets, (24 d age)	Improved digestibility of DM, LAM and LAM + FUC N, LAM Ash, LAM and LAM + FUC GE, LAM and LAM + FUC	 +7.9% -+4.5% +6.6% 58.0% - 42.6% +8.5% - +4.3%	Heim et al., 2014
Laminaria spp.	Extract (0.66 g/kg) [k]	Weaned piglets, (24 d age)	Improved digestibility of OM, N, ash NDF GE	 +8.8% +8.9% +82.4% +57.5% +10.9%	O'Shea et al., 2014

[x] 112 g/kg Laminarin, 89 g/kg Fucoidan and 799 g/kg ash.
[k] 455 g/kg Laminarin and 360 g/kg Fucoidan
[s] 112 g/kg Fucoidan
[z] 720 g/kg Fucoidan
[y] 990 g/kg Laminarin
FUC, Fucoidan;
LAM, laminarin;
LW, live weight;
DM, dry matter;
GE, gross energy;
N, nitrogen;
NDF neutral detergent fibre;
OM, organic matter.

3.7 PREBIOTIC FUNCTION

Seaweeds are rich in carboxylated and sulfated polysaccharides, such as alginates, ulvans and fucoidans which all act as prebiotics with positive effects on gut health. According to FAO (2007) a prebiotic is a 'non-viable food component that confers a health benefit on the host associated with the modulation of microbiota'. The health benefit is associated with the stimulated activity/growth of beneficial bacteria and the higher production of short chain fatty acids (SCFAs) with direct impact on gut health and also an immunomodulatory effect, as reported below. Several papers have analyzed the prebiotic effects of algae (O'Sullivan et al., 2010; Sardari and Norberg Karlsson,2018; Sweeney and O'Doherty, 2016; Evans and Critchley, 2014; Chen et al., 2018). In swine, 24 studies have been published in the last 10 years on the effects of supplementation with brown seaweeds, or their extracts, on gut health: *Ascophyllum nodosum* (Dierick et al., 2009; Michiels et al., 2012; Gardiner et al., 2008), *Ecklonia cava* (Choi et al., 2017), *Laminaria digitata* (O'Doherty et al., 2010; Mukhopadhya et al., 2012, Murphy et al., 2013), *Laminaria hyperborea* (Lynch et al., 2010a,b), *Laminaria digitata* and *Laminaria hyperborea* association (Reilly et al., 2008), *Laminaria* spp. (Bouwhuis et al., 2017a, b; Dillon et al., 2010; Heim et al., 2014 ; McDonnel et al., 2010; Walsh et al., 2013a; Leonard et al., 2012 ; McDonnel et al., 2016). Brown seaweeds titrated in alginic acid polysaccharides have also been studied (Choi et al., 2017). Most of the studies were carried out in weaned piglets (14 trials), considering that weaning phase is a critical period with high incidence of enteric pathologies. Some studies were carried out on growing pigs ranging between 14 and 65 kg LW, and some others on gestating and lactating sows. In general the compounds present in the brown seaweeds (in 20 trials the supplement is titrated in laminarin and/or fucoidans) stimulated the growth of *Lactobacilli* (Wan et al., 2016; O'Doherty et al., 2010; Dillon et al., 2010; O'Shea et al., 2014; Gardiner et al., 2008; Murphy et al., 2013; Reilly et al., 2008) and reduced the enterobacteria population or *Escherichia coli* (Dierick et al., 2009; Bouwhuis et al., 2017a; Wan et al., 2016; , Choi et al., 2017; O'Doherty et al., 2010; McDonnel et al., 2010; Walsh et al., 2013a; Gardiner et al., 2008; Lynch et al., 2010b; Leonard et al., 2012; , Heim et al., 2015a). Brown seaweed supplements supported the growth of *Bifidobacteria* species in the ileum in piglets (Wan et al., 2016; Mukhopadhya et al., 2012; Murphy et al., 2013). Gut health is modulated by laminarin and/or fucoidans, with the microbial production of short-chain fatty acids (SCFAs), in particular butyrate (Murphy et al., 2013; Reilly et al., 2008; Walsh et al., 2013b). Glucose are the main energy source for small intestinal epithelial cells, and SCFAs are the main energy source for caecum and colon cells, stimulating cell growth (Rossi et al., 2010). Several studies have reported that brown seaweed have a positive influence on gut morphology (Choi et al., 2017; Heim et al., 2015a; Heim et al. 2015b; Wan et al., 2018). Supplementation with *Ecklonia cava* (0.05 and 0.15% of dietary inclusion), linearly improved villi height in ileum (Choi et al., 2017). In weaning piglets, maternal dietary supplementation with laminarin and fucoidan (1 and 0.8 g/day) after 83 days of gestation and during lactation increased villi height in the jejunum and ileum (+43 and +88% respectively) (Heim et al., 2015a). According to Heim et al. (2015b) maternal dietary treatment with fucoidans (0.8 g/day) had no influence on the small intestine morphology, while laminarin increased the villus height in the ileum (+13%) at day 8 post-weaning. In vitro and in vivo experiments carried out by Dierick et al. (2009) revealed that native seaweeds *Ascophyllum nodosum* suppresed in vitro the gut flora counts and metabolic activity (production of organic acids), while in vivo, a significant better *lactobacilli/E.coli* ratio was found in the small intestine.

Michiels et al., 2012 on the other hand observed no significant effects on gut health with the use of the same seaweed in weaned piglets, most probably due to the already high digestible basal diet, including lactose. To probiotic activity algae associate bacteriostatic and antibacterial activities recently reviewed by Perez et al, 2016.
In particular potential application in acquaculture (Vatsos et al., 2015) and in the pharmaceutical and food industry (Eom et al., 2012) have been evaluated.

3.8 ANTIBACTERIAL FUNCTION

In addition to the probiotic action that positively modulates the intestinal microbiota, the seaweeds and their extracts show a specific anti-bacterial and / or bacteriostatic action (Kidgell et al., 2019; Pina-Pérez et al., 2017; Shannon and Abu-Ghannam, 2016). Phlorotannins, fatty acids, peptides, terpenes, polysaccharides and sulphated polysaccharides, and several other bioactive compounds have been reported as bacterial inhibitors (Table 5). A very interesting action of seaweed extracts is the effectiveness against methicillin resistant Staphylococcus aureus and vancomycin–resistant Enterococcus faecium (Lane et al., 2009).

Table 5. Antibacterial activity of seaweeds.

Strain	Seaweed	Functional Group	Seaweed	Ref.
Campylobacter jejuni	*Delisea pulchra*	Halogenated furanone	Red	Castillo et al., 2015
Enterococcus faecium vancomycin-resistant	*Callophycus serratus*	Diterpene-benzoate	Red	Lane et al., 2009
Escherichia coli	*Ascophyllum nodosum and Laminaria hyperborea*	Laminarin	Brown	Kadam et al., 2015
	Shaerococcus coronopifolius	Sphaerane bromoditerpenes	Red	Rodrigues et al.. 2015
	Pterocladia capillacea	Water-extracted polysaccharides	Red	Abou Zeid et al., 2014
	Sargassum swartzii	Sulphated polysaccharides	Red	Vijayabaskar et al., 2012
	Delisea pulchra	Halogenated furanone	Red	Ren et al., 2004
Laminaria monocytogenes	*Ascophyllum nodosum and Laminaria hyperborea*	Laminarin	Brown	Kadam et al., 2015
Pseudomonas aeruginosa	*Soliera filiformis*	Lectin	Red	Holanda et al., 2005
	Shaerococcus coronopifolius	Sphaerane bromoditerpenes	Red	Rodrigues et al., 2015
	Delisea pulchra	Halogenated furarone	Red	Brameyer and Heermann, 2015; Hentzer and Givskov, 2003
Propionibacterium	*Eisenia bicyclis*	Phlorofucofuroeckol	Brown	Lee et al., 2014; Lee et al., 2015
Salmonella typhimurium	*Ascophyllum nodosum and Laminaria hyperborea*	Laminarin	Brown	Kadam et al., 2015
Staphylococcus aureus	*Eisenia bicyclis*	Phlorofucofuroeckol	Brown	Eom et al., 2014
	Ascophyllum nodosum and Laminaria hyperborea	Laminarin	Brown	Kadam et al., 2015
	Pterocladia capillacea	Water-extracted polysaccharides	Red	Abou Zeid et al., 2014
Staphylococcus aureus methicillin resistant	*Saccharina longicruris*	Extracted peptides (>10 kDa)	Brown	Beaulieu et al, 2015
	Shaerococcus coronopifolius	Sphaerane bromoditerpenes	Red	Rodrigues et al., 2015
	Callophycus serratus	Diterpenes-benzoate	Red	Lane et al., 2009

Antibacterial activity is expressed across multiple mechanisms: inhibition of oxidative phosphorylation and link with compounds in the bacterial cell wall and increased permeability of the cytoplasmic membrane causing cell lysis (Shannon et al., 2016). At the same time some seaweed compounds, in particular polysaccharides, contribute significantly to the health and well-being of the animals by enhancing the in vivo immune response. The variability of bioactive compounds in seaweeds influences the lab techniques used to obtain antimicrobials ranging from traditional extraction techniques, solid–liquid extraction or liquid–liquid extraction, to the most modern process of supercritical fluid extraction using CO2 ultrasonically-assisted extraction (Pérez et al., 2016). The potential applications in aquaculture (Vatsos and Rebours, 2015) and in the pharmaceutical and food industry (Eom et al., 2012) have been evaluated, while studies on food producing animals, and in pigs in particular, are limited. Berri et al., 2016 evaluated the effect of marine-sulfated polysaccharide extract from the green macroalga Ulva armoricana against seven bacterial strains found in pigs (Table 6).

Table 6. Minimum inhibitory concentration (MIC) of marine-sulfated polysaccharides extract from *Ulva armoricana* (Berri et al., 2016).

Strain	MIC (mg/mL)
Pasteurella multocida	1.56
Pasteurella multocida subsp. *multocida*	3.125
Streptococcus suis	6.25
Trueperella pyogenes	50
Bordetella bronchiseptica	50
Escherichia coli K85	>50
Escherichia coli K88(F4)	>50

3.9 INFLUENCE ON ANTIOXIDANT FUNCTION

Seaweeds have antioxidant properties due to the presence of phenols, carotenoid fucoxanthin, tannins and phlorotannins, polysaccharides (fucoidans, laminarans in brown seaweeds, ulvans in green seaweeds and carrageenans, porphyrin and agar in red seaweeds) (Jacobsen et al., 2019). The highest concentrations of phenols and phlorotannins have been observed in brown seaweed, up to 12-14% DM in *Ascophhyllum nodosum*, *Fucus* spp. and *Sargassum* spp. (Holdt and Kraan, 2011). In green and red seaweeds concentrations lower than 1% have been reported (Holdt and Kraan, 2011; Farvin and Jacobsen, 2013). The carotenoid fucoxanthin has only been detected in brown seaweeds with concentrations of up to 5,000 mg/kg (Ramus et al., 1977; Narayani et al., 2016). Tocopherols are present in all seaweed, with variables concentrations. In brown seaweed higher values have been reported for *Fucus* spp. and *Ascophyllum nodosum* (up to 600 mg/kg DM), (Jensen et al., 1969a, b) than in *Laminaria* spp. (Wen et al., 2006). Lower concentrations were observed in red seaweeds (Wen et al., 2006; Naseri et al., 2019), and green seaweeds showed up to 1070 mg/kg DM in *Ulva* spp.) (Ortiz et al., 2006). The effects of dietary seaweeds are reported on serum and plasma antioxidant status, duodenum, jejunum and ileum antioxidant markers and *Longissimus dorsi* muscle oxidative stability (Table 7).

At the blood level, dietary supplementation with *Laminaria* spp. extract (laminarin 0.18 g/kg and fucoidans 0.33 g/kg) or of brown seaweed (alginates 100 mg/kg) has a strong antioxidant effect in growing pigs (Rajauria et al., 2016) and weaned piglets (Wan et al., 2016, 2017). The total

antioxidant capacity (TAS), superoxide dismutase (SOD), glutathione (GSH) and catalase activities increased from +14 to +37% with respect to the control group.

At the same time, there was a reduction in lipid oxidation with lower values of malondialdehyde (MDA) ranging from 10% to 26% than in the controls. Other authors reported the non-significant effects on serum MDA, using dietary *Ascophyllum nodosum* (Michiels et al., 2012) and brown seaweeds (Wan et al., 2017). Wan et al. (2018) observed a significant reduction in the MDA concentration in duodenum, jejunum and ileum of between -35 and -40% and an increase in catalase activity. Finally, some studies have evaluated the oxidative stability of pork during storage time by evaluating thiobarbituric acid-reactive substances (TBARS). The reduction in TBARS concentration for long refrigerated storage times of 14 d, ranged between -21% and -60%. The administration of an extract of *Laminaria digitata* in liquid form instead of spray-dried appears to affect the antioxidant potential of the extract. The liquid form better exploits the antioxidant potential of the extract, with reductions in TBARS production of -47% compared to -29% of spray-dried form (Moroney et al., 2012).

Table 7. Effects of seaweed on antioxidant capacity in pigs.

Algae Supplement	Dose g/Kg or g/day	Animal	Antioxidant effects	Treatment vs. Control, %	Ref.
A. nodosum	Dried seaweed 5-10 g/kg	Weaned piglets, 6.59 kg LW	Plasma TBARS [a], FRAP [b], α-tocopherol	NS	Michiels et al., 2012
Brown seaweed	Alginic acid olisaccharides (100 mg/kg)	Weaned piglets, 7.8 kg LW	Serum T-AOC [e] SOD [f] CAT [g] MDA [h]	 +14% +20% +37% -26%	Wan et al., 2016
Brown seaweed	Alginates olisaccharides (100 mg/kg)	Weaned piglets, 6.2 kg LW	Serum T-AOC CAT GSH[i] MDA	 +21% +28% +28% -10% NS	Wan et al., 2017
Brown seaweed	Alginates olisaccharides (100 mg/kg)	Weaned piglets, 6.2 kg LW	Duodenum T-AOC MDA Jejunum T-AOC CAT MDA Ileum T-AOC CAT MDA	 +45% -40% +39% +22% -36% +58% +72% -35%	Wan et al., 2018
Laminaria digitata	Wet (W) or spray dried (SD) seaweed LAM + FUC (0.5+0.4 g/kg)	Pigs, 14.5 kg LW	Plasma TAS [c] LD muscle TBARS (refrig. storage 14 d)	NS -29% SD -47% W	Moroney et al., 2012
Laminaria digitata	LAM + FUC (0.45 or 0.9 g/kg) 3 or 6 weeks pre slaughter	Pigs, 82 kg LW	Plasma TAS LD muscle (refrig. storage 14d) TBARS 0.45 for 3 weeks 0.90 for 3 weeks	NS -57% -60%	Moroney et al., 2015
Laminaria spp.	LAM + FUC (0.18+0.33 g/kg)	Pigs, 71 kg LW	Serum DPPH [d] LD muscle TBARS (refrig. storage 14 d)	 +400% -41%	Rajauria et al., 2016

LAM = Laminarin, FUC = Fucoidan

[a] TBARS, thiobarbituric acid-reactive substances; [b] FRAP, ferric reducing ability of plasma; [c] TAS, total antioxidant status; [d] DPPH, 2,2-diphenyl-1-picrylhydrazyl assay; [e] T-AOC, total antioxidant capacity; [f] SOD, superoxide dismutase; [g] CAT, catalase; [h] MDA, malondialdehyde; [i] GSH, glutathione.

3.10 ANTI-INFLAMMATORY FUNCTION

Many studies have evaluated the anti-inflammatory activity of the brown seaweeds, in particular *Laminaria digitata, Laminaria hyperborea* and *Laminaria* spp., usually titrated in laminarin and fucoidans (Bouwhuis et al., 2017a,b; Wan et al., 2016; Walsh et al., 2013a; Mukhopadhya et al., 2012; Leonard et al., 2012; Heim et al., 2015a,b; McDonnel et al., 2016;Wan et al., 2018). In addition, non-specific brown seaweeds titrated in alginic acid have been evaluated by Wan et al., (2016, 2018). Of these authors, 8/10 showed anti-inflammatory effects. Walsh et al. (2013a) reported a lower expression of pro-inflammatory cytokines in the colon of piglets after dietary supplementation with laminarin, but not with fucoidans. Dietary treatment of gestation and lactating sows with laminarin (1g/d) and fucoidans (0.8 g/d) reduced the ileal gene expression of IL-6, IL-8, IL-10, of piglets at weaning (Heim et al., 2015a). Similarly, in piglets born from sows fed diets supplemented with laminarin from 109 d gestation and during lactation, a reduction on the colon IL-6 concentration at weaning and of ileal IL-8 concentration eight days post weaning were observed (Heim et al., 2015b).

In a 28-days trial, Bouwhuis et al. (2017b) observed the effects of a diet supplemented with laminarin 0.18 g/kg and fucoidans 0.34 g/kg, in 30 kg LW female pigs. After 11-day adaptation period, pigs were orally challenged with *Salmonella Typhimurium*. Dietary treatment reduced colon cytokine expression (IL-6, IL-18, IL-22 and TNF-α) 17 days post challenge, thus revealing an anti-inflammatory effect. In addition, in 18 kg LW pigs, laminarin from *Laminaria digitata* dietary supplementation (0.6 g/kg) significantly increased gut mucins gene expression (MUC2 and MUC4) from 20% to 33% with a protective effect on epithelial cells (Smith et al, 2011). McDonnel et al. (2016) observed an increase of 16% in mucin gene expression (MUC2) in the ileal in female pigs fed diets supplemented with *Laminaria* spp. (0.18 g of laminarin and 0.34 g of fucoidan per kg of feed). A study using an in vitro system of porcine intestinal epithelial cells showed that ulvans from *Ulva armoricana*, a green seaweed, upregulated the gene expression of of cytokines such as IL1α, IL1β, L6, IL8, TNFα (Berri et al., 2016, 2017).

3.11 IMMUNOMODULATORY FUNCTION

Many studies have evaluated the immunomodulatory activity of seaweeds (Wan et al., 2016; Choi et al., 2017; Leonard et al., 2012; Katayama et al., 2011; Bussy et al., 2019). In addition to an anti-inflammatory action, laminarin also has an immunomodulatory function. Leonard et al. (2012) reported that the dietary supplementation of sows from 109 days of gestation until weaning with *Laminaria* spp. extract (1 g laminarin and 0.8 g fucoidans / day) increased immunoglobulin G (IgG) and immunoglobulin A (IgA) in sow colostrum by 19% to 25%, respectively. Consequently, an increase of piglet serum IgG was observed (10%).

In another study, the effects were evaluated of sow supplementation with 30 g/day of an extract of *Ascophyllum nodosum* and *Fucus* from the 85th day of gestation until weaning on liver and lymphoid organs of piglets. The relative population of CD4+CD8+ T cells was higher in piglets from treated sows in the thymus, spleen, mesenteric node, liver and in peripheral blood, thus suggesting an important effect of maternal diet on immune status of 40-day-old piglets (Azizi et al., 2018). The immunomodulatory effect of green seaweed extract (*Ulva armoricana*) was evaluated in sows by Bussy et al. (2019). Different levels of inclusion were tested: 2, 8 and 16 g/day during two periods of three days: 34 days before farrowing, before the last vaccine booster against *Bordetella bronchiseptica* and one week before farrowing. The higher dietary level increased *anti-Bordetella* IgG in sow's blood and colostrum, while with the middle dietary integration, the authors observed an increase in milk IgA. Wan et al. (2016) and Choi et al. (2017) evaluated the immunomodulatory effects of seaweed fed to weaned piglets. Alginic acid oligosaccharides from brown seaweed increased IgG and IgA concentrations in piglet serum by 20 and 53 % respectively after 21 days of treatment (Wan et al., 2016). No immunomodulatory effect was observed in weaned piglets fed a diet supplemented with different *Ecklonia cava* concentrations (Choi et al., 2017). According to the authors, the result may be consequence of the low dosage and consequently the low content of fucoidan in the diets (0.056 – 0.112 – 0.168 g/kg respectively). In growing pigs (29 kg LW), 0.8% seaweed enhanced the immune function. Pigs were sensitized with the subcutaneous inoculum of sheep red blood cells at days 42 and 49. Seaweed increased the saliva IgA production five times more than the control after 56 days (Katayama et al., 2011). The concentration of antigen-specific IgG in peripheral blood was higher in seaweed group, but not significantly due to the high standard deviation.

3.12 POTENTIAL TOXICITY

The use of seaweed in swine nutrition may have the following limitations: mineral elements and potentially toxic minerals content. Generally, seaweeds are introduced in pigs diet as ingredients/raw materials in a low percentage, thus there is no risk of potential toxicity in this case. In terms of microelements, the first limiting factor is the iodine content which, as reported in Table 1, can reach particularly high concentrations in brown seaweeds. According to NRC (2012), tolerance levels in growing pigs and sows are 400 and 1500-2500 mg/kg DM for iodine from iodine salt, respectively. Of the potentially toxic minerals, the first limiting element is arsenic (As) which is found in high concentrations in brown seaweed (Table 8). However, a low content of the most toxic form of arsenic, inorganic arsenic, has been observed in seaweeds. The arsenic content of green seaweed is below the maximum level of 40 mg/kg feed (12% moisture content) sets by European feed legislation (Commission Directive 2002/32/EC and amendments).

Table 8. Potentially toxic trace element concentrations in seaweed (mg/kg DM).

Trace Element	Brown seaweed[1]	Green seaweed[2]	Red seaweed[3]	Feed[4]	Feed ingredient[4]	Ref.
Cadmium	0.05-8	0.03-4	0.04-3.8	0.5 (1*)	1	Cabrita et al., 2016 Circuncisão et al.,2018, Maehre et al.,2014; Duinker et al., 2016
Mercury	<0.005-0.16	0.005-0.07	<0.005-0.03	0.1 (0.2*)	0.1	Cabrita et al., 2016., Circuncisão et al.,2018, Maehre et al.,2014; Duinker et al., 2016
Lead	0.01-7	0.05-7	0.01-19	5	10	Cabrita et al., 2016 Circuncisão et al.,2018, Duinker et al., 2016
Arsenic	8-120	0.8-18	1-50	2 (10*)	40	Cabrita et al., 2016., Circuncisão et al.,2018, Maehre et al.,2014 Duinker et al., 2016
Inorganic arsenic	0.03-7.7	0.2-0.4	0.03-0.6	-	-	Circuncisão et al.,2018, Duinker et al., 2016

[1]Brown seaweed: *Alaria esculenta, Ascophyllum nodosum, Fucus spiralis, Fucus vesicolosus, Himanthalia elongate, Laminaria digitata, Lamibaria hyperborea, Laminaria* spp., *Pelvetia canaliculata, Saccharina latissima Sargassum fusiformis, Undaria pinnatififda*

[2]Green seaweed: *Cladophora rupestris, Codium adhaerens, Codium vermilara, Enteromorpha intestinalis, Ulva lactuca, Ulva* spp.

[3]Red seaweed: *Chondrus crispus, Gigartina* spp., *Gracilaria vericulophylla, Gracilaria* spp., *Palmaria palmata, Polysiphonia lanosa, Porphyra* spp.

[4]UE directive 2002/32/EC and amendments

* Fish feed

3.13 CONCLUSIONS

The biological activities of brown seaweeds could be used to improve health and welfare of pigs. The prebiotic effects and the antimicrobial activities of laminarin and fucoidans may have beneficial effects in the prevention of gastrointestinal diseases and to enhance diet digestibility in the post-weaning piglets. Laminarin also has an anti-inflammatory activity, which reduce the pro-inflammatory cytokine response. The seaweed content of antioxidant molecules enhances the antioxidant status and meat oxidative stability. Dietary supplementation with brown seaweed may positively affect the immune system, enhancing immunoglobulin production and modulating cytokine production. In conclusion, brown seaweeds seem to be a promising dietary intervention in pigs in order to enhance immune system, antioxidant status and gut health. Data on the dietary supplementation with green seaweeds in pigs seem to be lacking at present and merit further investigations.

3.14 REFERENCES

Abou Zeid, A.H.; Aboutabl, E.A.; Sleem, A.A.; El-Rafie, H.M. Water soluble polysaccharides extracted from Pterocladia capillacea and Dictyopteris membranacea and their biological activities. *Carbohydr. Polym.* **2014**, 113, 62–66.

Anderson, M.J., Blanton, J.R., Gleghorn, J., Kim, S.W., Johnson, J.W. *Ascophyllum nodosum* supplementation strategies that improve overall carcass merit of implanted English crossbred cattle. *Asian-Aust. J. Anim. Sci*. **2006**, 19, 1514–1518.

Angell, A.R.; Angell, S.F.; DeNys, R.; Paul, N.A., Seaweed as a protein source for mono-gastric livestock. *Trends Food Sci. Technol.* **2016**, 54, 74–84.

Azizi, A.F.N.; Miyazaki, R.; Yumito, T.; Ohashi, Y.; Uno, S.; Miyajima, U.; Kumamoto, M.; Uchiyama, S.; Yasuda, M. Effect of maternal supplementation with seaweed powder on immune status of liver and lymphoid organs of piglets. *J. Vet. Med. Sci.* **2018,** 80, 8–12.

Balboa, E.M.; Conde, E.; Moure, A.; Falqué, E.; Domínguez, H. *In vitro* antioxidant properties of crude extracts and compounds from brown algae. *Food Chem.* **2013**, 138, 1764–1785.

Beaulieu, L.; Bondu, S.; Doiron, K.; Rioux, L.-E.; Turgeon, S.L. Characterization of antibacterial activity from protein hydrolysates of the macroalga *Saccharina longicruris* and identification of peptides implied in bioactivity. *J. Funct. Foods* **2015**, 17, 685–697.

Berri, M.; Olivier, S.C.; Olivier, M.; Helloin, E.; Jacques, I.; Salmon, H.; Demais, H.; Le Goff, M.; Nival Collen, P. Marine–sulfated polysaccharides extract of *Ulva armoricana* green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells. *J. Appl. Phycol.* **2016**, 28, 2999–3008.

Berri, M., Olivier, M., Holbert, S., Dupont, J., Demais, H., Le Goff, M., Collen, P.N. Ulvan from *Ulva armoricana* (Chlorophyta) activates the PI3K/Akt signalling pathway via TLR4 to induce intestinal cytokine production. *Algal Res.* **2017**, 28, 39–47.

Biancarosa, I.; Espe, M.; Bruckner, C.G.; Heesch, S.; Liland, N.; Waagbø, R.; Torstensen, B.; Lock, E.J. Amino acid composition, protein content, and nitrogen-toprotein conversion factors of 21 seaweed species from Norwegian waters. *Appl. Phycol.* **2017**, 29, 1001–1009.

Bouwhuis M.A., Sweeney T., Mukhopadhya A., Thornton K., McAlpine P.O., O'Doherty, J.V. Zinc methionine and laminarin have growth-enhancing properties in newly weaned pigs influencing both intestinal health and diarrhoea occurrence. *J. Anim. Physiol. Anim. Nutr*. **2016**, 101, 1273–1285.

Bouwhuis, M.A.; Sweeney, T.; Mukhopadhya, A.; McDonnell, M.J.; O'Doherty, J.V. Maternal laminarin supplementation decreases *Salmonella Typhimurium* shedding and improves intestinal health in piglets following an experimental challenge with *Salmonella Typhimurium* post-weaning. *Anim. Feed Sci. Technol.* **2017a**, 223, 156–168.

Bouwhuis, M.A.; McDonnell, M.J.; Sweeney, T.; Mukhopadhya, A.; O'Shea, C. J.; O'Doherty, J.V., Seaweed extracts and galacto-oligosaccharides improve intestinal health in pigs following *Salmonella Typhimurium* challenge. *Animal.* **2017b**, 11, 1488–1496.

Brameyer, S.; Heermann, R. Specificity of signal–binding via non–AHL LuxR–type receptors. *PLoS ONE* **2015**, 10, e0124093.

Burtin, P. Nutritional Value of Seaweeds. *Electron. J. Environ. Agric. Food Chem*. **2003**, 2, 498–503.

Bussy, F.; Le Goff, M.; Salmon, H.; Delaval, J.; Berri, M.; Pi, N.C. Immunomodulating effect of a seaweed extract from *Ulva armoricana* in pig: Specific IgG and total IgA in colostrum, milk, and blood. *Vet. Anim. Sci*. **2019**, 7, 10005.

Cabrita, A.R.J.; Maia, M.R.G.; Oliveira, H.M.; Sousa-Pinto, I.; Almeida, A.A.; Pinto, E.; Fonseca, A.J.M. Tracing seaweeds as mineral sources for farm-animals. *J. Appl. Phycol*. **2016**, 28, 3135–3150.

Castillo, S.; Heredia, N.; García, S. 2 (5H)–Furanone, epigallocatechin gallate, and a citric–based disinfectant disturb quorum–sensing activity and reduce motility and biofilm formation of Campylobacter jejuni. *Folia Microbiol*. **2015**, 60, 89–95.

Catarino, M.D.; Silva, A.M.S.; Cardoso, S.M. 2018. Phycochemical Constituents and Biological Activities of *Fucus* spp. *Mar. Drugs* **2018**, 16, 249.

Chandini, S.K.; Ganesan, P.; Bhaskar, N. *In vitro* antioxidant activities of three selected brown seaweeds of India. *Food Chem.* **2008**, 107, 707–713.

Chen, X.; Sun, Y.; Hu, L.; Yu, H.; Xing, R.; Li, R.; Wang, X.; Li, P. In vitro prebiotic effects of seaweed polysaccharides. *J. Ocean. Limnol*. **2018**, 36, 926–932.

Cherry, P.; Yadav, S.; Strain, C.R.; Allsopp, P.J.; McSorley, E.M.; Ross, R.P.; Stanton, C. Prebiotics from Seaweeds: An Ocean of Opportunity? *Mar. Drugs* **2019**, 17, 327.

Choi, Y.; Hosseindoust, A.; Goel, A.; Lee, S.; Jha, P. K.; Kwon, I. K.; Chae, B. J. Effects of *Ecklonia cava* as fucoidan-rich algae on growth performance, nutrient digestibility, intestinal morphology and caecal microflora in weanling pigs. *Asian-Australas J.Anim. Sci*. **2017**, 30, 64–70.

Cian, R.E.; Drago, S.R.; Sánchez de Medina F.; Martínez-Augustin, O. Proteins and carbohydrates from red seaweeds: Evidence for beneficial effects on gut function and microbiota. *Mar Drugs* **2015**, 13, 5358–5383.

Circuncisão, A.R.; Catarino, M.D.; Cardoso, S.M.; Silva, A.M.S., Minerals from macroalgae origin: Health benefits and risks for consumers. *Mar. Drugs* **2018**, 16, 11.

Cruz-Suárez, L.E.; Ricque-Marie, D.; Tapia-Salazar, M.; Guajardo-Barbosa, C. Uso de harina de kelp (*Macrocystis pyrifera*) en alimentos para camarón. In *Avances en Nutrición Acuícola, V. Memorias del Quinto Simposium Internacional de Nutrición Acuícola*; Cruz-Suárez L.E.; Ricque-Marie D., Tapia-Salazar M., Olvera-Novoa M.A., Cerecedo-Olvera R., 9-22 Noviembre. Universidad Autónoma de Nuevo León. Monterrey, Nuevo León, México. **2000**; pp. 227–266.

Cruz-Suarez, L.E.; Tapia-Salazar, M.; Nieto-López, M.G.; Guajardo, C. Comparison of *Ulva clathrata* and the kelps *Macrocystis pyrifera* and *Ascophyllum nodosum* as ingredients in shrimp feeds *Aquac. Nutr.* **2009**, 15, 421–430.

Dierick, N.; Ovyn, A.; De Smet, S. Effect of feeding intact brown seaweed *Ascophyllum nodosum* on some digestive parameters and on iodine content in edible tissues in pigs. *J. Sci. Food Agr.* **2009**, 89, 584–594.

Dillon, S.; Sweeney, T.; Figat, S.; Callan, J.J.; O'Doherty, J.V. The effects of lactose inclusion and seaweed extract on performance, nutrient digestibility and microbial populations in newly weaned piglets. *Livest. Sci*. **2010**, 134, 205–207.

Dominguez, H. *Functional Ingredients from Algae for Foods and Nutraceuticals*; Woodhead Publishing Limited: Cambridge, UK, **2013**, pp. 228–229.

Draper, J.; Walsh, A.M.; McDonnell, M.; O'Doherty, J.V. Maternally offered seaweed extracts improves the performance and health status of the post weaned pig. *J. Anim. Sci.* **2016**, 94, 391–394.

Duinker, A; Roiha, I.S.; Amlund, H.; Dahl, L.; Lock, E-J.; Kögel, T.; Måge, A.; Lunestad B.T. *Potential risks posed by macroalgae for application as feed and food - a Norwegian perspective*. National Institute of Nutrition and Seafood Research (NIFES): Trondheim, Norway, **2016**; p. 23.

Duis, K.; Inglis, V.; Beveridge, M.C.M.; Hammer, C. Leaching of four different antibacterials from oil- and alginate-coated fish-feed pellets. *Aquac. Res.* **1995**, 26, 549 –556.

Eom, S.H.; Kim, Y.M.; Kim, S.K. Antimicrobial effect of phlorotannins from marine brown algae. *Food Chem. Toxicol*. **2012**, 50, 3251–3255.

Eom, S.-H.; Lee, D.-S.; Jung, Y.-J.; Park, J.-H.; Choi, J.-I.; Yim, M.-J.; Jeon, J.-M.; Kim, H.-W.; Son, K.-T.; Je, J.-Y.; et al. The mechanism of antibacterial activity of phlorofucofuroeckol–A against methicillin–resistant *Staphylococcus aureus*. *Appl. Microbiol. Biotechnol*. **2014**, 98, 9795–9804.

Evans, F.D.; Critchley, A.T. Seaweeds for animal production use. *J. Appl. Phycol*. **2014**, 26, 891–899.

Farvin, K.H.S.; Jacobsen, C. Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. *Food Chem*. **2013**, 138, 1670–1681.

Fleurence, J. Seaweed proteins: Biochemical, nutritional aspects and potential uses. *Trends Food Sci. Technol*. **1999**, 10, 25–28.

Food and Agriculture Organization of the United Nations (FAO). *The State of World Fisheries and Aquaculture-2008* (SOFIA); FAO: Rome, Italy, **2009**.

Food and Agriculture Organization of the United Nations (FAO). *The Global State of Seaweed Production, Trade and Utilizatio*n. Globefish Research Program: Rome, Italy, **2018**; p.2.

Gahan, D.A.; Lynch, M.B.; Callan, J.J.; O'Sullivan, J.T.; O'Doherty, J.V. Performance of weanling piglets offered low-, medium- or high-lactose diets supplemented with a seaweed extract from *Laminaria* spp. *Animal* **2009**, 3, 24–31.

Gaillard, C.; Bhatti, H.S.; Novoa-Garrido, M.; Lind, V.; Roleda, M.Y.; Weisbjerg, M.R. Amino acid profiles of nine seaweed species and their in situ degradability in dairy cows. *Anim. Feed Sci. Techn.* **2018**, 241, 210–222.

Galland-Irmouli, A.V.; Fleurence, J.; Lamghari, R.; Lucon, M. ; Rouxel, C.; Barbaroux, O.; Bronowicki, J.P.; Villaume, C.; Gueant, J.L. Nutritional value of proteins from edible seaweed *Palmaria palmata* (Dulse). *J. Nutr. Biochem.* **1999**, 10, 353–359.

Ganesan, A.R.; Tiwari, U.; Rajauria, G. Seaweed nutraceuticals and their therapeutic role in disease prevention. *Food Sci.Hum. Wellness*, **2019**,8, 252–263.

García, J.L.; De Vicente, M.; Galán, B. Microalgae, old sustainable food and fashion nutraceuticals. *Microb. Biotechnol.* **2017**, 10, 1017–1024.

Gardiner, G.E.; Campbell, A.J.; O'Doherty, J.V.; Pierce, E.; Lynch, P.B.; Leonard, F.C.; Stanton, C.; Ross, R.P.; Lawlor, P.G. Effect of *Ascophyllum nodosum* extract on growth performance, digestibility, carcass characteristics and selected intestinal microflora populations of grower-finisher pigs. *Anim. Feed Sci. Techol*. **2008**, 141, 259–273.

Guedes, C.; Barbosa, A.; Amaro, C.R.; Pereira, H.M.; Malcata, X. Microalgal and cyanobacterial cell extracts for use as natural antibacterial additives against food pathogens. *Int. J. of Food Sci. Tech.* **2011**, 46, 862–870.

Hagen Rødde, R.; Vårum, K.M.; Larsen, B.A.; Myklestad, S.M. Seasonal and geographical variation in the chemical composition of the red alga *Palmaria palmata* (L.) Kuntze. *Bot. Mar.* **2004**, 47, 125–133.

Heim, G.; O'Doherty, J.V.; O'Shea, C.J.; Doyle, D.N.; Egan, A.M.; Thornton, K.; Sweeney, T. Maternal supplementation of seaweed-derived polysaccharides improves intestinal health and immune status of suckling piglets. *J. Nutr. Sci*. **2015a**, 4, e27.

Heim, G., Sweeney, T., O'shea, C.J., Doyle, D.N., O'doherty, J.V. Effect of maternal dietary supplementation of laminarin and fucoidan, independently or in combination, on pig growth performance and aspects of intestinal health. *Anim. Feed Sci. Technol*. **2015b**, 204, 28–41.

Heim, G.; Sweeney, T.; O'Shea, C.J.; Doyle, D.N.; O'Doherty, J.V., Effect of maternal supplementation with seaweed extracts on growth performance and aspects of gastrointestinal health of newly weaned piglets after challenge with enterotoxigenic *Escherichia coli* K88. *Br. J. Nutr*. **2014**, 112, 1955–1965.

Hentzer, M.; Givskov, M. Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. *J. Clin. Investig*. **2003**, 112, 1300–1307.

Holanda, M.L.; Melo, V.M.M.; Silva, L.M.C.M.; Amorim, R.C.N.; Pereira, M.G.; Benevides, N.M.B. Differential activity of a lectin from *Solieria filiformis* against human pathogenic bacteria. *Braz. J. Med. Biol. Res*. **2005**, 38, 1769–1773.

Holdt, S.L.; Kraan, S. Bioactive compounds in seaweed: functional food applications and legislation. *J. Appl. Phycol*. **2011**, 23, 543–549.

Jacobsen, C.; Sørensen, A.D.M.; Holdt S.L.; Akoh, C.C.; Hermund, D.B. Source, Extraction, Characterization, and Applications of Novel Antioxidants from Seaweed. *Annu. Rev. Food Sci. Technol.* **2019**, 10, 541–568.

Jensen, A. Tocopherol content of seaweed and seaweed meal: II. Individual, diurnal and seasonal variations in some Fucaceae. *J. Sci. Food Agric*. **1969a**, 20, 454–458.

Jensen, A. Tocopherol content of seaweed and seaweed meal. 3. Influence of processing and storage on content of tocopherols, carotenoids and ascorbic acid in seaweed meal. *J. Sci. Food Agric*. **1969b**, 20, 622–626.

Kadam, S.U.; O'Donnell, C.P.; Rai, D.K.; Hossain, M.B.; Burgess, C.M.; Walsh, D.; Tiwari, B.K. Laminarin from Irish brown seaweeds Ascophyllum nodosum and Laminaria hyperborea: Ultrasound assisted extraction, characterization and bioactivity. *Mar. Drugs* **2015**, 13, 4270–4280.

Katayama, M.; Fukuda, T.; Okamura, T.; Suzuki, E.; Tamura, K.; Shimizu, Y.; Suda, Y.; Suzuki, K. Effect of dietary addition of seaweed and licorice on the immune performance of pigs. *Anim. Sci. J.* **2011**, 82, 274–281.

Kidgell, J.T.; Magnusson, M.; de Nys, R.; Glasson, C.R.K. Ulvan: A systematic review of extraction, composition and function. *Algal Res.* **2019**, 39, 101422.

Koizumi, J.; Takatani, N.; Kobayashi, N.; Mikami, K.; Miyashita, K.; Yamano Y.; Wada, A.; Maoka, T.; Hosokawa, M. Carotenoid Profiling of a Red Seaweed Pyropia yezoensis: Insights into Biosynthetic Pathways in the Order Bangiales. *Mar. Drugs*. **2018**, 16, 426.

Kolb, N.; Vallorani, L.; Milanovic, N.; Stocchi, V. Evaluation of Marine Algae Wakame (Undaria pinnatifida) and Kombu (*Laminaria digitata japonica*) as Food Supplements. *Food Technol. Biotechnol*. **2004**, 42, 57–61.

Lane, A.L.; Stout, E.P.; Lin, A.-S.; Prudhomme, J.; le Roch, K.; Fairchild, C.R.; Franzblau, S.G.; Hay, M.E.; Aalbersberg, W.; Kubanek, J. Antimalarial bromophycolides J–Q from the Fijian red alga Callophycus serratus. *J. Org. Chem*. **2009**, 74, 2736–2742.

Lee, J.B.; Takeshita, A.; Hayashi, K.; Hayashi, T. Structures and antiviral activities of polysaccharides from *Sargassum trichophyllum*. *Carbohyd. Polym*. **2011**, 86, 995–999.

Lee, J.-H.; Eom, S.-H.; Lee, E.-H.; Jung, Y.-J.; Kim, H.-J.; Jo, M.-R.; Son, K.-T.; Lee, H.-J.; Kim, J.H.; Lee, M.-S. In vitro antibacterial and synergistic effect of phlorotannins isolated from edible brown seaweed *Eisenia bicyclis* against acne–related bacteria. *Algae* **2014**, 29, 47–55.

Lee, S.H.; Kim, S.K. Biological phlorotannins of *Eisenia bicyclis*. In *Marine Algae Extracts: Processes, Products, and Applications*; Kim, S.-K., Chojnacka, K., Eds.; Wiley: Oxford, UK, **2015**; pp. 453–464.

Leonard, S.G.; Sweeney, T.; Bahar, B; O'Doherty, J.V. Effect of maternal seaweed extract supplementation on suckling piglet growth, humoral immunity, selected microflora, and immune response after an ex vivo lipopolysaccharide challenge. *J. Anim. Sci.* **2012**, 90, 505–514.

Lorenzo, J.M.; Agregán, R.; Munekata, P.E.S.; Franco, D.; Carballo, J.; Sahin, S.; Lacomba, R.; Barba, F.J. Proximate Composition and Nutritional Value of Three Macroalgae: *Ascophyllum nodosum*, *Fucus vesiculosus* and *Bifurcaria bifurcate*. *Mar. Drugs*. **2017**, 15, 360.

Lynch, M.B.; Sweeney, T.; Callan, J.J.; O'Sullivan, J.T.; O'Doherty, J.V. The effect of dietary *Laminaria* derived laminarin and fucoidan on intestinal microflora and volatile fatty acid concentration in pigs. *Livest. Sci*. **2010a,** 133, 157–160.

Lynch, M.B.; Sweeney, T.; Callan, J.J.; O'Sullivan, J.T.; O'Doherty, J.V. The effect of dietary *Laminaria* derived laminarin and fucoidan on nutrient digestibility, nitrogen utilisation, intestinal microflora and volatile fatty acid concentration in pigs. *J. Sci. Food Agric.* **2010b,** *90*, 430–437.

Mæhre, H.K.; Malde, M.K.; Eilertsen, K.E.; Elvevoll, E.O. Characterization of protein, lipid and mineral contents in common Norwegian seaweeds and evaluation of their potential as food and feed. *J. Sci. Food Agric.* **2014**, 94, 3281–3290.

Maghin, F.; Ratti S.; Corino, C. Biological functions and health promoting effects of brown seaweeds in swine nutrition. *J. Dairy Vet. Anim. Res.* **2014**, 1, 14–16.

Makkar, H.P.S.; Tran, G.; Heuze, V.; Giger-Reverdin, S.; Lessire, M.; Lebas, F.; Ankers, P. Seaweeds for livestock diets: A review. *Anim. Feed Sci. Technol.* **2016**, 212, 1–17.

Marín, A.; Casas-Valdez, M.; Carrillo, S.; Hernández, H.; Monroy, A.; Sanginés, L.; Pérez-Gil, F. The marine algae Sargassum spp. (Sargassaceae) as feed for sheep in tropical and subtropical regions. *Rev. Biol. Trop*. **2009**, 57, 1271–1281.

Marsham, S.; Scott, G.W.; Tobin, M.I. Comparison of nutritive chemistry of a range of temperate seaweeds. *Food Chem*. **2007**, 100, 1331–1336.

McAlpine, P.; O'Shea, C.J.; Varley, P.F.; Flynn, B.; O'Doherty, J.V. The effect of seaweed extract as an alternative to zinc oxide diets on growth performance, nutrient digestibility, and fecal score of weaned piglets. *J. Anim. Sci.* **2012**, 90, 224–226.

McDonnell, M.; Bouwhuis, M.; Sweeney, T.; O'Shea, C.; O'Doherty, J. Effects of dietary supplementation of galactooligosaccharides and seaweed-derived polysaccharides on an experimental *Salmonella Typhimurium* challenge in pigs. *J. Anim.Sci.* **2016**, 94, 153–156.

McDonnell, P.; Figat, S.; O'Doherty, J.V. The effect of dietary laminarin and fucoidan in the diet of the weanling piglet on performance, selectedfaecal microbial populations and volatile fatty acid concentrations. *Animal* **2010,** 4, 579–585.

Michiels, J.; Skrivanova, E.; Missotten, J.; Ovyn, A.; Mrazek, J.; De Smet, S.; Dierick, N. Intact brown seaweed (*Ascophyllum nodosum*) in diets of weaned piglets: Effects on performance, gut bacteria and morphology and plasma oxidative status. *J. Anim Physiol. Anim. Nutr.* **2012**, 96, 1101–1111.

Moroney, N.C.; O'Grady, M.N.; O'Doherty, J.V.; Kerry, J.P. Addition of seaweed (*Laminaria digitata*) extracts containing laminarin and fucoidan to porcine diets: influence on the quality and shelf-life of fresh pork. *Meat Sci.* **2012**, 92, 423–429.

Moroney, N.C.; O'Grady, M.N.; Robertson R.C.; Stanton, C.; O'Doherty, J.V.; Kerry, J.P. Influence of level and duration of feeding polysaccharide (laminarin and fucoidan) extracts from brown seaweed (*Laminaria digitata*) on quality indices of fresh pork. *Meat Sci.* **2015**, 132–141.

Mukhopadhya, A.; O'Doherty, J.V.; Smith, A.; Bahar, B.; Sweeney, T. The microbiological and immunomodulatory effects of spray-dried versus wet dietary supplementation of seaweed extract in the pig gastrointestinal tract. *J.Anim. Sci.* **2012**, 90, 28–30.

Murphy, P.; Dal Bello, F.; O'Doherty, J.; Arendt, E.K.; Sweeney, T.; Coffey, A. Analysis of bacterial community shifts in the gastrointestinal tract of pigs fed diets supplemented with b-glucan from *Laminaria digitata*, *Laminaria hyperborea* and *Saccharomyces cerevisiae*. *Animal* **2013**, 7, 1079-1087.

Murty, U.S; Banerjee, A.K. Seaweeds: The wealth of oceans. In *Handbook of Marine Macroalgae*: *Biotechnology and Applied Phycology*; Se-Kwon, K., Ed.; John Wiley & Sons: Hoboken, NJ, USA, **2012**.

Narayani S.S.; Saravanan, S.; Bharathiaraja, S.; Mahendran, S. Extraction, partially purification and study on antioxidant property of fucoxanthin from *Sargassum cinereum* J. Agardh. *J. Chem. Pharm. Res*. **2016**, 8, 610–616.

Naseri, A.; Holdt, S.L.; Jacobsen, C. Nutritional value added compounds in industrial red seaweed used in carrageenan production. *J. Aquat. Food Prod. Technol*. **2019,** 28, 967–973.

Noviendri, D.; Jaswir, I.; Salleh, M.H.; Taher, M.; Miyashita, K.; Ramli, N. Fucoxanthin extraction and fatty acid analysis of *Sargassum binderi* and *S. duplicatum*. *J.Med. Plants Res.* **2011**, 5, 2405–2412.

NRC, National Research Council. Nutrient Requirements of Swine: Eleventh Revised Edition. The National Academies Press ,Washington, DC, USA, **2012**.

O'Doherty, J.V; McDonnell, P.; Figat, S. The effect of dietary laminarin and fucoidan in the diet of the weanling piglet on performance and selected faecal microbial populations. *Livest. Sci.* **2010**, 134, 208–210.

O'Sullivan, L.; Murphy, B.; McLoughlin, P.; Duggan, P.; Lawlor, P.G.; Hughes, H.; Gardiner, G.E. Prebiotics from marine macroalgae for human and animal health applications. *Mar. Drugs.* **2010**, 8, 2038–2064.

Okolie, C.L.; Rajendran, S.R.C.K.; Udenigwe, C.C.; Aryee, A.N.A.; Mason, B. Prospects of brown seaweed polysaccharides (BSP) as prebiotics and potential immunomodulators. *J. Food Biochem.* **2017**, 41, e12392.

Ortiz, J.; Romero, N.; Robert, P.; Araya, J.; Lopez-Hernández, J.; Bozzo, C.; Navarrete, E.; Osorio, A.; Rios, A. Dietary fiber, amino acid, fatty acid and tocopherol contents of the edible seaweeds *Ulva lactuca* and *Durvillaea antarctica*. *Food Chem*. **2006**, 99, 98–104.

O'Shea, C.J.; McAlpine, P.; Sweeney, T.; Varley, P.F.; O'Doherty, J.V., Effect of the interaction of seaweed extracts containing laminarin and fucoidan with zinc oxide on the growth performance, digestibility and faecal characteristics of growing piglets. *Br. J. Nutr.* **2014,** 111, 798–807.

Øverland, M.; Mydland, L.T.; Skrede, A. Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. *J. Sci. Food Agric*. **2019**, 99, 13–24.

Pangestuti, R.; Kurnianto, D. Green Seaweeds-Derived Polysaccharides Ulvan: Occurrence, Medicinal Value and Potential Applications. In *Seaweed Polysaccharides, Isolation, Biological and Biomedical Applications*, 1st ed.; Jayachandran V., Sukumaran A., Se-Kwon K., Eds.; Elsevier Science Publishing Co. Inc.: New York, NY, USA, **2017**; pp. 205–221.

Park, Y.H.; Jang, D.S.; Kim, S.B. *Utilization of Marine Products*, 2nd ed.; Hyoungsul Press: Seoul, Korea, 1997; pp. 283-336.

Peng, J.; Yuan, J.P.; Wu, C.F.; Wang, J.H. Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. *Mar. Drugs*. **2011**, 9, 1806–1828.

Pérez, M.J.; Falqué, E.; Domínguez, H. Antimicrobial action of compounds from Marine Seaweed. *Mar. Drugs* **2016**, 14, 52.

Pina-Pérez, M.C.; Rivas, A., Martínez, A., Rodrigo, D. Antimicrobial potential of macro and microalgae against pathogenic and spoilage microorganisms in food. *Food Chem*. **2017,** 235, 34–44.

Pomin, V.H. *Seaweed: Ecology, Nutrient Composition and Medicinal Uses*, 1st ed.; Nova Science Publishers, Inc.: Hauppauge, NY, USA, **2011**; pp. 15–47.

Rajauria, G.; Cornish, L.; Ometto, F.; Msuya, F.E.; Villa, R. Identification and selection of algae for food, feed, and fuel applications. In Seaweed Sustainability, Food and Non-Food Applications, 1st ed; Academic Press, UK, **2015**; pp. 315–345.

Rajauria, G.; Draper, J; McDonnel, M.; O'Doherty, J.V. Effect of dietary seaweed extracts, galactooligosaccharide and vitamin E supplementation on meat quality parameters in finisher pigs. *Innov. Food Sci. Emerg. Technol.* **2016**, 37, 269–275.

Ramus, J.; Lemons, F.; Zimmerman, C. Adaptation of light-harvesting pigments to downwelling light and the consequent photosynthetic performance of the eulittoral rockweeds *Ascophyllum nodosum* and *Fucus vesiculosus*. *Mar. Biol*. **1977**, 42, 293–303.

Reilly, P.; Sweeney, T.; Pierce, K.M.; Callan, J.J.; Julka, A.; O'Doherty, J.V. The effect of seaweed extract inclusion on gut health and immune status of the weaned pig. *Animal* **2008**, 2, 1465–1473.

Ren, D.; Bedzyk, L.A.; Ye, R.W.; Thomas, S.M.; Wood, T.K. Differential gene expression shows natural brominated furanones interfere with the autoinducer–2 bacterial signaling system of Escherichia coli. *Biotechnol. Bioeng*. **2004**, 88, 630–642.

Rodrigues, D.; Alves, C.; Horta, A.; Pinteus, S.; Silva, J.; Culioli, G.; Thomas, O.P.; Pedrosa, R. Antitumor and antimicrobial potential of bromoditerpenes isolated from the red alga, Sphaerococcus coronopifolius. *Mar. Drugs* **2015**, 13, 713–726.

Rossi, R.; Pastorelli, G.; Cannata, S.; Corino, C. Recent advances in the use of fatty acids as supplements in pig diets: A review. *Anim. Feed Sci.Technol*. **2010**, 162, 1–11.

Ruiz, Á.R.; Gadicke, P.; Andrades, S.M.; Cubillos, R. Supplementing nursery pig feed with seaweed extracts increases final body weight of pigs. *Austral J. Vet Sci.* **2018,** 50, 83–87.

Rupérez, P.; Saura-Calixto, F. Dietary fibre and physicochemical properties of edible Spanish seaweeds. Eur. *Food Res. Technol*. **2001**, 212, 349–354.

Rupérez, P.; Toledano, G. Indigestible fraction of edible marine seaweeds. *J. Sci. Food Agric*. **2003**, 83, 1267–1272.

Saini, R.; Badole, S.L.; Zanwar, A.A. *Bioactive dietary factors and plant extracts in dermatology*; Watson, R.R., Zibadi, S., Eds.; Humana Press: Totowa, NJ, USA. **2013**; pp. 73–82.

Sanz-Pintos, N.; Perez-Jimenez, J.; Buschmann, A.H.; Vergara-Salinas, J.R.; Perez-Correa, J.C.; Saura-Calixto, F. Macromolecular antioxidants and dietary fiber in edible seaweeds. *J. Food Sci*. **2017**, 2, 289–295.

Saravana, P.S; Yin, S.; Choi, J.H.; Park, Y.B.; Woo, H.C.; Chun, B.S. Evaluation of the chemical composition of brown seaweed (*Saccharina japonica*) hydrolysate by pressurized hot water extraction. *Algal Res.* **2016**, 13, 246–254.

Sardari, R.R.R.; Nordberg Karlsson, E. Marine Poly- and Oligosaccharides as Prebiotics. *J. Agric. Food Chem.* **2018**, 66, 11544–11549.

Schiener, P.; Black, K.D.; Stanley, M.S.; Green, D.H. The seasonal variation in the chemical composition of the kelp species *Laminaria digitate, Laminaria hyperborean, Saccharina latissima* and *Alaria esculenta*. *J. Appl. Phycol*. **2015**, 27, 363–373.

Shannon, E.; Abu–Ghannam, N. Antibacterial derivatives of marine algae: An overview of pharmacological mechanisms and applications. *Mar. Drugs* **2016**, 14, 81.

Smith, A.G.; O'Doherty, J.V.; Reilly, P.; Ryan, M.T.; Bahar, B.; Sweeney, T. The effects of laminarin derived from *Laminaria digitata* on measurements of gut health: selected bacterial populations, intestinal fermentation, mucin gene expression and cytokine gene expression in the pig. *Br. J. Nutr*. **2011**, 105, 669–677.

Sweeney, T.; O'Doherty, J.V. Marine macroalgal extracts to maintain gut homeostasis in the weaning piglet. *Domest. Anim. Endocrinol*. **2016**, 56, S84–S89.

Tanna, B.; Mishra, A. Nutraceutical Potential of Seaweed Polysaccharides: Structure, Bioactivity, Safety, and Toxicity. *Compr. Rev. Food Sci. Food Saf.* **2019**, 18, 817–831.

Tibbetts, S.M.; Milley, J.E.; Lall, S.P. Nutritional quality of some wild and cultivated seaweeds: Nutrient composition, total phenolic content and *in vitro* digestibility. *J. Appl. Phycol.* **2016**, 28, 3575–3585.

Usov, A. Polysaccharides of the red algae. *Adv. Carbohydr. Chem Biochem.* **2011**, 65, 115-217.

Vatsos, I.N.; Rebours, C. Seaweed extracts as antimicrobial agents in in aquaculture. *J. Appl. Phycol*. **2015**, 27, 2017–2035.

Vijayabaskar, P.; Vaseela, N.; Thirumaran, G. Potential antibacterial and antioxidant properties of a sulfated polysaccharide from the brown marine algae Sargassum swartzii. *Chin. J. Nat. Med*. **2012**, 10, 421–428.

Walsh, A.M.; Sweeney, T.; O'Shea, C.J.; Doyle, D.N.; O 'Doherty, J.V. Effect of supplementing varying inclusion levels of laminarin and fucoidan on growth performance, digestibility of diet components, selected faecal microbial populations and volatile fatty acid concentrations in weaned pigs. *Anim. Feed Sci.Technol.* **2013a,** 183, 151–159.

Walsh, A.M.; Sweeney, T.; O'Shea, C.J.; Doyle, D.N.; O'Doherty, J.V. Effect of dietary laminarin and fucoidan on selected microbiota, intestinal morphology and immune status of the newly weaned pig. *Br. J. Nutr*. **2013b**, 110, 1630–1638.

Wan, J.; Jiang, F.; Xu, Q.S.; Chen, D.W.; He, J. Alginic acid oligosaccharide accelerates weaned pig growth through regulating antioxidant capacity, immunity and intestinal development. *RSC Adv.* **2016**, 6, 87026–87035.

Wan, J.; Zhang, J.; Chen, D.W.; Yu, B.; He, J. Effects of alginate oligosaccharide on the growth performance, antioxidant capacity and intestinal digestion-absorption function in weaned pigs. *Anim. Feed Sci. Technol.* **2017**, 234, 118–127.

Wan, J., Zhang J., Chen D., Yu B., Huang Z., Mao X., Zheng P., Yu, J., He, J. Alginate oligosaccharide enhances intestinal integrity of weaned pigs through altering intestinal inflammatory responses and antioxidant status. *RSC Adv*. **2018**, 8, 13482–13492.

Wen, X.; Peng, C.; Zhou, H.; Lin, Z.; Lin, G.; Chen, S.; Li, P. Nutritional composition and assessment of *Gracilaria lemaneiformis Bory*. *J. Integr. Plant Biol*. **2006**, 48, 1047–1053.

Wong, K.H.; Cheung, P.C.K. Nutritional evaluation of some subtropical red and green seaweeds: Part I — proximate composition, amino acid profiles and some physico-chemical properties. *Food Chem.* **2000**, 71, 475–482.

Wong, K.H.; Cheung, P.C.K. Nutritional evaluation of some subtropical red and green seaweeds Part II. *In vitro* protein digestibility and amino acid profiles of protein concentrates. *Food Chem*. **2001**, 72, 11–17.

Yaich, H.; Garna, H.; Besbes, S.; Paquot, M.; Blecker, C.; Attia, H. Chemical composition and functional properties of *Ulva lactuca* seaweed collected in Tunisia. *Food Chem*. **2011**, 128, 895–901.

Xu, S.-Y.; Huang, X.; Cheong, K.-L. Recent Advances in Marine Algae Polysaccharides: Isolation, Structure, and Activities. *Mar. Drugs* **2017**, 15, 388.

CHAPTER 4

Trial 1. Dietary biotechnological *Ajuga Reptans* extract in post-weaning piglets: effects on growth performance, oxidative status and immune parameters

Published in:

Annals of Animal Science, Vol. 19, No. 3(2019) 793-806

4.1 ABSTRACT

The effect of dietary supplementation with a biotechnological extract of *Ajuga Reptans* on growth performance, oxidative status and immune parameters was evaluated in post weaning piglets. At weaning, 120 piglets with an average live weight of 8.1 ± 1.3 kg, were assigned to one of three experimental groups. The first group was fed a control diet (C). The second and third groups were fed the same diet supplemented with 5 mg (T1) and 10 mg (T2) of teupolioside/kg feed from a biotechnological plant extract. Growth performances were recorded and blood samples were collected at the beginning, at 14 days, and at the end of the trial (56 days). Serum biochemical parameters, oxidative status and immunoglobulin titres were determined. Average daily gain tended to be higher ($P=0.057$) and live weight was higher in piglets ($P<0.05$) fed with different amounts of plant extract (T1 and T2) than the controls. The production of reactive oxygen metabolites (ROMs) was higher ($P<0.05$) in the control group than in the groups receiving teupolioside (T1 and T2). Concentration of serum immunoglobulin of class G improved ($P<0.001$) in piglets fed the T1 and T2 diets than the controls. Overall, the results suggested that the biotechnological extract of *Ajuga Reptans* containing teupolioside has an antioxidant and immunomodulant effect.

4.2 INTRODUCTION

Antimicrobial resistance is an important concern for both animal and human health (WHO, 2017). Approximately, 75% of the antimicrobials sold, including those for human consumption, are intended for animals (67% in USA, FDA 2013). Antimicrobials are used in intensively farmed animals for disease prevention and growth promotion effect. The use of antibiotics has been linked with the development of resistant bacteria in chickens and swine gut microbiota (Brüssow, 2017; Looft et al., 2012). Van Boeckel et al. (2015) estimated that the global average annual consumption of antimicrobials per kilogram of animal produced was: 45 $mg \cdot kg^{-1}$ for cattle, 148 $mg \cdot kg^{-1}$ for chickens and 172 $mg \cdot kg^{-1}$ for pigs. Italy, where the present study was carried out, has the third highest use of antibiotics in livestock in Europe (ESVAC, 2016).

According to the World Health Organization, the reduction in antimicrobial consumption cannot be delayed. It is thus essential to find new strategies to support animal health and growth performances. Dietary integration with bioactive molecules from natural sources is a sustainable way to reduce the use of antimicrobial and synthetic additives.

Some studies have reported that dietary supplementation with natural extracts in swine and poultry production lead to a better growth performances compared with antimicrobials (Kamel, 2001; Gheisar and Kim, 2018). Phenylpropanoid glycosides (PPGs) belong to the largest group of bioactive molecules in plants and derive mainly from phenylalanine synthesized by a metabolic pathway, which is efficient only in microorganisms and plants (Sangwan et al., 2001). Phenylpropanoid glycosides have shown several biological activities such as antitumoral, antiviral, anti-inflammatory, antibacterial, antioxidative, and free radical scavenging (Dembitsky, 2005). PPGs are thus a very interesting group of molecules for producing immunostimulant, hepatoprotective, antimicrobial, and antinflammatory phyto-preparations (Korkina et al., 2006).

In the group of PPGs, teupolioside (also known as Lamiuside A) is a promising biologically active compound from *Ajuga reptans* (Di Paola et al., 2009).

Teupolioside is structurally characterized by caffeic acid and 4,5 hydroxyphenylethanol bound to a β-[D]-glucopyranoside through ester and glycosidic links respectively. Two other carbohydrates, i.e. rhamnose and galactose, are linked in sequence to the glucose molecule (Figure 1).

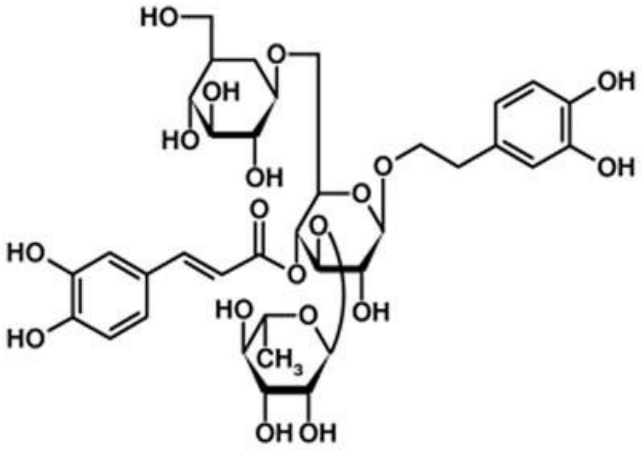


Figure 1. Chemical structure of teupolioside (Di Paola et al., 2009).

Teupolioside is a secondary metabolite produced in order to protect the plant from UV radiation. From a technological point of view, it is difficult to extract this molecule using industrial methods due to its low content in the whole plant. In fact, the chemical synthesis of the molecule, due to the structure of teupolioside, is complex and very expensive. The Biotecnological Research Institute (I.R.B. S.p.A, Altavilla Vicentina, Italy) has developed a platform for the production of teupolioside using cell cultures in suspension of *Ajuga reptans*. The biotechnological processes also improve the safety profile and guarantee the highly standardized composition of the extract, thus reducing the environmental impact (Dal Toso and Melandri, 2009). The aim of the present study was to investigate the effects of dietary supplementation with teupolioside produced by cell cultures of *Ajuga reptans* (*Laminaceae spp.*) on growth performance, oxidative status and immunological parameters in post weaning piglets.

4.3 MATERIALS AND METHODS

Animals and experimental design

The animals used in this experiment were cared for following the European Union guidelines (No. 2010/63/EU) and approved by the Italian Ministry of Health. One hundred and twenty weaned piglets (Goland), half castrated males and half females, aged 24 ± 2 days, were randomly selected and divided into three experimental groups, balanced for body weight (BW) and gender. During the period of adaptation (7 days), the piglets received a commercial diet for *ad libitum* consumption. After 7 days of adaptation, at an average body weight of 8.1 ± 1.3 kg, the piglets were assigned to three dietary treatments (8 piglets per pen; 5 pens per treatment) and reared in an environmentally-controlled room. The control group (C) received a commercial diet, and groups T1 and T2 received the same diet supplemented with the biotechnological plant extract in order to ensure 5 mg or 10 mg teupolioside per kg feed.The plant extract is produced by cell cultures of *Ajuga reptans* (*Laminaceae spp.*) and prepared on an industrial scale by a standardised procedure (I.R.B. s.r.l., Altavilla Vicentina, Vicenza, Italy). To prevent oxidation, the supplement was microencapsulated within a protective matrix of hydrogenated vegetable lipids using spray cooling technology (Sintal Zootecnica, Isola Vicentina, Vicenza, Italy). The experimental diets were formulated to meet the requirements for all nutrients (NRC 1998). The composition of the experimental diets is reported in Table 1.The animals had free access to water and were fed *ad libitum*. The experimental trial lasted 56 days. Piglets were weighed at the beginning and at the end of the trial. The amounts of feed offered and refused were recorded daily to calculate the feed intake. These data were used to calculate the average daily gain (ADG), gain feed ratio (G : F), and average daily feed intake (ADFI) of each pen.

Table 1. Composition of the experimental diets (g/kg, as-fed basis).

Live weight range	8-15 kg	15-30 kg
Ingredient:		
Steam-rolled corn	280	180
Corn yellow	150	200
Barley	150	200
Wheat middings	80	80
Dried whey	50	20
Soy protein concentrate, 64% CP	40	44
Soybean meal, 48% CP	60	80
Fish meal, 70% CP	28	-
Rice protein meal, 65% CP	24	20
Dextrose	25	10
Wheat bran	30	80
Soy oil	30	30
Experimental supplement [1]	1	1
Vitamin- mineral premix [2]	34	34
Dicalcium phosphate	10	14
L-Lysine · HCl	5	4
Preservative [3]	3	3
Calculated chemical composition [4]		
Crude protein	206.1	195.4
Ether extract	83.3	75.6
Crude fiber	30.9	39.9
Ash	61.3	65.9
Lysine	13.1	11.9
Methionine + cysteine	7.9	7.2
Threonine	8.5	7.7
Tryptophan	2.6	2.4

[1]quantities of plant extract standardized for teupolioside provided per 1 kg of complete diet: 0 (maltodextrins), for control, 5 mg teupolioside (T1), and 10 mg teupolioside (T2) groups respectively.

[2]provided per 1 kg of complete diet: Ca 2.8 g, P 0.14 g, Na 1.33 g, vit. A 16 000 IU, vit. D3 2000 IU, vit. E 175 IU, vit. K (menadione sodium bisulfite) 3.8 mg, vit. B1 4.9 mg, vit. B2 9.8 mg, calcium D-pantothenate 40 mg, niacin 57.8 mg, vit. B12 0.09 mg, vit. B6 7.7 mg, folic acid 3.4 mg, biotin 0.33 mg, choline chloride 1000 mg, Zn (ZnO) 85 mg, Cu (CuSO4) 85 mg, Mn (MnO) 108 mg, Fe (FeCO3) 470 mg, I (KI) 3.85 mg, Co (CoSO4) 1.4 mg, Se (as Na2SeO3) 490 μg. Premix containing calcium formiate, Saccharomices cerevisiae, sodium chloride, barley, butyric acid, dL-tryptophan, dL-methionine, L-treonine.

[3]composition per 1 kg of complete feed: formic acid 0.3 g, lactic acid 1.1 g, colloidal silica carrier 1.6g.

[4]calculation based on INRA (2004).

Sample Collection

Blood samples were obtained, by anterior vena cava puncture before the morning feeding, from 10 randomly selected castrated male piglets per treatment (2 piglets/pen), at the beginning of the trial, and at days 14 and 56. The blood samples were collected in 10 mL vacutainer glass tubes (Venoject®, Terumo Europe N.V., Leuven, Belgium) and immediately placed on ice. Serum was harvested by centrifugation (8,500 x g for 15 min. 4 °C) and stored at -80 °C pending analysis.

Determination of antioxidant activity of plant extract

The antioxidant activities of the biotechnological extract of *Ajuga reptans*, was evaluated using the Kit Radicaux Libres (KRL) biological test (Prost, 1992). This *ex-vivo* test is based on free radical-induced haemolysis and tests the antioxidant capacity of several molecules in a biological condition. A control blood sample was used as the biological medium (Astra for medics S.R.L, Milan, Italy). The direct effect of the phenolic compounds on the control blood was tested without free radical addition, also verifying that the blank did not present any interference (cytotoxic assays). The phenolic compounds were dissolved in aqueous solution at different concentrations. Blood solutions were incubated at 37 °C with different ranges of concentration (from 0 to 1 mg by liter of reaction medium) of the extract for 15 min before being submitted to free radicals produced by a final 50 mM solution of 2,20-azobis (2-amidinopropane) dihydrochloride (AAPH). Diluted blood samples without and in the presence of different amounts of the extract were submitted to organic free radicals produced at 37°C under air atmosphere from the thermal decomposition of a 50 mM solution of AAPH. Hemolysis was recorded using a 96-well microplate reader by measuring the optical density decay at 450 nm (Laboratoires Spiral, France). The results were expressed as the percentage increase in KRL value, which is the time required to reach 50% haemolysis compared to the control blood. A range from 0 to 100 mg/L of Trolox® (MW 250.29 g/mole), a water-soluble analogue of vitamin E, enabled us to standardize the global antioxidant capacity of the product compared to vitamin E. Mean values of three independent determinations were used for the calculation.

Haematochemical parameters

Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose and urea were determined by enzymatic spectrophotometric assay (Alfa Wasserman, Milano, Italy). The concentration of low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation according to Johnson et al., (1997).

Oxidative status

The serum reactive oxygen metabolite (ROM) was determined using a spectrophotometric assay ("D-Roms test" Diacron s.r.l., Grosseto, Italy). The *D-Roms* test is based on the concept that the amount of organic hydroperoxides present in serum is related to the free radicals from which they are formed (Cesarone et al., 1999; Alberti et al., 2000). When the serum sample is dissolved in an acidic buffer, the hydroperoxides react with the transition metal ions freed from the proteins in the acidic medium and are converted to alkoxy and peroxy radicals. These newly-formed radicals are able to oxidize an additive (*N*,*N*-diethyl-*para*-phenylendiamine) to the corresponding radical cation. The concentration of this persistent species can be easily determined through spectrophotometric procedures (absorption at 505 nm). The results are expressed in U CARR (Carratelli Units) where 1 U CARR corresponds to 0.024 mmol/l H_2O_2 or 0.8 mg/L H_2O_2.

Serum Immunoglobulin Concentration

Serum concentrations of class G (IgG) and A (IgA) immunoglobulin were measured at T0 and T56 using the ELISA method, as described by the manufacturer (Bethyl, Montgomery, TX). Briefly, plasma samples were diluted 1:4000 and 1:60,000 to detect IgA and IgG, respectively, in Tris–buffered saline and added to plates coated with class specific immunoglobulin pig antibody. The different subsets were detected with the appropriate peroxidase anti-pig IgA or IgG (Bethyl) and were quantified with reference to standard curves constructed with known amounts of pig immunoglobulin subsets. Absorbance was read at 450 nm using an ELISA plate reader (Spectra thermo, Tecan, NC, USA).

4.4 STATISTICAL ANALYSES

All parameters were analyzed using SPSS (SPSS/PC Statistics 24 SPSS Inc., IBM). Live weight data were analysed by GLM (General Linear Model) procedure with treatment as the main effect and the value at the beginning of the trial entered as a covariate. Biochemical parameters and serum oxidative status were analysed by repeated measure analysis of variance (ANOVA) to assess the main effect of treatment and time and value at the beginning of the trial entered as a covariate. Pen was the experimental unit for the productive performance. Piglet was considered the experimental unit of all serum metabolites and immunological variables. Data are presented as means ± SEM, and a value of $P \leq 0.05$ is used to indicate statistical significance.

4.5 RESULTS

Antioxidant activity of plant extract

The results underline that teupolioside from the cell culture of *Ajuga Reptans* has an important antioxidant capacity *in vitro*, which increases linearly with the dose of the extract up to a concentration of 100 mg/L (Figure 2). The plant extract from the cell culture of *Ajuga reptans* (standardized at 50% in teupolioside) showed an antioxidant activity equivalent to 3.73 μmoles of Trolox per mg of extract.

Growth Performance

The piglets' growth parameters in relation to dietary treatments are reported in Table 2. At the end of the experimental trial, group T2 showed a higher ($P<0.05$) body weight compared to the others (C and T1). Therefore, also the ADG tended to be higher ($P<0.10$) in piglets fed with the highest dosage of Teupolioside (T2). The gain to feed ratio and ADFI were unaffected ($P>0.05$) by dietary treatments.No differences among experimental group were observed in piglet's health status during all experimental trial.

Table 2. Growth performances of piglets fed control diet or diet supplemented with biotechnological plant extract containing teupolioside.

	Dietary Treatment[2]			
Parameters[1,3]	Control	T1	T2	*P-value*
Final BW, kg	29.8 ± 0.54[a]	29.1 ± 0.55[a]	31.4 ± 0.56[b]	0.016
ADG, g/d	386 ± 9.4	380 ± 10.6	414 ± 11.5	0.057
G:F, kg/kg	0.495 ± 0.04	0.509 ± 0.05	0.513 ± 0.06	0.853
ADFI, kg/day	0.782 ± 0.06	0.747 ± 0.08	0.748 ± 0.07	0.134

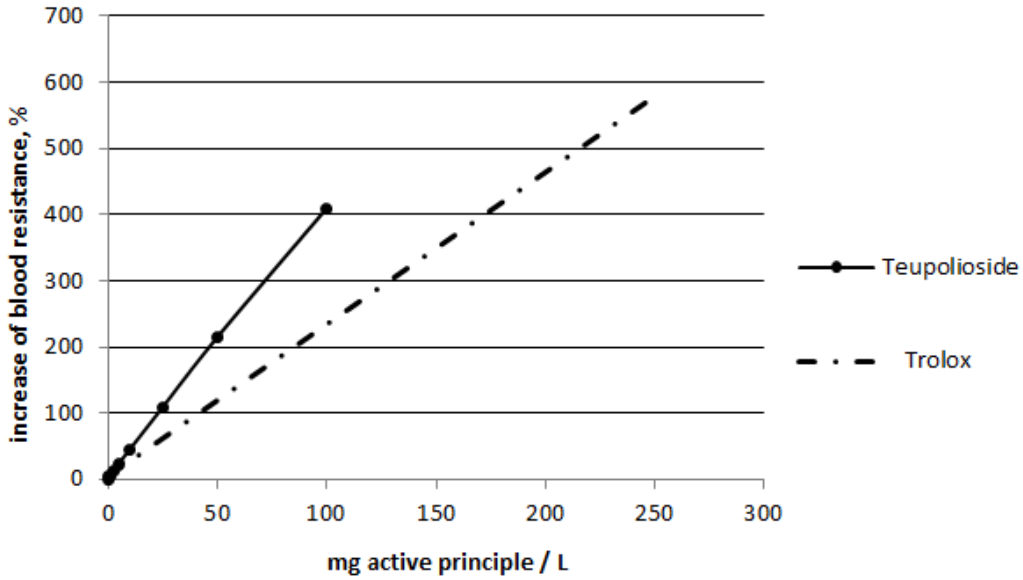
[1]Data are reported as mean ± standard error of the mean. *n=5*;

[2] T1, diet supplemented with plant extract to supply 5 mg teupolioside /kg feed, T2, diet supplemented with plant extract to supply 10 mg teupolioside /kg feed.

[3]BW, body weight; ADG, average daily gain; G:F, gain to feed ratio; ADFI, average daily feed intake;

[a, b] values in rows with different superscript letters differ significantly ($P\leq0.05$).

Figure 2. Antioxidant activity of teupolioside measured by biological test KRL.



Oxidative status

A significant difference in the effect of dietary treatment (P=0.037) was found in d-ROM production between the control and treatment groups at the end of the trial (Table 3). No significant differences were observed for the time effect (P=0.860) and the time x treatment effect (P=0.868).

Immune parameters

Dietary supplementation with the plant extract increased (P=0.001) the IgG serum concentration in T1 and T2 groups compared to control (8.38± 0.61 g/L in control *vs* 11.77±0.62 g/L and 11.79±0.56 g/L in groups T1 and T2, respectively). Figure 3 provides the descriptive analysis (box plot) of IgG serum concentration after 56 days of dietary supplementation with biotechnological plant extract. The IgA serum concentration was unaffected (P=0.260) by dietary treatments (0.64 ± 0.09 g/L in control *vs* 0.65±0.01 g/L and 0.83 ± 0.09 g/L in groups T1 and T2, respectively).

Table 3. Serum biochemical parameters of piglets fed control diet or diet supplemented with biotechnological plant extract containing teupolioside (T1 and T2).

Measure[1]	Dietary Treatment[2]			*P-value*	
	Control	T1	T2	Time	Treatment
HDL cholesterol mmol/L[3]					
Day 14	0.341 ± 0.021	0.380 ± 0.021	0.383 ± 0.020		
Day 56	0.431 ± 0.013	0.413 ± 0.013	0.415 ± 0.012	0.810	0.793
Total cholesterol mmol/L					
Day 14	0.944 ± 0.033	0.972 ± 0.034	0.909 ± 0.033		
Day 56	1.192 ± 0.084	1.200 ± 0.084	1.117 ± 0.083	0.661	0.463
LDL cholesterol, mmol/L[3]					
Day 14	0.462 ± 0.041	0.448 ± 0.040	0.438 ± 0.035		
Day 56	0.672 ± 0.024	0.701 ± 0.023	0.718 ± 0.020	0.942	0.147
Glucose mmol/L					
Day 14	4.78 ± 0.26	4.30 ± 0.26	4.19 ± 0.26		
Day 56	3.87 ± 0.30	4.11 ± 0.30	3.62 ± 0.30	0.234	0.341
Triglycerides mmol/L					
Day 14	0.67 ± 0.05	0.70 ± 0.05	0.51 ± 0.05		
Day 56	0.48 ± 0.03	0.52 ± 0.35	0.52 ± 0.03	0.495	0.130
Urea mmol/L					
Day 14	2.84 ± 0.73	2.94 ± 0.73	3.09 ± 0.73		
Day 56	7.55 ± 1.22	7.35 ± 1.22	5.92 ± 1.22	0.152	0.703
d-Rom (U carr)					
Day 14	433 ± 9.0	415 ± 8.1	414 ± 7.2	0.860	0.037
Day 56	528 ± 8.2	471 ± 8.4	485 ± 8.4		

[1] Data are reported as mean ± standard error of the mean; $n = 10$.

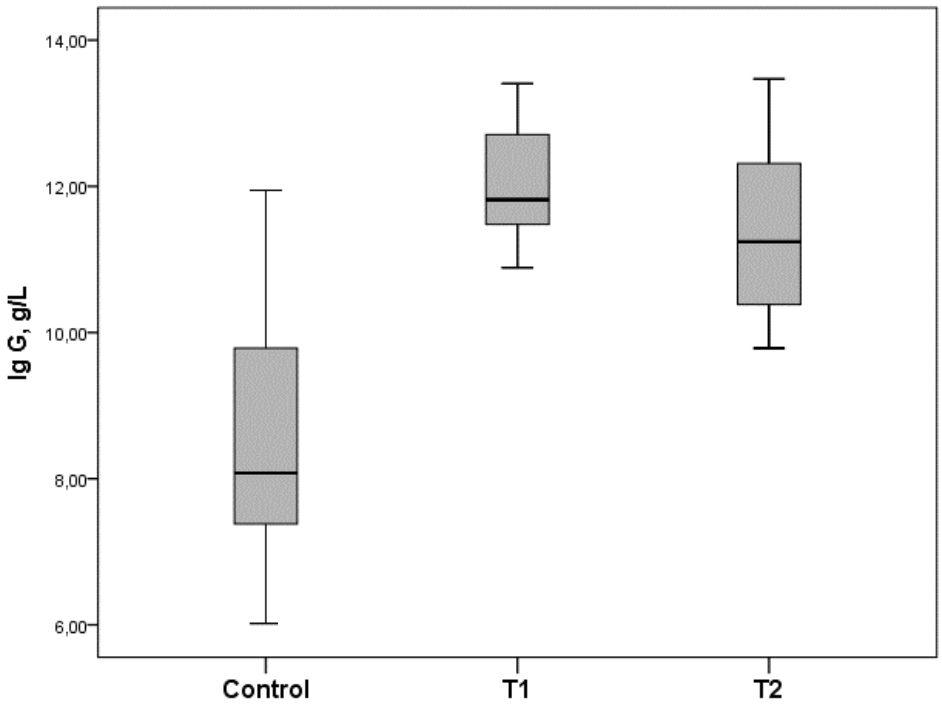
[2]T1, diet supplemented with plant extract to supply 5 mg teupolioside/kg feed, T2, diet supplemented with plant extract to supply 10 mg teupolioside /kg feed.

[3]HDL, high density lipoprotein, LDL low density lipoprotein.

Biochemical parameters

The serum biochemical parameters of piglets fed the control or plant extract supplemented diet are reported in Table 3. Dietary treatment and sampling time did not affect (P>0.05) HDL, LDL and total cholesterol, glucose, triglycerides and urea. No significant differences were observed in the time x treatment effect (P>0.05). Data fall within the reference values for all the parameters analysed.

Figure 3. Boxplot of serum IgG in piglets fed control diet or diet supplemented with biotechnological plant extract titrated in Teupolioside (T1 and T2) after 56 d of dietary treatment[1].



n=10; T1, diet supplemented with plant extract to supply 5 mg teupolioside /kg feed, T2, diet supplemented with plant extract to supply 10 mg teupolioside /kg feed.
Treatment effect, P <0.001.

4.6 DISCUSSION

Regarding phenylpropanoids, many studies have been conducted on several bioactive principles in livestock, but few data are available on plant extracts containing teupolioside. An *in vitro* study reported that teupolioside has demonstrated anti-inflammatory, anti-oxidant and chelating properties (Korkina et al., 2006). Pastore et al., (2009) also reported that teupolioside could decrease the expression of proinflammatory cytokine which was correlated with the antioxidant, scavenging, and iron-chelating activities of this polyphenol (Korkina et al., 2006). In the present study a higher antioxidant activity of teupolioside was observed in comparison with the vitamin E soluble analogue (Trolox). The KRL test could be a promising approach which allows a dynamic evaluation of the overall antioxidant activity of the plant extract against oxidative stress in a biological system (Maghin et al., 2016).

At weaning, piglets have to deal with many changes and nutritional, environmental and immunological stresses (Le Dividich and Sève 2000; Montagne et al., 2007). The use of natural supplements in animal nutrition is a good way to improve piglet health and performance as well as to increase the sustainability of the pig sector. In recent years, plants extracts have attracted interest as an innovative dietary strategy to replace antimicrobials (Cheng et al., 2014). No previous study has reported the effects of dietary supplementation with a biotechnological extract of *Ajuga Reptans* containing teupolioside.

The present results showed an improvement in final weight and ADG in piglets fed the high dosage of biotechnological plant extract (T2). These results agree with other studies reporting that dietary natural extracts containing polyphenols improve growth performance in post-weaning piglets (Maass et al., 2005; Devi et al., 2015). The effects of PPG dietary supplementation on growth performance in livestock are conflicting. In previous studies, supplementation with a water-soluble extract of Verbenaceae (Lippia spp.) leaves containing the PPG verbascoside, had a positive effect on growth performance in suckling lambs (Casamassima et al., 2009). On the other hand, no effect on growth performance was reported in pigs, broilers, hares and horses with a supplementation of 5 mg verbascoside/kg feed (De Marco et al., 2015; Rossi et al., 2013; Rossi et al., 2017; Vizzarri et al., 2014).

Weaning is a crucial phase in pig husbandry. A sudden dietary change from milk to solid feed induces transient anorexia, intestinal inflammation and unbalanced gut microbiota, which are important causes of post weaning diarrhea and associated infections in piglets (Gresse et al., 2017). In this situation, an imbalance between reactive oxygen species production and their neutralization by the antioxidant system of the organism has also been observed, leading to oxidative stress which negatively affects piglet health (Rossi et al., 2009; Buchet et al., 2017). This situation is more serious in the presence of post weaning diarrhea. In fact it has been reported that inflammation increases oxidative stress in animals (Lykkesfeldt et al., 2007). In addition, a study performed by Sauerwein et al., (2007) highlighted that the high d-ROMs values in the first week after weaning is associated with decreasing growth rates.

The present data show that serum d-ROMs in piglets fed with teupolioside was lower than the controls, thus demonstrating the antioxidant activity of the active principle. These data agree with the literature that reports a reduction in d-ROM production or an enhancement in antioxidant status in livestock after dietary supplementation with a natural extract containing PPG (Casamassima et al., 2012; Casamassima et al., 2013; Rossi et al., 2013). In fact, polyphenols prevent the oxidation

of low-molecular-weight antioxidants, such as vitamin A and E, thus increasing their amount of serum (Palazzo et al., 2011; Paszkiewicz et al., 2012). Additionally, previous studies in piglets have reported that dietary supplementation with verbascoside can restore liver antioxidant status induced by the high intake of n-6 PUFA, leading to oxidative stress (Di Giancamillo et al., 2015). As recently reported in the literature, polyphenols protect cells against oxidative stress caused by free radicals thought several mechanisms, thus reducing the risk of associated diseases (Lipiński et al., 2017). The polyphenol metabolism has not been fully clarified and their availability is related to its chemical characteristics and functional groups (Landete, 2012). The present data showed that blood biochemical parameters were not affected by dietary integration with the biotechnological extract, in agreement with a previous study in weaned piglets fed a natural extract containing verbascoside (Pastorelli et al., 2012). On the other hand, other studies in livestock have reported that dietary supplementation with a plant extract containing verbascoside improved the lipid blood profile, decreasing total cholesterol, LDL cholesterol and triglycerides (Palazzo et al., 2011; Casamassima et al., 2014; D'Alessandro et al., 2017).

Several studies have reported that dietary polyphenols from several sources have an immunomodulatory effect on immune cell populations (Cuevas et al., 2013). One important lymphocytes class are B cells which are involved in humoral immunity and produce immunoglobulins. In the literature, the effects of a natural extract containing polyphenols on immunoglobulin production have not been described sufficiently in depth. An *in vitro* study on human peripheral blood mononuclear cells reported that resveratrol did not affect IgM and IgG production (Zunino et al., 2009). Other *in vitro* studies have reported that polyphenols from several sources are able to the modulate B cell function, downregulating IgE and IgG production (Sanbongi et al., 1997; Hassanain et al., 2010).

Our data showed that dietary supplementation with a biotechnological plant extract increased serum IgG without affecting IgA production. The modulation in immunoglobulin production by a dietary supplement with a natural extract in post weaning piglets has been reported by Pastorelli et al. (2012). The authors reported that dietary supplementation with different amounts of plant extract titrated in verbascoside increased both serum IgG and IgA concentrations, thus reflecting the active synthesis of antibodies by the piglets' immune system (Kanitz et al., 2004).

4.7 CONCLUSION

Overall, the results suggest that dietary supplementation with a biotechnological extract of *Ajuga Reptans* containing teupolioside in the post-weaning phase improves piglet health. In fact, piglets fed the high dosage of teupolioside showed a higher body weight at the end of the trial. In addition, dietary supplementation with teupolioside in piglets positively affected serum d-ROM production and IgG title, without affecting other biochemical parameters. These data demonstrate the antioxidant and immunomodulant effects of the biotechnological extract of *Ajuga Reptans* containing teupolioside. Further studies are needed to explore the mechanism of action of teupolioside and to clarify the optimal length and dosage of the dietary treatment.

4.8 REFERENCES

Alberti A., Bolognini L., Macciantelli D., Caratelli M. (2000). The radical cation of N, N-diethyl-para-phenylendiamine: a possible indicator of oxidative stress in biological samples. Res. Chem. Intermed., 26: 253–267.

Brüssow H. (2017). Adjuncts and alternatives in the time of antibiotic resistance and in-feed antibiotic bans. Microb. Biotechnol., 10: 674–677.

Buchet A., Belloc C., Leblanc-Maridor M., Merlot E. (2017). Effects of age and weaning conditions on blood indicators of oxidative status in pigs. PLoS One 12: e0178487.

Casamassima D., Palazzo M., Presutti T., Colella G.E. (2009). Productive performances, plasmatic oxidative status and some blood parameters in suckling lambs supplemented with verbascoside. It. J. Anim. Sci., 8: 668.

Casamassima D., Palazzo M., Martemucci G., Vizzarri F., Corino C. (2012). Effects of verbascoside on plasma oxidative status and blood and milk production parameters during the peripartum period in lacaune ewes. Small Ruminant Res., 105: 1–8.

Casamassima D., Palazzo M., Vizzarri F., Cinone M., Corino C. (2013). Effect of dietary phenylpropanoid glycoside-based natural extracts on blood parameters and productive performance in intensively-reared young hares. Czech J. Anim. Sci. 58: 270–278.

Casamassima D., Nardoia M., Palazzo M., Vizzarri F., D'Alessandro A.G., Corino C. (2014). Effect of dietary extruded linseed, verbascoside and vitamin E supplements on yield and quality of milk in Lacaune ewes. J. Dairy Res., 81: 485–493.

Cesarone M.R., Belcaro G., Caratelli M., Cornelli U., De Sanctis M.T., Incandela L., Barsott R., Terranova R., Nicolaides A. (1999). A simple test to monitor oxidative stress. Intern. Angiol., 2: 127–130.

Cheng G., Hao H., Xie S., Xu W., Dai M., Huang L., Yuan Z. (2014). Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front. Microbiol., 5: 217.

Cuevas A., Saavedra N., Salazar L.A., Abdalla D.S.P. (2013). Modulation of Immune Function by Polyphenols: Possible Contribution of Epigenetic Factors. Nutrients, 5: 2314–2332.

Dal Toso R., Melandri F. (2009). Biotechnologically produced botanical ingredients Ajuga reptans Extract Titrated in Teupolioside. Nutrafoods, 8: 29–36.

D'Alessandro A.G., Vizzarri F., Palazzo M., Martemucci G. (2017). Dietary verbascoside supplementationin donkeys: effects on milk fatty acid profile during lactation, and serum biochemical parameters and oxidative markers. Animal, 11: 1505–1512.

De Marco M., Salcedo W.L., Pastorelli G., Rossi R., Corino C., Bergagna S., Mellia E., Gennero M.S., Biasibetti E., Capucchio M.T., Nurisso S., Tarantola M., Forneris G., Schiavone A. (2015). Effects of verbascoside supplemented diets on growth performance, blood traits, meat quality, lipid oxidation and histological features in broiler chickens. It. J Anim. Sci. 14: 172–178.

Dembitsky V.M. (2005). Astonishing Diversity of Natural Surfactants: 5. Biologically Active Glycosides of Aromatic Metabolites. Lipids, 40: 869–900.

Devi S.M., Lee S.I., Kim I.H. (2015). Effect of phytogenics on growth performance, fecal score, blood profiles, fecal noxious gas emission, digestibility, and intestinal morphology of weanling pigs challenged with *Escherichia coli* K88. Pol. J. Vet. Sci., 18: 557–564.

Di Giancamillo A., Rossi R., Pastorelli G., Deponti D., Carollo V., Casamassima D., Domeneghini C., Corino C. (2015). The effects of dietary verbascoside on blood and liver oxidative stress status induced by a high n-6 polyunsaturated fatty acids diet in piglets. J. Anim. Sci., 93: 284–959.

Di Paola R., Esposito E., Mazzon E., Riccardi L., Caminiti R., Dal Toso R., Pressi G., Cuzzocrea S. (2009). Teupolioside, a phenylpropanoid glycosides of Ajuga reptans, biotechnologically produced by IRB plant cell line, exerts beneficial effects on a rodent model of colits. Biochem. Pharmacol., 77: 845–857.

European Surveillance of Veterinary Antimicrobial Consumption (2016). Sales of veterinary antimicrobial agents in 29 European countries in 2014. Trends from 2011 to 2014. VI ESVAC report. 14.10. 2016.

European Patent EP 1 997 501 A2. Extracts from *Ajuga reptans* cell lines, their preparation and use. Date of publication: 03.12.2008 Bulletin 2008/49.

Food and Drug Administration (2013) Animal Drug User Fee Act (ADUFA) public meeting. Food and Drug Administration Center for Veterinarian Medicine.18.12 2012.

Gheisar M.M., Kim M. (2018). Phytobiotics in poultry and swine nutrition – a review. It. Anim. Sci., 17: 92–99.

Gresse R., Chaucheyras-Durand F., Fleury M.A., Van de Wiele T., Forano E., Blanquet-Diot S. (2017). Gut microbiota dysbiosis in postweaning piglets: understanding the keys to health. Trends Microbiol., 25: 851–873.

Hassanain E., Silverberg J.I., Norowitz K.B., Chice S., Bluth M.H., Brody N., Joks R., Durkin H.G., Smith-Norowitz T.A. (2010). Green tea (*Camelia sinensis*) suppresses B cell production of igE without inducing apoptosis. Ann .Clin. & Lab. Sci., 40: 135–143.

Johnson R., McNutt P., MacMahon S., Robson R. (1997). Use of the Friedewald formula to estimate LDL-cholesterol in patients with chronic renal failure on dialysis. Clin. Chem., 11: 2183–2184.

Kamel C. (2001). Tracing modes of action and the roles of plant extracts in non-ruminants. In: Recent Advances in Animal Nutrition, Garnsworthy P.C., Wiseman J. (eds), pp. 135–150.

Kanitz E., Tuchscherer M., Puppe B., Tuchscherer A., Stabenow B. (2004). Consequences of repeated early isolation in domestic piglets (Sus scrofa) on their behavioural, neuroendocrine, and immunological responses. Brain Behav. Immunol., 18: 35–45.

Korkina L.G., Mikhalchik E., Suprun M.V., Pastore S., Dal Toso R. (2006). Molecular mechanisms underlying wound healing and anti-inflammatory properties of naturally occurring biotechnologically produced phenylpropanoid glycosides. Cell. Mol. Biol., 53: 78–83.

Landete J.M. (2012). Updated knowledge about polyphenols: functions, bioavailability, metabolism, and health. Crit. Rev. Food Sci. Nutr., 52: 936–948.

Le Dividich J., Sève B. (2000). Effects of under feeding during the weaning period on growth, metabolism, and hormonal adjustments in the piglet. Domestic Anim. Endocrinol., 19: 63–74.

Lipiński K., Mazur M., Antoszkiewicz Z., Purwin C. (2017). Polyphenols in monogastric nutrition - a review. Annals Anim. Sci., 17: 41–58.

Looft T., Johnson T.A., Allen K.H., Bayles D.O., Alt D.P., Stedtfeld R.D., Sul W.J., Stedtfeld T.M., Chai B., Cole J.R., Hashsham S.A., Tiedje J.M., Stanton T.B. (2012). In-feed antibiotic effects on the swine intestinal microbiome. Proc. Natl. Acad. Sci., 109: 1691–1696.

Lykkesfeldt J., Svendsen O. (2007). Oxidants and antioxidants in disease: Oxidative stress in farm animals. Vet. J., 173: 502–511.

Looft, T., Johnson T.A., Allen K.H., Bayles D.O., Alt D.P., Stedtfeld R.D., Sul W.J., Stedtfeld T.M., Chai B., Cole J.R., Hashsham S.A., Tiedje J.M., Stanton T.B. (2012). In-feed antibiotic effects on the swine intestinal microbiome. Proc. Natl. Acad. Sci., 109: 1691–1696.

Maass N., Bauer J., Paulicks B.R., Böhmer B.M., Roth-Maier D.A. (2005). Efficiency of Echinacea purpurea on performance and immune status in pigs. J. Anim. Physiol. Anim. Nutr., 89: 244–252.

Maghin F., Rossi R., Prost M., Corino C. (2016). Biological system to assess the antioxidant capability of plant extracts. Proc. Nutriox meeting. Kaiserslautern, Germany. 21-23.09.2016

Montagne L., Boudry G., Favier C., Le Huërou-Luron I., Lallès J.P., Sève B. (2007). Main intestinal markers associated with the changes in gut architecture and function in piglets after weaning. Br. J Nutr., 97: 45–57.

National Research Council (1998). Nutrition Requirements of Swine. (10th rev Eds., National Academy Press: Washington, DC.

Palazzo M., Vizzarri F., Cinone M., Corino C., Casamassima D. (2011). Assessment of a natural dietary extract, titrated in phenylpropanoid glycosides, on blood parameters and plasma oxidative status in intensively reared italian hares (*Lepus Corsicanus*). Animal, 5: 844–850.

Pastore S., Potapovich A., Kostyuk V., Mariani V., Lulli D., De Luca C., Korkina L. (2009). Plant polyphenols effectively protect HaCaT cells from ultraviolet C–triggered necrosis and suppress inflammatory chemokine expression. Ann. New York Acad. Sci., 1171: 305-313.

Pastorelli G., Rossi R., Corino C. (2012) Influence of *Lippia citriodora* verbascoside on growth performance, antioxidant status, and serum immunoglobulins content in piglets. Czech J. Anim. Sci., 57: 312–322.

Paszkiewicz M., Budzynska A., Rozalska B., Sadowska B. (2012). The immunomodulatory role of plant polyphenols(in Polish). Postep. Hig. Med. Dosw., 66: 637–646.

Prost M. (1992). Process for the determination by means of free radicals of the antioxidant properties of a living organism or potentially aggressive agents. In: Us patent 5,135,850.

Rossi R., Pastorelli G., Cannata S., Corino C. (2009). Effect of weaning on total antiradical activity in piglets. Proc. of the XVIII Animal Science and Production Association Congress, Palermo, Italy 9-12.06.2009. p. 673.

Rossi R., Pastorelli G., Corino C. (2013). Application of KRL test to assess total antioxidant activity in pigs: sensitivity to dietary antioxidants. Res. Vet. Sci., 94: 372–377.

Rossi R., Ratti S., Pastorelli G., Maghin F., Martemucci G., Casamassima D., D'Alessandro, A.G., Corino C. (2017). Effect of dietary plant extract on meat quality and sensory parameters of meat from Equidae. J. Sci. Food Agric., 97: 4690–4696.

Sanbongi C., Suzuki N., Sakane T. (1997). Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans *in vitro*. Cell. Immunol., 177: 129–136.

Sangwan N.S., Farooqi A.H.A., Shabih F., Sangwan R.S. (2001). Regulation of essential oil production in plants. Plant Growth Reg. 34: 3–21.

Sauerwein H., Schmitz S., Hiss S. (2007). Effects of a dietary application of a yeast cell wall extract on innate and acquired immunity, on oxidative status and growth performance in weanling piglets and on the ileal epithelium in fattened pigs. J. Anim. Physiol. Anim. Nutr., 91: 369–380.

Van Boeckel T. P., Brower C., Gilbert M., Grenfell B. T., Levin S. A., Robinson T. P., Teillant A., Laxminarayan R. (2015). Global trends in antimicrobial use in food animals. Proc. Nat. Acad. Sci. 112: 5649–5654.

Vizzarri F., Nardoia M., Palazzo M. (2014). Effect of dietary Lippia citriodora extract on productive performance and meat quality parameters in hares (*Lepus europaeus* Pall.). Archiv. Tierzucht, 57: 1–7.

World Health Organization (WHO). (2017). Stop using antibiotics in healthy animals to prevent the spread of antibiotic resistance, 07 November, Geneve.

Zunino S.J., Storms D.H. (2009). Resveratrol alters proliferative responses and apoptosis in human activated B. Lymphocytes *in vitro*. J. Nutr., 139:1603–1608.

CHAPTER 5

Trial 2. Dietary supplementation with natural extracts mixture: effects on reproductive performances, blood biochemical and antioxidant parameters in rabbit does.

Pubblished in:

Annals of Animal Science, (2019), DOI: 10.2478/aoas-2019-0084

5.1 ABSTRACT

The present study evaluates the effects of natural extracts on reproductive performances, haematochemical parameters, and antioxidant status of rabbit does. A total of sixty New Zealand White second parity does were divided into three groups: the first group was fed a control diet (CON), the second (T1) and the third groups (T2), were fed the same diet supplemented with prebiotic polysaccharides from brown seaweeds (*Laminaria* spp.) plus phenolic acid, hydroxycinnamic acids, tannins, flavonoids from plant extracts (0.3% and 0.6% respectively). The trial was conducted for two consecutive reproductive cycles (75 days). Reproductive performance was recorded. Blood samples were collected before the first insemination, 10 d after the first kindling and 10 d after the second one. At the first reproductive cycle, productive parameters were negatively affected ($P < 0.05$) by high dosage of dietary supplement (T2 group). At the second reproductive cycle no difference ($P > 0.05$) between dietary treatments on reproductive and productive performances were observed. Bilirubin was affected by dietary treatment ($P < 0.001$) and decreased in relation to sampling time ($P < 0.001$) and decreased in relation to sampling time ($P < 0.001$). The HDL cholesterol decreased by dietary treatment ($P < 0.01$). All the plasma antioxidant markers were positively affected ($P < 0.001$) by dietary supplementation and sampling time. No previous study has reported the effects of brown seaweeds and polyphenols in rabbit does and the present data shows that this natural extract supplement improved the antioxidant status of rabbits does.

5.2 INTRODUCTION

There has been increasing global concern regarding the development of antimicrobial resistance and the transfer of resistance genes from animal to human strains (Devirgiliis et al., 2013). Due to the ban on using antibiotic growth promoter in animal feed (1831/2003/EC EU), natural alternatives to support animal health and performance have been studied (Lillehoj et al., 2018). Phytogenic and plant extracts are an effective strategy to support a sustainable animal production (Pastorelli et al., 2012; Yang et al., 2015; Casamassima et al., 2014; Attia et al., 2017a & Attia et al., 2017b; Valenzuela-Grijalva et al., 2017; Attia et al., 2018). Brown seaweeds are an excellent source of vitamins, minerals (Descamps, 2006). It also contains sulfur polysaccharides, phlorotannin, catechins, carotenoids, tocopherols and diterpenes, which are characterized by antimicrobial, antioxidant, antinflammatory and immunomodulatory activities (Maghin et al., 2014). These properties make these compounds promising in livestock for the improvement of animal health and welfare. As reviewed by Makkar et al. (2016) dietary supplementation with brown seaweed in rabbits has been shown to have different effects. In particular, dietary *Laminaria s*pp. appears to improve the blood lipid profile, however *Ascophillum nodosum* supplementation should be avoided because it was shown to have a toxic effect. Previous studies have reported that tannins, a heterogeneous group of polyphenols, show antibacterial and antioxidant activities (Huang et al., 2018). Rabbit production is based on high reproductive efficiency, growth rate, feed utilization and meat nutritional parameters (Djakalia et al., 2012). Enteric pathologies are one of the main causes of mortality (Grilli et al., 2006). Natural extract supplementation could thus enhance rabbit doe health and performances during pregnancy and lactation. These phases are critical in does and are characterized by several physiological changes and an increase in the production of reactive oxygen species production (Abdel-Khalek et al., 2008). The number of weaned rabbits needs to increase to enhance the does'productive performance, and nutrition has been largely recognized as a key factor in pregnancy and lactation phases (Chavatte-Palmer et al., 2016). The productive performance of does thus needs to be improved using sustainable dietary supplement (Okab et al., 2013; Casamassima et al., 2017; Uhlirova and Volek 2019). Having focused attention on the animals livestock welfare and the fully expression of their productivity, the present study aims to evaluate the effects of dietary natural supplementation with a brown seaweed and polyphenol extract mixture on reproductive performance, biochemical parameters, and antioxidant markers of New Zealand White rabbit does.

5.3 MATERIAL AND METHODS

Animals and experimental design

Does were handled following the guidelines for animal experiments, indicated in EU Directive 2010/63/EU, and national guidelines for the care and use of animals were followed. All experimental procedures involving animals were approved by ethical committee (No. NPPC 18-10-2016). The trial was performed during January–May period in the experimental rabbit farm at the National Agricultural and Food Centre (Nitra, Slovak Republic). Second parity New Zealand White does (n = 60) were enrolled for two consecutive reproductive cycles (75 days). Lactating does were artificially inseminated at 12 days after kindling. Fourteen days after artificial insemination, the does were tested for pregnancy by palpation, and non-pregnant does were discarded from the experiment. Does were individually housed in wire cages arranged in flat-decks measuring 600 x 500 x 330 mm high on one level. Cages were equipped with a hopper for feed and an automatic nipple drinking system. A Lighting cycle of 16h of light and 8h of dark was used throughout the trial. Heating and forced ventilation systems maintained the building temperature within 18 ± 4°C. Relative humidity was about 70 ± 5%. For an adaptation period of one week, does were fed a commercial diet and the insemination was at the beginning of the trial, in which does were randomly assigned to one of three experimental groups (*n* = 20 replicates per treatment) homogeneous for body weight (4.83 ± 0.19 kg) and parity order (second). The first group (CON) received a control diet, and groups T1 and T2 received the same diet supplemented with 0.3% and 0.6% of a natural feed additive consisting of prebiotic polysaccharides from brown seaweeds (*Laminaria spp*.) plus phenolic acid, hydroxycinnamic acids, tannins, flavonoids from plant extracts. The diets did not include anticoccidials, antibiotics or any other medications. The two dosages of the natural extract were chosen after an *in vitro* evaluation of the minimal inhibitory concentration (MIC) against *Clostridium* spp, *Staphylococcus* spp and *Escherichia coli* spp. (Tosi, personal communication). The ingredients and the chemical composition of experimental diets are reported in Table 1. The chemical composition of experimental diet and the brown seaweeds and the polyphenols extract mixture was performed in accordance with the methods of the Association of Analytical Chemists (AOAC, 2000). The quantitative analysis of the phenolic compounds of the dietary plant supplement was performed by HPLC-UV–DAD (Russo et al., 2017). The chemical and phenolic compositions of the feed supplement are reported in Table 2. Does were fed *ad libitum*, and the average daily feed intake (ADFI) of does was recorded. The body weight of the does was recorded the days before insemination.

Table 1. Ingredients and chemical composition of experimental diets (g/kg).

	Experimental diet [1]		
Ingredients	CON	T1	T2
Maize	282	279	276
Alfalfa hay	305	305	305
Sunflower meal	135	135	135
Palm seed oil	8	8	8
Soybean oil	7	7	7
Wheat	80	80	80
Cane molasses	20	20	20
Carob bean meal	90	90	90
Oat	53	53	53
Calcium carbonate	7	7	7
Sodium Chloride	3	3	3
Dicalcium phosphate	2	2	2
DL-Methionine (99%)	2.5	2.5	2.5
L-Lysine HCl (78.5%)	1.6	1.6	1.6
Choline (75%)	1.4	1.4	1.4
Vitamin and mineral premix*	2.5	2.5	2.5
Dietary supplement	0	3	6
Chemical composition, [2]			
Crude protein	184	183.6	183.5
Ether extract	35.7	35.5	35.5
Crude fibre	187	186.8	187
Ash	86	85.7	85.8
Nitrogen free extract	507	507.1	506.9
NDF	302.1	301.5	301.7
ADF	195.8	195.4	195.3
ADL	39.9	39.5	39.5

[1] CON= control group; T1= group supplemented with 0.3% of brown seaweed and plant polyphenols; T2= group supplemented with 0.6% of brown seaweed and plant polyphenols ;
*Supplied per kg diet: 13,500 I.U. vitamin A (trans-retinyl acetate);
800 I.U. vitamin D3 (cholecalciferol);
35 mg vitamin E (α-tocopherol min 91%),
35 mg copper (cupric sulphate pentahydrate);
[2] analyses determined in triplicate.

Table 2. Chemical composition and polyphenols content of the dietary supplement.

Item [1]	% on dry matter
Dry matter	93.58 ± 5.05
Crude Protein	7.21 ± 0.99
Ether extract	0.32 ± 0.01
Carbohydrates	60.84 ± 3.18
Ash	32.68 ± 1.38
Compounds, mg/kg dry weight	
Phenolic Acid:	
Dihydroxybenzoic acid	≤ LOD [2]
Syringic acid	1059.79 ± 62.82
Hydroxycinnamic acids:	
Neochlorogenic acid	7979.23 ± 468.11
Rosmarinic acid	126.54 ± 8.67
Trans sinapic acid	105.54 ± 8.09
Chlorogenic acid	21.45 ± 3.65
Tannins:	
Ellagic acid	2440.88 ± 148.29
Rutin	272.37 ± 20.82
Flavonoids:	
Myricetin	53.88 ± 5.68
Kaempferol	≤ LOD

[1] values are expressed as means (n= 4) ± standard deviation.

[2] Limit of detection;

Reproductive performances

Cross-fostering was applied within groups with a maximum of eight offspring/litter. The number of offspring born alive and stillborn, the number of weaned offspring per litter, and the body weight of offspring at birth and at weaning per doe were recorded for two reproductive cycle (75 days).

Blood sampling

The first blood sampling was performed after 12 h fasting, at the beginning of the dietary supplementation (t0). After two days, the rabbit does were artificially inseminated. The second blood sampling was performed 10 days after the first kindling (t1). The third blood sampling (t2) was performed et 10 days after the second kindling. Blood samples were taken from the *vena auricolaris marginalis* and were collected in 5 mL vacutainer glass tubes (Venoject®, Terumo Europe N.V., Leuven, Belgium) with lithium heparin. The blood samples were immediately stored at 4°C. All blood analyses were performed at the laboratory of Animal Physiology Department at the Slovak University of Agriculture in Nitra, Slovak Republic, where samples were then centrifuged for 20 min at 3000 rpm at 4°C to obtain plasma.

Biochemical parameters

Triglycerides, total cholesterol, HDL cholesterol and LDL cholesterol, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), were determined in blood plasma using a semi-automatic clinical chemistry Analyzer Arco model (Biotechnical Instruments, S.p.A., Italy).

Plasma oxidative markers

The superoxide dismutase (SOD) was determined using a colorimetric assay (Zhou and Prognon, 2006). The SOD activity was expressed in units per milligram of protein (U/mg). The ferric ion reducing antioxidant power (FRAP) test was determined using Benzie and Strain (1996) method, which measures the antioxidant capacity of plasma. One unit FRAP is expressed in mmol/mL and indicates the number of moles of ferric ion (FeIII) reduced to ferrous ion (FeII) from one mol of tested antioxidants. The total antioxidant *status* (TAS) was measured on blood plasma by 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical cation decolorization assay, following Re et al. (1999). Trolox was used as the standard. The TAS value of samples was defined as the concentration of Trolox with an equivalent activity as units per liter of plasma.

The determination of thiobarbituric acid reactive substances (TBARS) was spectrophotometrically performed according to Esterbauer and Zollner (1989), using a standard curve with 1,1,3,3-tetra-methoxypropane (Sigma Aldrich, St. Louis, USA). The results were expressed as μg of malondialdehyde (MDA)/mL of plasma. Vitamins A and E were extracted from plasma samples with chloroform, according to Zhao et al. (2004). The amount of vitamins was detected by HPLC (Kontron Instruments, Italy), which consisted of an automatic auto-sampler (HPLC Autosampler 360) with a loop of 20 μl, pump system (HPLC Pump 422), a column C18, 5 μm, 250 × 4.60 mm, (Phenomenex, Torrance, Ca, USA). The mobile phase consisted of a mixture of acetonitrile and methanol (85:15 v/v) with a flow value of 1 mL/min. Vitamins A and E were identified by comparing the retention time of the samples with the retention time of pure standards (> 97%) purchased by Sigma Aldrich (St. Louis, USA). The quantification was performed using the Gyminix system (version 1.8.1) by comparing the peak of the area with that of the reference standard curve. Results were expressed as μg/mL of plasma.

5.4 STATISTICAL ANALYSES

Statistical analyses of the data were performed using SPSS (SPSS/24 PC Statistics 24.0 IBM). After assessing whether the frequency distribution assumed normality with the Shapiro-Wilk Test, data on reproductive performances were analyzed by one-way analysis of variance (ANOVA) to evaluate the effects of dietary treatments at first and second partum. Data on biochemical parameters and antioxidant status were submitted to a repeated measure ANOVA to assess the main effect of treatment and time and their interaction. Rabbit does were considered as experimental unit for all parameters. Data were reposted as means ± pooled SEM. Differences were considered statistically significant at level of $P<0.05$.

5.5 RESULTS

Productive and reproductive parameters

During the experimental trial, 30% of does in the CON and T1 groups and 35% in the T2 group were removed from the experiment due to lack of occurring pregnancy after artificial insemination and the data were removed from the analyses. No difference in body weight of the does at the second and third insemination was observed ($P > 0.05$). The average feed intake of does during pregnancy and lactation were not affected ($P > 0.05$) by the dietary treatment. During pregnancy, the average daily feed intake was 318 ± 8.5 g in the CON group, and 314 ± 8.7 g and 320 ± 9.7 g in groups T1 and T2 respectively. During lactation the average daily feed intake was 364 ± 7.5 g in CON group and 395 ± 9.9 g and 395 ± 10.6 g in groups T1 and T2 respectively.

Tables 3 and 4 reported the reproductive parameters of rabbit does evaluated at the first and second kindling cycle respectively. The dietary treatments did not influence ($P > 0.05$) the number of kits per litter, the mortality and weight of kits at birth and weaning in the first reproductive cycle. Although there was a difference in the number of offspring after cross-fostering and at 14 days of lactation (lower number in T2 than in CON and T1; $P < 0.05$) there was a greater mortality and a lower weight of the animals in T2 at weaning, although not significant. At weaning (35 days) the number of offspring per litter tended to be lower ($P = 0.055$) in T2 than in the other groups.

Table 3. Productive parameters at first reproductive cycle of rabbit does fed control diet (CON) and diets supplemented with two levels of brown seaweed and plant polyphenols (0.3% and 0.6% in T1 and T2 groups respectively).

	Diet				
Item [1]	CON	T1	T2	SEM	P-value
Number of offspring per litter:					
total born	10.38	9.47	9.93	0.463	0.732
born alive	9.81	9.07	9.40	0.487	0.827
born dead	0.56	0.40	0.53	0.203	0.944
after cross-fostering	7.73[a]	7.94[a]	7.13[b]	0.128	0.021
14 days of lactation	7.60[a]	7.63[a]	6.69[b]	0.189	0.022
35 days (weaning)	7.27	7.31	6.25	0.220	0.055
Dead, no.	0.47	0.63	0.88	0.126	0.425
Mortality during lactation, %	6.05	7.94	12.35	2.04	0.187
Weight of the litter, kg:					
after cross-fostering	0.596	0.514	0.557	0.028	0.508
14 days	2.17	2.12	1.81	0.099	0.072
35 days (weaning)	6.08	6.03	5.08	2.202	0.073
Weight of offspring, g:					
birth	64.67	58.00	59.33	1.75	0.264
35 days (weaning)	841.9	820.7	810.9	12.92	0.676
ADG [2], g/d	22.10	21.55	21.03	0.410	0.580

[1] data are reported as mean ± pooled standard error of means.

[a,b] Within the same row, means with different letters differ significantly ($P < 0.05$);

[2] Average daily gain.

Table 4. Productive parameters at second reproductive cycle of rabbit does fed control diet (CON) and diets supplemented with two levels of brown seaweed and plant polyphenols (0.3% and 0.6% in T1 and T2 groups respectively).

	Diet			SEM	P-value
Item [1]	CON	T1	T2		
Number of offspring per litter:					
total born	8.31	8.64	9.46	0.504	0.648
born alive	7.85	8.00	8.77	0.477	0.714
born dead	0.46	0.64	0.69	0.133	0.771
after cross-fostering	7.00	7.14	7.08	0.169	0.943
14 days lactation	6.93	7.00	7.08	0.189	0.942
35 days (weaning)	6.71	7.00	6.85	0.162	0.776
Dead, no.	0.29	0.14	0.23	0.082	0.777
Mortality during lactation, %	4.14	1.97	3.25	1.030	0.775
Weight of litter, kg:					
birth	0.523	0.475	0.500	0.023	0.704
14 days	2.02	1.97	2.08	0.702	0.824
35 days (weaning)	7.28	7.12	6.94	0.203	0.798
Weight of offspring, g:					
birth	66.15	62.14	60.00	2.32	0.164
35 days (weaning)	1075	1028	1015	18.49	0.381
ADG [2], g/d	28.72	27.44	27.22	0.515	0.447

[1] data are reported as mean ± pooled standard error of means, [2] Average daily gain.

Biochemical parameters

Table 5 shows the data on does' plasma biochemical parameters in relation to dietary treatments and sampling time. Bilirubin values were affected by dietary treatments ($P<0.01$) and decreased in relation to sampling time ($P<0.001$). Comparing dietary treatments at the last sampling, bilirubin values resulted lower ($P<0.05$) in T2 than T1 and CON rabbit does. An increase (P=0.005) in HDL cholesterol was observed in T1 group at the second and third sampling time. No other biochemical parameters were affected ($P>0.05$) by dietary supplementation. Triglycerides values decreased in relation to sampling time ($P<0.001$). No other parameters were affected by dietary treatments and sampling time.

Table 5. Blood values of rabbit does fed control diet (CON) and diets supplemented with two levels of brown seaweed and plant polyphenols (0.3% and 0.6% in T1 and T2 groups respectively) in relation to sampling time.

	Diet				P-value [3]		
Item [1]	CON	T1	T2	SEM	D	T	T*D
Bilirubin, mg/dL [3]							
t0 [2]	0.72	0.72	0.71	0.011			
t1	0.64	0.63	0.57	0.014			
t2	0.66	0.55	0.46	0.017	<0.001	<0.001	<0.001
Tryglicerides, mg/dL							
t0	66.43	64.03	67.96	1.165			
t1	65.44	60.47	63.82	0.977			
t2	63.44	59.86	63.03	1.139	0.195	<0.001	0.753
Total cholesterol, mg/dL							
t0	56.68	54.69	53.15	0.727			
t1	55.28	54.14	51.16	0.904			
t2	57.79	53.43	53.17	0.873	0.072	0.103	0.333
LDL cholesterol, mg/dL							
t0	35.91	35.36	37.34	0.624			
t1	36.72	34.65	36.75	0.650			
t2	36.43	34.18	37.97	0.674	0.145	0.957	0.537
HDL cholesterol, mg/dL							
t0	31.50	33.47	31.15	0.714			
t1	29.58	36.80	32.69	0.732			
t2	30.03	36.00	32.80	0.896	0.005	0.311	0.039
Aspartate aminotransferase, UI/L							
t0	26.90	25.14	25.11	0.589			
t1	27.42	24.55	27.77	0.554			
t2	27.30	26.38	27.20	0.424	0.134	0.116	0.199
Alanine aminotransferase, UI/L							
t0	41.60	38.82	43.61	0.857			
t1	40.56	40.28	42.71	0.779			
t2	41.37	41.56	40.07	0.892	0.574	0.886	0.009

[1] data are reported as mean values ± pooled standard error of means.

[2] t0, beginning of the dietary supplementation; t1, 10 days after the first kindling; t2 10 days after the second kindling.

[3] D=fixed effect of dietary supplementation; T=fixed effect of time; D x T=interaction dietary supplementation x time;

Plasma antioxidant markers

The plasma antioxidant status of does in relation to dietary treatment and sampling time is reported in Table 6. All the parameters were affected by dietary treatments and sampling time (P<0.001). An interaction between time and treatment effects was also observed (P<0.01). The antioxidant parameters SOD, FRAP and TAS resulted higher (P<0.001) in groups fed seaweeds and the polyphenols mixture and increased in relation to sampling time (P<0.001). Vitamin A and E significantly increased in T1 and T2 groups and in relation to sampling time (P<0.001). The MDA decreased in relation to sampling time in groups fed the natural extract mixture (P<0.001).

Table 6. Plasma antioxidant markers* of rabbit does fed control diet (CON) and diets supplemented with two levels of brown seaweed and plant polyphenols (0.3% and 0.6% in T1 and T2 groups respectively) in relation to sampling time.

	Diet				P-value [3]		
Item[1]	CON	T1	T2	SEM	D	T	T*D
Superoxide dismutase, U/mg							
t0 [2]	41.57	41.41	41.94	0.177			
t1	41.60	40.13	44.93	0.306	<0.001	<0.001	<0.001
t2	41.91	56.54	59.88	1.056			
FRAP, µmol Fe2+/L [4]							
t0	365.70	367.30	381.74	1.339			
t1	360.90	448.95	464.05	6.516	<0.001	<0.001	<0.001
t2	362.10	486.85	489.11	8.077			
Total antioxidant status, U/L							
t0	11.45	11.99	12.07	0.110			
t1	11.86	19.92	22.86	0.632	<0.001	<0.001	0.038
t2	11.82	20.59	23.18	0.654			
Malondialdehyde, µg/mL							
t0	2.83	2.84	2.87	0.012			
t1	2.97	2.41	2.50	0.035	<0.001	<0.001	<0.001
t2	3.01	2.11	2.16	0.056			
Vitamin A, µg/mL							
t0	0.32	0.32	0.34	0.006			
t1	0.31	0.34	0.38	0.006	<0.001	<0.001	0.003
t2	0.33 [a]	0.35	0.40	0.007			
Vitamin E, µg/mL							
t0	1.65	1.71	1.71	0.008			
t1	1.69	1.95	2.04	0.022	<0.001	<0.001	<0.001
t2	1.69	2.33	2.28	0.039			

[1] data are reported as mean values ± pooled standard error of means;

[2] t0, beginning of the dietary supplementation; t1, 10 days after the first kindling; t2, 10 days after the second kindling

[3] D=fixed effect of dietary supplementation; T=fixed effect of time; D x T=interaction dietary supplementation x time.

[4] ferric ion reducing antioxidant power

5.6 DISCUSSION

Productive and reproductive parameters

The lower number of offspring per litter in the T2 group, compared with the other two groups, could indicate an adverse effect of the high dosage of the natural extract. In fact, up to weaning, milk is the main feed of kits, and the number of kits in a litter is closely related to the does'milk production. In addition, the physiological mechanisms that regulate the milk secretion can be influenced by natural bioactive compounds (Albert-Puleo, 1980). It is possible that the high dosage of natural extract negatively affected milk production and resulted in a lower survival rate and weaning weight. However, in the second reproductive cycle, no effect of dietary supplementation with brown seaweed and polyphenols extract mixture was observed on the productive parameters, thus suggesting that tolerance increases with the advanced age of does. In the present experiment a high prebiotic activity from brown seaweeds was expected, however it is possible that the feed additive has no effects on productive and reproductive performances due to the good breeding conditions and low pathogen pressure (Attia et al., 2017c). Thus, studies in field conditions are needed in order to validate the present data. In a similar study on rabbit does, Okab et al. (2013) observed an improvement in kindling rate, litter size, and offspring ratio, after supplementation of 2% of brown seaweed. The authors linked the results with an enhancement of sexual receptivity, highlighting the positive correlation between fertility and prolificacy in artificially inseminated rabbits. The difference between our data and the literature could be related to the different feed supplement and lengh of the dietary supplementation.

Biochemical parameters

The feed additive decreased the bilirubin value in the plasma of does in the treated groups (T1 and T2), which could be related to the antioxidant activity of polyphenols. In fact, inflammatory and oxidative injuries can up-regulate the cellular antioxidant status by generating antioxidants such as bilirubin. The low bilirubin plasma concentration at the last sampling time should be indicative of a better defense from oxidative damage (Aliyu et al., 2007).

An increase in plasma HDL values in the supplemented groups was observed at the end of the trial. Brites et al. (2017) reported that the HDL values showed antioxidant and antiatherogenic activities that suggest it protects LDL from oxidation. The improved blood lipid profile may be related to the effects of polyphenols, which are involved in the regulation of lipid and glucose metabolism (Attia et al., 2018). According to some authors (Bursill and Roach, 2007), this bioactive compounds activate the PPAR-α receptor, with an increased stimulation effect in the liver of the expression of key proteins involved in the metabolism of HDL. Triglycerides also seem to be involved in the same mechanism of activation of PPAR-α by polyphenols, with an induction in lipoprotein lipase expression in peripheral tissues and increased lipolysis, but in our study, no dietary effect on the triglyceride was observed.

Our previous study in sheep, hare and piglets fed with polyphenols revealed a significant reduction in triglycerides, total cholesterol, and LDL cholesterol along with an increased HDL cholesterol (Corino et al. 2007; Palazzo et al. 2011; Casamassima et al. 2012). The present data suggest that natural extracts contain several hypocholesterolemic agents that might prove valuable for the modulation of lipid metabolism and prevention of cardiovascular diseases (Attia et al., 2018).

Plasma antioxidant markers

The dietary supplementation with the brown seaweed and plant polyphenols mixture improved the markers of plasma oxidative status. The bioactive compounds contained in the feed additive (phenolic acid, hydroxycinnamic acids, tannins and flavonoids), are redox-active molecules, and can be oxidised and reduced without becoming highly reactive-radical; they thus, protect against free radicals (Attia et al., 2018). A consequent reduction in lipid peroxidation was observed, as also highlighted by the improvement in the enzymatic marker levels. The reduction in lipid peroxidation could be related to the direct capture of free radicals due to the antioxidant activity of bioactive molecules during the propagation phase of the chain reaction. In addition, the initial oxidative process may be blocked through the inhibition of the pro-oxidant enzymes that produce free radicals (Kamiloglu et al., 2006). The increase in plasma liposoluble vitamins may also be attributed to the ability of the natural compounds to strengthen the endogenous antioxidant system. This is achieved by controlling the oxidative metabolism, by reducing the production of reactive oxygen radicals, and by inducing enzymes with antioxidant activities (Zhu et al., 1999). Comparable data have been obtained in previous studies on hares, on naturally milk-fed lambs and ewes, all fed a diet supplemented with a natural extract rich in polyphenols (Palazzo et al., 2011; Casamassima et al., 2012, 2013a). Also, in pigs (Rossi et al., 2013, 2017) and broilers (Attia et al., 2017a; 2017b; 2018), a dietary supplementation with natural extracts increased the blood antioxidant activity, which in pigs was measured with a biological KRL test.

5.7 CONCLUSION

Our data on the productive and reproductive performances suggest that the lower dosage of dietary supplement containing prebiotic polysaccharides from brown seaweeds (*Laminaria spp*.) plus phenolic acid, hydroxycinnamic acids, tannins, flavonoids from plant extracts positively affect the antioxidant status of does without influence other parameters. An environmentally-friendly dietary integration seems to be promising in supporting the does' health, by enhancing the antioxidant status. Further studies *in field* condition are needed to evaluate the effects of feed supplements on rabbit does' zootechnical parameters an to explore the mechanism of action on gut health.

5.8 REFERENCES

Abdel-Khalek, A.M., Selim, N.A., El-Medany, Sha A., Nada, S.A. (2008). Response of doe rabbits to dietary antioxidant vitamins E and C during pregnancy and lactation. Nutrition and Digestive Physiology, 9thWorld Rabbit Congress, Verona, Italy, 519–524.

Albert-Puleo, M. (1980). Fennel and anise as estrogenic agents. J. Ethnopharmacol. 2(4), 337-44.

Aliyu, R., Gatsing, D., Jaryum K.H. (2007). The effects of *Boswellia dalzielii* (Burseraceae) aqueous bark extract on rat liver function. Asian J. Biochem. 2, 359–363. doi:10.3923/ajb.2007.359.363.

AOAC (2000). Official methods of analysis (17th edition) Gaithersburg, MD: Association of Analytical Communities.

Attia Y.A., Al-Harthi M.A., Hassan S.S. (2017a). Turmeric (Curcuma longa Linn.) as a phytogenic growth promoter alternative for antibiotic and comparable to mannan oligosaccharides for broiler chicks. Rev. Mex. Cienc. Pecu. 8(1): 11-21.

Attia Y.A., Bakhashwain Ahmed A., Bertu Nehal K. (2017b). Thyme oil (Thyme vulgaris L.) as a natural growth promoter for broiler chickens reared under hot climate. Ita J. Anim. Sci. 16: 275–282.

Attia Y.A., Hamed R.S., Bovera F., Abd El-Hamid A.E., Al-Harthi M.A., Shahba H.A. (2017c). Semen quality, antioxidant status and reproductive performance of rabbits bucks fed milk thistle seeds and rosemary leaves. Anim. Reprod. Sci. 184: 178-186.

Attia Y.A., Bakhashwain A.A., Bertu N.K. (2018). Utilisation of thyme powder (Thyme vulgaris L.) as a growth promoter alternative to antibiotics for broiler chickens raised in a hot climate. Europ. Poult. Sci. 82: 15 pp, doi:10.1399/eps.2018.238.

Benzie, F.F., Strain J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239, 70-76.

Brites, F., Martin, M., Guillas, I., Kontush, A. (2017). Antioxidative activity of high-density lipoprotein (HDL): Mechanistic insights into potential clinical benefit. BBA Clinical 8, 66–77. https://doi.org/10.1016/j.bbacli.2017.07.002.

Bursill, C., Roach, P. (2007). A green tea catechin extract upregulates the hepatic low density lipoprotein receptor in rats. Lipids 42, 621-627. https://doi.org/10.1007/s11745-007-3077-x.

Casamassima, D., Nardoia, M., Palazzo, M., Vizzarri, F., D'Alessandro, A.G., Corino, C. (2014). Effect of dietary extruded linseed, verbascoside and vitamin E supplements on yield and quality of milk in Lacaune ewes. J. Dairy Res. 81, 485-493. https://doi.org/10.1017/S0022029914000387.

Casamassima, D., Palazzo, M., Martemucci, G., Vizzarri, F., Corino, C. (2012). Effects of verbascoside on plasma oxidative status and blood and milk production parameters during the peripartum period in Lacaune ewes. Small Ruminant Res. 105, 1-8. doi:10.1016/j.smallrumres.2011.12.001.

Casamassima, D., Palazzo, M., Vizzarri, F., Ondruska, L., Massanyi, P., Corino C. (2017). Effect of dietary Lippia citriodora extract on reproductive and productive performance and plasma biochemical parameters in rabbit does. Anim. Prod. Sci. 57, 65–73. https://doi.org/10.1071/AN14845.

Casamassima, D., Palazzo, M., D'Alessandro, A.G., Colella, G.E., Vizzarri, F, Corino, C. (2013). The effects of lemon verbena (*Lippia citriodora*) verbascoside on productive performance, plasma oxidative status, and some blood metabolites in suckling lambs. J. Anim. Feed Sci. 22, 204-212. https://doi.org/10.22358/jafs/65989/2013

Chavatte-Palmer, P., Tarrade, A., Kiefer, H., Duranthon, V., Jammes, H. (2016). Breeding animals for quality products: not only genetics. Reprod. Fert. Develop. 28, 94-111. doi:org/10.1071/rd15353.

Corino, C., Cannata, S., Musella, M., Pastorelli, G., Rossi, R. (2007). Influence of verbascoside on lipid profile in weaned piglets. Ann. Nutr. Metab. 51, 270. doi: 10.1159/000105121.

Descamps, V., Colin, S., Lahaye, M., Jam, M., Richard, C., Potin, P., Barbeyron, T., Yvin, J.C., Kloareg, B. (2006). Isolation and culture of a marine bacterium degrading the sulfated fucans from marine brown algae. Mar. Biotechnol. 8, 27-39. doi: 10.1007/s10126-005-5107-0.

Devirgiliis, C., Zinno, P., Perozzi G., (2013). Update on antibiotic resistance in foodborne *Lactobacillus* and *Lactococcus* species. Front. Microbiol. 4, 301. doi: 10.3389/fmicb.2013.00301.

Djakalia, B., Guichard, B.L., Fernande, A.E., (2012). Effect of palm oil and soya bean oil on growth performance and health of rabbit before and after weaning. J. Appl. Anim. Res. 40, 56-62. https://doi.org/10.1080/09712119.2011.627137.

Esterbauer, H., Zollner, H., (1989). Methods for determination of aldehydic lipid peroxidation products. Free Radical Bio. Med. 7, 197-203.

Grilli, G., Ferrazzi, V., Agnoletti, F., Piccirillo, A., Pisoni, A.M., Gallazzi, D. (2006). La patologia enterica nel coniglio da carne allevato in Italia. Rivista di zootecnia e veterinaria 34, 51-56.

Huang, Q., Liu, X., Zhao, G., Hu, T., Wang, Y. (2018). Potential and challenges of tannins as an alternative to in-feed antibiotics for farm animal production. Animal Nutrition 4, 137-150. https://doi.org/10.1016/j.aninu.2017.09.004.

IBM Corp. Released (2016). IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.

Kamiloglu, N.N., Beytut, E., Aksakal, M. (2006). Alteration in antioxidant status and lipid peroxidation of sheep previously treated with vitamin A and b-carotene during breeding and periparturient period. B. Vet. I. Pulawy 50, 171–177.

Lillehoj, H., Liu, Y., Calsamiglia, S., Fernandez Miyakawa, M.E., Chi, F., Cravens, R.L., Oh, S., Gay, G.C. (2018). Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Vet. Res. 49:76. doi: 10.1186/s13567-018-0562-6.

Makkar, H.P.S., Tran, G., Heuzé, V., Reverdin, S.G., Lessire, M., Lebas, F., Ankers, P. (2015). Seaweeds for livestock diets: A review. Anim. Feed Sci. Technol. 212, 1–17. doi.org/10.1016/j.anifeedsci.2015.09.018.

Maghin, F., Ratti, S., Corino, C. (2014). Biological functions and health promoting effects of brown seaweeds in swine nutrition. Journal of Dairy, Veterinary & Animal Research 1, 14-16. 10.15406/jdvar.2014.01.00005.

Okab, A.B., Samara, E.M., Abdoun, K.A., Rafay, J., Ondruska, L., Parkanyi, V., Pivko, J., Ayoub, M.A., Al-Haidary, A.A., Aljumaah, R.S., Peter, M., Lukac, N. (2013). Effects of dietary seaweed (*Ulva lactuca)* supplementation on the reproductive performance of buck and doe rabbits. J. Appl. Anim. Res. 41, 347–355. doi:10.1080/09712119.2013.783479.

Palazzo, M., Vizzarri, F., Cinone, M., Corino, C., Casamassima, D. (2011). Assessment of a natural dietary extract titrated in phenylpropanoid glycosides, on blood parameters and plasma oxidative status in intensively reared Italian hares (*Lepus Corsicanus*). Animal 5, 844–850. doi10.1017/S1751731110002569.

Pastorelli G., Rossi, R., Corino C. (2012). Influence of Lippia citriodora verbascoside on growth performance, antioxidant status, and serum immunoglobulins content in piglets. Czech Journal of Animal Science, 57(7): 312–322. doi: 10.17221/6006-CJAS.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C.A. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio. Med. 26, 1231-1237.

Rossi, R., Pastorelli, G., Corino, C. (2013). Application of KRL test to assess total antioxidant activity in pigs: sensitivity to dietary antioxidants. Res. Vet. Sci. 94, 372–377. doi: 10.1016/j.rvsc.2012.08.005.

Rossi, R., Stella, S., Ratti, S., Maghin, F., Tirloni, E., Corino, C. (2017). Effects of antioxidant mixtures in the diet of finishing pigs on the oxidative status and shelf life of *Longissimus dorsi* muscle packaged under modified atmosphere. J. Anim. Sci. 95, 4986-4997. doi: 10.2527/jas2017.1603.

Russo, R., Pucci, L., Giorgetti, L., Árvay, J., Vizzarri, F., Longo, V., Pozzo, L. (2017). Polyphenolic characterisation of plant mixture (Lisosan® Reduction) and its hypocholesterolaemic effect in high fat diet-fed mice. Nat. Prod. Res. doi.org/10.1080/14786419.2017.1402328.

Yang, C., Chowdhury, M.A., Huo, Y., Gong J. (2015). Phytogenic compounds as alternatives to in-feed antibiotics: potentials and challenges in application. Pathogens 23, 137-56. doi: 10.3390/pathogens4010137.

Uhlířová L., Volek Z. (2019). Effect of dehulled white lupine seeds on the milk production and milk composition in rabbit does and the growth performance of their litters before weaning. J. Anim. Feed Sci. 29: article in press, https://doi.org/10.22358/jafs/110371/2019.

Valenzuela-Grijalva, N.V., Pinelli-Saavedra, A., Muhlia-Almazan, A., Domínguez-Díaz, D., González-Ríos, H. (2017). Dietary inclusion effects of phytochemicals as growth promoters in animal production Journal of Animal Science and Technology. 59: 8. doi: 10.1186/s40781-017-0133-9.

Zhao, B., Tham, S.Y., Lu, J., Lai M.H., Lee, L.K.H. Moochhala, S.M. (2004). Simultaneous determination of vitamins C, E, and β carotene in human plasma by high-performance liquid chromatography with photodiode-array detection. J. Pharm. Pharm. Sci. 7, 200-204.

Zhou, J.Y., Prognon, P. (2006). Raw material enzymatic activity determination: A specific case for validation and comparison of analytical methods-the example of superoxide dismutase (SOD). J. Pharmaceut. Biomed. 40, 1143-1148. doi: 10.1016/j.jpba.2005.09.022.

Zhu, Q.Y., Huang, Y., Tsang, D., Chen, Z.Y. (1999). Regeneration of a-tocopherol in human low density lipoprotein by green tea catechin. J. Agr. Food Chem. 47, 2020–2025.

CHAPTER 6

Trial 3. Effects of dietary levels of brown seaweeds and plant polyphenols on growth and meat quality parameters in growing rabbit.

Pubblished in:

Meat Science, 161(2020) 107987

6.1 ABSTRACT

Growth performances, carcass characteristics and meat quality parameters from growing rabbit fed with two levels of dietary brown seaweed (*Laminaria spp*) and plant polyphenols were investigated. One hundred and forty-four New Zealand White rabbits were allotted into three dietary treatments containing 0 (C), 0.3% (T1), and 0.6% (T2) of brown seaweed and plant polyphenols mixture for 42 days. Growth performances and carcass weight were improved in T1 group. Vitamin A and E content in *Longissimus thoracis and lumborum* (LTL) and *Semimembranosus* (SM) muscle were enhanced in the treated groups. In the SM muscle, the oxidative stability was improved in rabbit fed with both dosages of dietary supplement, and the cholesterol content tended to be lower in T1 than in T2 and C groups. The LTL and SM muscle sensory characteristics were improved. In conclusion, dietary integration with a low dosage of brown seaweed and plant polyphenols is a valid strategy for enhance growth performance and produce healthier rabbit meat.

6.2 INTRODUCTION

Animal welfare and farm sustainability are key factors in animal production systems (Dawkins, 2016). Consumers increasingly demand products of animal origin that come from production chains certified for animal welfare. Production institutions also restrict antibiotic use to prevent antibiotic resistance (Roca et al., 2015). Therefore, sustainable nutritional strategies able to support animal health and enhance product quality are required.

In recent years, herbs and spices containing polyphenols have been investigated as feed supplements to improve rabbit health and meat quality parameters, due to their effects on the digestive function and growth performance and their antioxidant and antimicrobial properties (Dalle Zotte, Celia & Szendrő, 2016). In this context, seaweeds are also potentially important in animal nutrition due to their high content of bioactive molecules (Makkar et al., 2016). In particular, brown seaweed has been of interest as a functional dietary ingredient, due to its various health benefits related to its sulfated polysaccharides, phlorotannin, diterpenes, minerals and vitamins content (Maghin, Ratti & Corino, 2014).

Rabbit meat is particularly appreciated by consumers due to its healthy properties (Wang, Su, Elzo, Jia, Chen, & Lai, 2016). Compared to other meats, rabbit has low fat and cholesterol content and high levels of protein with essential amino acids, and with a high digestibility value (Dalle Zotte, 2002). The high degree of unsaturation of fatty acids makes this meat particularly susceptible to oxidative processes during storage, with negative effects on sensory parameters and nutritional value (Dal Bosco et al., 2014). Previous studies have reported that in rabbit meat lipid oxidation can be prevented using vitamin E or natural extract supplements, which are good sources of dietary antioxidants (Corino, Pastorelli, Pantaleo, Oriani, & Salvatori, 1999; Dal Bosco et al., 2014; Dalle Zotte et al., 2016; Vizzarri, Palazzo, D'Alessandro, & Casamassima, 2017).

There is a growing interest in the use of natural supplements in rabbit nutrition to enhance productive performance, thus improving rabbit health and meat quality parameters (Hassan, Mahrose, & Basyony, 2016). Dalle Zotte et al., (2016) reported that several herbs and spices containing polyphenols have shown positive effects such as being growth promoters, antimicrobials and antioxidants in rabbit species. Makkar et al. (2016) reported that dietary supplementation with brown seaweed in rabbit has different effects: *Laminaria spp.* improved blood lipid profiles, but the use of *Ascophillum nodosum* should be avoided because it had a toxic effect. No previous studies have reported the effects of dietary brown seaweed in association with plant polyphenols in growing

rabbit and on growth performances and meat quality parameters. Thus, the aim of the study was to investigate the effects of a dietary brown seaweeds and plant polyphenols mixture on productive performance, carcass characteristics, and meat quality parameters in growing rabbits.

6.3 MATERIAL AND METHODS

Animal and experimental treatments

Rabbits were handled following the guidelines for animal experiments, indicated in the EU Directive 2010/63/EU and national guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the National Agricultural and Food Centre ethical committee (No. NPPC 18-10-2016).

A total of 144 New Zealand White rabbits, half males and half females, were housed at the National Agricultural and Food Centre, Nitra (Slovak Republic). At weaning, the 35-day-old rabbits were randomly allotted into three experimental groups balanced for sex (48 rabbits per treatment).

Rabbits were housed in cages (2 females and 2 males cage) and the trial lasted 42 days. The cages were equipped with a hopper for feed and an automatic nipple drinking system. The lighting cycle throughout the trial was 16h of light and 8h of dark. Heating and forced ventilation system allowed the building temperature to be maintained within 18 ± 4° C. The relative humidity was about 70 ± 5%. Rabbits were fed a control diet (C) or T1 and T2 diets, which were supplemented with 0.3% and 0.6% of feed additive consisting of prebiotic polysaccharides from brown seaweeds (*Laminaria Digitate* and *Hyperborea*, ratio 1:1) plus phenolic acid, hydroxycinnamic acids, tannins, and flavonoids from plant extracts.

The diets included no anticoccidials, antibiotics or other medications. The supplement was included in the basal mashed diet. All the experimental diets were pelleted. The two dosages of the natural extract were chosen after an *in vitro* evaluation of the minimal inhibitory concentration (MIC) against *Clostridium* spp., *Staphylococcus* spp., and *Escherichia coli* spp. (Tosi, personal communication). The ingredients and chemical composition of the experimental diets are reported in Table 1.

The chemical composition of the diets and feed supplement were in accordance with the methods of the Association of Analytical Chemists (AOAC, 2002). The quantitative analysis of the phenolic compounds of the supplement was performed using HPLC-UV-DAD, according to Russo et al. (2017). The quantification of beta-carotene of the feed supplement was performed in accordance with the method proposed by Rakusa, Srecnik, & Roskar (2017). The chemical composition, phenolic composition and carotenoid content of the feed supplement is reported in Table 2.

Throughout the study feed was available *ad libitum* and animals were monitored daily to assess their health conditions. They were weighted at the beginning (0 day), at 21 days and at the end of the experiment trial (42 days). The daily feed intake was calculated from the amounts of feed offered and refused weekly.These data were used to calculate the average daily gain (ADG), average daily feed intake (ADFI), and feed convertion ratio (FCR).

At 77 days old all rabbits were weighted, and after 6 h fasting, 12 animals per group (1 male rabbit/cage) were randomly selected and slaughtered at the research center slaughterhouse. Rabbits were subjected to electrical stunning and sacrificed by bleeding according with the guidelines established by the European Community (1099/2009/EC) for the protection of animals during slaughter. Carcasses were chilled for 24 h at +4°C and then dissected, according to the

recommendations of the WRSA (Blasco & Ouhayoun, 1996), discarding the skin, the distal part of the limbs, genitals, bladder, and gastrointestinal tract, and carcass measurements and meat quality analyses were conducted. The *Longissimus thoracis* and *lumborum* (LTL) muscle and thighs (n = 12) were removed from each carcass. Samples were vacuum packed and stored at -20°C until lab analyses. The S*emimembranosus* (SM) and the LTL muscles (n = 12) were subjected to lab analyses to investigate their meat quality parameters.

Table 1. Ingredients and chemical composition of experimental diets (g/kg).

	Experimental diet [a]		
Ingredients	CON	T1	T2
Maize	282	279	276
Alfalfa hay	305	305	305
Sunflower meal	135	135	135
Palm seed oil	8	8	8
Soybean oil	7	7	7
Wheat	80	80	80
Cane molasses	20	20	20
Carob bean meal	90	90	90
Oat	53	53	53
Calcium carbonate	7	7	7
Sodium Chloride	3	3	3
Dicalcium phosphate	2	2	2
DL-Methionine (99%)	2.5	2.5	2.5
L-Lysine HCl (78.5%)	1.6	1.6	1.6
Choline (75%)	1.4	1.4	1.4
Vitamin and mineral premix [c]	2.5	2.5	2.5
Dietary supplement	0	3	6
Chemical composition, [b]			
Crude protein	184	183.6	183.5
Ether extract	35.7	35.5	35.5
Crude fibre	187	186.8	187
Ash	86	85.7	85.8
Nitrogen free extract	507	507.1	506.9
NDF	302.1	301.5	301.7
ADF	195.8	195.4	195.3
ADL	39.9	39.5	39.5

[a] CON= control group; T1= group supplemented with 0.3% of brown seaweed and plant polyphenols ; T2= group supplemented with 0.6% of brown seaweed and plant polyphenols ;
[b] Analyses determined in triplicate.
[c] Supplied per kg diet: 13,500 I.U. vitamin A (trans-retinyl acetate); 800 I.U. vitamin D3 (cholecalciferol); 35 mg vitamin E (α-tocopherol min 91%), 35 mg copper (cupric sulphate pentahydrate).

Table 2. Chemical composition and polyphenols content of the dietary supplement.

Item	% on dry matter
Dry matter	93.58 ± 5.05
Crude Protein	7.21 ± 0.99
Ether extract	0.32 ± 0.01
Crude fibre	11.20 ± 1.02
Carbohydrates	60.84 ± 3.18
Ash	32.68 ± 1.38
Compounds: [b]	mg/kg dry weight
β-Carotene	402 ± 30.89
Phenolic Acid:	
Dihydroxybenzoic acid	≤ LOD [a]
Syringic acid	1059.79 ± 62.82
Hydroxycinnamic acids:	
Neochlorogenic acid	7979.23 ± 468.11
Rosmarinic acid	126.54 ± 8.67
Trans sinapic acid	105.54 ± 8.09
Chlorogenic acid	21.45 ± 3.65
Tannins:	
Ellagic acid	2440.88 ± 148.29
Rutin	272.37 ± 20.82
Flavonoids:	
Myricetin	53.88 ± 5.68
Kaempferol	≤ LOD

[a] Limit of detection;
[b] value are expressed as means (n= 4) ± standard deviation.

Physical parameters

The pH and color parameters were measured 24 h after slaughter. The pH was performed using a pH meter (HI98191 microcomputer; Hanna Instruments, Vila do Conde, Portugal). The pH probe was calibrated using standard buffers of pH 4.0 and 7.0 and the maintenance of calibration was monitored between samples.

The International Commission on Illumination's (CIE) lightness (L*), redness (a*), and yellowness (b*) values were measured for the samples using a CR-300 Chroma Meter (Minolta Camera, Co., Osaka, Japan). The instrument was calibrated on the Commission Internationale d'Eclairage (CIE) LAB color space system using a white calibration plate (Calibration Plate CR-A43; Minolta Camera Co.). The colorimeter had an 8-mm measuring area and was illuminated with a pulsed Xenon arc lamp (illuminat C) at a viewing angle of 0°. Reflectance measurements were obtained at a viewing angle of 0° and the spectral component was included. The color variables were measured at three different points on the central part of the samples. Moreover, total color differences (TDC) were calculated using the following equation: $\Delta E^* = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$.

Chemical parameters

Dry matter (DM), protein, ether extract (EE), and ash contents of the LTL and SM muscles were determined according to AOAC (2002) methods. Dry matter was determined by the oven drying method at 105°C until constant weight (method 950.46), protein by the Kjeldahl method (method 990.03) using a 6.25 factor to convert the nitrogen content into total protein, the ether extract by Soxhlet extraction (method 920.39), and ash by using a muffle furnace for 12 h at 550°C (method 920.153).

The cholesterol content of the LTL and SM muscles was determined in accordance with the procedure of Du and Ahn (2002). Lipids were extracted from 1.5 g of minced meat homogenate with 33% KOH (ratio of 94:6), using Ultra-Turrax T18 Homogenizer (IKA, Cincinnati, USA) and keeping in ice to avoid oxidation processes. Cholesterol was extracted with 5 ml of hexane, and 1 µl was injected into the gas chromatograph. The cholesterol was identified based on the retention time of the standard (Sigma Aldrich, St. Louis, USA), and quantified with the Chrom Card Data System (version 1.17) software by comparing the peak area with the reference standard curve. All samples were analyzed in triplicate.

Oxidative stability

Meat oxidative stability was measured by evaluating the thiobarbituric acid-reactive substances (TBARS) content of 4°C chilling SM meat samples at 0 h, and then at 24 h and 72 h, in accordance with Meineri, Cornale, Tassone, and Peiretti (2010). The implemented method was as follows: 500 mg of meat was homogenized with 10 mL of distilled water using a Homogenizer Ultra Turrax T25 (IKA, Cincinnati, USA), and 2.5 mL of 25% trichloroacetic acid was added to the homogenized sample, cooled at 4°C for 15 min, and then centrifuged at 4000 g at 4°C for 5 min. The supernatant was filtered through Whatman 52 filter paper, and an aliquot of 3.5 mL was added to 1.5 mL of 0.67% thiobarbituric acid and incubated at 70°C for 30 min.

Immediately after cooling, the absorbance of the sample was read in a spectrophotometer at 532 nm and compared to a standard curve of malondialdehyde (MDA; Sigma Aldrich, St. Louis, USA). All analyses were performed in duplicate and the results were expressed as mg of MDA per kg of meat.

Vitamin E and A content

Alpha-tocopherol and retinol were determined in both muscles using a procedure modified from Zaspel and Csallany (1983). The muscles were analyzed using an HPLC system (Kontron Instruments, Milan, Italy) consisting of an autosampler (HPLC autosampler 360, Kontron Instruments, Milan, Italy) with a loop of 20 μL, a high-pressure pump and a C18 column 5 μm, 150 mm x 4.6 mm (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile and methanol (75: 25 v/v) and a flow rate of 1 mL per min was used. The alpha-tocopherol and retinol were identified using a fluorimeter detector and comparing the samples' retention time with the pure standards (97%) purchased from Sigma Aldrich (St. Louis, USA). The quantification was carried out using the Geminyx system (version 1.91) by comparing the area sample peak with that of the reference standards curve.

Sensory evaluation

The LTL muscle and thigh preparation for sensory analysis was conducted after thawing for 24h at 4°C. Both samples were then prepared as single pieces in an uncovered stainless-steel dish in a conventional oven (REX, Italy) at 180°C. A thermocouple (Pentronic AB, Gunnebobruk, Sweden) was inserted into the center of each piece of meat to register the core temperature. The samples were removed from the oven at 75-80°C to allow for post-heating rise. After cooling the entire LTL muscle and thigh were cut into 1.5cm thick slices (Electrolux 50, 220-24, kW0.2). The slices were warmed to 60°C before the evaluation.

A trained sensory panel, consisting of eight members familiar with descriptive analysis procedures (EN ISO 13299, 2010), was established. All assessments were carried out in a sensory laboratory equipped according to EN ISO 8598, (1989) recommendations. The list of descriptors, definitions, and standards are reported in Palazzo, Vizzarri, Nardoia, Ratti, Pastorelli, and Casamassima (2015). The sensory profile was assessed according to EN ISO 13299 (2010) and the panel evaluated the two samples (thigh and LTL) on different days in triplicate. Within each session the design was balanced for order and carry-over effects (MacFie, Bratchell, Greenhoff, & Vallis, 1989). During training and sampling, the panelists had access to unlimited water and unsalted crackers. They were requested to evaluate the intensity of each attribute by assigning a score between 1 (absence of the sensation) and 9 (extremely intense).

6.4 STATISTICAL ANALYSIS

Data on productive performance and slaughter parameters, were analyzed using one-way analysis of variance (ANOVA), with diet as fixed effect and cage as random effect (SPSS/PC Statistics 25.0 SPSS Inc., IBM). Meat physical and chemical parameters were processed with a one way ANOVA, with diet as fixed effect. A repeated measure ANOVA with diet, storage time and their interaction as fixed effects, was used to analyzed TBARS data. Means were compared according to the Duncan's test. The sensory data were submitted to ANOVA with samples, judges, replicates, and their interactions as effects (EN ISO 13299, 2010). The significance of these effects was tested with F tests. Post-hoc pairwise contrasts were evaluated by Duncan's test. The cage was considered as the experimental unit for growth performance and the rabbit for the meat quality parameters. Data are reported as mean ± SEM. Differences among treatments were considered significant at $P < .05$.

6.5 RESULTS AND DISCUSSION

Productive performance and carcass characteristics

The data of the productive performance of growing rabbits are reported in Table 3. The live weight was improved at 21 days ($P < .05$) and tended to be higher at 42 days ($P = .06$) in rabbit fed a lower dosage of the dietary supplement (T1 group) than the other two groups. Therefore, the ADG (0-42 d) tended to be higher ($P = .06$) in the T1 group. The feed conversion ratio was also improved ($P < .01$) in rabbits fed with a diet containing 0.3% of the natural extract mixture. The ADFI was lower in T2 than in C groups ($P < .05$) in the first period of the trial (0-21 d). Considering the ADFI in the first period of the trial, it is possible that the high dosage of bioactive compounds of the supplement negatively affected diet palatability and consequently growth as previously observed in rat fed high dosage of ellagic acid (Cerdá, Cerón, Tomás-Barberán & Espín 2003).

The slaughter weight (2.71 ± 0.067 kg C *vs* 2.91 ± 0.035 kg T1 *vs* 2.80 ± 0.052 kg T2; $P = .013$) and carcass weight (1.61 ± 0.041 kg C *vs* 1.75 ± 0.026 kg T1 *vs* 1.66 ± 0.029 kg T2; $P = .012$) of the sampled rabbits were higher in the T1 group than in the others. The dressing percentage was not affected by the dietary treatments (59.4 ± 0.434 % C *vs* 59.6 ± 0.327 % T1 *vs* 59.7 ± 0.446 % T2; $P = .929$). The data is thus in agreement with previous studies of post-weaning piglets that reported an improvement in ADG due to dietary supplementation with *Laminaria* spp. extract (Gahan, Lynch, Callan, O'Sullivan, & O'Doherty, 2009; McDonnell, Figat, & O'Doherty, 2010). An enhancement of growth performance was also observed in broilers with a dietary supplementation of *Ascophillum nodosum* meal (Evans & Critchley, 2014). Brown seaweed contains a high amount of water-soluble polysaccharides such as laminarins, fucoidans, and alginates. These constituents have been shown to have prebiotic effects and to reduce pathogenic microorganisms in the gastrointestinal tract in both *in vitro* and *in vivo* studies (Chen et al., 2018; Sweeney et al., 2011). *In vitro* prebiotic activity of fucoidans and alginates from brown seaweed has been found with an increase in the growth rate of *Lactobacillus* spp. (Okolie, Rajendran, Udenigwe, Aryee, & Mason, 2017). In addition, Lynch, Sweeney, Callan, O'Sullivan, and O'Doherty (2010) reported that laminarins have antibacterial properties, stimulate *Bifidobacteria*, and increase the production of short chain fatty acid (SFA) in the gut.

Studies have reported conflicting data on plant polyphenols supplementation and the enhancement of growth performance. Zhao, Xu, Du, Li, and Zhang (2005) found an improvement in feed intake

and growth performance in growing rabbits fed traditional Chinese herbs, which contain polyphenols. In another study, dietary supplementation with microalgae spirulina and thyme was not found to affect rabbit performance (Dalle Zotte, Sartori, Bohatir, Remignon, & Ricci, 2013). Palazzo et al. (2015) also reported no differences in ADG and the final weight of rabbit fed *Lippia citriodora* extract.

In the present study, the active principles of brown seaweed and plant polyphenols had a positive effect on antioxidant status, as shown in the TBARS values and the vitamin E content of the muscles, and probably on gut bacteria populations, due to more efficient feed utilization and consequently an improvement in growth performance. Indeed, positive effects on digestibility of nitrogen (N), gross energy (GE), fibers (NDF), and ash have been reported in post-weaning weaned piglets (O'Doherty, Dillon, Figat, Callan, & Sweeney, 2010; O'Shea, McAlpine, Sweeney Varley & O'Doherty, 2014).

Table 3. Productive performances of growing rabbits fed control diet and diets supplemented with 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively).

Item	**Dietary treatment**				
	C	**T1**	**T2**	**SEM**	***P-value***
Live weight, g					
0d	830.2	846.0	789.4	21.02	0.161
21d	1860.9 [b]	1996.3 [a]	1825.3 [b]	44.15	0.024
42d	2655.9	2834.8	2725.2	52.40	0.066
ADG [c], g/d					
0d-21d	49.1	54.8	49.3	1.84	0.062
21d-42d	37.9	39.9	42.9	2.19	0.284
0d-42d	43.5	47.4	46.1	1.15	0.067
ADFI [d], g/d					
0d-21d	154.9[a]	142.0 [ab]	136.8 [b]	5.07	0.046
21d-42d	188.8	175.6	192.6	8.99	0.382
0d-42d	171.8	158.8	164.7	6.54	0.379
FC [e], kg/kg					
0d-21d	3.20	2.59	2.89	0.17	0.057
21d-42d	5.03 [a]	4.41 [b]	4.58 [ab]	0.18	0.049
0d-42d	3.94 [a]	3.35 [b]	3.60 [ab]	0.11	0.003

Data are reported as mean ± pooled SEM n=12 (cages with 4 rabbits per cage)

C= Control; T1 = dietary supplementation of 0.3% of polyphenols and seaweeds mixture and T2 = dietary supplementation of 0.6% of polyphenols and seaweeds

[a] Values in the same row are different at $P < .05$.

[b] Values in the same row are different at $P < .05$.

[c] ADG= average daily gain;

[d] ADFI= feed intake;

[e] FC = feed conversion ratio.

Meat quality parameters of LTL and SM muscles

The data on the physical and chemical parameters of the LTL and SM muscles are reported in Table 4 and 5, respectively. The pH values were affected by dietary treatments in both LTL and SM muscles ($P < .05$), but the ranges fall within the values reported in previous studies (Maj, Bieniek, & Łapa, 2008; Carrilho, López, & Campo, 2009).

No difference ($P > .05$) was observed for the color indexes in either muscle in terms of dietary treatments, in agreement with the data reported for the LTL and SM muscles (Daszkiewicz, Gugolek, Janiszewski, Kubiak, & Czoik, 2012; Tůmová, Bízková, Skřivanová, Chodová, Martinec, & Volek, 2014). The TCD values resulted 1.41 and 1.33 for T1 and T2 treatment respectively, indicating small difference in perceivable colour (TCD < 1.5) (Adekunte, Tiwari, Cullen, Scannell, & O'donnell, 2010). The chemical composition of the LTL muscle was not affected by the dietary treatment, except that the ash content was lower ($P < .001$) in muscles from rabbit in the T1 group than those in the C and T2 groups. These data are in line with the results of previous studies on growing rabbit (Dal Bosco, Castellini, Bianchi, & Mugnai, 2004; Daszkiewicz et al., 2012).

Table 4. Physical parameters of *Longissimus thoracis and lumborum* and *Semimembranosus muscle* of rabbits fed control diet (C) or diet supplemented with 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively).

	Dietary treatment				
Item	**C**	**T1**	**T2**	**SEM**	***P-value***
Longissimus thoracis and lumborum					
pH, 24 h	5.86 [b]	5.92 [a]	5.86 [b]	0.010	0.020
Color indexes:					
L*	55.91	55.38	57.41	0.460	0.180
a*	4.01	3.90	3.96	0.252	0.944
b*	11.89	11.71	11.58	0.212	0.845
Semimembranosus muscle					
pH, 24 h SM	5.75 [a]	5.75 [a]	5.84 [b]	0.015	0.021
Color indexes:					
L*	64.24	63.44	64.31	3.391	0.994
a*	5.46	6.25	6.31	0.523	0.776
b*	3.97	4.23	2.95	0.392	0.394

n=12; data are reported as mean ± pooled SEM;
[a] Values in the same row are different at $P < 0.05$.
[b] Values in the same row are different at $P < 0.05$.

Table 5. Chemical composition of *Longissimus thoracis and lumborum* and *Semimembranosus* muscle of rabbits fed control diet (C) or diet supplemented with 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively).

	Dietary treatment				
Item	**C**	**T1**	**T2**	**SEM**	***P-value***
Longissimus thoracic and lumborum					
Moisture, %	72.82	73.02	73.41	0.182	0.412
Crude protein, %	24.50	23.76	24.40	0.176	0.210
Ether extract, %	1.17	0.90	0.95	0.064	0.123
Ash, %	1.20^{A}	0.94^{B}	1.04^{B}	0.030	<0.001
Cholesterol, mg/100g	32.72	27.22	34.62	2.210	0.373
Semimembranosus muscle					
Moisture, %	73.79	73.67	73.67	0.269	0.533
Crude protein, %	22.62	22.82	22.81	0.176	0.803
Ether extract, %	1.52	1.91	1.52	0.015	0.981
Ash, %	1.21	1.19	1.15	0.030	0.329
Cholesterol, mg/100g	53.25	30.47	42.08	4.052	0.056

n=12; data are reported as mean ± pooled SEM;
$^{A, B}$ values in the same row are different at $P < 0.01$.

In the SM muscle, the cholesterol content tended to be lower (P = .052) in rabbit fed with the low dosage of dietary supplement (T1) than in those in the T2 and C groups. A previous study showed that dietary *Laminaria* spp. (1 g/d for 14 days) lowered cholesterol and triglycerides in rabbits with experimental hyperlipoproteinemia (Tang & Shen, 1989). In addition, Vizzarri et al. (2017) reported a lower cholesterol content in rabbit LTL muscle due to dietary supplementation with plant polyphenols, which can modulate the activity of enzyme 5-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which regulates the metabolic pathway for cholesterol synthesis (Kowalska & Bielański, 2009).

In other animal species such as pigs, donkeys, horses and lambs dietary supplementation with natural extracts containing plant polyphenols was found to slightly affect the pH, color indexes and muscle chemical composition (Rossi, Pastorelli, Cannata, Tavaniello, Maiorano, & Corino, 2013; Rossi et al., 2017a; Valenti et al., 2019).

Vitamin content of LTL and SM muscles

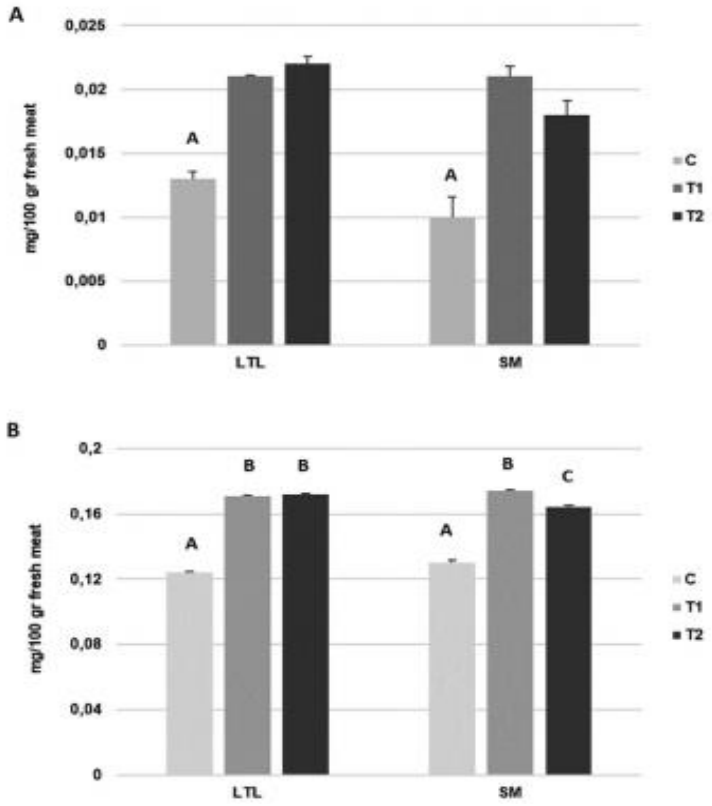
Figure 1 (A, B) shows the vitamin A and E content of LTL and SM muscles in terms of the dietary treatments. The vitamin A content was higher ($P < .001$) in the LTL muscle of rabbit fed brown seaweed and plant polyphenols (T1 and T2 groups) than in the control. In the SM muscle a higher content ($P < .001$) of vitamin A was observed in the T1 group than in the C and T2 groups. Vitamin E content was higher in the LTL muscles of rabbit in the T1 and T2 groups than in the control group. In the SM muscle a higher content ($P < .001$) of vitamin E was observed in the T1 group than in the C and T2 groups.

The higher content of vitamin A in muscles from rabbit of T1 and T2 groups than control should be related to the carotene content and the several antioxidant compounds from the dietary supplement. The carotenoids are present in *Laminaria* spp. in amount variable from 468 to 1065 mg/kg DM as reported by Jacobsen, Sorensen Holdt, Akoh, & Hermund (2019).

The dietary supplement contained several polyphenol compounds such as neochlorogenic acid, syringic acid and ellagic acids that possess a high antioxidant activity. As observed in rat the dietary hydroxycinnamic acid derivatives protecting vitamin E from oxidation in all tissues (Frank, Kamal-Eldin, Razdan, Lundh, & Vessby, 2003). Moreover, it is reported by Kumar, Prahalathan, & Raja (2012) that dietary supplementation with syringic acid in hypertensive rat positively affect vitamin E and C serum and tissue, reducing oxidative stress.

A previous study reported that *Ascophyllum nodosum* extract increases serum vitamin A in lamb and liver vitamin E in beef (Allen et al., 2001). Other studies of pigs reported that dietary supplementation with brown seaweed (*Ascophyllum* spp.; *Laminaria* spp.) increased the antioxidant status of piglets, measured as plasma superoxide dismutase, catalase, and muscle TBARS (Wang et al., 2016; Moroney, O'Grady, Robertson, Stanton, O' Doherty, & Kerry, 2015). The present data agree with Palazzo et al. (2015), who reported an increase in vitamin E content in the LTL muscle of New Zealand white rabbit fed a high dosage of *Lippia Citriodora* extract. Palazzo, Schiavitto, Cinone, and Vizzarri (2019) recently reported an increase in fat-soluble vitamins (vitamin A and vitamin E) in LTL muscles of rabbit fed natural extract containing the hydroxycinnamic ester derivative widely distributed in plants, called verbascoside, with a high antioxidant activity (Gil, Enache, & Oliveira-Brett, 2013). Thus, the higher muscle vitamin A and E content is related to the content of phenols, carotenoid fucoxanthin, tannins and phlorotannins and polysaccharides in brown seaweed as fucoidans, laminarans and vitamins (Jacobsen, Sorensen Holdt, Akoh, & Hermund, 2019) and to the antioxidant activity of plant polyphenols that preserve vitamin E oxidation through several well-known mechanisms (Cimmino et al., 2018).

Figure 1. Vitamin A (A) and Vitamin E (B) content of *Longissimus thoracis and lumborum* (LTL) and *Semimembranosus* muscle (SM) of rabbits fed control diet (C) or diet supplemented with 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively; n=12). Data are reported as mean ± SEM; $^{A, B, C}$ values with differ superscript letters are different at P < .001. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

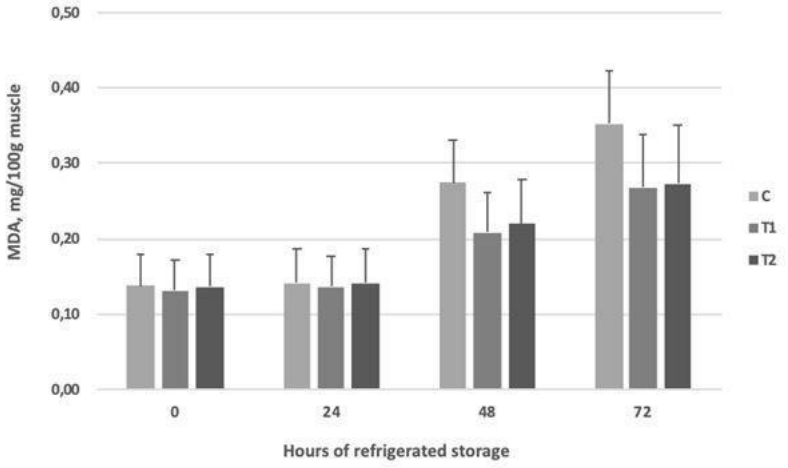


Oxidative stability of SM muscle

The oxidative stability of the SM muscle in terms of the dietary treatments and time of storage is reported in Figure 2. We analyzed the SM muscle to verify the antioxidant activity of the natural mixture on a muscle with a higher fat content than the LTL muscle and a different oxidative metabolism (Gondret et al., 2004). The oxidative stability of the SM muscle was affected (P < .001) by dietary treatments and storage time. A significant interaction between storage time and dietary treatment was also observed (P < .001). The oxidative stability at both sampling times was higher in groups fed the brown seaweed and polyphenols mixture than in the control. In agreement with the present data, Moroney, O' Grady, O'Doherty, and Kerry (2012) reported a high oxidative stability in the *Longissimus dorsi* muscle of pigs fed with seaweed extract (*Laminaria digitata*). In beef fed *Ascophyllum nodosum* extract a high muscle oxidative stability was also observed (Allen et al., 2001). A high oxidative stability in the LTL muscles of animals fed plant polyphenols was also reported in pigs, *Equidae*, goats and rabbit (Cimmino et al., 2018; Rossi et al., 2017a; Rossi, Stella, Ratti, Maghin, Tirloni, & Corino, 2017b; Palazzo et al., 2015; Palazzo et al., 2019). Usually natural antioxidant can reduce lipid oxidation, enhancing meat shelf-life, since they are able to block the oxidative chain propagation reactions.

Polyphenols have a high antioxidant activity, through several mechanisms: as a scavenger of free radicals (Zheng et al., 2009), as transition metal chelators (Andjelković et al., 2006) and as quencher of free singlet oxygen (Mukai, Nagai, & Ohara 2003).

Figure 2. Oxidative stability of *Semimembranosus* muscle of rabbits fed control diet (C) or diet supplemented with 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively) in relation to dietary treatments and time of refrigerated storage at 4°C.



Results are expressed as mean values ± SEM (n=12). Time effect for $P < .001$, Treatment effect for $P < .001$, interaction between time x Treatment for $P < .001$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Sensory profile

The F values for the aroma, taste, flavor and texture parameters of the LTL sensory profile are reported in Table 6. The results indicate that dietary supplementation with brown seaweed and plant polyphenols affected the aroma and flavor of the LTL muscle (P <. 05) The F values for replicates and interactions were not affected (P > .05) in all the descriptors, while judges presented differences (P < .01) for aroma and flavor. Differences between judges are common in sensory evaluations, due to the different use of the scale (Lea, Naes, & Rodbotten, 1997). No interaction between panelists × replicates and samples × replicates were observed.

In Table 7, the F values for aroma, taste, flavor, and texture parameters of the thigh sensory profile are reported. The data showed that dietary supplementation with brown seaweed and plant polyphenols affected the aroma and texture of the thigh (P <. 05). The F values for replicates were not affected (P > .05) for all the descriptors, while judges presented differences (P < .05) for aroma, flavor and texture. No interaction between judges × replicates and samples × replicates were observed in LTL, while in thigh, the interaction judges × samples was significant (P < .05). It is probably due to the type of samples analyzed. In fact, the thigh is more variable from a chemical point of view than LTL muscle that is more homogeneous, where we did not observe any statistical

interaction. These results did not influence the overall sensory evaluation, in fact no differences were reported for the same attributed perceived as flavour.

The F values for aroma, taste, flavor, and texture parameters in both the LTL muscle and the thigh highlight the excellent reproducibility of the scores given by the panelists and the homogeneity of samples during replicates.

The least squares mean of the different attributes for the LTL muscle and thigh are reported in Table 8 and Table 9, respectively. The data shows that taste and texture parameters were comparable in all the experimental groups. In the LTL muscle, a difference ($P < .05$) for aroma (rabbit, liver and rancid) and rabbit flavor were observed. The intensity of rabbit aroma and flavour was higher ($P < .05$) in the T1 and T2 groups than in the control, and the same result was observed for liver aroma. The rancid aroma was higher ($P < .05$) in the T2 group than in the T1 and control groups.

In the thigh, dietary treatments affected ($P < .05$) the aroma (rabbit, liver, and metallic) and the texture parameters. The rabbit aroma and flavor were higher ($P < .01$) in the T1 and T2 groups than in the controls and the same result was observed for liver aroma. The rancid aroma was higher ($P < .05$) in the T2 group than in T1 and the control groups. In terms of texture data, higher scores for tenderness and juiciness were observed in thighs from animals fed the natural supplement (T1 and T2) than in the control, while a lower score was given for stringiness ($P < .05$) in T1 and T2 than in the control. As previously observed in rabbit fed natural antioxidants, the high values for tenderness may be a result of the protection against the oxidation process (Palazzo et al., 2015). In our previous study of *Equidae*, dietary supplementation with an extract containing plant polyphenols enhanced meat texture parameters (Rossi et al., 2017a). This parameter is important for consumers' eating habits and is closely linked with consumer expectations of rabbit meat quality. The results of the present study indicate that the mean scores for each descriptor could be assumed to be satisfactory for the sensory profile of rabbit meat. The sensory evaluation showed that dietary supplementation with natural extract mixture containing brown seaweeds and plant polyphenols affects aroma in the LTL muscle and the aroma and texture in the thigh.

Table 6. Sensory evaluation of *Longissumus thoracis and lumborum* muscle: F value and statistical significance of treatments (n=3), judges (n = 8), replicates (n = 3) and their interaction for each sensory descriptor.

	F value					
Descriptors	Samples	Judges	Replicates	SxJ [a]	SxR	JxR
Aroma						
Rabbit	10.75***	1.40	0.92	1.21	1.16	0.64
Liver	7.88**	3.65**	0.31	2.86	0.12	0.27
Rancid	5.97*	4.17**	1.05	1.65	1.83	1.20
Taste						
Sweet	1.25	1.80	0.90	1.08	1.66	0.57
Salty	1.55	1.30	1.88	0.50	1.11	0.94
Flavour						
Rabbit	11.56***	4.07**	0.34	2.43	1.51	0.85
Liver	2.77	2.39**	0.81	1.49	0.60	0.31
Rancid	1.74	4.26**	0.32	2.20	0.39	0.56
Texture						
Tender	1.57	1.37	0.50	0.81	0.78	1.11
Juicy	0.24	1.39	1.89	0.76	0.83	1.33
Stringy	2.80	1.66	1.12	0.87	2.00	1.20

Significant: ***= 99,9%; ** = 99%; * = 95%; n.s. = no significant

[a] SxJ = Samples x Judges; SxR= Samples x Replicates; JxR= Judges x Replicates.

Table 7. Sensory evaluation of thigh: F value and statistical significance of treatments, judges (n = 8), replicates (n = 3) and their interaction for each sensory descriptor.

	F value					
Descriptors	Samples	Judges	Replicates	SxJ [a]	SxR	JxR
Aroma						
Rabbit	4.88*	1.51	1.54	0.60	0.67	1.13
Liver	3.92*	11.73***	1.38	5.31***	0.53	1.44
Metallic	3.61*	9.96***	0.20	4.90***	0.61	2.00
Taste						
Sweet	0.63	1.10	1.04	1.21	0.62	1.17
Salty	2.16	0.76	1.43	0.28	0.51	1.65
Flavour						
Rabbit	2.04	0.67	1.52	0.25	0.57	2.00
Liver	0.25	4.20	0.21	1.30	0.54	0.75
Metallic	0.29	4.26**	1.44	1.25	0.43	0.79
Texture						
Tender	3.30*	5.34	0.19	1.78	0.43	1.40
Juicy	8.70**	2.95*	1.30	0.84	0.69	0.97
Stringy	3.38*	4.26**	1.88	2.45*	0.97	1.09

Significant: ***= 99,9%; ** = 99%; * = 95%; n.s. = no significant.

[a] SxJ = Samples x Judges; SxR= Samples x Replicates; JxR= Judges x Replicates.

Table 8. Mean values of sensory attributes of Longissimus thoracis and lumborum and Semimembranosus muscle of rabbits fed control diet (C) or diet supplemented with 0.3 or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively).

	Dietary treatment		
Descriptors	C	T1	T2
Aroma			
Rabbit	5.0 [a]	5.8 [b]	6.4 [b]
Liver	5.0 [a]	5.4 [b]	5.7 [b]
Rancid	5.0 [a]	5.1 [a]	5.5[b]
Taste			
Sweet	5.0	4.4	5.0
Salty	5.0	5.4	5.4
Flavour			
Rabbit	5.0 [a]	5.8 [b]	6.1 [b]
Liver	5.0	5.4	5.6
Rancid	5.0	5.2	5.3
Texture			
Tender	5.0	5.3	5.5
Juicy	5.0	4.8	5.0
Stringy	5.0	5.6	5.7

[a, b] means within rows with different superscript letters differ significantly for $P < 0.05$.

Table 9. Mean values of sensory attributes of thigh of rabbits fed control diet (C) or diet supplemented with 0.3% or 0.6% of brown seaweed and plant polyphenols mixture (T1 and T2 respectively).

	Dietary treatment		
Descriptors	C	T1	T2
Aroma			
Rabbit	5.0 [a]	5.0 [a]	5.8 [b]
Liver	5.0 [b]	4.5 [a]	4.9 [b]
Rancid	5.0 [b]	4.6 [a]	5.0 [b]
Taste			
Sweet	5.0	5.1	5.3
Salty	5.0	5.4	5.4
Flavour			
Rabbit	5.0	5.4	5.5
Liver	5.0	4.8	4.8
Rancid	5.0	4.9	4.8
Texture			
Tender	5.0 [a]	5.3 [ab]	5.5 [b]
Juicy	5.0 [a]	4.8 [b]	5.8 [b]
Stringy	5.0 [b]	4.2 [a]	4.4 [ab]

[a, b] means within rows with different superscript letters differ significantly for $P < 0.05$.

6.6 CONCLUSION

Dietary supplementation with the low dosage of brown seaweeds and plant polyphenols mixture can be considered a useful nutritional strategy in rabbit meat production, since an improvement of feed conversion ratio and muscles vitamin A and E content was achieved. The higher muscle vitamin content enhances both nutritional quality and oxidative stability. Sensory parameters related to aroma, flavour and texture are positively affected by dietary treatment. In the present experimental condition, the low dosage of the natural extract mixture seems to be useful for enhancing rabbit meat production. The higher dosage of supplement does not produce any adverse effects on rabbit performance or meat quality parameters and should be utilized in a more stressful breeding condition.

6.7 REFERENCES

Adekunte, A. O., Tiwari, B. K., Cullen, P. J., Scannell, A. G. M., & O'donnell, C. P. (2010). Effect of sonication on colour, ascorbic acid and yeast inactivation in tomato juice. Food Chemistry, 122, 500-507.

Allen, V. G., Pond, K. R., Saker, K. E., Fontenot, J. P., Bagley, C.P., Ivy, R. L., Evans R. R., Schmidt, R.E., Fike, J. H., Zhang, X., Ayad, J. Y., Brown, C. P., Miller, M. F., Montgomery, J. L., Mahan, J., Wester, D. B., & Melton, C. (2001). Tasco: Influence of a brown seaweed on antioxidants in forages and livestock—A review. *Journal of Animal Science*, 79, E21–E3.

Andjelković, M., Van Camp, J., De Meulenaer, B., Depaemelaere, G., Socaciu, C., Verloo, M., & Verhe, R. (2006). Iron-chelation properties of phenolic acids bearing catechol and galloyl groups. Food Chemistry 98, 23–31.

AOAC. (2002). Official methods of analysis. 17th edition. *Journal - Association of Official Analytical Chemists*, Arlington, VA.

Blasco, A., & Ouhayoun, J. (1996). Harmonization of criteria and terminology in rabbit meat research. Revised proposal. *World Rabbit Science*, 4, 93–99.

Carrilho, M. C., López, M., & Campo, M. M. (2009). Effect of the fattening diet on the development of the fatty acid profile in rabbits from weaning. *Meat Science*, 83(1), 88–95.

Cerdá, B., Cerón, J. J., Tomás-Barberán, F. A., & Espín, J. C. (2003). Repeated oral administration of high doses of the pomegranate ellagitannin punicalagin to rats for 37 days is not toxic. Journal of Agricultural and Food Chemistry 51(11), 3493–3501.

Chen, X., Sun, Y., Hu, L., Liu, S., Yu, H., Xing, R., Li, R., Wang X., & Li, P. (2018). *In vitro* prebiotic effects of seaweed polysaccharides. *Journal of Oceanology and Limnology* 36(3), 926–932.

Cimmino, R., Barone, C. M. A., Claps, S., Varricchio, E., Rufrano, D., Caroprese, M., Albenzio, M., De Palo, P., Campanile, G., & Neglia, G. (2018). Effects of dietary supplementation with polyphenols on meat quality in Saanen goat kids. *BMC Veterinary Research*, 14(1), 181.

Corino, C., Pastorelli, G., Pantaleo, L., Oriani, G., & Salvatori, G. (1999). Improvement of color and lipid stability of rabbit meat by dietary supplementation with vitamin E. *Meat Science*, 52, 285–289.

Dal Bosco, A., Castellini, C., Bianchi, L., & Mugnai C. (2004). Effect of dietary α-linolenic acid and vitamin E on the fatty acid composition, storage stability and sensory traits of rabbit meat. *Meat Science*, 66, 407–413.

Dal Bosco, A., Mugnai, C., Roscini, V., Mattioli, S., Ruggeri, S., & Castellini, C. (2014). Effect of dietary alfalfa on the fatty acid composition and indexes of lipid metabolism of rabbit meat. *Meat Science*, 96, 606–609.

Dalle Zotte, A. (2002). Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livestock Production Science,* 75(1), 11–32.

Dalle Zotte, A., Sartori, A., Bohatir, P., Remignon, H., & Ricci R. (2013). Effect of dietary supplementation of Spirulina (Arthrospira platensis) and Thyme (Thymus vulgaris) on growth performance, apparent digestibility and health status of companion dwarf rabbits. *Livestock Science*, 152, 182–191.

Dalle Zotte, A., Celia, C., & Szendrő, Zs. (2016). Herbs and spices inclusion as feedstuff for additive in growin grabbit diets and as additive in rabbit meat: A review. *Livestock Science*, 189, 82–90.

Daszkiewicz, T., Gugolek, A., Janiszewski, P., Kubiak, D., & Czoik, M. (2012). The effect of intensive and extensive production systems on carcass quality in New Zealand White rabbits. *World Rabbit Science*, 20, 25–32.

Dawkins, M. S. (2016). Animal welfare and efficient farming: is conflict inevitable?. *Animal Production Science*, 57, 201–208.

Du, M., & Ahn, D. U. (2002). Simultaneous analysis of tocopherols, cholesterol, and phytosterols using gas chromatography. *Journal of Food Science,* 67(5), 1696–1700.

European Community. (2010). Council Directive 2010/63/EU of 22 September 2010 on the protection of animals used for scientific purposes. – [accessed May 23, 2019]. https://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:EN:PDF

European Community. (2009). Council Directive 1099/2009/EC of of 24 September 2009 on the protection of animals at the time of killing. - [accessed May 23, 2019]. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:303:0001:0030:EN:PDF.

Evans, F. D., & Critchley, A. T. (2014). Seaweeds for animal production use. *Journal of Applied Phycology,* 26, 891–899.

Frank, J., Kamal-Eldin, A., Razdan, A., Lundh, T., & Vessby B. (2003). The dietary hydroxycinnamate caffeic acid and its conjugate chlorogenic acid increase vitamin E and cholesterol concentrations in sprague−dawley rats. Journal of Agricultural and Food 473 Chemistry 519, 2526-2531.

Gahan, D. A., Lynch, M. B., Callan, J. J., O'Sullivan, J. T., & O'Doherty, J. V. (2009). Performance of weanling piglets offered low-, medium-or high-lactose diets supplemented with a seaweed extract from *Laminaria* spp. *Animal*, 3, 24–31.

Gil, E. S., Enache, T. A., & Oliveira-Brett A. M. (2013). Redox Behaviour of Verbascoside and Rosmarinic Acid. *Combinatorial Chemistry & High Throughput Screening*, 16(2), 92–97.

Gondret, F., Damon, M., Jadhao, S., Houdebine, L. M., Herpin, P., & Hocquette, J. F. (2004). Age-related changes in glucose utilization and fatty acid oxidation. *Journal of Muscle Research and Cell Motility*, 25, 405–410.

Hassan, F. A., Mahrose, K. M., & Basyony, M. M. (2016). Effects of grape seed extract as a natural antioxidant on growth performance, carcass characteristics and antioxidant status of rabbits during heat stress. *Archives of Animal Nutrition*, 70, 141–154.

ISO 8598. (1989). Sensory analysis: General guidance for design of test rooms, International Organization for Standardization, Geneva, Switzerland.

ISO 13299. (2010). International Organization for Standardization: Sensory analysis-Methodology-General guidance to establish a sensory profile, Geneva, Switzerland.

Jacobsen, C., Sorensen A. M., Holdt, S. L., Akoh, C. C., & Hermund, D. B. (2019). Source, Extraction, Characterization, and Applications of Novel Antioxidants from Seaweed. *Annual Review of Food Science and Technology*, 10, 541–68.

Kowalska, D. & Bielański, P. (2009). Meat quality of rabbits fed a diet supplemented with fish oil and antioxidant. *Animal Science Papers and Reports*, 27, 139–148.

Kumar, S., Prahalathan, P., & Raja B. (2012). Syringic acid ameliorates l-NAME-induced hypertension by reducing oxidative stress Naunyn-Schmiedeberg's Archives of Pharmacology, 385, 1175–1184.

Lea, P., Naes, T., & Rodbotten, M. (1997). Analysis of variance for sensory data. New York: Chirchester John Wilej & Sons Ltd.

Lynch, M. B., Sweeney, T., Callan, J. J., O'Sullivan, J. T., & O'Doherty, J. V. (2010). The effect of dietary Laminaria-derived laminarin and fucoidan on nutrient digestibility, nitrogen utilisation, intestinal microflora and volatile fatty acid concentration in pigs. *Journal Science of Food and Agriculture*, 90, 430–437.

MacFie, H. J., Bratchell, N., Greenhoff, K., & Vallis, L. V. (1989). Designs to Balance the Effect of Order of Presentation and First-Order Carry-Over Effect in Halls Tests. *Journal of Sensors and Sensor Systems*, 4, 129–148.

Makkar, H. P. S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F., & Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, 212, 1–17.

Maghin, F., Ratti, S., & Corino, C. (2014). Biological functions and health promoting effects of brown seaweeds in swine nutrition. *Journal of Dairy Veterinary & Animal Research,* 1(1), 00005.
Maj, D., Bieniek, J., & Łapa, P. (2008). Meat quality of New Zealand White and Californian rabbits and their crosses. *Medycyna Weterynaryjna*, 64, 351–353.

McDonnell, P., Figat, S., & O'Doherty, J.V. (2010). The effect of dietary laminarin and fucoidan in the diet of the weanling piglet on performance, selectedfaecal microbial populations and volatile fatty acid concentrations. *Animal,* 4, 579–585.

Meineri, G., Cornale, P., Tassone, S., & Peiretti, P. G. (2010). Effects of Chia (Salvia hispanica L.) seed supplementation on rabbit meat quality, oxidative stability and sensory traits. *Italian Journal of Animal Science,* 9, 45–49.

Moroney, N. C., O' Grady, M. N., O'Doherty, J. V., & Kerry, J. P. (2012). Addition of seaweed (*Laminaria digitata*) extracts containing laminarin and fucoidan to porcine diets: Influence on the quality and shelf-life of fresh pork. *Meat Science,* 92(4), 423–429.

Moroney, N. C., O'Grady, M. N., Robertson, R. C., Stanton, C., O'Doherty, J. V., & Kerry, J. P. (2015). Influence of level and duration of feeding polysaccharide (laminarin and fucoidan) extracts from brown seaweed (*Laminaria digitata*) on quality indices of fresh pork. *Meat Science,* 99, 132–141.

Mukai, K., Nagai, S., & Ohara, K. (2005). Kinetic study of the quenching reaction of singlet oxygen by tea catechins in ethanol solution. Free Radical Biology and Medicine 39, 752–761.

O'Doherty, J. V., Dillon, S., Figat, S., Callan, J. J., & Sweeney, T. (2010). The effects of lactose inclusion and seaweed extract derived from Laminaria spp. on performance, digestibility of diet components and microbial populations in newly weaned pigs. *Animal Feed Science and Technology,* 157, 173–180.

Okolie, C. L., Rajendran, S. R. C. K., Udenigwe, C. C., Aryee, A. N. A., & Mason, B. (2017). Prospects of brown seaweed polysaccharides (BSP) as prebiotics and potential immunomodulators. *Journal of Food Biochemestry*, 41, e12392.

O'Shea, C. J., McAlpine, P., Sweeney T., Varley P. F., & O'Doherty, J. V. (2014). Effect of the interaction of seaweed extracts containing laminarin and fucoidan with zinc oxide on the growth performance, digestibility and faecal characteristics of growing piglets. *British Journal of Nutrition,* 111, 798–807.

Palazzo, M., Vizzarri, F., Nardoia, M., Ratti, S., Pastorelli, G., & Casamassima, D. (2015). Dietary Lippia citriodora extract in rabbit feeding: effects on quality of carcass and meat. *Archives Animal Breeding*, 58, 355–364.

Palazzo, M., Schiavitto, M., Cinone, M., & Vizzarri, F. (2019). Rabbit metabolic response and selected meat quality traits: Evaluation of dietary PLX® 23 and LycoBeads® feed supplement. *Journal of Animal Physiology and Animal Nutrition*, 103, 383–394.

Rakusa, Z. T., Srecnik, E., & Roskar, R. (2017). Novel HPLC-UV method for simultaneous determination of fat-soluble vitamins and coenzyme Q10 in medicines and supplements. Acta Chimica Slovenica, 64, 523-529

Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., Cohen, J., Findlay, D., Gyssens, I., Heure, O. E., Kahlmeter, G., Kruse, H., Laxminarayan, R., Liébana, E., López-Cerero, L., MacGowan, A., Martins, M., Rodríguez-Baño, J., Rolain, J. M., Segovia, C., Sigauque, B., Taconelli, E., Wellington, E., & Vila, J. (2015). The global threat of antimicrobial resistance: science for intervention. *New Microbes New Infect*, 6, 22–9.

Rossi, R., Pastorelli, G., Cannata, S., Tavaniello, S., Maiorano, G., & Corino, C. (2013). Effect of long term dietary supplementation with plant extract on carcass characteristics meat quality and oxidative stability in pork. *Meat Science*, 95(3), 542–548.

Rossi, R., Ratti, S., Pastorelli, G., Maghin, F., Martemucci, G., Casamassima, D., D'Alessandro, A. G. & Corino, C. (2017a). Effect of dietary plant extract on meat quality and sensory parameters of meat from Equidae. *Journal of the Science of Food and Agricolture*, 97(14), 4690–4696.

Rossi, R., Stella, S., Ratti, S., Maghin, F., Tirloni, E., & Corino, C. (2017b). Effects of antioxidant mixtures in the diet of finishing pigs on the oxidative status and shelf life of Longissimus dorsi muscle packaged under modified atmosphere. *Journal of Animal Science*, 95(11), 4986–4997.

Russo, R., Pucci, L., Giorgetti, L., Árvay, J., Vizzarri, F., Longo, V., & Pozzo, L. (2017). Polyphenolic characterisation of plant mixture (Lisosan® Reduction) and its hypocholesterolaemic effect in high fat diet-fed mice. *Natural Product Research*.

Sweeney, T., Dillon, S., Fanning, J., Egan, J., O'Shea, C. J., Figat, S., Gutierrez, J. J M. Mannion C, Leonard, F., & O'Doherty, J. V. (2011). Evaluation of seaweed-derived polysaccharides on indices of gastrointestinal fermentation and selected populations of microbiota in newly weaned pigs challenged with Salmonella Typhimurium. *Animal Feed Science and Technology*, 165, 85–94.

Tang, Z. L., & Shen, S. F. (1989). A study of *Laminaria digitata* powder on experimental hyperlipoproteinemia and its hemorrheology. *Chinese journal of modern developments in traditional medicine*, 9(4), 223–5.

Tůmová, E., Bízková, Z., Skřivanová, V., Chodová, D., Martinec, M., & Volek, Z. (2014). Comparisons of carcass and meat quality among rabbit breeds of different sizes, and hybrid rabbits. *Livestock Science*, 165, 8–14.

Valenti, B., Natalello, A., Vasta, V., Campidonico, L., Roscini, V., Mattioli, S., Pauselli, M., Priolo, A., Lanza, M & Luciano, G. (2019). Effect of different dietary tannin extracts on lamb growth performances and meat oxidative stability: comparison between mimosa, chestnut and tara. *Animal*, 13(2), 435–443.

Vizzarri, F., Palazzo, M., D'Alessandro, A. G., & Casamassima, D. (2017). Productive performance and meat quality traits in growing 1 rabbit following the dietary supplementation of *Lippia citriodora, Raphanus sativus* and *Solanum lycopersicum* extracts. *Livestock Science,* 200, 53–59.

Wang, J., Su, Y., Elzo, M. A., Jia, X., Chen, S., & Lai, S. (2016). Comparison of carcass and meat quality traits among three rabbit breeds. *Korean Journal of Food Science of Animal Resources,* 36, 84–89.

Zaspel, B. J., & Csallany, A. S. (1983). Determination of alpha-tocopherol in tissues and plasma by high-performance liquid chromatography. *Analitycal Biochemestry*, 1,146–150.

Zhao, H. Q., Xu, H. J., Du, W. B., Li, L., & Zhang, D. F. (2005). Research and applications of Chinese herbal additives, *Chinese Journal Rabbit Farming*, 1, 31–33.

Zheng, G., Xu, L., Wua, P., Xie, H., Jiang, Y., Chen, F., & Wie, X. (2009). Polyphenols from longan seeds and their radical-scavenging activity. Food Chemistry, 116, 433–436.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

This thesis has focused on verifying the effects of different natural extracts in animal nutrition on reproductive and growth performance, physiological parameters and meat quality. It highlights the effects of plant and seaweed extracts as dietary treatments in piglets and rabbits in different physiological periods.

In the first experiment, *Ajuga Reptans* containing teupolioside improved piglet health in the post-weaning phase. Our group found that plant extracts containing teupolioside have a greater antioxidant activity compared to other phenolic compounds. Further studies are needed to specify the mechanism of action and find the optimal dosage and length of experiment in order to improve the antioxidant and immunomodulant effects of teupolioside.

In the second experiment, dietary supplementation with brown seaweeds and polyphenols showed positive effects on the antioxidant status in rabbit does. No significant effects of the supplemented diet were observed on the reproductive performance, thus further studies are needed with a greater number of animals targeted for treatment and in standard breeding conditions.

In the last experiment, results showed that the natural mixture positively affected the growth and meat quality parameters in growing rabbits. This mixed dietary supplementation could include antioxidants, prebiotics, immunomodulators and antimicrobial compounds that enhance not only the growth and animal health of growing rabbits but also without affecting the nutritional characteristics of the food and consumers' preferences.

In conclusion, I believe that this thesis improves knowledge on the beneficial effects of various plant extracts and seaweed. The dietary inclusion of bioactive components contained in natural extracts is an innovative nutritional approach that improves rabbit and piglets production without negative effects on the animals. Our study on growing rabbits presented important and interesting results, and the use of seaweeds and polyphenols could be considered as an innovative nutritional strategy.

To the best of our knowledge, this topic has never been investigated before in the scientific literature.

Increased consumer awareness and the consumption of safe, natural foods have prompted research into alternative animal feeding strategies that replace antibiotic growth promoters (AGPs) and synthetic antioxidants.

Suggestions for future studies: the following topics may be of interest for future research:

- More studies to evaluate the efficacy of algae and polyphenols at different inclusion levels in different species at different physiological periods aimed at enhancing animal health and productivity;

- Researchers should focus on the polyphenol content to clarify the optimal length of the plant extract dietary supplementation in order to enhance quality parameters in rabbit meat;

- Seaweeds have been shown to offer a wide range of activities, including improving animal performance and increasing nutrient availability. It is thus important to share the results of new experiments that show the effects of botanically and chemically well-characterized seaweed .

CHAPTER 8

ACKNOWLEDGEMENTS

At the end of my PhD experience I would like to thank Professor Carlo Corino, who supported me during the last three years. He gave me the possibility to travel to Europe for conferences and meet nice people.
I'd like to thank Francesco Vizzarri and Lubomir Ondruska who helped me during my period abroad in Slovak.
A big thank you also to Raffaella Rossi. She is a fantastic woman, a hard worker and very kind. I had a fantastic time with her during my PhD period, she helped me a lot.
A special thank to my lovely husband Luca, my family in particular my mum, gatto and my brothers Fulvio, Filippo and Matteo.
I wish to thank my colleagues Sabrina Ratti and Federica Maghin, the Trentacoste group, in particular Carlotta, Raffaella, Antonio and my new friends Erica, Lin from Taipei and Federica Ceriani. The last three years of my life was a great period, full of achievements and great deal of satisfaction. Now I'm ready for a new experience.