UNIVERSITY OF IOANNINA

School of Agriculture

Department of Agriculture

Study of the use of agro-food by-products and essential oils in pig nutrition and their effect on animal performance, health and meat quality

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Doctoral Thesis

Arta

2026

UNIVERSITY OF IOANNINA

A thesis submitted to the University of Ioannina, Department of Agriculture:

The present record describes the experimental work developed to obtain the

Doctor of Philosophy (Ph.D.) degree under the title:

Study of the use of agro-food by-products and essential oils in pig nutrition and their effect on animal performance, health and meat quality

Μελέτη της χρήσης υποπροϊόντων της αγροδιατροφής και αιθέριων ελαίων στη διατροφή των χοίρων και η επίδρασή τους στις ζωοτεχνικές αποδόσεις, στην υγεία και στην ποιότητα του κρέατος

by

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January 2026

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Co-financed by Greece and the European Union

Interreg Greece-Italy INNO.TRITION European Regional Development Fund

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To my wife, Alexandra, and my two children,
Vaggelis and Valia, for their support,
encouragement, and love

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Plagiarism Statement

As a Ph.D. student at the University of Ioannina (Greece), specifically in the Department of Agriculture, where my experimental work has been primarily conducted in the Laboratory of Animal Science, Nutrition, and Biotechnology, Department of Agriculture, University of Ioannina (Greece).

I CERTIFY:

The present dissertation records the progress made for the proposed Ph.D. thesis entitled *"Study of the use of agro-food by-products and essential oils in pig nutrition and their effect on animal performance, health, and meat quality"*.

This thesis is entirely the product of my own work, and I have not obtained a degree in this university or elsewhere based on this work.

For the record and for any applicable purposes, I sign this document in Arta (Greece), **21st of January 2026.**

Signed: Georgios Magklaras

List of Abbreviations

Abbreviation	Full Form / Definition
ADFI	Average Daily Feed Intake
ADG	Average Daily Gain
ADWG	Average Daily Weight Gain
AFI	Average Feed Intake
AIBP	Agro-Industrial By-Products
ALA	Alpha-Linolenic Acid
ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANC	Anaerobe and Corynebacterium
ANFs	Anti-Nutritional Factors
ANOVA	One-way Analysis of Variance
T-AOC	Total Antioxidant Capacity
AST	Aspartate Aminotransferase
BBGK	Balkan Botanic Garden of Kroussia
BHT	Butylated Hydroxytoluene
BOD	Biochemical Oxygen Demand
BW	Body Weight
CARB	Protein Carbonyl
CBC	Clostridium spp. Biochemical Card
CCDA	Charcoal Cefoperazone Deoxycholate Agar
CFU	Colony Forming Units

CHOL	Cholesterol
CK	Creatine Kinase
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
CONT	Control treatment group
CP	Crude Protein
CVD	Cardiovascular Disease
CW	Cheese Whey
DM	Dry Matter
EAAP	European Federation of Animal Science
EDTA	Ethylenediaminetetraacetic Acid
EFSA	European Food Safety Authority
ELSTAT	Hellenic Statistical Authority
E. coli	*Escherichia coli*
E. fergusonii	*Escherichia fergusonii*
E. durans	*Enterococcus durans*
E. hirae	*Enterococcus hirae*
EO	Essential Oil
FA	Fatty Acid
FAME	Fatty Acid Methyl Esters
FCR	Food Conversion Ratio
FEEDAP	Panel on Additives and Products or Substances used in Animal Feed
FI	Feed Intake
FID	Flame Ionization Detector

FXU	Fungal Xylanase Unit
FYT	Fungal Phytase Unit
GAE	Gallic Acid Equivalents
GC	Gas Chromatography
g/dL	Grams per deciliter
GGT	Gamma-Glutamyl Transferase
GHG	Greenhouse Gas
GLU	Blood Glucose
GP	Grape Pomace
GSH-Px	Glutathione Peroxidase
HB	Hemoglobin
HCT	Hematocrit
HSD	Honestly Significant Difference test
IL-6	Interleukin-6
IPB & GR	Institute of Plant Breeding and Genetic Resources
IPEN	International Plant Exchange Network
IPVS	International Pig Veterinary Society
ISO	International Organization for Standardization
IU	International Units
KAA	Kanamycin Aesculin Azide Agar
LCA	Life Cycle Assessment
LPS	Lipopolysaccharide
M17	Medium 17
MDA	Malondialdehyde

mL	Milliliter
mg/dL	Milligrams per deciliter
MRD	Maximum Recovery Diluent
MRS	De Man, Rogosa and Sharpe agar
MUFA	Monounsaturated Fatty Acids
NIR	Near-Infrared Spectroscopy
NRC	National Research Council
OMWW	Olive Mill Waste Water
PCA	Plate Count Agar
PCV2	Porcine Circovirus Type 2
PDO	Protected Designation of Origin
PM	Phytobiotic Mixture
PUFA	Polyunsaturated Fatty Acids
R^2	Coefficient of Determination
RBC	Red Blood Cells
RCB	Randomized Complete Block design
SD	Standard Deviation
SDG	Sustainable Development Goals
SEM	Standard Error of the Mean
SFA	Saturated Fatty Acids
SOP	Standard Operating Procedure
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
TBIL	Total Bilirubin
TBX	Tryptone Bile X-Glucuronide

TCA	Trichloroacetic Acid
THR	Transient Hyperemic Response
TNF-α	Tumor Necrosis Factor alpha
TOS	Transoligosaccharide Propionate Agar
TP	Total Phenolic
TRIG	Triglycerides
T-SOD	Total Superoxide Dismutase
U/L	Units per Liter
UV	Ultraviolet
WBC	White Blood Cells
µL	Microliter

Acknowledgements

This Doctoral study has been both challenging and rewarding, and I feel truly fortunate to have had the support, encouragement, and kindness of many people along the way. I am deeply grateful to all who stood by me during this time, offering guidance, inspiration, and patience, without which the completion of this thesis would not have been possible.

At the heart of this journey stands my family. To my wife, Alexandra, who has been my constant source of love, encouragement, and patience, and to my children, Vaggelis and Valia, who have filled my life with meaning and inspiration. Their love has been my foundation and my greatest source of strength.

I would like to acknowledge with deep respect the late Prof. Anastasios Tsinas, whose guidance and wisdom, as a member of my advisory committee, greatly influenced the early stages of this work. Although he sadly passed away before the completion of this thesis, his intellectual contribution and encouragement remain an enduring source of inspiration.

Above all, one of the most important acknowledgements of this thesis is owed to my supervisor, Prof. Ioannis Skoufos. His mentorship, expertise, and unwavering encouragement have been a constant source of strength throughout this journey. Our many years of collaboration in the Department of Agriculture, the countless conversations we shared about animal science—and especially pig production—and the friendship that grew through them, have shaped not only this research but also my personal and academic path. For his guidance, his trust, and his companionship, I am profoundly grateful.

I am also deeply indebted to the other two members of my advisory committee, Prof. Athina Tzora and Prof. Eleftherios Bonos, experts in the fields of microbiology and animal nutrition, respectively, for their valuable feedback, insightful suggestions, and continuous commitment to supporting my academic progress. Their guidance and encouragement have been instrumental in enhancing the quality of this research and in fostering my development as a scholar.

My heartfelt thanks go to my colleagues and fellow researchers in the Laboratories of Animal Science, Nutrition and Biotechnology, and Animal Health, Hygiene and Food Quality, Department of Agriculture, University of Ioannina, with whom I have shared not only knowledge and collaboration but also camaraderie and friendship. In particular, I am grateful to Prof. Chrysa Voidarou, Asst. Prof. Lambros Chatzizisis, Asst. Prof. Sotiria Vouraki, and Dr. Konstantina

Nikolaou for their valuable support and collegial spirit throughout this journey. A special thanks is due to Dr. Konstadina Fotou and Dr. Evangelia Gouva, as well as to PhD candidates Mr. Christos Zacharis and Mr. Achilleas Karamoutsios, for their support and assistance during the writing of this thesis and over the years, which I deeply appreciate. Their presence has made this journey both rewarding in knowledge and meaningful on a personal level. I would also like to warmly thank the Department's administrative staff, Ms. Giota Filippa, Ms. Evangelia Tsoumpa, and Ms. Kalliopi Tsitra, for their unfailing kindness and readiness to assist whenever needed.

I gratefully acknowledge the financial and institutional support provided by:

Acknowledgments I: This research has been funded by the European Union and by National Greek Funds, under the research program "Bilateral and Multilateral Cooperation Greece - China", project Code: T7ΔΚΙ-00313 (MIS5050735), Acronym: GreenPro, which made this research possible.

Acknowledgments II: This research has been co-funded by European Union, European Regional Development Funds (E.R.D.F.) and by National Funds of Greece and Italy. Interreg V-A Greece-Italy, 2014-2020. Project number (MIS) 5003778, Acronym: «Inno.trition», which made this research possible.

Abstract

In recent years, livestock production systems have been increasingly affected by unsustainable management practices and by the impacts of climate change. Antibiotics have been widely used in swine and poultry production to enhance growth rates, improve feed efficiency, and reduce disease incidence. However, excessive antibiotic use poses a significant risk for the development of antimicrobial resistance, endangering animal health and, through the One Health continuum, human health as well. Addressing these challenges while ensuring global food security remains a pressing concern and requires sustainable solutions.

Consequently, there has been growing research interest in identifying alternative food-production methods capable of reducing environmental impacts and improving the sustainability of production systems. In this context, the search for alternative protein sources, cereal substitutes, and naturally derived feed additives that can support the health of farm animals has intensified. Moreover, the incorporation of agro-industrial by-products into animal diets has been considered both a strategy for reducing waste-disposal costs and improving feeding practices, as well as an important component of environmental sustainability in livestock protein production. These by-products contain an array of bioactive compounds, such as specific fatty acids and polyphenols, which can act as functional ingredients and enhance meat quality.

The valorization of agro-industrial by-products as potential feed resources in primary production—particularly in intensive monogastric systems—provides opportunities to implement circular-economy principles through the reutilization of "waste" streams and the reintroduction of valuable nutrients into the livestock food chain. In parallel, phytobiotics derived from medicinal plants and their essential oils—especially oregano essential oil, *Crithmum maritimum* essential oil, garlic meal, and camelina meal—have gained attention as natural feed additives due to their antimicrobial, antioxidant, and immunomodulatory properties, supporting their use in swine nutrition as a strategy for health management.

This doctoral dissertation investigated, for the first time, the simultaneous inclusion of agro-food by-products—winery grape pomace, olive-mill wastewater, and deproteinized whey—in the form of an innovative silage in swine diets at different developmental stages. Emphasis was placed on its capacity to enhance zootechnical performance, modulate gut health, improve meat-quality traits, and potentially reduce the need for chemotherapeutic interventions during critical production periods. In addition, the study evaluated the use of a mixture of four

aromatic/medicinal plant extracts and essential oils to determine their effects on productive parameters of weaned pigs, on the microbial flora of the gastrointestinal tract, and on the differentiation of meat quality traits. In this way, the dissertation contributes to the development of production systems oriented simultaneously toward animal welfare, reduced environmental footprint, minimized antibiotic use, conservation of natural nutritional resources, and the enhancement of product quality attributes with a distinct Greek identity in the final product—pork meat. The dissertation also addresses existing knowledge gaps, reports limitations, and outlines future perspectives for implementing holistic, large-scale production strategies with a focus on environmentally and economically sustainable pig farming.

Three experimental trials examined the effects of incorporating the novel silage—produced from agro-industrial by-products [olive-mill effluents (olive-mill wastewater), winery residues (grape pomace), and cheese-dairy by-products (deproteinized whey from feta production)]—as well as a phytobiotic mixture of four native medicinal plant extracts and essential oils [oregano essential oil (*Origanum vulgare* subsp. *hirtum*), *Crithmum maritimum L.* essential oil, garlic meal (*Allium sativum* L.), and camelina meal (*Camelina sativa* L. Crantz)] on performance parameters, health indicators, and meat quality of weaned and growing–finishing pigs. Zootechnical indicators (Average Daily Gain, Feed Conversion Ratio, Carcass Yield, Final Body Weight), along with biochemical and hematological markers of health, were assessed. Intestinal content from the ileum and caecum was collected for microbiological analysis using culture-based methods and proteomic identification to determine shifts in gut-health indicators resulting from silage and phytobiotic supplementation. Muscle samples [shoulder (triceps brachii), abdominal region (external abdominal), and ham (biceps femoris)] were analyzed chemically and microbiologically, and pH, total phenols, fatty-acid profile, oxidation indices, and colour parameters were determined to evaluate the effects of the innovative feed components on pork quality traits.

The inclusion of the innovative silage improved growth performance, increased carcass coverage, and contributed to a more favourable gut-microbial profile enriched with eubiotic microorganisms, supporting the observed zootechnical outcomes and promoting animal health and welfare. It also altered meat-quality characteristics by improving the n-6/n-3 fatty-acid ratio in muscle tissue and increasing total phenolic content and antioxidant capacity. In the third trial, the phytobiotic mixture—used for the first time as a dietary additive—proved suitable for

inclusion in weaned-pig diets, producing beneficial effects on gut microbiota composition, enhancing oxidative stability of the meat, modifying fatty-acid profiles, and improving colour attributes, thereby increasing consumer desirability.

Περίληψη

Τα τελευταία χρόνια, τα συστήματα ζωικής παραγωγής έχουν επηρεαστεί σε αυξανόμενο βαθμό από μη βιώσιμες πρακτικές διαχείρισης και την κλιματική αλλαγή/κρίση. Επιπλέον, τα αντιβιοτικά είχαν χρησιμοποιηθεί ευρέως στη χοιροτροφία και την πτηνοτροφία με σκοπό την αύξηση των ρυθμών ανάπτυξης, τη βελτίωση της αποδοτικότητας της τροφής και τη μείωση της συχνότητας εμφάνισης ασθενειών. Ωστόσο, η υπερβολική χρήση των αντιβιοτικών ενέχει τον κίνδυνο ανάπτυξης ανθεκτικότητας στα αντιβιοτικά διακυβεύοντας την υγεία των ζώων, αλλά και δια μέσω της ενιαίας υγείας την υγεία του ανθρώπου. Η αντιμετώπιση αυτών των προκλήσεων αλλά και η διασφάλιση της παγκόσμιας επισιτιστικής ασφάλειας παραμένουν ζητήματα διαρκούς ανησυχίας, αλλά επιζητούν και τρόπους επίλυσης.

Ως εκ τούτου, έχει αναπτυχθεί σημαντικό ερευνητικό ενδιαφέρον για την αναζήτηση εναλλακτικών μεθόδων παραγωγής τροφίμων, οι οποίες θα μπορούσαν να μειώσουν τις περιβαλλοντικές επιπτώσεις και να ενισχύσουν τη βιωσιμότητα των συστημάτων παραγωγής. Στο πλαίσιο αυτό, η αναζήτηση εναλλακτικών πρωτεϊνούχων πηγών αλλά και δημητριακών στη διατροφή των ζώων όπως και συστατικών ή πρώτων υλών που μπορεί να συμβάλουν με φυσικό τρόπο στην υγεία των παραγωγικών ζώων έχει αναδειχθεί σε κεντρικό πεδίο έρευνας. Επιπλέον, η χρήση υποπροϊόντων της αγροδιατροφής στα σιτηρέσια των ζώων έχει θεωρηθεί αφενός ως μέσο μείωσης του κόστους διάθεσης των αποβλήτων επεξεργασίας και ως στρατηγική βελτίωσης των διατροφικών πρακτικών στην εκτροφή ζώων και αφετέρου σημαντικός σύμμαχος στην περιβαλλοντική στρατηγική της βιωσιμότητας των συστημάτων παραγωγής ζωικής πρωτεΐνης. Αξίζει να σημειωθεί ότι τα υποπροϊόντα αυτά περιέχουν πλήθος βιοδραστικών ενώσεων, όπως ορισμένα λιπαρά οξέα και/ή πολυφαινόλες, οι οποίες μπορούν να λειτουργήσουν ως λειτουργικά συστατικά, βελτιώνοντας επιπρόσθετα και τα ποιοτικά χαρακτηριστικά του κρέατος.

Η αξιοποίηση αγροβιομηχανικών υποπροϊόντων ως ενδυνάμει διατροφικών πόρων στην πρωτογενή παραγωγή και ιδίως σε εντατικές εκτροφές μονογαστρικών, προσφέρει τη

δυνατότητα εφαρμογής των αρχών της κυκλικής οικονομίας μέσω της επαναχρησιμοποίησης «αποβλήτων» και της επανεισαγωγής πολύτιμων θρεπτικών συστατικών που εμπεριέχονται σε αυτά στην τροφική αλυσίδα της ζωικής παραγωγής με πολλαπλούς στόχους και με κύριο τη διατήρηση των φυσικών πόρων του πλανήτη. Παράλληλα, φυτοβιοτικά που προέρχονται από φαρμακευτικά φυτά και τα αιθέρια έλαιά τους —ιδίως το αιθέριο έλαιο ρίγανης, το αιθέριο έλαιο κρίταμου, το άλευρο σκόρδου και της καμελίνας— έχουν προσελκύσει το ενδιαφέρον ως φυσικά πρόσθετα ζωοτροφών λόγω επίδειξης αντιμικροβιακών, αντιοξειδωτικών και ανοσοτροποποιητικών ιδιοτήτων και τη χρήση τους στη διατροφή των χοίρων ως στρατηγική διαχείρισης της υγείας των ζώων.

Η παρούσα μελέτη της διδακτορικής διατριβής διερεύνησε για πρώτη φορά την ταυτόχρονη ενσωμάτωση υποπροϊόντων της αγροδιατροφής, όπως στέμφυλων οινοποιίας, υγρών αποβλήτων ελαιουργείων και αποπρωτεϊνωμένου τυρογάλακτος υπό μορφή καινοτόμου ενσιρώματος στα σιτηρέσια χοίρων, σε διαφορετικές ηλικίες, με έμφαση στη δυνατότητα της ενίσχυσης των ζωοτεχνικών αποδόσεων των ζώων, της ρύθμισης της υγείας του εντέρου και της βελτίωσης ποιοτικών χαρακτηριστικών του κρέατος, καθώς και στον πιθανό τους ρόλο στη μείωση της ανάγκης χρήσης χημειοθεραπευτικών σε συγκεκριμένες κρίσιμες περιόδους της παραγωγικής ζωής των χοίρων. Επιπρόσθετα, μελετήθηκε η χρήση ενός μείγματος τεσσάρων αρωματικών/ φαρμακευτικών φυτών με στόχο τη διερεύνηση της επίδρασής τους σε παραγωγικές παραμέτρους χοίρων απογαλακτισμού, στην μικροβιακή χλωρίδα του πεπτικού συστήματος καθώς και στη διαποροποίηση ποιοτικών χαρακτηριστικών του κρέατος. Με τον τρόπο αυτό, η διατριβή συμβάλλει στην ανάπτυξη προτύπων συστημάτων παραγωγής προσανατολισμένων ταυτόχρονα στην ευζωία, στη μείωση του περιβαλλοντικού αποτυπώματος, στην ελαχιστοποίηση χρήσης αντιβιοτικών, στην διαφύλαξη των φυσικών διατροφικών πόρων, αλλά και στην ανάδειξη ποιοτικών σταθερών με Ελληνική ταυτότητα στο τελικό προϊόν παραγωγής, το χοιρινό κρέας. Τέλος, στην παρούσα διατριβή καλύπτονται υπάρχοντα κενά γνώσης και αναφέρονται τυχόν περιορισμοί, και προσδιορίζονται οι μελλοντικές προοπτικές εφαρμογής ολιστικών στρατηγικών παραγωγής σε ευρεία κλίμακα, με έμφαση στη συμβολή τους σε μία περιβαλλοντικά και οικονομικά βιώσιμη χοιροτροφία.

Συγκεκριμένα, σε τρεις πειραματικές δοκιμές αξιολογήθηκε η ενσωμάτωση ενός καινοτόμου ενσιρώματος που παρήχθη από υποπροϊόντα της αγροβιομηχανίας [υποπροϊόντα ελαιοτριβείων (κατσίγαρος), οινοποιείων (στέμφυλα) και τυροκομείων (αποπρωτεϊνωμένος ορός γάλακτος από

παραγωγή φέτας)], καθώς και ενός φυτοβιοτικού μείγματος τεσσάρων εγχώριων φαρμακευτικών φυτικών εκχυλισμάτων και αιθέριων ελαίων [αιθέριο έλαιο ρίγανης (*Origanum vulgare* subsp. *hirtum)*, αιθέριο έλαιο κρίταμου (*Crithmum maritimum* L.), άλευρο σκόρδου (*Allium sativum* L.) και άλευρο καμελίνας (*Camelina sativa* L. Crantz)] σε παραμέτρους απόδοσης, σε δείκτες υγείας και στην ποιότητα του κρέατος απογαλακτισμένων και παχυνόμενων χοίρων. Συγκεκριμένα, μελετήθηκαν συγκεκριμένοι παραγωγικοί/ζωοτεχνικοί δείκτες (Μέση Ημερήσια Αύξηση Βάρους, Δείκτης Μετατρεψιμότητας της Τροφής, Απόδοση σε Σφάγιο, Τελικό Βάρος) καθώς και βιοχημικοί και αιματολογικοί δείκτες μετά από αιμοληψία των χοίρων που προσδιορίζουν δείκτες υγείας των ζώων. Ταυτόχρονα, ελήφθη εντερικό περιεχόμενο από τον ειλεό και το τυφλό για μικροβιολογική ανάλυση με καλλιεργητικές μεθόδους και ταυτοποίησης των μικροβιακών οργανισμών μέσω πρωτεωμικών τεχνολογιών, ώστε να διαπιστωθούν οι παράμετροι μεταβολής δεικτών υγείας που προέρχονται από τη χρήση του ενσιρώματος και του μείγματος των φυτοβιοτικών. Ακολούθησε η χημική και μικροβιολογική ανάλυση τεμαχίων μυικών ιστών [ωμοπλάτη (triceps brachii), κοιλιακή χώρα (external abdominal), χοιρομήριο (biceps femoris)] καθώς και ο προσδιορισμός του pH, των ολικών φαινολών, των λιπαρών οξέων, των δεικτών οξείδωσης και του χρώματος με στόχο να αξιολογηθεί η επίδραση των καινοτόμων διατροφικών συστατικών και προσθετικών στις ποιοτικές παραμέτρους του χοιρινού κρέατος.

Η χρήση και ενσωμάτωση του καινοτόμου ενσιρώματος είχε ως αποτέλεσμα την βέλτιστη ανάπτυξη των χοίρων, αύξηση του ποσοστού κάλυψης του σφάγιου, συνέβαλε στη διαμόρφωση ενός ευνοϊκότερου μικροβιακού προφίλ με παρουσία ευβιοτικών μικροοργανισμών στον εντερικό σωλήνα, το οποίο συνδέεται και εξηγεί τα ζωοτεχνικά ευρήματα και προάγει την υγεία και την ευζωία των χοίρων, ενώ διαφοροποίησε και τα ποιοτικά χαρακτηριστικά του κρέατος, βελτιώνοντας την αναλογία n-6 προς n-3 λιπαρών οξέων στον μυϊκό ιστό και αυξάνοντας τη συγκέντρωση ολικών φαινολών και την αντιοξειδωτική του ικανότητα. Στον τρίτο πειραματισμό, το υπό εξέταση μείγμα φυτοβιοτικών που χρησιμοποιήθηκε για πρώτη φορά ως προσθετικό διατροφής, έδειξε ότι μπορεί να χρησιμοποιηθεί στη διατροφή των απογαλακτισμένων χοίρων, με θετικά αποτελέσματα στο εντερικό μικροβίωμα και τη διαφοροποίηση των μικροβιακών κοινοτήτων προς ένα ευβιοτικό πρότυπο, την αύξηση της οξειδωτικής σταθερότητας του παραγόμενου κρέατος, τη μεταβολή του προφίλ των λιπαρών οξέων και τη χρωματική του αποτύπωση, ούτως ώστε να γίνει πιο επιθυμητό για τον καταναλωτή.

Chapter 1 : Introduction

Introduction

Pig production is among the most important livestock sectors globally as it contributes significantly to the supply of high-quality animal protein for human consumption [1]. Yet, intensive swine farming has come under increasing criticism due to its environmental burden, its reliance on conventional feed resources such as soybean and cereals, and the widespread use of antibiotics for disease prevention and treatment [2,3]. These practices are linked to challenges, including food–feed competition, greenhouse gas emissions, antimicrobial resistance, and animal welfare concerns [4,5]. Consequently, there is a need for innovative feeding strategies that adhere to sustainability principles while maintaining animal performance and obviously meat quality. One promising direction is the utilization of agro-industrial by-products in livestock nutrition, since the agro-industry generates large volumes of residues rich in nutrients and bioactive compounds that are often underutilized or disposed of as waste, contributing to environmental pollution [6,7]. Valorizing these materials enables the integration of pig production into a circular economy framework in which waste streams are transformed into functional feed ingredients. Examples include grape pomace from wine-making, olive mill wastes from olive oil production and cheese whey from dairy processing, each of which carries unique nutritional and functional properties that can promote pig growth, support gut health, and affect meat quality [8–10].

Alongside this approach, increasing attention has been given to phytobiotics which are bioactive plant-derived compounds, such as essential oils and plant flours, proposed as sustainable alternatives to growth promoters [11,12]. Compounds like oregano essential oil from *Origanum vulgare subsp.* hirtum, rock samphire essential oil from *Crithmum maritimum* L., garlic flour from *Allium sativum* L. and false flax flour from *Camelina sativa* L. Crantz, are of particular interest due to their antimicrobial, antioxidant and immunomodulatory activities, which contribute to balanced gut microbiota, reduced oxidative stress and improved resilience in pigs [13–15]. The integration of agro-industrial by-products with phytobiotics in pig diets therefore has the potential to achieve multiple objectives, including valorization of waste within circular bio-economy systems, reduction of environmental impacts, decreased reliance on antibiotics, improved animal health and welfare and production of meat with superior nutritional and functional attributes. This synthesis provides an overview of recent advances in this field, with emphasis on their relevance to modern pig production, while also identifying research gaps and outlining perspectives for scaling up these strategies.

The Greek agro-industrial sector generates substantial quantities of nutrient- and fiber-rich by-products that remain underexploited or are often discarded, simply by incinerating or sending them to landfills, leading to environmental pollution [16]. Such residues represent both a disposal challenge and an opportunity to reinforce the circular economy, by reintroducing them into livestock feeding systems where they can replace conventional feedstuffs, reduce costs, and enhance the environmental sustainability of production. Among the most promising of these by-products are grape pomace, olive mill waste and cheese whey. Grape pomace is the solid residue of winemaking composed of skins seeds and stems and it is particularly rich in polyphenols such as flavonoids, tannins, and anthocyanins, as well as dietary fiber and residual oils rich in unsaturated fatty acids [17,18]. The polyphenol fraction has attracted attention for its antioxidant, antimicrobial, and anti-inflammatory properties which can improve gut health and meat quality in pigs [19]. Studies have demonstrated that dietary inclusion of grape pomace at levels ranging from 2 to 10 percent can enhance antioxidant status by reducing oxidative stress markers [20], alter gut microbiota composition by stimulating beneficial species such as *Lactobacillus* spp. [21] improve pork quality through greater oxidative stability of lipids and increased polyphenol content [22] and reduce malodorous compounds in manure, thereby contributing to environmental sustainability [23], although the high fiber and tannin content may reduce digestibility and growth performance when used excessively [24]. Processing methods, including enzymatic treatment, fermentation or polyphenol extraction, have therefore been proposed to improve its nutritional value before dietary inclusion [25]. Olive mill waste is another key by-product of the Hellenic agro-industry, generated during olive oil extraction, and consisting of olive cake vegetation, water, and liquid effluents. These residues are notable for their high content of phenolic compounds such as hydroxytyrosol and oleuropein, monounsaturated fatty acids and fibers [26]. However, their disposal can be environmentally problematic due to their high organic load and phenolic content which pollutes soil and water if unmanaged [27]. When incorporated into pig diets, derivatives of olive mill wastes have been shown to improve lipid profiles of pork by increasing monounsaturated fatty acids and lowering saturated fats [28], enhance meat oxidative stability through the antioxidant activity of phenolic compounds [29], positively influence gut microbiota populations [30] and have been shown to lower ammonia emissions and modify methane emission potential from manure, thereby contributing to environmental sustainability. [31]. Yet, the variability in composition depending on extraction

methods and storage conditions represents a challenge, and detoxification treatments such as drying, ensiling, or fermentation are often necessary to ensure safe inclusion in feed [32]. Cheese whey mainly from feta production, is another abundant residue produced after milk coagulation in cheese manufacturing. It contains lactose, whey proteins, such as β-lactoglobulin, α-lactalbumin, and immunoglobulins, bioactive peptides and minerals [33]. With millions of tons produced annually, its disposal is problematic due to its high biological oxygen demand [34]. Nevertheless cheese whey is a highly digestible protein and lactose source for pigs and has been successfully applied in both liquid and dried forms with positive effects, including improved growth performance and feed efficiency, especially in weaned piglets [35], favorable modulation of gut microbiota with lactose acting as a prebiotic substrate [36], enhanced nitrogen utilization and reduced pollutant excretion [37] giving opportunities for enhancing its functional and nutritional properties [38]. Though practical limitations such as storage, transportation, and compositional variability remain, with drying and processing technologies increasingly applied to stabilize it for consistent feed use [39]. In summary the incorporation of grape pomace, olive mill wastes and cheese whey in pig diets, provides nutritional, functional, environmental, and economic benefits, as their proper use can reduce environmental burdens of the agroindustry. Furthermore, they can supply bioactive compounds that support pig health and meat quality and reinforce circular economy objectives, although optimization of inclusion levels, processing and safety standards is critical for effective large-scale implementation.

Parallel to agro-industrial by-products produced in the country, phytobiotics have gained strong interest in pig nutrition as they include plant-derived compounds, such as essential oils, extracts, spices and flours, with antimicrobial, antioxidant, and immunomodulatory properties [40]. With restrictions or ban on antibiotic growth promoters increasing globally, research has accelerated on their potential as natural feed additives [41,42]. Among the most extensively studied phytobiotics are oregano and garlic, but less data is available for camelina or rock samphire. Oregano essential oil contains high concentrations of phenolic monoterpenes such as carvacrol and thymol, which are primarily responsible for its strong antimicrobial and antioxidant effects [43], by disrupting bacterial membranes, modifying enzyme activity and scavenging free radicals [44]. Its dietary use has been shown to improve feed efficiency and growth in weaned piglets by stabilizing gut microbiota [45], reducing post-weaning diarrhea through suppression of pathogens including *Escherichia coli* and *Clostridium perfringens* [46]. It enhances antioxidant

capacity in plasma and tissues, thereby reducing lipid peroxidation in pork [47] and potentially enriching sensory attributes, such as flavor [48], with optimal dosages generally reported between 100 and 300 mg per kg of feed, though higher levels may impair palatability [12]. Rock samphire essential oil is derived from the halophytic plant *Crithmum maritimum* L., native to Mediterranean coasts, and characterized by high levels of monoterpenes limonene and γ-terpinene, phenolics and polyacetylenes with known antimicrobial, antioxidant, and anti-inflammatory functions [49,50]. Although studies on pigs are limited, evidence from in vitro and other animal models suggests antimicrobial activity against a wide range of bacteria [51,52], potential to improve oxidative stability of tissues and feed lipids [53], and immunomodulatory properties that enhance disease resilience [54], making it a promising but underexplored phytobiotic requiring further investigation. Garlic flour contains sulfur-based compounds such as allicin, diallyl sulfide, and ajoene, which are primarily responsible for its antimicrobial and antioxidant effects [55], as allicin interferes with microbial metabolism and supports immune function [56]. Garlic supplementation has been associated with improved growth and feed efficiency [57,58], reduced serum cholesterol and triglycerides, thereby potentially enhancing cardiovascular health and modifying pork lipid composition [59]. Also reported are improvements in gut microbiota balance with reductions in pathogens and increases in beneficial species [60] as well as subtle sensory modifications in pork that may be desirable or undesirable depending on dosage [61], although challenges include the instability of allicin and strong odor that may reduce feed intake at high levels. Camelina flour, derived from *Camelina sativa* L.Crantz, is rich in polyunsaturated fatty acids especially α-linolenic acid as well as tocopherols and glucosinolates [62]. Its use in pig diets enriches pork with omega-3 fatty acids improving its nutritional value [63], provides natural antioxidants that enhance oxidative stability [64], may improve immune function and reduce inflammation [65]. It represents a sustainable alternative to fish oil and soy products, thereby reducing food–feed competition [66]. Nevertheless, the glucosinolate content may limit inclusion rates if not managed through optimized processing [62]. Overall, phytobiotics such as oregano, rock samphire, garlic, and camelina represent natural multifunctional feed additives capable of improving pig health, reducing antibiotic use, and enhancing pork quality. Their bioactive compounds could act synergistically with agro-industrial by-products by providing complementary antimicrobial, antioxidant, and immunomodulatory

effects, though further studies are needed to standardize dosages, ensure consistency in bioactive contents, and evaluate long-term commercial applications.

The use of agro-industrial by-products and phytobiotics in pig diets might represent a multifactorial strategy, that could enhance animal health performance and environmental sustainability, with potential synergies greater than the effects of individual additives. These benefits are based on complementary nutritional and functional contributions, since by-products mainly provide nutrients such as proteins, fibers, lipids and polyphenols, while phytobiotics contribute concentrated antimicrobial, antioxidant, and immune-boosting molecules [7,11]. Examples could include the additive antioxidant effects from combining grape pomace polyphenols with oregano essential oil, synergistic improvements in gut integrity when whey peptides are combined with the antimicrobial action of garlic, and functional pork products resulting from olive mill phenolics acting as natural preservatives, alongside camelina-derived omega-3 fatty acids. This integrative strategy is particularly relevant for reducing antibiotic reliance in pig production as prebiotic fibers from by-products stimulate beneficial bacteria, phytobiotics suppress pathogens, bioactive peptides strengthen gut barriers, and phytochemicals from oregano, garlic, and camelina enhance immunity, collectively reducing disease incidence and limiting the need for medical interventions [14,67]. Beyond health benefits, these combinations may also provide environmental gain as valorization of grape pomace, olive mill waste and whey, also reducing industrial pollution [68]. Furthermore, improved feed efficiency and gut health diminish nutrient excretion [30] and certain essential oils can mitigate methane and ammonia emissions from manure [42]. Meat quality and consumer value are likewise enhanced, since polyphenols and essential oils delay lipid oxidation, extending shelf life [41]. Camelina enriches meat with omega-3 fatty acids, garlic and oregano influence flavor positively and consumer demand for antibiotic-free, eco-friendly and naturally enriched products creates additional market potential. Despite these advantages, challenges persist including lack of standardization of inclusion rates, variability in bioactive content due to plant origin or processing, and the need for more long-term in vivo studies under commercial conditions [69]. Nonetheless, the combined use of by-products and phytobiotics may provide a holistic feeding strategy that improves performance, reduces antibiotic dependence, mitigates pollution, and delivers value-added pork products in alignment with sustainability and innovation.

From a broader perspective, this approach contributes to the circular economy and environmental sustainability, as global livestock production faces mounting pressure to reduce its ecological footprint while sustaining profitability. The circular economy framework emphasizes closing nutrient and energy loops, and the use of agro-industrial by-products and phytobiotics in pig diets fits this model, by valorizing waste, reducing emissions and improving resource efficiency [70,71]. Agro-industrial residues such as grape pomace, olive mill wastes, and cheese whey are often pollutants when mismanaged, but when reintegrated into feed systems they reduce waste disposal, recover nutrients, and decrease reliance on human-edible feed sources. Reusing whey for instance prevents environmental damage from its high biochemical oxygen demand while supplying high-quality protein [72]. This closed-loop approach also supports the circular bioeconomy, by converting agro-industrial residues into feed, and manure into renewable energy or organic fertilizer via anaerobic digestion and composting, thereby minimizing the need for synthetic fertilizers and fossil energy inputs [73]. From an economic perspective these strategies reduce feed costs, lower disposal costs for agro-industries, and foster partnerships between food processors and farmers, while generating premium-value pork marketed as sustainable [74,75]. Furthermore, these practices align with global sustainability objectives, particularly the United Nations Sustainable Development Goals including SDG 2 on Zero Hunger, SDG 12 on Responsible Consumption and Production, SDG 13 on Climate Action, and SDG 15 on Life on Land, thus embedding pig production within broader global sustainability frameworks [76,77].

The integration of by-products and phytobiotics also has direct implications for animal health, welfare, and meat quality. By-products such as whey and grape pomace supply highly digestible proteins, lactose, and prebiotic fibers that enhance nutrient utilization, while phytobiotics such as oregano and garlic provide antimicrobial and gut-modulating functions. Together, these could contribute to stabilized gut microbiota with reduced pathogens and increased beneficial bacteria, improved intestinal morphology, including villus height and crypt depth, and enhanced digestibility that translates into better feed conversion efficiency, ultimately lowering the risk of gastrointestinal disorders, especially during the weaning phase [19,78,]. Immune function and disease resistance are likewise strengthened as polyphenols, from grape pomace and olive residues, act as immunomodulators, enhancing leukocyte activity and lowering inflammation [79-81]. Similarly, sulfur compounds in garlic stimulate immune cells [82,83], and oregano essential oil reduces inflammatory markers including TNF-α and IL-6 [84], thus collectively

reducing antibiotic dependence. Animal welfare benefits further from lower oxidative stress, improved antioxidant enzyme activity, and reduced stress-related behaviors through microbiota–gut–brain interactions [85,86]. These welfare benefits translate into measurable improvements in meat quality, since bioactive compounds such as polyphenols and tocopherols protect lipids and proteins against oxidation [87], camelina flour enriches pork with omega-3 fatty acids and improves the n-6 to n-3 ratio [88], phytobiotics such as garlic and oregano influence sensory attributes positively, while grape pomace improves meat color stability [78,89]. Furthermore, the functional value of meat is enriched with antioxidants and polyunsaturated fatty acids, providing added health benefits to consumers [88,90].

Consumer perceptions are central to market success and there is growing awareness of the connections between feeding practices, sustainability and meat quality. Products marketed as antibiotic-free, eco-friendly, and naturally enriched, resonate strongly with consumers and often achieve higher acceptance and price premiums [91,92]. By reducing disease risks, enhancing meat stability and producing functional pork products enriched with bioactive compounds, the use of agro-industrial by-products and phytobiotics, positions pig production as a system that addresses sustainability consumer trust and product quality simultaneously, thereby contributing to a transition toward more resilient and welfare-oriented livestock systems.

Overall, the integration of agro-industrial by-products and phytobiotics into pig diets enhances digestive health, immune function, and overall welfare, while simultaneously improving pork quality and consumer value. By reducing disease incidence, increasing oxidative stability, and promoting the production of functional meat products, these strategies not only reinforce the role of pig production within sustainable and welfare-oriented farming systems but also support the transition toward a circular, low-emission, and resource-efficient model. Through the valorization of agro-industrial residues and the incorporation of phytobiotics, modern production practices can minimize environmental pollution, strengthen the interconnection between agriculture, food processing, and the energy sector, and ultimately enhance both the resilience and profitability of pig farming.

In summary, the potential outcomes of these nutritional strategies and approaches, as supported by international literature, may be described as follows:

1.1. Agro-industrial By-Products in Pig Diets

Studies have demonstrated that dietary inclusion of Grape Pomace (GP) (typically 2–10% of the diet) can:

- Enhance the antioxidant status of pigs, reducing oxidative stress markers.
- Modify gut microbiota composition, favoring beneficial bacteria such as *Lactobacillus* spp.
- Improve meat quality, including higher oxidative stability of lipids and enriched polyphenol content.
- Reduce malodorous compounds in manure, thereby contributing to environmental sustainability.

Incorporating Olive Mill Waste Water (OMWW) derivatives into pig diets has been shown to:

- Improve lipid profiles of pork by increasing MUFA and decreasing saturated fatty acids.
- Enhance oxidative stability of meat due to phenolic compounds acting as antioxidants.
- Positively affect gut health by modulating microbiota populations.
- Reduce methane and ammonia emissions from manure, contributing to lower environmental pollution.

Cheese whey is a highly digestible source of protein and lactose for pigs and has been used successfully as a dietary ingredient in both liquid and dried forms. Reported effects include:

- Enhanced growth performance and feed efficiency, particularly in weaned piglets.
- Positive modulation of gut health, with lactose serving as a prebiotic substrate for beneficial bacteria.
- Improved nitrogen utilization and reduced excretion of pollutants.
- Potential for generating functional pork products enriched with bioactive peptides.

1.2. Phytobiotics in Pig Diets

Oregano essential oil, with optimal dosages typically ranging from 100–300 mg/kg feed in pig diets, has been shown to:

- Improve feed efficiency and growth performance, particularly in weaned piglets, by stabilizing gut microbiota.

- Reduce the incidence of post-weaning diarrhea by suppressing pathogenic bacteria such as *Escherichia coli* and *Clostridium perfringens*.
- Enhance antioxidant capacity in plasma and tissues, thereby reducing lipid peroxidation in pork.
- Enriching the sensory qualities of pork, positively influencing flavor attributes.

Although research on pigs with Rock Samphire (*Crithmum maritimum* L.) essential oil is limited, extrapolations from in vitro studies and other species suggest that it may:

- Exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria.
- Enhance the oxidative stability of animal tissues and feed lipids.
- Possess immunomodulatory properties that improve resilience against infections.

Garlic (*Allium sativum* L.) flour in pig diets is suggested to:

- Improve growth performance and feed efficiency in growing pigs.
- Reduce serum cholesterol and triglycerides, potentially improving cardiovascular health in animals and altering pork lipid profiles.
- Enhance gut microbiota balance by reducing pathogenic bacteria while stimulating beneficial species.
- Impart subtle sensory changes to pork, which may be either desirable or undesirable depending on dosage.

False flax (*Camelina sativa* L. Crantz) flour in pig diets is reported to:

- Enrich pork with n-3 fatty acids, thereby improving its nutritional profile for human consumers.
- Provide natural antioxidants (tocopherols), enhancing the oxidative stability of meat.
- Potentially improve immune response and reduce inflammation.
- Offer a sustainable alternative to fish oil and soybean products, reducing feed–food competition.

1.3. Project rationale, aims, hypothesis and objectives

Modern pig production systems face growing challenges, including the need to enhance productivity while minimizing environmental pollution, ensuring animal welfare, and reducing reliance on antibiotic usage. Valorization of agro-industrial by-products, such as grape pomace, olive mill wastes, and cheese whey, provides an opportunity to promote circular economy principles by reducing waste streams and reintroducing valuable nutrients into the food chain. In parallel, phytobiotics derived from medicinal plants and their essential oils - particularly oregano (*Origanum vulgare* subsp. *hirtum*) essential oil, rock samphire (*Crithmum maritimum* L.) essential oil, garlic (*Allium sativum* L.) flour, and false flax (*Camelina sativa* L. Crantz) flour - have attracted interest as natural feed additives with antimicrobial, antioxidant, and immunomodulatory properties.

This thesis aimed to examine how abandoned feed resources can be integrated into pig diets, focusing on their potential to improve feed efficiency, promote gut health, and enhance meat quality. Special attention was given to their role during key phases of the pig's life cycle, such as weaning, considering the possibility of reducing reliance on synthetic antibiotics in the future. This supports the development of innovative, sustainable, and welfare-oriented animal production systems. A three-phase experimental approach was used to evaluate alternative nutritional strategies in pigs. Phase I (Chapter 2), for the first time worldwide, tested the inclusion of a novel silage made from Greek olive, winery, and feta cheese by-products in the diets of weaned pigs, examining its effects on growth performance, health, and meat quality traits. Phase II (Chapter 3) expanded this research by assessing the same silage in fattening pigs, focusing on production metrics, health indicators, and carcass quality. Finally, Phase III (Chapter 4) evaluated the effectiveness of a phytobiotic mixture (PM) containing oregano (*Origanum vulgare* subsp. *hirtum*) essential oil, rock samphire (*Crithmum maritimum* L.) essential oil, garlic (*Allium sativum* L.) flour, and false flax (*Camelina sativa* L. Crantz) flour as a potential feed additive with functional properties. The thesis also discusses knowledge gaps, limitations, and future prospects for implementing these strategies on a commercial scale, emphasizing their potential to support environmentally sustainable and economically viable pig production.

1.3.1. Phase I (Chapter 2): EXPERIMENT 1_Innovative Silage In Weaned Pig Diets

Overall aim: To test the efficacy of feeding a novel silage, created from Greek agro-industrial residues (olive mill, winery and cheese-making by-products), and assess their synergistic application, on weaned pigs' performance, health and meat quality indices.

Hypothesis: These by-products often contain significant levels of nutrients and bioactive compounds but are underutilized and frequently disposed of as waste, creating environmental burdens. They may be used in pig diets with no adverse effects on productivity, health, and meat quality, and at the same time could reduce production costs.

Objectives:

- Maintain or enhance productivity, product quality, and animal welfare indices in pigs fed with a novel silage formulation enriched with antioxidants and other bioactive compounds derived from three commonly produced in Greece, agro-industrial wastes.
- Improve the overall valorization of agro-industrial wastes to reduce environmental impacts while maximizing bioactivity through their synergistic use.
- Demonstrate that the successful application of the novel silage can provide tangible benefits in terms of animal welfare, productivity, and the quality of the resulting livestock products.
- Achieve the overarching goal of producing higher-quality products from low-cost agro-industrial residues through an innovative approach, thereby increasing the competitiveness of the Greek pig production sector and food agroindustry.

1.3.2. Phase II (Chapter 3): EXPERIMENT 2_Innovative Silage In Fattening Pig Diets

Overall aim: To evaluate the effectiveness of a novel silage, produced from Greek agro-industrial residues (olive mill, winery, and cheese-making by-products), and assess their synergistic application on the performance, health, and meat quality of finishing pigs.

Hypothesis: These by-products are rich in nutrients and bioactive compounds but are often underexploited and discarded as waste, contributing to environmental challenges. Incorporating them into finishing pig diets may have no negative impact on productivity, health, or meat quality, while potentially lowering production costs. If the results are consistent with those observed in the weaned pig trial (Phase I), the potential benefits could be even greater, particularly when taking into account the feed consumption and associated costs during this stage of pig production.

Objectives:

- Maintain or enhance productivity, product quality, and animal welfare indices in pigs fed with a novel silage formulation enriched with antioxidants and other bioactive compounds derived from three commonly produced in Greece, agro-industrial wastes.
- Improve the overall valorization of agro-industrial wastes to reduce environmental impacts while maximizing bioactivity through their synergistic use.
- Demonstrate that the successful application of the novel silage can provide tangible benefits in terms of animal welfare, productivity, and the quality of the resulting livestock products.
- Achieve the overarching goal of producing higher-quality products from low-cost agro-industrial residues through an innovative approach, thereby increasing the competitiveness of the Greek pig production sector and food agroindustry.
- To take the first step toward the production of pork with modified organoleptic characteristics, aiming to meet modern consumer demands for livestock products produced through environmentally sustainable practices, while ensuring both animal welfare and the quality and safety of the final food products.

1.3.3. Phase III (Chapter 4): EXPERIMENT 3_Phytobiotic Mixture In Weaned Pig Diets

Overall aim: The present study aimed to evaluate the effects of Greek medicinal plant extracts and essential oils on performance parameters, health indicators, and meat quality of piglets. Specifically, a mixture was used consisting of oregano essential oil (*Origanum vulgare subsp*. hirtum), sea fennel essential oil (*Crithmum maritimum* L.), garlic meal (*Allium sativum* L.), and camelina meal (*Camelina sativa* L. Crantz).

Hypothesis: The substances contained in the above phytobiotics, including oregano essential oil, rock samphire essential oil, garlic flour, and camelina flour, have been studied for their ability to enhance gut microbiota balance, reduce oxidative stress, modulate immunity, and even enrich pork with health-promoting fatty acids. There is a possibility that the mixture of these bioactive compounds may show a synergistic effect on the pigs' zootechnical, health, and meat quality parameters.

Objectives:

- To examine the productivity, product quality, and animal health indices in pigs fed a phytobiotic mixture of medicinal plant extracts and essential oils.
- To identify novel strategies that synergistically enhance feed use efficiency while reducing the ecological footprint of pig production systems.
- To advance innovative feeding methodologies that may enable the future production of pork without the use of synthetic therapeutic agents or anticoccidials.
- To evaluate the potential for producing pork with distinct nutritional characteristics by supplementing animal diets with bioactive ingredients derived from essential oils and extracts of aromatic and medicinal plants.

1.4 References

1. Kim SW, Gormley A, Jang KB, Duarte ME. - Invited Review - Current status of global pig production: an overview and research trends. Anim Biosci. 2024;37(4):719-729. doi:10.5713/ab.23.0367
2. Lekagul, A.; Tangcharoensathien, V.; Yeung, S.; Guha, N.; Chatfield, J.; Tangcharoensathien, N. Patterns of antibiotic use in global pig production: A systematic review. Vet. Anim. Sci. 2019, 7, 100058. doi:10.1016/j.vas.2019.100058.
3. Pereira GR, Portes AB, Conte CA Junior, Brandão MLL, Spisso BF. Antimicrobial resistance in bacteria from pig production chain: a systematic review and meta-analyses focused on the Brazilian context. Crit Rev Food Sci Nutr. 2025;65(32):7987-8005. doi: 10.1080/10408398.2025.2489531. Epub 2025 Apr 13. PMID: 40222024.
4. Elbehiry, A.; Marzouk, E. From Farm to Fork: Antimicrobial-Resistant Bacterial Pathogens in Livestock Production and the Food Chain. Vet. Sci. 2025, 12, 862. https://doi.org/10.3390/vetsci12090862.
5. Verkuijl, C.; Smit, J.; Green, J.M.H.; Nordquist, R.E.; Sebo, J.; Hayek, M.N.; Hötzel, M.J. Climate change, public health, and animal welfare: Towards a One Health approach to reducing animal agriculture's climate footprint. Front. Anim. Sci. 2024, 5, 1281450. doi:10.3389/fanim.2024.1281450.
6. Vastolo, A.; Calabrò, S.; Cutrignelli, M.I. A review on the use of agro-industrial co-products in animals' diets. Ital. J. Anim. Sci. 2022, 21, 577–594. https://doi.org/10.1080/1828051X.2022.2039562.
7. Saini, R.K.; Khan, M.I.; Kumar, V.; Shang, X.; Lee, J.-H.; Ko, E.-Y. Bioactive Compounds of Agro-Industrial By-Products: Current Trends, Recovery, and Possible Utilization. Antioxidants 2025, 14(6), 650. https://doi.org/10.3390/antiox14060650.
8. Mhalhel, K.; Cavallaro, M.; Pansera, L.; Ledesma, L.H.; Levanti, M.; Germanà, A.; Sutera, A.M.; Tardiolo, G.; Zumbo, A.; Aragona, M.; et al. Histological Assessment of Intestinal Changes Induced by Liquid Whey-Enriched Diets in Pigs. Vet. Sci. 2025, 12, 716. https://doi.org/10.3390/vetsci12080716.
9. da Silveira Almeida, B.C.; Ludke, M.C.M.M.; Bertol, T.M.; Ludke, J.V.; Bernardi, D.M.; Cunha Jr., A.; Coldebella, A. Growth Performance, Meat Quality, and Lipid Oxidation in

Pigs Fed Diets Containing Grape Pomace. Appl. Biosci. 2024, 3, 25. https://doi.org/10.3390/applbiosci3030025.

10. Paié-Ribeiro, J.; Pinheiro, V.; Guedes, C.; Gomes, M.J.; Teixeira, J.; Teixeira, A.; Outor-Monteiro, D. From Waste to Sustainable Animal Feed: Incorporation of Olive Oil By-Products into the Diet of Bísaro Breed Pigs. Sustainability 2025, 17, 3174. https://doi.org/10.3390/su17073174.
11. Pandey, S.; Kim, E.S.; Cho, J.H.; Song, M.; Doo, H.; Kim, S.; Keum, G.B.; Kwak, J.; Ryu, S.; Choi, Y.; Kang, J.; Choe, J.; Kim, H.B. Cutting-edge knowledge on the roles of phytobiotics and their proposed modes of action in swine. Front. Vet. Sci. 2023, 10, 1265689. doi:10.3389/fvets.2023.1265689.
12. Madesh, M.; Yan, J.; Jinan, G.; Hu, P.; Kim, I.H.; Liu, H.-Y.; Ennab, W.; Jha, R.; Cai, D. Phytogenics in swine nutrition and their effects on growth performance, nutrient utilization, gut health, and meat quality: a review. Stress Biol. 2025, 5, 11. doi:10.1007/s44154-024-00209-2.
13. ·Magklaras, G.; Tzora, A.; Bonos, E.; Zacharis, C.; Fotou, K.; Wang, J.; Grigoriadou, K.; Giannenas, I.; Jin, L.; Skoufos, I. Nutritional Use of Greek Medicinal Plants as Diet Mixtures for Weaned Pigs and Their Effects on Production, Health and Meat Quality. Appl. Sci. 2025, 15, 9696. https://doi.org/10.3390/app15179696.
14. Wang, J.; Deng, L.; Chen, M.; Che, Y.; Li, L.; Zhu, L.; Chen, G.; Feng, T. Phytogenic Feed Additives as Natural Antibiotic Alternatives in Animal Health and Production: A Review of the Literature of the Last Decade. Anim. Nutr. 2024, 17, 244–264.
15. Skoufos, I.; Bonos, E.; Anastasiou, I.; Tsinas, A.; Tzora, A. Effects of Phytobiotics in Healthy or Disease-Challenged Animals. In Feed Additives; Academic Press: Oxford, UK, 2020; pp. 311–337.
16. Carrillo-Rodríguez, L. A., Romero, M. C., & Galindo Larrahondo, J. A. (2025). The use of agro-industrial waste towards a sustainable circular economy: A systematic review. Detritus, 31, 45–61. https://doi.org/10.31025/2611-4135/2025.19491.
17. Blasi, F.; Pollini, L.; Fondi, M.; Petrocchi, R.; Mancinelli, R.; Valiani, A.; Trabalza-Marinucci, M. Grape Pomace for Feed Enrichment to Improve the Quality of Animal Products: A Review. Foods 2024, 13, 3541. https://doi.org/10.3390/foods13223541.

18. Alfaia, C.M.; Lopes, P.A.; Madeira, M.S.; Pestana, J.M.; Coelho, D.; Moreira, O.; Prates, J.A.M. Use of Grape By-Products to Enhance Meat Quality and Nutritional Value in Monogastrics: A Review. Foods 2022, 11, 2754. https://doi.org/10.3390/foods11182754.
19. Proca, A.C.; Horodincu, L.; Solcan, C.; Solcan, G. The Potential of Grape Polyphenols Additive in Pig Nutrition: Chemical Structure, Bioavailability and Their Effect on Intestinal Health of Pigs. Agriculture 2024, 14, 1142. https://doi.org/10.3390/agriculture14071142.
20. Chedea, V.S.; Palade, L.M.; Pelmus, R.S.; Dragomir, C.; Taranu, I. Red Grape Pomace Rich in Polyphenols Diet Increases the Antioxidant Status in Key Organs—Kidneys, Liver, and Spleen of Piglets. Animals 2019, 9, 149. https://doi.org/10.3390/ani9040149
21. Wang R, Yu H, Fang H, Jin Y, Zhao Y, Shen J, Zhou C, Li R, Wang J, Fu Y, Zhang J. Effects of dietary grape pomace on the intestinal microbiota and growth performance of weaned piglets. Arch Anim Nutr. 2020 Aug;74(4):296-308. doi: 10.1080/1745039X.2020.1743607. Epub 2020 Apr 19. PMID: 32308036.
22. Kafantaris, I.; Stagos, D.; Kotsampasi, B.; Hatzis, A.; Kypriotakis, A.; Gerasopoulos, K.; Makri, S.; Goutzourelas, N.; Mitsagga, C.; Giavasis, I.; Petrotos, K.; Kokkas, S.; Goulas, P.; Christodoulou, V.; Kouretas, D. Grape pomace improves performance, antioxidant status, fecal microbiota and meat quality of piglets. Animal 2018, 12, 246–255. doi:10.1017/S1751731117001604.
23. Cho, S.; Cho, J.; Hwang, O.; Yang, S.; Park, K.; Choi, D.; Yoo, Y.; Kim, I. Effects of fermented diets including grape and apple pomace on amino acid digestibility, nitrogen balance and volatile fatty acid (VFA) emission in finishing pigs. J. Anim. Vet. Adv. 2012a, 11, 3444–3451.
24. Costa, M.M.; Alfaia, C.M.; Lopes, P.A.; Pestana, J.M.; Prates, J.A.M. Grape By-Products as Feedstuff for Pig and Poultry Production. Animals 2022, 12, 2239. https://doi.org/10.3390/ani12172239.
25. Meini, M., Cabezudo, I., Boschetti, C. E., & Romanini, D. (2019). Recovery of phenolic antioxidants from syrah grape pomace through the optimization of an enzymatic extraction process. Food Chemistry, 283, 257-264. https://doi.org/10.1016/j.foodchem.2019.01.037.
26. Leite, A.; Vasconcelos, L.; López, S.; Outor-Monteiro, D.; Pinheiro, V.; Rodrigues, S.; Teixeira, A. Incorporating Olive By-Products in Bísaro Pig Diets: Effect on Dry-Cured Product Quality. Foods 2024, 13(16), 2579. https://doi.org/10.3390/foods13162579.

27. Varricchio, E.; Coccia, E.; Orso, G.; Lombardi, V.; Imperatore, R.; Vito, P.; Paolucci, M.; Vito, P.; Paolucci, M. Influence of polyphenols from olive mill wastewater on the gastrointestinal tract, alveolar macrophages and blood leukocytes of pigs. Ital. J. Anim. Sci. 2019, 18(1), 574-586. https://doi.org/10.1080/1828051X.2018.1548911.
28. Joven, M., Pintos, E., Latorre, M.A., Suárez-Belloch, J., Guada, J.A. and Fondevila, M. 2014. Effect of replacing barley by increasing levels of olive cake in the diet of finishing pigs: Growth performances, digestibility, carcass, meat and fat quality. Animal Feed Science and Technology, 197, 185-193. DOI: 10.1016/j.anifeedsci.2014.08.007.
29. Ferlisi F, Tang J, Cappelli K and Trabalza-Marinucci M (2023) Dietary supplementation with olive oil co-products rich in polyphenols: a novel nutraceutical approach in monogastric animal nutrition. Front. Vet. Sci. 10:1272274. doi: 10.3389/fvets.2023.1272274.
30. Belloumi, D.; García-Rebollar, P.; Calvet, S.; Francino, M.P.; Reyes-Prieto, M.; González-Garrido, J.; Piquer, L.; Jiménez-Belenguer, A.I.; Bermejo, A.; Cano, C.; Cerisuelo, A. Impact of including two types of destoned olive cakes in pigs' diets on fecal bacterial composition and study of the relationship between fecal microbiota, feed efficiency, gut fermentation, and gaseous emissions. Front. Microbiol. 2024, 15, 1359670. https://doi.org/10.3389/fmicb.2024.1359670.
31. Ferrer, P.; García-Rebollar, P.; Cerisuelo, A.; Ibáñez, M.A.; Rodríguez, C.A.; Calvet, S.; De Blas, C. Nutritional Value of Crude and Partially Defatted Olive Cake in Finishing Pigs and Effects on Nitrogen Balance and Gaseous Emissions. Anim. Feed Sci. Technol. 2018, 236, 131–140. https://doi.org/10.1016/j.anifeedsci.2017.12.014.
32. Paié-Ribeiro, J.; Outor-Monteiro, D.; Guedes, C.; Gomes, M.J.; Teixeira, J.; Teixeira, A.; Pinheiro, V. Effects of Olive Cake on the Performance, Digestibility, Blood Parameters, and Intestinal Villi of Bísaro Pigs. Animals 2025, 15, 1131. https://doi.org/10.3390/ani15081131.
33. Valta, K.; Damala, P.; Angeli, E.; Antonopoulou, G.; Malamis, D.; Haralambous, K.J. Current treatment technologies of cheese whey and wastewater by Greek cheese manufacturing units and potential valorisation opportunities. Waste Biomass Valor. 2017, 8, 1649-1663. https://doi.org/10.1007/s12649-017-9862-8.
34. Pires, A.F.; Marnotes, N.G.; Rubio, O.D.; Garcia, A.C.; Pereira, C.D. Dairy By-Products: A Review on the Valorization of Whey and Second Cheese Whey. Foods 2021, 10, 1067. https://doi.org/10.3390/foods10051067.

35. Jang, K.; Purvis, J.M.; Kim, S.W. Dose–Response and Functional Role of Whey Permeate as a Source of Lactose and Milk Oligosaccharides on Intestinal Health and Growth of Nursery Pigs. J. Anim. Sci. 2021, 99, skab008. https://doi.org/10.1093/jas/skab008.
36. Floridia, V.; Giuffrè, L.; Giosa, D.; Arfuso, F.; Aragona, F.; Fazio, F.; Chen, C.; Song, C.; Romeo, O.; D'Alessandro, E. Comparison of the Faecal Microbiota Composition Following a Dairy By-Product Supplemented Diet in Nero Siciliano and Large White × Landrace Pig Breeds. Animals 2023, 13, 2323. https://doi.org/10.3390/ani13142323.
37. Mahan, D.C. Efficacy of dried whey and its lactalbumin and lactose components at two dietary lysine levels on postweaning pig performance and nitrogen balance. J. Anim. Sci. 1992, 70, 2182–2187. https://doi.org/10.2527/1992.7072182x.
38. Kafantaris, I.; Stagos, D.; Kotsampasi, B.; Kantas, D.; Koukoumis, V.; Moschonas, N.D.; Skaperda, Z.; Gerasopoulos, K.; Makri, S.; Goutzourelas, N.; Mitsagga, C.; Giavasis, I.; Petrotos, K.; Kokkas, S.; Kouretas, D. Whey protein concentrate improves antioxidant capacity, faecal microbiota and fatty acid profile of growing piglets. J. Agric. Sci. 2019, 157, 72–82. https://doi.org/10.1017/S0021859619000224.
39. Delgado-Macuil, R.J.; Perez-Armendariz, B.; Cardoso-Ugarte, G.A.; Tolibia, S.E.M.; Benítez-Rojas, A.C. Recent Biotechnological Applications of Whey: Review and Perspectives. Fermentation 2025, 11, 217. https://doi.org/10.3390/fermentation11040217.
40. Garavito-Duarte Y, Deng Z, Kim SW. Literature Review: Opportunities with Phytobiotics for Health and Growth of Pigs. Annals of Animal Science. National Research Institute of Animal Production, 2025;25(4): 1237-1247. https://doi.org/10.2478/aoas-2024-0119.
41. Zhai, H.; Liu, H.; Wang, S.; Wu, J.; Kluenter, A.-M. Potential of essential oils for poultry and pigs. Anim. Nutr. 2018, 4, 179–186. doi:10.1016/j.aninu.2018.01.005.
42. Nhara, R.B.; Marume, U.; Nantapo, C.W.T. Potential of Organic Acids, Essential Oils and Their Blends in Pig Diets as Alternatives to Antibiotic Growth Promoters. Animals 2024, 14, 762. https://doi.org/10.3390/ani14050762.
43. Duarte, M.E.; Kim, S.W. Phytobiotics from Oregano Extracts Enhance the Intestinal Health and Growth Performance of Pigs. Antioxidants 2022, 11, 2066. https://doi.org/10.3390/antiox11102066.
44. Mohammadi Gheisar, M.; Kim, I.H. Phytobiotics in poultry and swine nutrition—A review. Ital. J. Anim. Sci. 2018, 17, 92–99. https://doi.org/10.1080/1828051X.2017.1350120.

45. Serrano-Jara, D.; Rivera-Gomis, J.; Tornel, J.A.; Jordán, M.J.; Martínez-Conesa, C.; Pablo, M.J.C. Oregano Essential Oil and Purple Garlic Powder Effects on Intestinal Health, Microbiota Indicators and Antimicrobial Resistance as Feed Additives in Weaning Piglets. Animals 2024, 14, 111. https://doi.org/10.3390/ani14010111.
46. Henn, J.D.; Bertol, T.M.; Moura, N.F.; Coldebella, A.; de Brum, P.A.R.; Casagrande, M. Oregano essential oil as food additive for piglets: antimicrobial and antioxidant potential. Rev. Bras. Zootec. 2010, 39, 1761–1767.
47. Forte, C.; Ranucci, D.; Beghelli, D.; Branciari, R.; Acuti, G.; Todini, L.; Cavallucci, C.; Trabalza-Marinucci, M. Dietary integration with oregano (Origanum vulgare L.) essential oil improves growth rate and oxidative status in outdoor-reared, but not indoor-reared, pigs. J. Anim. Physiol. Anim. Nutr. 2017, 101, e352–e361. https://doi.org/10.1111/jpn.12612.
48. Janacua-Vidales, H.; Peña-González, E.; Alarcón-Rojo, A.D.; Ortega-Gutiérrez, J.; Aguilar-Palma, N. Determination of carcase yield, sensory and acceptance of meat from male and female pigs with dietary supplementation of oregano essential oils. Ital. J. Anim. Sci. 2019, 18, 668–678. https://doi.org/10.1080/1828051X.2018.1553507.
49. Dzhoglova, V.; Ivanova, S.; Shishmanova-Doseva, M.; Saracheva, K. Crithmum maritimum L.: Phytochemical Profile, Biological Activities, and Therapeutic Potential. Molecules 2025, 30, 2832. https://doi.org/10.3390/molecules30132832.
50. Šunić, L.; Ilić, Z.S.; Stanojević, L.; Milenković, L.; Lalević, D.; Stanojević, J.; Milenković, A.; Cvetković, D. Essential Oil Content, Composition and Free Radical Scavenging Activity from Different Plant Parts of Wild Sea Fennel (Crithmum maritimum L.) in Montenegro. Plants 2024, 13, 2003. https://doi.org/10.3390/plants13142003.
51. Correia, I.; Antunes, M.; Tecelão, C.; Neves, M.; Pires, C.L.; Cruz, P.F.; Rodrigues, M.; Peralta, C.C.; Pereira, C.D.; Reboredo, F.; et al. Nutritive Value and Bioactivities of a Halophyte Edible Plant: Crithmum maritimum L. (Sea Fennel). Plants 2024, 13, 427. https://doi.org/10.3390/plants13030427.
52. Bonos, E.; Skoufos, I.; Giannenas, I.; Sidiropoulou, E.; Fotou, K.; Stylianaki, I.; Tsiftsoglou, O.; Lazari, D.; Venardou, B.; Galamatis, D.; et al. Effect of an Herbal Mixture of Oregano, Garlic, Sage and Rock Samphire Extracts in Combination with Tributyrin on Growth Performance, Intestinal Microbiota and Morphology, and Meat Quality in Broilers. Sustainability 2022, 14, 13565. https://doi.org/10.3390/su142013565.

53. Ismail, A., El-Shibani, F.A., Mohammed, H.A. et al. Chemical composition, antioxidant, and enzyme inhibition activities of Crithmum maritimum essential oils: the first chemo-biological study for species grown in North Africa. Sci Rep 14, 25318 (2024). https://doi.org/10.1038/s41598-024-74544-9.
54. Alemán, A.; Marín-Peñalver, D.; de Palencia, P.F.; Gómez-Guillén, M.d.C.; Montero, P. Anti-Inflammatory Properties, Bioaccessibility and Intestinal Absorption of Sea Fennel (Crithmum maritimum) Extract Encapsulated in Soy Phosphatidylcholine Liposomes. Nutrients 2022, 14, 210. https://doi.org/10.3390/nu14010210.
55. Liu Y, Espinosa CD, Abelilla JJ, Casas GA, Lagos LV, Lee SA, Kwon WB, Mathai JK, Navarro DMDL, Jaworski NW, Stein HH. Non-antibiotic feed additives in diets for pigs: A review. Anim Nutr. 2018 Jun;4(2):113-125. doi: 10.1016/j.aninu.2018.01.007. Epub 2018 Feb 8. PMID: 30140751; PMCID: PMC6103469.
56. Huang RH, Qiu XS, Shi FX, Hughes CL, Lu ZF, Zhu WY. Effects of dietary allicin on health and growth performance of weanling piglets and reduction in attractiveness of faeces to flies. Animal. 2011;5(2):304–11.
57. Sánchez, C.J.; Martínez-Miró, S.; Ariza, J.J.; Madrid, J.; Orengo, J.; Aguinaga, M.A.; Baños, A.; Hernández, F. Effect of Alliaceae Extract Supplementation on Performance and Intestinal Microbiota of Growing-Finishing Pig. Animals 2020, 10, 1557. https://doi.org/10.3390/ani10091557.
58. Cong, O.N.; Dang, P.K.; Ton, V.D. Effect of garlic powder supplementation on growth performance and meat quality of pork. KHKT Chăn Nuôi 2021, 269, (Vietnam National University of Agriculture).
59. Ogbuewu IP, Okoro VM, Mbajiorgu EF, Mbajiorgu CA. Beneficial Effects of Garlic in Livestock and Poultry Nutrition: A Review. Agric Res 2019;8(4):411–26. Available from: https://doi.org/10.1007/s40003-018-0390-y.
60. Rabelo-Ruiz, M.; Teso-Pérez, C.; Peralta-Sánchez, J.M.; Ariza, J.J.; Martín-Platero, A.M.; Casabuena-Rincón, Ó.; Vázquez-Chas, P.; Guillamón, E.; Aguinaga-Casañas, M.A.; Maqueda, M.; et al. Allium Extract Implements Weaned Piglet's Productive Parameters by Modulating Distal Gut Microbiota. Antibiotics 2021, 10, 269. https://doi.org/10.3390/antibiotics10030269.

61. Omojola, A.B.; Fagbuaro, S.S.; Ayeni, A.A. Cholesterol Content, Physical and Sensory Properties of Pork from Pigs Fed Varying Levels of Dietary Garlic (Allium sativum). World Appl. Sci. J. 2009, 6, 971–975.
62. Delver, J.J.; Smith, Z.K. Opportunities for Camelina Meal as a Livestock Feed Ingredient. Agriculture 2024, 14, 116. https://doi.org/10.3390/agriculture14010116.
63. Li, Y.Z.; Lazarus, W.F.; Reese, C.; Hilbrands, A.M.; Cox, R.B.; Forcella, F.; Gesch, R.W.; Johnston, L.J. Integrating camelina into organic pig production—Impact on growth performance of pigs, costs, and returns. Front. Sustain. Food Syst. 2021, 5, 759721. https://doi.org/10.3389/fsufs.2021.759721.
64. Taranu, I.; Gras, M.; Pistol, G.C.; Motiu, M.; Marin, D.E.; Lefter, N.; Ropota, M.; Habeanu, M. ω-3 PUFA Rich Camelina Oil By-Products Improve the Systemic Metabolism and Spleen Cell Functions in Fattening Pigs. PLoS ONE 2014, 9(10), e110186. doi:10.1371/journal.pone.0110186.
65. Luise D, Correa F, Cestonaro G, Sattin E, Conte G, Mele M, Archetti I, Virdis S, Negrini C, Galasso I, Stefanelli C, Mazzoni M, Nataloni L, Trevisi P, Costanzo E. Effect of different doses of camelina cake inclusion as a substitute of dietary soyabean meal on growth performance and gut health of weaned pigs. Br J Nutr. 2024 Jun 28;131(12):1962-1974. doi: 10.1017/S0007114524000722.
66. Tallaksen, J.; Johnston, L.; Gesch, R.; Forcella, F.; Li, Y. Organic Camelina Meal as a Replacement for Soybean Meal in Swine Finishing Diets: A Life Cycle Analysis Perspective. Sustainability 2025, 17, 1443. https://doi.org/10.3390/su17041443.
67. Grigore-Gurgu, L.; Dumitrașcu, L.; Aprodu, I. Aromatic Herbs as a Source of Bioactive Compounds: An Overview of Their Antioxidant Capacity, Antimicrobial Activity, and Major Applications. Molecules 2025, 30, 1304. https://doi.org/10.3390/molecules30061304.
68. Georganas, A.; Kyriakaki, P.; Giamouri, E.; Mavrommatis, A.; Tsiplakou, E.; Pappas, A. C. Mediterranean agro-industrial by-products and food waste in pig and chicken diets: Which way forward? Livestock Science 2024, 289, 105584. doi:10.1016/j.livsci.2024.105584.
69. Nantapo, C.W.T.; Marume, U. Strategic technologies to improve phytogenic feed additive efficacy in pigs and poultry. Animal Nutrition 2025, xx(xx), xx–xx. doi:10.1016/j.aninu.2024.06.010.

70. Yang, K.; Qing, Y.; Yu, Q.; Tang, X.; Chen, G.; Fang, R.; Liu, H. By-Product Feeds: Current Understanding and Future Perspectives. Agriculture 2021, 11, 207. https://doi.org/10.3390/agriculture11030207.
71. Mosolov AA, Starodubova Y V., Miroshnik AS, Slozhenkina MI, Komlatsky VI, Struk AN. Phytobiotics in pig feeding architecture for the organic animal husbandry development. IOP Conf Ser Earth Environ Sci. 2022;1112(1).
72. Sutera AM, Arfuso F, Tardiolo G, Riggio V, Fazio F, Aiese Cigliano R, Paytuví A, Piccione G, Zumbo A. Effect of a Co-Feed Liquid Whey-Integrated Diet on Crossbred Pigs' Fecal Microbiota. Animals (Basel). 2023 May 25;13(11):1750. doi: 10.3390/ani13111750. PMID: 37889679; PMCID: PMC10252047.
73. Papatsiros, V. G.; Tsekouras, N.; Papakonstantinou, G. I.; Kamvysi, K.; Eliopoulos, C.; Fotos, L.; Arapoglou, D.; Meletis, E.; Michailidis, G.; Gougoulis, D. The Use of Food Industry By-Products in Pig Diets as a Strategy to Reduce Environmental Footprint. Preprints 2025, 2025102026. https://doi.org/10.20944/preprints202510.2026.v1
74. Pinotti, L. Circular Feed Production and Consumption in the Context of Smart Animal Nutrition. In Smart Livestock Nutrition; Kyriazakis, I., Ed.; Springer International Publishing: Cham, Switzerland, 2023; pp. 55–70. doi:10.1007/978-3-031-22584-0_3.
75. Sossidou, E.N.; Banias, G.F.; Batsioula, M.; Termatzidou, S.-A.; Simitzis, P.; Patsios, S.I.; Broom, D.M. Modern Pig Production: Aspects of Animal Welfare, Sustainability and Circular Bioeconomy. Sustainability 2025, 17, 5184. https://doi.org/10.3390/su17115184.
76. United Nations. Transforming our world: The 2030 Agenda for Sustainable Development. UN General Assembly Resolution A/RES/70/1, 25 September 2015. United Nations; 2015. Available online: https://sdgs.un.org/2030agenda.
77. M. C. Ogwu, C. E. Hills, S. Pietrosemoli, The Piggy Solution: Harnessing Food Waste for Sustainable Hog Farming. Global Challenges 2025, 9, e00073. https://doi.org/10.1002/gch2.202500073.
78. Rivera-Gomis, J.; Peres Rubio, C.; Martínez Conesa, C.; Otal Salaverri, J.; Cerón, J.J.; Tortosa, D.E.; Cubero Pablo, M.J. Effects of Dietary Supplementation of Garlic and Oregano Essential Oil on Biomarkers of Oxidative Status, Stress and Inflammation in Postweaning Piglets. Animals 2020, 10, 2093. https://doi.org/10.3390/ani10112093.

79. Horodincu, L.; Proca, A.C.; Șlencu, B.G.; Trifan, A.; Pavel, G.; Solcan, G.; Solcan, C. Modulating Effects of Grape Pomace on the Intestinal Antioxidative and Inflammatory Status in Fattening Pigs. Agriculture 2025, 15, 740. https://doi.org/10.3390/agriculture15070740.
80. Varricchio, Ettore, Elena Coccia, Graziella Orso, Vittoria Lombardi, Roberta Imperatore, Pasquale Vito, and Marina Paolucci. 2019. "Influence of Polyphenols from Olive Mill Wastewater on the Gastrointestinal Tract, Alveolar Macrophages and Blood Leukocytes of Pigs." Italian Journal of Animal Science 18 (1): 574–86. doi:10.1080/1828051X.2018.1548911.
81. Williams, A.R.; Krych, Ł.; Fauzan Ahmad, H.; Nejsum, P.; Skovgaard, K.; Nielsen, D.S.; Thamsborg, S.M. A polyphenol-enriched diet and Ascaris suum infection modulate mucosal immune responses and gut microbiota composition in pigs. PLoS ONE 2017, 12(10), e0186546. doi:10.1371/journal.pone.0186546.
82. Biswas, S.; Kim, I.H. Determination of garlic extract efficacy on growth, nutrient digestibility, and fecal score of growing pigs via diet supplementation. Korean J. Agric. Sci. 2023, 50(3), 391–398. doi:10.7744/kjoas.500304.
83. Guillamón, E.; Andreo-Martínez, P.; Mut-Salud, N.; Fonollá, J.; Baños, A. Beneficial Effects of Organosulfur Compounds from Allium cepa on Gut Health: A Systematic Review. Foods 2021, 10, 1680. https://doi.org/10.3390/foods10081680.
84. Cappelli K, Sabino M, Trabalza-Marinucci M, Acuti G, Capomaccio S, Menghini L, Verini-Supplizi A. Differential Effects of Dietary Oregano Essential Oil on the Inflammation Related Gene Expression in Peripheral Blood Mononuclear Cells From Outdoor and Indoor Reared Pigs. Front Vet Sci. 2021 Feb 25;8:602811. doi: 10.3389/fvets.2021.602811. PMID: 33718464; PMCID: PMC7946822.
85. Wang H, Xu R, Zhang H, Su Y, Zhu W. Swine gut microbiota and its interaction with host nutrient metabolism. Anim Nutr. 2020 Dec;6(4):410-420. doi: 10.1016/j.aninu.2020.10.002. Epub 2020 Oct 10. PMID: 33364457; PMCID: PMC7750828.
86. Hao Y, Xing M, Gu X. Research Progress on Oxidative Stress and Its Nutritional Regulation Strategies in Pigs. Animals (Basel). 2021 May 13;11(5):1384. doi: 10.3390/ani11051384. PMID: 34068057; PMCID: PMC8152462.

87. Galassi, G.; Battelli, M.; Verdile, N.; Rapetti, L.; Zanchi, R.; Arcuri, S.; Petrera, F.; Abeni, F.; Crovetto, G.M. Effect of a Polyphenol-Based Additive in Pig Diets in the Early Stages of Growth. Animals 2021, 11, 3241. https://doi.org/10.3390/ani11113241.
88. Smit MN, Beltranena E. Effects of feeding camelina cake to weaned pigs on safety, growth performance, and fatty acid composition of pork. J Anim Sci. 2017;95(6):2496–508.
89. Bertol T, Ludke J V, Campos R, Kawski V, Jr A, Figueiredo E. Inclusion of grape pomace in the diet of pigs on pork quality and oxidative stability of omega-3 enriched fat. Ciência Rural. 2017;47.
90. Bonos, E.; Florou-Paneri, P.; Christaki, E.; Giannenas, I.; Skoufos, I.; Tsinas, A.; Tzora, A.; Peng, J. Pork Meat as a Functional Food. In Proceedings of the II International Congress "Food Technology, Quality and Safety", Novi Sad, Serbia, 28-30 October 2014; pp. 34-39.
91. Bradford H, McKernan C, Elliott C, Dean M. Consumers' perceptions and willingness to purchase pork labelled 'raised without antibiotics'. Appetite. 2022 Apr 1;171:105900. doi: 10.1016/j.appet.2021.105900. Epub 2021 Dec 27. PMID: 34968563.
92. Denver, S.; Christensen, T.; Lund, T.B.; Olsen, J.V.; Sandøe, P. Willingness-to-pay for reduced carbon footprint and other sustainability concerns relating to pork production – A comparison of consumers in China, Denmark, Germany and the UK. Livest. Sci. 2023, 276, 105337. doi:10.1016/j.livsci.2023.105337.

Chapter 2 : Inclusion of agro-industrial by-products, in a silage form, in weaned pig diets and effects on performance, health, and meat quality

Sections of this chapter have been published in:

Innovative Use of Olive, Winery and Cheese Waste By-Products as Novel Ingredients in Weaned Pigs Nutrition. **Magklaras G**, Skoufos I, Bonos E, Tsinas A, Zacharis C, Giavasis I, Petrotos K, Fotou K, Nikolaou K, Vasilopoulou K, Giannenas I, Tzora A. Vet Sci. 2023 Jun 16;10(6):397. doi: 10.3390/vetsci10060397.

2.1. Introduction

During the last decades, farmers have been facing multiple challenges concerning their productions systems. One of the most important issues is the constantly increasing cost of feed due global effects such as climate change, the reduction of natural feed sources and the extensive deforestation [1-3]. On the other hand, consumers nowadays show a preference for healthy foods with advanced nutritional properties that are produced sustainably. Additionally, the goal for reduction of microbial resistance by minimizing use of antibiotics on farm animals, has intensified the pursuit for novel feed ingredients and supplements of potential value [4]. Thus, researchers are seeking economical and innovative feeds for farm animals that could lead to the production of functional foods of animal origin, that could replace conventional feed ingredients that are produced with excessive global environmental footprint, and that could, potentially, improve animal and consumer health and welfare. Recently, unpredictable developments in the European feed trade market have attenuated the availability and have vastly increased prices of traditional protein and carbohydrate feed sources, such as soybean meal and cereals [5]. However, in many countries, especially those of Southern Europe, large amounts of agro-industrial wastes are produced annually as by-products of various agro-industries such as olive oil production, cheese production and viniculture. These wastes are considered as heavy environmental pollutants, not only because of their physicochemical status, but mostly because of the inappropriate way they are discarded by the industries to the ecosystem. Representative examples of these agro-industrial wastes in Greece are olive mill wastewaters, cheese whey (from feta cheese production) and grape pomace, all delivered in vast quantities [6-8]. However, it is well known that these agro-industrial waste have significant concentrations of potential useful bio-functional and nutritive components, including dietary fiber, unsaturated fatty acids, carotenoids, polyphenols, flavonoids, and useful biomass [9-11]. Since the methods and technologies of agro-industrial waste recycling are constantly evolving, incorporating such by-products in the diets of farm animals, should have high priority because it could provide substantial possibilities to the prospect of creating novel animal feeds with real benefits, for example the acceleration of sustainability models, the protection of natural animal health and welfare systems and the improvement of quality indexes of the animal products, especially concerning the antioxidant activity [12,13] and the lipid content [9,14,15].

Solid substrate fermentation, ensiling, and high solid or slurry processes are among the technologies available for protein enrichment of these wastes. Technologies for reprocessing these wastes must take into account the characteristics of individual wastes as well as the environment in which they are generated, reprocessed, and used [16,17]. It is typically impractical to include these wastes into feed formulas and production systems because of their physicochemical characteristics which impair feed digestibility and storage time. Even though silages are more frequently incorporated in ruminant diets, silages made from waste by-products are also being tested in monogastric farm animals, such as poultry and pigs, with encouraging results [13,18-21]. The present study tested a novel silage that has been created by a Greek scientific research group [9], facing the common challenge of using agro-industry wastes of local produced products such as olive mill waste water solids, grape pomace solids and de-proteinized feta cheese way solids. This silage was created through testing for the optimal combinations of the three by-products [9] and has already been studied in broiler chickens [21]. In this work, this silage was tested for the first time in weaned pigs by evaluating its effects on production parameters, animal health and meat quality characteristics (Fig.2.1.)

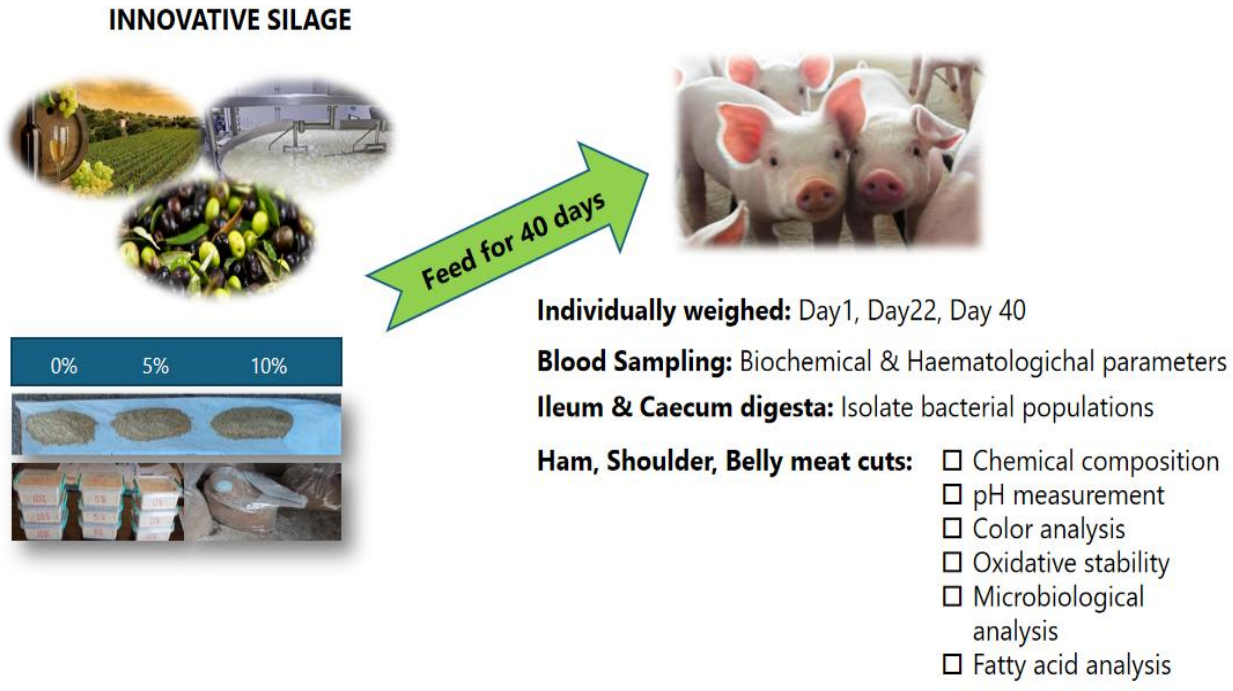


Figure 2.1. Experimental summary schematic illustration of Chapter 2 (exp. No1).

2.2. Materials and methods

2.2.1. Animals, Diets, Experimental Design

Petrotos et al. [9] provide a comprehensive description of the design of the innovative silage made from agro-industrial by-products in their study. In brief, advanced statistics and mathematical models were used to evaluate for the determination of the optimal mixing ratio of the three wastes: olive mill wastewater solids, grape pomace solids and de-proteinized feta cheese way solids. After fermentation, an optimized silage was produced, with a low pH (pH=4.37), a high lactic acid content (total acidity=2.52), a high lactic acid bacteria count (total Lactic acid bacteria=6.9 cfu/g) and a low yeast and mold count (total yeast and mold count=0.1 cfu/g). Then this silage was produced in large amounts, sufficient for the dietary trial with the pigs. Table 2.1 presents the chemical analysis of the tested silage.

All experimental procedures were in accordance with the National guidelines for animal trials (PD, 2013) and the authorities of the School of Agriculture of the University of Ioannina, Greece (UOI University Research Committee research registration: 61291). A veterinary surgeon and an animal scientist, both from the Department of Agriculture of the University of Ioannina, supervised the farming conditions and the weaned pigs during the whole experimental period. A total of 45 crossbreed weaned pigs (¼ Large White × ¼ Landrace × ½ Duroc) 34 days old (with average initial mean body weight 8.3 ± 0.12kg) were selected from a commercial pig farm in the Region of Epirus Greece and were randomly allocated to one of three treatment groups (Silage-0%, Silage-5% or Silage-10%). The initial body weights of the pigs were similar in the three groups. Each pigs was individually marked with numbered plastic ear tags. The pigs of each treatment (N=15) were housed in pens with slatted plastic floors, under controlled environmental conditions (ambient temperature, average humidity, ventilation rate, animal density) according to their production stage. All pigs were vaccinated according to the standard management procedures of the farm. Ad libitum access to feed and water was available.

The control treatment (Silage-0%) was fed a commercial maize-based diet appropriate for weaned pigs, based on the recommended values from the National Research Council [22] and the database of Premier Nutrition [23]. The other two treatments were fed diets that incorporated either 5% (Silage-5%) or 10% (Silage-10%) of the tested novel silage, respectively. All three

diets were formulated to be isonitrogenous and isocaloric. Table 2.2 presents the ingredients and chemical composition of the experimental diets.

Table 2.1. Chemical composition of the examined silage**.**

Silage chemical analysis (as fed basis)	
Moisture (%)	42.89
Dry matter (%)	57.11
Ash (%)	1.15
Crude fat (%)	3.21
Crude fiber (%)	2.63
Crude protein (%)	5.51
Total Ca (%)	0.05
Total P (%)	0.18
Mn (mg/kg)	16.95
Fe (mg/kg)	82.48
Cu (mg/kg)	3.21
Zn (mg/kg)	30.43

The trial lasted 40 days (from 34 to 74 days of age) and during the experimental period, pigs were individually weighed on the 1st, 22nd and 40th day, while feed intake and mortality data were recorded daily. To estimate the effects of dietary treatments on the pigs' zootechnical performance indices, average daily gain (ADG, kg/day), average daily feed intake (ADFI, kg feed intake/day) and feed conversion ratio (FCR, kg feed intake/kg live weight gain) were calculated. On the last day of the trial blood samples were taken from 6 pigs per group and then these pigs were slaughtered under humanitarian conditions in a modern abattoir, close to the experimental farm.

Table 2.2. Composition and calculated proximate analysis of experimental diets

Ingredients (g/kg)	**Diets**		
	Silage-0%	**Silage-5%**	**Silage-10%**
Maize	436.000	369.714	303.427
Silage	0.000	50.000	100.000
Soybean meal (47% CP)	158.000	163.687	169.374
Barley	200.000	200.000	200.000
Fish-meal 62% CP	30.000	30.000	30.000
Wheat middlings	30.000	30.000	30.000
Soybean oil	20.000	30.600	41.199
Commercial premix 6%*	60.000	60.000	60.000
Whey permeate **(**4,5% CP)	60.000	60.000	60.000
Zinc oxide	3.000	3.000	3.000
Benzoic acid	3.000	3.000	3.000
Total	**100.000**	**100.000**	**100.000**
Calculated chemical analysis			
Digestible energy, MJ/kg	13.865	13.862	13.860
Crude protein, %	17.641	17.641	17.640
Dry matter, %	89.029	87.676	86.322
Ash, %	5.450	5.453	5.456
Crude fat, %	4.500	5.434	6.367
Crude fiber, %	2.900	2.860	2.819
ADF, %	3.300	3.259	3.217
NDF, %	9.864	9.613	9.362
Ca, %	0.576	0.577	0.578
Total P, %	0.485	0.480	0.475
Lysine, %	1.177	1.185	1.193
Methionine+Cystine, %	0.740	0.736	0.731

*Provided per kg of diet: 15000 IU vitamin A, 50 mcg 25-hydroxycholecalciferol, 9.96 mg vitamin E, 10.02 mg vitamin K3, 3 mg vitamin B1, 10.02 mg vitamin B2, 6 mg pantothenic acid, 6 mg vitamin B6, 40.02 mcg vitamin B12, 100 mg vitamin C, 35 mg niacin, 300 mcg biotin, 1.5 mg folic acid, 375 mg choline chloride, 200 mg ferrous sulfate monohydrate, 90 mg copper sulfate pentahydrate, 60 mg manganese sulfate monohydrate, 100 mg zinc sulfate monohydrate, 2 mg calcium iodate, 300 mg sodium selenide, 150 mg L- selenomethionine – Selenium, 1500 FYT 6-phytase, 80 U β-1,4-endoglucanase, 70 U β-1,3 (4)-endoglucanase, 270 U β-1,4-endoxylanase, 5000 mg benzoic acid, 40,8 mg butylated hydroxytoluene, 3.5 mg propyl gallate.

2.2.2. Isolation, enumeration and identification of bacterial populations

Fresh digesta samples from the ileum, and caecum were collected from 6 animals per treatment immediately after slaughter. Initially, 1 g of intestinal content was homogenized with 9 ml of sterile peptone water solution 0.1%. For bacterial enumeration Miles and Misra Plate Method

(surface drop) was used, and each sample was diluted serially via 12-fold dilutions (from 10-1 to 10-12) using standard 96-well plates for microdilutions. 10 µl of each dilution was inoculated on media and incubated properly. MacConkey and Kanamycin aesculin azide (KAA) agar (Merck, Darmstadt, Germany) were respectively used for the isolation of Enterobacteriaceae. All plates were incubated aerobically at 37°C for 24 h-48 h on De Man, Rogosa and Sharpe (MRS) agar (Oxoid, Basingstoke, UK), M17 agar (Lab M Limited, Lancashire, UK) was used for the isolation and enumeration of Lactobacillaceae, while media were incubated at 37°C for 48 h in anaerobic conditions. Bifidobacteriaceae isolation and enumeration were performed on Transoligosaccharide propionate agar medium (TOS) (Merck, Darmstadt, Germany) supplemented with glacial acetic acid (1%, v/v) and mupirocin (100 µl/ml), incubated anaerobically at 37°C for 72 h. Total aerobic and anaerobic bacterial counts were determined using plate count agar medium (Oxoid, Basingstoke, UK), while plates were incubated at 30°C aerobically for 48 h and at 37°C anaerobically for 48-72 h respectively. For bacterial counts, typical colonies from an appropriate dilution were counted on microbial colony counter instrument and counts were expressed as colony forming units (CFU) x log per 1 g wet weight sample. Typical colonies grown on different media were then described and subcultured. All bacterial populations were identified on family level by the automated Vitek 2 compact system (bioMérieux, Marcy l'Etoile, France), which provide reliable and accurate results for a big range of Gram-positive and Gram-negative bacteria [24]. For the identification of *Enterobacteriaceae, Enterococcaceae, Lactobacillaceae and Bifidobacteriaceae*, the VITEK 2 Gram-Negative identification card (ID-GN) (bioMérieux, Marcy l'Etoile, France), the VITEK 2 Gram-Positive identification card (ID-GP) (bioMérieux, Marcy l'Etoile, France), the CBC and ANC identification cards (bioMérieux, Marcy l'Etoile, France) and the VITEK 2 ANC ID card (bioMérieux, Marcy l'Etoile, France) were used respectively.

2.2.3. Blood Parameters Analysis

For the determination of hematological and biochemical parameters, blood samples were taken from six pigs per treatment, prior to slaughter, on the last day of the trial (day 40). Feed was removed from the feeders 4 hours before blood sampling. 4 ml of blood was collected from the pigs' jugular vein and placed in vacutainer tubes with ethylenediamine tetra acetic acid (EDTA).

Haematological parameters (Haemoglobin; Erythrocytes; Haematocrit, HCT; Leucocytes; Lymphocytes) were determined using an automated analyser MS4 (Melet Schloesing Lab, Osny, France) and biochemical parameters (Albumine, ALB; Alanine aminotransferase, ALT; Aspartate aminotransferase, AST; Cholesterol, CHOL; Creatine kinase, CK; Glucose, GLU; Total bilirubin, TBIL; Triglycerides; TRIG) in serum using the IDEXX VETTEST 8008 (IDEXX LAB, USA).

2.2.4. Meat Chemical Analysis, pH measurement and Meat Color Analysis

Meat samples detached from the ham (Biceps femoris muscles), shoulder (Triceps branchii muscles) and belly (External abdominal muscles), were collected and stored at -20°C for the meat chemical analysis. Each meat sample, weighing 200g, was ground using an industrial large meat grinder (Bosch, Gerlingen, Germany). Moisture, crude protein, fat, collagen and ash content were determined by near infra-red spectroscopy using a FoodScanTM Lab (FOSS, Hilleroad, Denmark) in transmittance mode, according to AOAC 2007.04 for meat and meat products [25].

The pH measurement of shoulder, ham and belly samples was performed using a portable Hanna HI981036 instrument (Hanna Instruments, Woonsocket, RI) pH meter for solid samples, measuring 6 pig meat samples from each group by inserting the stainless probe deep in the tissue. Average values from each group were estimated.

The color of the shoulder, ham and belly meat samples was evaluated according to Hunter scale (L*, A*, B* values) by using the CAM - System 500 Chromatometer (Lovibond, Amesbury,UK).

2.2.5. Meat Oxidative Stability Analysis

For the measurement of the total polyphenols of the pig meat samples, a modified Folin - Ciocalteu method was used. According to this method, 0.2 g/L of gallic acid (Merck, Germany) was diluted in 100 ml of distilled water. The stock solution was used to prepare the standards solutions 0.005, 0.01, 0.05, 0.1, 0.25, 0.5 and 1 g/L of gallic acid. From each standard solution 0.2 ml was transferred into a 50 ml falcon tube and mixed with 10.8 ml of distilled water, 8 ml of Na_2CO_3 (75 g Na_2CO_3 in 1 L distilled water) (Penta Chemicals, Prague) and 1 ml of the Folin – Ciocalteu reagent (PanReac Applichem, Germany). A control sample was prepared in which 0.2 ml of distilled water was added instead of a standard solution to calibrate the UV - Vis

spectrophotometer (DR 5000, Hach Lange). All tubes were homogenized in vortex and they were placed in a dark cabinet for 1 hour at room temperature. After the incubation, the control was used to calibrate the UV – Vis spectrophotometer (DR 5000, Hach Lange) at 750nm and then all the standards solutions were measured. A standard curve of concentration of gallic acid and absorbance was constructed using the Microsoft Excel software while the R^2 was 0.9989. The above procedure was followed to measure the total polyphenols of the pigmeat. Then, 5 g of shoulder, belly or ham meat were homogenized in a blender with 10 ml of distilled water and filtered with a filter paper. 0.2 ml of the filtrate were transferred in 50 ml falcon tubes and mixed with 10.8 ml distilled water, 8 ml of Na_2CO_3 (75 g/L solution) and 1 ml of the Folin - Ciocalteu reagent. A blank sample was prepared in which 0.2 ml was added instead of sample to calibrate the UV - Vis spectrophotometer. All tubes were mixed in vortex and placed in a dark cabinet at room temperature for 1 hour. After incubation, the blank sample was used to calibrate the spectrophotometer at 750 nm and then all the samples were measured.

For the measurement of lipid oxidation of pig meat samples, a modified version of the 2-thiobarbituric acid method (TBARS, Thiobarbituric acid reactive substances) described by Dias et al. [26] was applied. In brief, 5 g of shoulder, ham or belly meat was homogenized with 25 ml of trichloroacetic acid in a blender and transferred in glass bottle and left there for 20 minutes. Then, the samples were filtered with filter paper and 5 ml of the filtrate was transferred in glass tubes with 5 ml of 2-thiobarbituric acid. A blank sample was prepared replacing the sample with 5 ml of trichloroacetic acid. All tubes were mixed in vortex and placed in water bath at 60°C for 15min. The samples were measured in a UV - Vis spectrophotometer after the calibration with the blank sample at 532nm.

2.2.6. Microbiological analysis of meat

For the enumeration of the bacterial count in meat the average values from six individual pigs from each group were estimated for each microbial population of meat samples. More specifically, 10 g of shoulder, belly or ham meat were homogenized in Bagmixer 400 (Interscience, France) with 90 ml of sterile Maximum Recovery Diluent (MRD) (Oxoid, Basingstoke, UK). Each sample was 10-fold diluted using glass tubes with 9 ml of sterile MRD. From the appropriate dilution was inoculated either 1 ml or 0,1 ml in petri dishes for the

enumeration of the bacterial count. The tested microorganisms were Escherichia coli which was cultivated on Tryptone Bile X- Glucuronide (TBX) agar (Oxoid, Basingstoke, UK) and incubated aerobically at 37°C for 24 h, Sulfite Reducing Clostridia were counted on Perfringens Agar Base (Oxoid, Basingstoke, UK) incubated at 37°C for 48 h under anaerobic conditions using anaerobic jars with the addition of Anaerocult A (Oxoid, Basingstoke, UK). Staphylococcus aureus and Staphylococcus spp. were spread on Baird Parker agar (Oxoid, Basingstoke, UK) which was supplemented with egg yolk tellurite (50 ml/ 1 l substrate) and incubated under aerobic conditions at 37°C for 48 h. Total Mesophilic Count was measured in Plate Count Agar (PCA) (Oxoid, Basingstoke, UK) at 30°C for 48 h under aerobic conditions and Campylobacter jejuni was spread on Campy Blood Free Selective Medium (CCDA) (Acumedia – Lab M, Lansing, USA) with Campylobacter selective supplement under microaerophilic conditions in incubator with 10 % CO_2 at 37°C for 72 h.

All samples were examined for the presence of Salmonella spp. and Listeria monocytogenes per 25g of shoulder, ham and belly meat using the ISO 6579:2002 and ISO 4833:2001 methods, respectively. The petri dishes were incubated in Binder BD 115 thermostable incubators.

2.2.7. Meat Fatty Acid Analysis

For shoulder and belly meat fatty acid analysis, samples were processed as recommended by O'Fallon et al. [27]. Methyl esters' separation and quantification was performed according the method described by Skoufos et al. [28], with the use of TraceGC (Model K07332, Thermofinigan, Thermoquest, Milan, Italy) equipped with a flame ionization detector.

2.2.8. Statistical Analysis

The basic study design was RCB (random complete block design), and each ear tagged pig was considered as the experimental unit. Microbiology data were log-transformed (Log10) prior to analysis. Data homogeneity was tested using Levene's test. Experimental data were analysed by one-way analysis of variance (one-way ANOVA) or Krushar-Wallis Test, depending on the data format, using SPSS v20 statistical package [29]. Tukey's test was used for post-hoc comparisons between the three treatments. Significance level for all tests was set at 5% ($p \leq 0.05$).

2.3. Results

2.3.1. Performance Parameters

The effects of the dietary use of the novel silage on pig performance parameters are presented in Table 2.3. Final body weight of the pigs did not differ (P>0.05) between the three treatments. It was noted that the weight gain was higher for treatment Silage 10% (P≤0.05) compared to the Silage 5% treatment during period of 22-40 days, however the weight gain for the overall period (1-40 days) did not differ significantly (P>0.05) between the three treatments, despite that the pigs were in the crucial post-weaning period of their life. Feed intake and feed conversion ratio were within the expected ranges for the commercial pig farm that housed the experimental trial. Concerning the carcass parameters, carcass weight and dressing percentage did not differ (P>0.05) between the three treatments.

Table 2.3. Effect of silage supplementation on pig performance parameters.

Body weight on day (kg)	**Diets**			**SEM**	**p-value**
	Silage-0%	**Silage-5%**	**Silage-10%**		
1	8.30	8.32	8.40	0.177	0.975
22	17.46	18.51	18.37	0.404	0.525
40	26.47	26.38	27.64	0.494	0.518
Weight gain for days (kg)					
1-22	9.16	10.18	9.97	0.291	0.322
22-40	9.01 [ab]	7.87 [a]	9.27 [b]	0.240	0.038
1-40	18.16	18.06	19.24	0.391	0.401
Feed intake per pig for days (kg)					
1-22	14.54	15.97	16.09	NA	NA
22-40	16.86	14.10	15.12	NA	NA
1-40	31.39	30.07	31.21	NA	NA
FCR for days (g feed / g weight gain)					
1-22	1.587	1.568	1.613	NA	NA
22-40	1.871	1.791	1.632	NA	NA
1-40	1.728	1.665	1.622	NA	NA
Carcass parameters					
Carcass weight (kg)	20.33	20.22	20.87	0.732	0.936
Carcass percentage (%)	74.24	74.44	74.52	0.326	0.941

NA, Non applicable.
[a,b] Values (n = 15 per treatment) with no common superscript differ significantly (P≤0.05)

2.3.2. Intestinal Microflora

Intestinal microflora populations were affected by the dietary use of the tested silage (Table 2.4). In the ileum digesta it was noted that total anaerobes were increased (P≤0.05) in treatment Silage 10%, compared to treatment Silage 5%. *Lactobacillaceae* (P≤0.001) were increased in the control Silage 0% and Silage 10% treatments compared to the Silage 5% treatment. In the caecum digesta, total anaerobes were lower (P≤0.001) in treatments Silage 5% and Silage 10% compared to the control treatment, while *Lactobacillaceae* were lowest (P≤0.001) in treatment Silage 10%, intermediate in treatment Silage 5% and highest in the control treatment Silage 0%. The other evaluated microbial species did not differ (P>0.05) between the three treatments.

Table 2.4. Effect of silage supplementation on pig intestinal microflora populations.

Ileum microbes (Log_{10} CFU/g)	**Diets**			**SEM**	**p-value**
	Silage-0%	**Silage-5%**	**Silage-10%**		
Total aerobes	5.831	6.467	6.444	0.187	0.304
Total anaerobes	7.138 ^{ab}	6.310 ^{a}	7.598 ^{b}	0.203	0.020
Enterobacteriaceae	5.150	4.258	5.347	0.334	0.388
Enterococcaceae	0.884	0.000	2.100	0.460	0.164
Lactobacillaceae	7.006 ^{b}	6.085 ^{a}	7.585 ^{b}	0.180	<0.001
Bifidobacteriaceae	2.985	0.616	1.146	0.431	0.052
Caecum microbes (Log_{10} CFU/g)					
Total aerobes	8.893	8.006	7.810	0.322	0.366
Total anaerobes	10.172 ^{b}	8.083 ^{a}	7.355 ^{a}	0.354	<0.001
Enterobacteriaceae	5.013	4.167	5.464	0.315	0.245
Enterococcaceae	2.333	0.000	1.984	0.520	0.133
Lactobacillaceae	10.878 ^{c}	9.788 ^{b}	8.117 ^{a}	0.290	<0.001
Bifidobacteriaceae	3.533	1.435	2.271	0.435	0.139

^{a,b} Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)

2.3.3. Blood parameters

The effect of the novel silage on pig blood hematological and biochemical parameters is presented in Table 2.5. Concerning hematological values, the monocyte levels were significantly higher (P≤0.01) in the Silage 10% treatment, compared to the other two treatments. Also, Hematocrit (Hct) and Hemoglobin (Hb) levels were lower (P≤0.05) in the Silage 10% group, compared to the control Silage 0% treatment. The other hematological parameters did not differ (P>0.05) between the treatments. Furthermore, regarding the biochemical parameters blood glucose (GLU) was lower (P≤0.05) in the Silage 10% treatment, compared to the control Silage 0% treatment. The other blood biochemical parameters did not differ between the three treatments.

Table 2.5. Effect of silage supplementation on pig blood hematological and biochemical parameters.

Hematological parameters	**Diets**			**SEM**	**p-value**
	Silage-0%	**Silage-5%**	**Silage-10%**		
WBC (10^3/μL)	23.66	19.83	18.52	1.488	0.366
Lymphocytes (%)	32.82	38.63	39.50	2.090	0.388
Monocytes (%)	8.07 ^{a}	7.35 ^{a}	13.03 ^{b}	0.583	0.002
RBC (10^6/μL)	5.92	5.66	5.48	0.193	0.347
Hct (%)	33.30 ^{b}	30.55 ^{ab}	28.85 ^{a}	0.535	0.013
Hb (g/dL)	12.20 ^{b}	11.88 ^{ab}	10.93 ^{a}	0.175	0.025
Blood biochemical parameters					
ALB (g/dL)	2.58	2.52	2.40	0.036	0.146
ALP (u/L)	327.17	277.83	355.50	13.628	0.094
ALT (u/L)	104.00	78.67	97.50	4.035	0.055
AST (u/L)	67.00	56.83	61.67	3.176	0.445
CHOL (mg/dL)	70.17	77.17	72.00	2.413	0.487
GLU (mg/dL)	97.17 ^{b}	80.67 ^{ab}	64.00 ^{a}	3.037	0.002
TRIG (mg/dL)	39.00	49.67	53.67	3.077	0.197

WBC: white blood cells; RBC: Red blood cells; HCT: hematocrit; HB: hemoglobin; ALB: Albumin; ALP: Alkaline Phosphatase; ALT: Alanin Aminotransferase; AST: Aspartate Aminotransferase; CHOL: Cholesterol; GLU: Glucose; TRIG: Triglycerides

^{a,b} Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)

2.3.4. Meat Analysis

As shown in Table 2.6, shoulder meat (Triceps branchii) ash content was increased (P≤0.05) in treatment Silage 10% compared to treatment Silage 5 %, however the other chemical composition parameters (fat, moisture, protein and collagen) did not differ (P>0.05). Regarding ham meat (Biceps femoris), no differences were noted in any of examined parameters. In the belly meat (External abdominal) ash content was increased (P≤0.01) in treatments Silage 5% and Silage 10%, compared to the control treatment Silage 0%, however the other chemical composition parameters (fat, moisture, protein and collagen) did not differ (P>0.05). The pH values of all meat samples did not differ between the treatments (P>0.05).

Table 2.6. Effect of silage supplementation on pig shoulder, ham and belly meat chemical composition and pH.

Shoulder meat (Triceps brachii) chemical composition (%)	**Diets**			**SEM**	**p-value**
	Silage-0%	**Silage-5%**	**Silage-10%**		
Fat	5.12	5.97	4.17	0.372	0.143
Moisture	76.18	75.48	77.14	0.307	0.078
Protein	17.92	18.04	18.06	0.154	0.935
Collagen	1.48	1.50	1.51	0.058	0.985
Ash	1.03 ^{ab}	0.97 ^{a}	1.14 ^{b}	0.2646	0.019
pH	5.57	5.66	5.61	0.051	0.787
Ham meat (Biceps femoris) chemical composition (%)					
Fat	3.97	3.33	4.16	0.219	0.281
Moisture	76.72	76.86	77.05	0.244	0.873
Protein	18.60	19.38	18.61	0.203	0.198
Collagen	1.19	1.04	1.19	0.032	0.081
Ash	1.02	1.07	1.07	0.028	0.709
pH	5.54	5.48	5.52	0.017	0.384
Belly meat (External abdominal) chemical composition (%)					
Fat	9.85	8.74	9.81	0.333	0.325
Moisture	72.18	72.32	71.96	0.328	0.916
Protein	17.35	17.67	17.06	0.114	0.087

Collagen	1.65	1.55	1.48	0.052	0.402
Ash	0.91 [a]	1.04 [b]	1.01 [b]	0.016	0.003
pH	5.52	5.50	5.54	0.014	0.397

[a,b] Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)

Regarding the color measurements (Table 2.7), there were no significant statistical differences (P>0.05) in the shoulder and the ham meat samples.

Table 2.7. Effect of silage supplementation on pig shoulder, ham and belly meat color.

	Diets			SEM	p-value
Shoulder meat color	**Silage-0%**	**Silage-5%**	**Silage-10%**		
L*	62.81	61.99	60.28	0.630	0.263
A*	10.60	11.22	12.01	0.301	0.165
B*	9.10	10.13	9.85	0.303	0.386
Ham meat color					
L*	64.46	65.49	61.23	1.315	0.416
A*	9.18	9.31	11.42	0.644	0.301
B*	9.85	10.81	9.58	0.381	0.416
Belly meat color					
L*	64.39	67.63	66.47	0.802	0.264
A*	11.61	9.27	9.11	0.536	0.094
B*	9.76	10.40	10.36	0.349	0.731

L*: Lightness; A*: redness; B*: yellowness.
a,b Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)

Table 2.8 presents the results of the meat microbiological analysis. In shoulder and ham meat samples, no significant differences (P>0.10) were identified between the three treatments. In the belly meat the Sulfite Reducing Clostridium (which includes the pathogenic clostridia C. perfringens and C. botulinum) counts were lower (P<0.05) in the Silage 10% treatment compared to the other two treatments, whereas the other examined microbial populations did not

differ between the treatments. Finally, in all samples there was absence of Salmonella spp. and Listeria monocytogenes (per 25 g of sample).

Table 2.8. Effect of silage supplementation on pig shoulder, ham and belly meat microbial populations.

	Diets			SEM	p-value
Shoulder meat microbes (Log CFU/g)	**Silage-0%**	**Silage-5%**	**Silage-10%**		
Total viable count	6.426	6.591	6.639	0.0922	0.647
E. coli	3.992	4.023	4.227	0.0893	0.540
S. aureus	3.213	3.107	3.137	0.1340	0.953
Staphylococcus spp	4.461	4.167	3.958	0.1548	0.443
Clostridium spp	4.235	4.054	3.776	0.1228	0.329
C. jejuni	3.830	3.832	3.773	0.0717	0.935
Ham meat microbes (Log CFU/g)					
Total viable count	6.677	6.203	5.713	0.1838	0.092
E. coli	4.091	4.183	4.279	0.0928	0.741
S. aureus	3.204	2.990	3.123	0.1279	0.813
Staphylococcus spp	4.344	3.590	3.802	0.1537	0.113
Clostridium spp	4.172	2.053	2.065	0.3836	0.066
C. jejuni	3.857	3.457	3.735	0.0771	0.085
Belly meat microbes (Log CFU/g)					
Total viable count	6.710	6.838	6.792	0.0820	0.833
E. coli	4.248	3.801	3.954	0.0991	0.178
S. aureus	3.384	3.186	3.145	0.1158	0.699
Staphylococcus spp	4.356	4.145	4.113	0.1329	0.750
Clostridium spp	4.382 [b]	4.352 [b]	3.690 [a]	0.1188	0.013
C. jejuni	3.828	3.837	4.026	0.0741	0.725

[a,b] Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)

Data on total phenolic content and oxidative stability of meat samples are presented in Table 2.9. In the shoulder meat, the phenolic content was significantly higher (P≤0.05) in Silage 10% treatment compared to the control Silage 0% treatment, whereas the TBARS analysis results did not differ between the treatments. In the ham meat, the phenolic content was significantly higher (P≤0.05) in the Silage 5% and Silage 10% treatments compared to the control Silage 0%

treatment. Also, the TBARS content was significantly higher (P≤0.05) in Silage 10% treatment compared to the control Silage 0% treatment. In the belly meat, the phenolic content was highest (P≤0,001) in the Silage 10% treatment, intermediate in the Silage 5% treatment and lowest in the control Silage 0% treatment.

Table 2.9. Effect of silage supplementation on pig ham, shoulder and belly meat oxidative stability.

	Diets			SEM	p-value
Shoulder meat	**Silage-0%**	**Silage-5%**	**Silage-10%**		
Total phenols (g / L)	0.86 [a]	1.79 [ab]	2.32 [b]	0.247	0.022
TBARS (mg MDA / kg)	0.088	0.053	0.047	0.0010	0.390
Ham meat					
Total phenols (g / L)	1.75 [a]	2.24 [b]	2.36 [b]	0.091	0.043
TBARS (mg MDA / kg)	0.123 [b]	0.081 [ab]	0.068 [a]	0.0099	0.045
Belly meat					
Total phenols (g / L)	1.55 [a]	2.15 [b]	2.79 [c]	0.096	0.001
TBARS (mg MDA / kg)	0.082 [b]	0.046 [ab]	0.036 [a]	0.0074	0.015

TBARS: Thiobarbituric acid reactive substances; MDA: Malondialdehyde.
[a,b,c] Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)

Fatty acid analysis of the shoulder meat cuts is presented in Table 2.10. The dietary supplementation of the examined silage modified (P<0.05) the fatty acid composition of most examined fatty acids, compared to the control 0% Silage treatment. Overall, the total saturated fatty acids (SFA) were lowest (P≤0.001) in the Silage 10% treatment, intermediate in the Silage 5% treatment and highest in the silage 0% treatment. The total monounsaturated fatty acids (MUFA) were highest (P≤0.001) in the Silage 5% treatment, intermediate in the Silage 0% treatment and lowest in the Silage 10% treatment. The total polyunsaturated fatty acids (PUFA) were higher (P≤0.001) in the supplemented Silage 5% and Silage 10% treatments compared to the control. The total omega-3 fatty acids were highest (P≤0.001) in the Silage 10% treatment, intermediate in the Silage 5% treatment and lowest in the silage 0% treatment. The total omega-6 fatty acids were highest (P≤0.001) in the Silage 0% treatment, intermediate in the Silage 5%

treatment and lowest in the silage 10% treatment. Finally, the ratio of omega-6/omega-3 fatty acids was lowest (P≤0.001) in the Silage 5% treatment, intermediate in the Silage 10% treatment and highest the silage 0% treatment.

The results of the fatty acid analysis of belly meat cuts are shown in Table 2.11. The dietary supplementation of the examined silage modified (P<0.05) the fatty acid composition of most examined fatty acids, compared to the control 0% Silage treatment. Overall, the total SFA were lowest (P≤0.001) in the Silage 10% treatment, intermediate in the Silage 5% treatment and highest in the silage 0% treatment. The total MUFA was lowest (P≤0.001) in the Silage 5% treatment, intermediate in the Silage 10% treatment and highest the silage 0% treatment. The total PUFA was highest (P≤0.001) in the Silage 5% treatment, intermediate in the Silage 10% treatment and lowest the silage 0% treatment. The total omega-3 fatty acids were highest (P≤0.001) in the Silage 10% treatment, intermediate in the Silage 5% treatment and lowest in the silage 0% treatment. The total omega-6 fatty acids were highest (P≤0.001) in the Silage 5% treatment, intermediate in the Silage 10% treatment and lowest in the silage 0% treatment. Finally, the ratio of omega-6/omega-3 fatty acids was lowest (P≤0.001) in the Silage 10% treatment, intermediate in the Silage 5% treatment and highest the silage 0% treatment.

Table 2.10. Effect of silage supplementation on pig shoulder meat fatty acid composition.

Shoulder meat FA (%)	**Diets**			**SEM**	**p-value**
	Silage-0%	**Silage-5%**	**Silage-10%**		
(C10:0) Capric	0.10	0.12	0.10	0.009	0.614
(C12:0) Lauric	0.10[a]	0.13 [ab]	0.18 [b]	0.119	0.011
(C14:0) Myristic	1.41[c]	1.07 [b]	0.76 [a]	0.065	0.001
(C16:0) Palmitic	22.80[c]	18.22[b]	15.78 [a]	0.706	0.001
(C16:1 cis) Palmitoleic	4.10 [c]	2.74[b]	2.13 [a]	0.200	0.001
(C17:0) Heptadecanoic	0.29[b]	0.21 [a]	0.22 [a]	0.012	0.006
(C17:1 cis-10) Heptadecenoic cis	0.21[b]	0.17[b]	0.11 [a]	0.013	0.002
(C18:0) Stearic	11.07[c]	6.08 [a]	7.20[b]	0.519	0.001
(C18:1 cis n9) Oleic	32.66 [a]	36.68 [c]	34.52[b]	0.399	0.001
(C18:1 n7) Vaccenic	3.24 [c]	2.67 [b]	1.94 [a]	0.129	0.001
(C18:2 n6c) Linoleic	19.21 [a]	22.76 [b]	24.49 [c]	0.533	0.001
(C20 :1 cis n9) cis-11-Eicosenoic	1.52 [a]	1.68 [b]	1.78 [c]	0.027	0.001
(C18:4n3) Stearidonic	0.64 [c]	0.53 [b]	0.46 [a]	0.020	0.001
(C20 :2 cis n6) cis-11,14-Eicosadienoic	0.65 [a]	0.67 [a]	0.79 [b]	0.017	0.001
C20:3 cis n6) cis-8,11,14-Eicosatrienoate	0.19 [a]	0.52 [b]	0.64 [c]	0.047	0.001

(C22:5 cis n3) cis-7,10,13,16,19-Docosapentaenoic acid	0.00[a]	0.66[b]	0.68[b]	0.078	0.001
(C20:3 cis n3) cis-11-14-17-Eicosatrienoate	0.00[a]	0.72[b]	0.88[c]	0.093	0.001
(C20:4 cis n6) Arachidonic	0.00[a]	0.99[b]	1.10[c]	0.121	0.001
(C20:5 cis n3) Cis-5,8,11,14,17-Eicosapentaenoic	0.32[a]	0.41[b]	0.42[b]	0.014	0.001
(C21:5 n3) Heneicosapentaenoate	0.22[a]	0.41[b]	0.47[c]	0.027	0.001
(C22 :6 cis n3) cis-4,7,10,13,16,19-Docosahexaenoic	0.00[a]	2.02[b]	1.98[b]	0.229	0.001
Σ SFA (Total Saturated FA)	35.77[c]	25.83[b]	24.23[a]	1.238	0.001
Σ MUFA (Total Monounsaturated FA)	41.91[b]	44.11[c]	40.49[a]	0.361	0.001
Σ PUFA (Total Polyunsaturated FA)	21.22[a]	29.69[b]	34.54[b]	1.331	0.001
Σ n3 (Total omega-3 FA)	1.18[a]	4.75[b]	4.89[c]	0.416	0.001
Σ n6 (Total omega-6 FA)	20.05[a]	24.94[b]	27.07[c]	0.709	0.001
Ratio n6/n3 FA	16.99[c]	5.25[a]	5.53[b]	1.327	0.001

FA: Fatty acids
[a,b] Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)

Table 2.11. Effect of silage supplementation on pig belly meat fatty acid composition.

Belly meat FA (%)	**Diets**			**SEM**	**p-value**
	Silage-0%	**Silage-5%**	**Silage-10%**		
(C10:0) Capric	0.07	0.07	0.10	0.009	0.348
(C12:0) Lauric	0.08	0.08	0.09	0.009	0.882
(C14:0) Myristic	1.32	1.30	1.32	0.009	0.614
(C16:0) Palmitic	22.94[c]	22.58[b]	20.96[a]	0.210	0.001
(C16:1 cis) Palmitoleic	2.99[b]	2.91[a]	3.13[c]	0.024	0.001
(C17:0) Heptadecanoic	0.38[c]	0.30[b]	0.22[a]	0.018	0.001
(C17:1 cis-10) Heptadecenoic cis	0.24[b]	0.14[a]	0.13[a]	0.015	0.001
(C18:0) Stearic	12.30[c]	11.54[b]	11.02[a]	0.128	0.001
(C18:1 cis n9) Oleic	34.51[c]	27.21[a]	31.28[b]	0.724	0.001
(C18:1 n7) Vaccenic	2.31[a]	2.59[b]	2.72[c]	0.042	0.001
(C18:2 trans n6) Linelaidic	0.11[b]	0.05[a]	0.06[ab]	0.010	0.043
(C18:2 n6c) Linoleic	18.19[a]	23.55[c]	20.47[b]	0.533	0.001
(C18:3 cis n6) g-Linolenic	0.14[a]	0.51[c]	0.44[b]	0.040	0.001
(C20 :1 cis n9) cis-11-Eicosenoic	0.12[b]	0.03[a]	0.07[ab]	0.013	0.002
(C18:3 trans n3) Linolenic	1.36[a]	2.02[b]	2.11[c]	0.082	0.001
(C18:4 n3) Stearidonic	0.57	0.55	0.54	0.009	0.434
(C20 :2 cis n6) cis-11,14-Eicosadienoic	0.56[a]	0.63[b]	0.72[c]	0.018	0.001
(C20:3 cis n6) cis-8,11,14-Eicosatrienoate	0.17[a]	0.55[b]	0.18[a]	0.044	0.001
(C22:5 cis n3) cis-7,10,13,16,19-Docosapentaenoic acid	0.25[a]	0.61[b]	0.64[b]	0.043	0.001
(C20:3 cis n3) cis-11-14-17-Eicosatrienoate	0.13[a]	0.33[b]	0.41[c]	0.299	0.001

(C20:4 cis n6) Arachidonic	0.49 [a]	0.97 [b]	0.98 [b]	0.561	0.001
(C20:5 cis n3) Cis-5,8,11,14,17-Eicosapentaenoic	0.12 [a]	0.22 [b]	0.47 [c]	0.037	0.001
(C21:5 n3) Heneicosapentaenoate	0.18 [a]	0.41 [b]	0.45 [b]	0.031	0.001
(C22 :6 cis n3) cis-4,7,10,13,16,19-Docosahexaenoic	0.37 [a]	0.82 [b]	1.39 [c]	0.102	0.001
Σ SFA (Total Saturated FA)	37.18 [c]	35.94 [b]	33.77 [a]	0.342	0.001
Σ MUFA (Total Monounsaturated FA)	40.17 [c]	32.84 [a]	37.33 [b]	0.732	0.001
Σ PUFA (Total Polyunsaturated FA)	22.65 [a]	31.23 [c]	28.86 [b]	0.877	0.001
Σ n3 (Total omega-3 FA)	2.98 [a]	4.96 [b]	6.01 [c]	0.305	0.001
Σ n6 (Total omega-6 FA)	19.66 [a]	26.26 [c]	22.85 [b]	0.654	0.001
Ratio n6/n3 FA	6.60 [c]	5.29 [b]	3.80 [a]	0.278	0.001

FA: Fatty acids
[a,b] Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)

2.4. Discussion

The increasing future demand for livestock products, driven by global population growth and consequent urbanization, will impose an increasing shortage of available feed resources by 2050 [30]. The efficient and sustainable development of the livestock sector should include the reduction of wastage and furthermore a feasible reduction of production costs, by delivering an enlargement of the feed resource base. Such tasks can be achieved at least partially through the development of novel animal feeds, particularly those not competing with human foods.

In this trial, a novel silage made from olive, winery, and cheese waste by-products was fed to weaned pigs in a commercial farm in Greece to investigate its effect on their performance, health and meat quality characteristics. Cheese waste was incorporated in the initial design of the biofunctional silage mainly due to its high caloric and lactose content [31], grape pomace due to its abundance in phenolic compounds, unsaturated fatty acids, dietary fiber, and beneficial microorganisms [32], while olive mill waste water was included due to the presence of several compounds with demonstrated antioxidant and radical scavenging action, including hydroxytyrosol, oleuropein, tyrosol, caffeic acid, p-cumaric acid, verbascoside, and elenolic acid [33]. Similar agro-industrial by-product have been previously individually tested in several trials as ingredients of pig diets with encouraging results regarding the economical and biological effects: olive mill waste water solids [18,34,35], grape pomace solids [12,13,15,20] or cheese way solids [36-39]. To the best of our knowledge, it is the first time that such a combination of the previously mentioned three agro-industrial wastes, in a silage form, has been evaluated in weaned pig diets. It has been reported that partially delactosed whey in the feed of nonruminants

can lead to increased body weight gain, enhanced feed efficiency and ameliorated protein and fat digestibility [31]. On the other hand, Martins et. al [38] found no significant differences ($P>0.05$) in the performance and carcass indices of growing pigs which were fed with cheese whey that substituted 0%, 20% or 30% of the dry substance of their rations. In trials using only grape pomace solids in pig diets, Hao et al. [12] and Wang et al. [20] reported no significant effects ($P>0.05$) between control and grape pomace enriched dietary pig groups in their growth performance, average daily feed intake and feed conversion ratio. In contrast, Kafantaris et al. [15] noticed a significant increase ($P<0.05$) in average daily gain of the piglets that consumed the grape pomace experimental diet, while Liehr et al. [35] demonstrated refined piglet growth due to improvements in intestinal integrity after consumption of olive-oil bioactive extracts. Our results, with the use of the bioactive silage, are partly in agreement with the above findings since it was noted that body weight gain was higher for treatment Silage 10% ($P\leq0.05$) compared to the other two treatments during period 22-40 days, but with neither positive nor negative effects on the other performance and carcass parameters.

One of the key variables influencing the content and operation of the pigs' gut microbiota is their diet [40]. The composition of the gut microbiome and, as a result, the products of bacterial metabolism have an impact on host health. Kafantaris et al. [15] reported a significant increase ($P<0.05$) of lactic acid bacteria and a significant reduction ($P< 0.05$) of Enterobacteriaceae in piglets feces that were fed a grape pomace experimental diet for 30 days. The grape pomace fed group's intestinal microbiota species did not change when compared to the control group, but the ratio of beneficial bacteria increased [20]. In our study, intestinal microflora populations were affected by the dietary use of the novel silage fed at 5% or 10% concentrations. A significant ($P<0.05$) reduction in total anaerobes in the pig's ileum (Silage 5%) and caecum (both Silage 5% and Silage 10%) was recorded. In addition, the dietary silage supplementation modified the Lactobacillae counts both the ileum and the caecum. Research on pigs fed diets containing grape pomace revealed that this supplementation can attenuate the number of *Lactobacillaceae* counts in the proximal colon [41] while other researchers [42] reported a positive impact of feeding apple pomace and red-grape pomace on *Lactobacillaceae* numbers and a tendency to attenuate total anaerobes [43]. In addition, the overall balance of other intestinal microbial families could potentially be modified such as *Enterobacteriaceae, Enterococcaceae,* and *Bifidobacteriaceae,* although such effects were not apparent in the present trial.

In the present study, hematological and biochemical parameters were analyzed as indications of general health status. Concerning hematological values, the monocyte levels were significantly higher in the 10% Silage treatment, while both Hematocrit (Hct) and Hemoglobin (Hb) levels were the lowest in this treatment. These results are in accordance with other studies where piglets were fed apple or red-grape pomace enriched diet and reported decreased Hematocrit (Hct) and Hemoglobin (Hb) levels and elevated monocytes [43]. Biochemical parameters were similar for all treatments except for blood glucose (GLU), which was lowest in the Silage-10% group. Similarly, Formigoni et al. [37] determined a significant reduction of plasma glucose and urea after feeding pigs with liquid whey and theorised that this could be a side effect of reduced glucose absorption in the small intestine.

The chemical composition of the meat in monogastric animal can be modified to a noticeable decree by dietary changes. In the present study, the dietary supplementation with the silage did not affect main meat chemical composition values, as fat, protein, collagen, moisture and pH did not differ significantly between the three treatments for all meat cuts. The only apparent difference was a small increase in the total ash content of the shoulder and belly, especially in the Silage 10% treatment. Moreover, the carcasses and meat cuts quality parameters were within acceptable limits for commercial use. The ash level of the meat is a significant component in determining its nutritional value, quality, and physicochemical characteristics while its content, along with protein, varies depending on water content [44]. Ash content has been positively correlated and intramuscular fat negatively correlated to increased lean meat percentage in pigs [45]. Our findings suggest that not only can the novel silage be utilized without affecting meat attributes, but it may also be a promising option for further research and use, to better understand the biological impacts of mineral deposition in muscle.

Color of meat is a major quality characteristic and a key factor for consumer preference. Marbling and color are used to assess the 'value' or quality of a meat cut. In the present study, there were no apparent differences in any of the examined color parameters (lightness, redness, yellowness). It has been reported that color can be affected by some feed ingredients, such as the carotenoids and other pigments that can be found in plant material or the feed iron levels [46]. Moreover, meat color can be modified during storage through the combined effects of water loss, maturation and lipid oxidation. Previously, Tian et al. [47] indicated no significant effects on meat color parameters of pigs fed a 6% dried grape pomace powder, while other researchers

noticed an increase of A* value which led to redness of pork meat [46] or even a rise in both A* and B* values (20% and 31%, respectively), for 21 day old piglets fed a 3% grape pomace solids inclusion rate [48].

Microbial growth in meat cuts is closely related to their quality and safety. In the present study. In the present study, the identified microbial populations were low and within acceptable levels, and in all meat samples there was absence of Salmonella spp. and Listeria monocytogenes (per 25 g). The only apparent statistically significant effect was the reduction of sulphite-reducing clostridia (which include the pathogenic clostridia *C. perfringens* and *C. botulinum*) especially in the belly meat samples. The antimicrobial activity of grape pomace has been reported and is attributed to its flavonoid content and non-flavonoid (phenolic acids and stilbenes) compounds [49,50]. There is a link between gut microbiota, development and function of skeletal muscle and meat quality, implying that diet can influence microbial populations, bacterial metabolites, and meat quality [51,52]. The microbiota heredity has been estimated for carcass composition and meat quality traits in pigs, and positive microbial correlations have been found among different traits, particularly those related to meat color and firmness score [53]. It should be noted that in a previous trial that tested the same novel silage in broiler diets at the same inclusion levels (5% and 10%), the effect of this supplementation on broiler meat microbial populations was more noticeable [21]. This variability potentially highlights the biological and physiological differences in digestion, growth and tissue composition between different animal species, as well as the need to extensively test new products in different animal production systems.

Weaning is a critical event that can cause physiological, environmental, and social stress in pigs, increasing their risk of intestinal dysfunction and oxidative stress [54,55]. Lipid oxidation is closely related to the control of meat pathogenic or spoilage microflora, as well as to the meat products' quality and organoleptic properties. In the present study, total phenol content was elevated in meat cuts of the supplemented treatments and especially in the Silage 10% treatment that consumed the highest amount of silage. Respectively, the TBARS levels were reduced in the most meat cuts and to a higher amount in the Silage 10% treatment. It seems that there was a correlation between dietary phenol content and meat resistance to oxidation. This is an important finding since lipid oxidation and rancidity directly affect meat quality and storability, especially during refrigeration or freezing of the meats. Polyphenols' ability to act as antioxidants or free radical scavengers, as well as their ability to inhibit some enzymes involved in free radical

production and thus stimulate an immune response, have been previously described [56]. Gerasopoulos et al. [18], fed a diet enriched with olive mill waste water solids to piglets, and reported downregulated oxidative stress-induced lipid and protein damage, as demonstrated by decrease in TBARS and CARB levels, respectively. Piglet diets containing 5% grape pomace were proven beneficial for overall normal blood constituent metabolism and health maintenance by increasing polyphenol content in blood plasma and antioxidant activity in the liver, spleen, and kidneys [13]. Piglets fed an experimental diet containing 9% grape pomace solids showed less oxidative stress-induced damage to lipids and proteins, as evidenced by lower levels of TBARS and CARB in the grape pomace solids group compared to the control [15].

It has been well established that the fatty acid composition of the meat lipids in monogastric animals is directly affected by dietary lipids [21,57,58]. In the present trial, the meat fatty acid composition was modified to an extensive degree by the silage supplementation. Overall, the supplemented treatments had elevated amounts of polyunsaturated fatty acids and especially the desirable omega-3 (n3) fatty acids (Docosapentaenoic acid, Docosahexaenoic acid), which resulted to reduced omega-6/omega-3 (n6/n3) ratios. It has been documented that the inclusion of olive mill waste water solids in ruminants diets, increases MUFA levels while decreasing SFA levels in dairy and meat products and this effect is conducive to consumer health [9,59]. Additionally, Gerasopoulos et al. [34] described decreased n6/n3 ratios in the plasma and tissues of piglets fed ensilaged byproducts of olive mill waste water solids, while other researchers indicate significant increases of unsaturated fatty acids (MUFAs and PUFAs) in the meat of finishing pigs [60,61]. Similarly, Kafantaris et al. [15] reported that the inclusion of grape pomace in piglet diets increased significantly the omega-3 fatty acids and decreased significantly omega-6/omega-3 ratio compared with control diets ($P<0.05$). In contrast there are studies that report dissimilar results such a no effects of grape pomace solids inclusion (at a 5% rate in finishing pig diets) on meat SFA, MUFA, PUFA and n-6 PUFA fatty acids or an increase in omega-3 PUFA and a decrease in omega-6/omega-3 ratio [62].

2.5. Conclusions

This experiment evaluated for the first time the effects of a novel dietary silage created by combining olive mill, winery and cheese-making by-products of the Greek agro-industry sector on the productive, health and meat quality parameters of young, weaned pigs. The results

indicate that the tested silage containing various bioactive compounds, had no adverse effects on growth performance, zootechnical and health traits of the weaned pigs. In addition, the tested silage did not appear to have adverse effects in gut function and microbial balance. Notable effects were identified on the meat oxidative stability and fatty acid profile. Further research is certainly needed to test the bioactivity of these types of silages in pigs in different ratios and age periods so as to positively influence the environmental impact of pig production in relation to the environmental pollution imposed by the improper disposal of the silage's main ingredients.

2.6. References

1. Makkar, H.P.S. Review: Feed demand landscape and implications of food-not feed strategy for food security and climate change. *Animal* 2018, *12*, 1744-1754, doi:10.1017/S175173111700324X.
2. Harding, T.; Herzberg, J.; Kuralbayeva, K. Commodity prices and robust environmental regulation: Evidence from deforestation in Brazil. *J. Environ. Econ. Manage.* 2021, *108*, 102452, doi:10.1016/j.jeem.2021.102452.
3. Allipour Birgani, R.; Kianirad, A.; Shab Bidar, S.; Djazayery, A.; Pouraram, H.; Takian, A. Climate change and food price: a systematic review and meta-analysis of observational studies, 1990-2021. *Am. J. Clim. Change* 2022, *11*, 103-132, doi:10.4236/ajcc.2022.112006.
4. Sidiropoulou, E.; Marugán-Hernández, V.; Skoufos, I.; Giannenas, I.; Bonos, E.; Aguiar-Martins, K.; Lazari, D.; Papagrigoriou, T.; Fotou, K.; Grigoriadou, K.; et al. In vitro antioxidant, antimicrobial, anticoccidial, and anti-inflammatory study of essential oils of oregano, thyme, and sage from Epirus, Greece. *Life (Basel)* 2022, *12*, 1783, doi:10.3390/life12111783.
5. FAO. The importance of Ukraine and the Russian federation for global agricultural markets and the risks associated with the war in Ukraine, June, 1–47. Available online: https://www.fao.org/3/cb9013en/cb9013en.pdf. 2022.
6. Foti, P.; Romeo, F.V.; Russo, N.; Pino, A.; Vaccalluzzo, A.; Caggia, C.; Randazzo, C.L. Olive Mill Wastewater as Renewable Raw Materials to Generate High Added-Value Ingredients for Agro-Food Industries. *Appl. Sci.* 2021, *11*, doi:10.3390/app11167511.
7. Eurostat. Annual Production Series of Dairy products, 2 March. Available online: https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/farming/documents/eu-dairy-historical-production-stocks-series_en.pdf. 2021.
8. Chowdhary, P.; Gupta, A.; Gnansounou, E.; Pandey, A.; Chaturvedi, P. Current trends and possibilities for exploitation of Grape pomace as a potential source for value addition. *Environ. Pollut.* 2021, *278*, 116796, doi:10.1016/j.envpol.2021.116796.
9. Petrotos, K.; Papaioannou, C.; Kokkas, S.; Gkoutsidis, P.; Skoufos, I.; Tzora, A.; Bonos, E.; Tsinas, A.; Giavasis, I.; Mitsagga, C. Optimization of the composition of a novel bioactive silage produced by mixing of ground maize grains with olive mill waste waters, grape

pomace and feta cheese whey. *AgriEngineering* 2021, *3*, 868-893, doi:10.3390/agriengineering3040055.

10. Brenes, A.; Viveros, A.; Chamorro, S.; Arija, I. Use of polyphenol-rich grape by-products in monogastric nutrition. A review. *Anim. Feed Sci. Technol.* 2016, *211*, 1-17, doi:10.1016/j.anifeedsci.2015.09.016.
11. Hassen, I.; Casabianca, H.; Hosni, K. Biological activities of the natural antioxidant oleuropein: Exceeding the expectation – A mini-review. *J. Funct. Foods* 2015, *18*, 926-940, doi:10.1016/j.jff.2014.09.001.
12. Hao, R.; Li, Q.; Zhao, J.; Li, H.; Wang, W.; Gao, J. Effects of grape seed procyanidins on growth performance, immune function and antioxidant capacity in weaned piglets. *Livest. Sci.* 2015, *178*, 237-242, doi:10.1016/j.livsci.2015.06.004.
13. Chedea, V.S.; Palade, L.M.; Pelmus, R.S.; Dragomir, C.; Taranu, I. Red Grape Pomace Rich in Polyphenols Diet Increases the Antioxidant Status in Key Organs—Kidneys, Liver, and Spleen of Piglets. *Animals* 2019, *9*, doi:10.3390/ani9040149.
14. Mahfuz, S.; Shang, Q.; Piao, X. Phenolic compounds as natural feed additives in poultry and swine diets: a review. *J. Anim. Sci. Biotechnol.* 2021, *12*, 48, doi:10.1186/s40104-021-00565-3.
15. Kafantaris, I.; Stagos, D.; Kotsampasi, B.; Hatzis, A.; Kypriotakis, A.; Gerasopoulos, K.; Makri, S.; Goutzourelas, N.; Mitsagga, C.; Giavasis, I.; et al. Grape pomace improves performance, antioxidant status, fecal microbiota and meat quality of piglets. *Animal* 2018, *12*, 246-255, doi:10.1017/S1751731117001604.
16. Ajila, C.M.; Brar, S.K.; Verma, M.; Tyagi, R.D.; Godbout, S.; Valéro, J.R. Bio-processing of agro-byproducts to animal feed. *Crit. Rev. Biotechnol.* 2012, *32*, 382-400, doi:10.3109/07388551.2012.659172.
17. Georganas, A.; Giamouri, E.; Pappas, A.C.; Zoidis, E.; Goliomytis, M.; Simitzis, P. Utilization of agro-industrial by-products for sustainable poultry production. *Sustainability* 2023, *15*, doi:10.3390/su15043679.
18. Gerasopoulos, K.; Stagos, D.; Kokkas, S.; Petrotos, K.; Kantas, D.; Goulas, P.; Kouretas, D. Feed supplemented with byproducts from olive oil mill wastewater processing increases antioxidant capacity in broiler chickens. *Food Chem. Toxicol.* 2015, *82*, 42-49, doi:10.1016/j.fct.2015.04.021.

19. Makri, S.; Kafantaris, I.; Stagos, D.; Chamokeridou, T.; Petrotos, K.; Gerasopoulos, K.; Mpesios, A.; Goutzourelas, N.; Kokkas, S.; Goulas, P.; et al. Novel feed including bioactive compounds from winery wastes improved broilers' redox status in blood and tissues of vital organs. *Food Chem. Toxicol.* 2017, *102*, 24-31, doi:10.1016/j.fct.2017.01.019.
20. Wang, R.; Yu, H.; Fang, H.; Jin, Y.; Zhao, Y.; Shen, J.; Zhou, C.; Li, R.; Wang, J.; Fu, Y.; et al. Effects of dietary grape pomace on the intestinal microbiota and growth performance of weaned piglets. *Arch. Anim. Nutr.* 2020, *74*, 296-308, doi:10.1080/1745039X.2020.1743607.
21. Bonos, E.; Skoufos, I.; Petrotos, K.; Giavasis, I.; Mitsagga, C.; Fotou, K.; Vasilopoulou, K.; Giannenas, I.; Gouva, E.; Tsinas, A.; et al. Innovative use of olive, winery and cheese waste by-products as functional ingredients in broiler nutrition. *Vet. Sci.* 2022, *9*, doi:10.3390/vetsci9060290.
22. NRC. *National Research Council, Nutrient Requirements of Swine*; The National Academies Press: Washington, DC, 2012.
23. PremierNutrition. *Premier Atlas 2014*; Brereton, UK, 2014.
24. Hernández-Durán, M.; López-Jácome, L.; Colín-Castro, C.; Cerón-González, G.; Ortega-Peña, S.; Vanegas-Rodríguez, E.; Mondragón-Eguiluz, J.; Franco-Cendejas, R. Comparison of the MicroScan WalkAway and VITEK 2 Compact systems for the identification and susceptibility of clinical Gram-positive and Gram-negative bacteria. *Investigación en Discapacidad* 2017, *6*.
25. Anderson, S. Determination of fat, moisture, and protein in meat and meat products by using the FOSS FoodScan near-infrared spectrophotometer with FOSS artificial neural network calibration model and associated database: collaborative study. *J. AOAC Int.* 2007, *90*, 1073-1083, doi:10.1093/jaoac/90.4.1073.
26. Dias, F.F.G.; Augusto-Obara, T.R.; Hennebelle, M.; Chantieng, S.; Ozturk, G.; Taha, A.Y.; Vieira, T.; Leite Nobrega de Moura Bell, J.M. Effects of industrial heat treatments on bovine milk oxylipins and conventional markers of lipid oxidation. *Prostaglandins Leukot. Essent. Fatty Acids* 2020, *152*, 102040, doi:10.1016/j.plefa.2019.102040.
27. O'Fallon, J.V.; Busboom, J.R.; Nelson, M.L.; Gaskins, C.T. A direct method for fatty acid methyl ester synthesis: application to wet meat tissues, oils, and feedstuffs. *J. Anim. Sci.* 2007, *85*, 1511-1521, doi:10.2527/jas.2006-491.

28. Skoufos, I.; Giannenas, I.; Tontis, D.; Bartzanas, T.; Kittas, C.; Panagakis, P.; Tzora, A. Effects of oregano essential oil and attapulgite on growth performance, intestinal microbiota and morphometry in broilers. *South African Journal of Animal Science* 2016, *46*, 77-88.
29. SPSS. *SPSS Statistics for Windows*; IBM: Armonk, NY, USA, 2018.
30. FAO; WHO. Sustainable healthy diets guiding principles. 2019.
31. Schingoethe, D.J. Nutritional supplements for animals: Dairy by-productstheir origin, manufacture, composition, uses, dosage, and effect. In CRC Handbook of Nutritional Supplements: Volume II Agricultural Use. 2018.
32. Hassan, Y.I.; Kosir, V.; Yin, X.; Ross, K.; Diarra, M.S. Grape pomace as a promising antimicrobial alternative in feed: a critical review. *J. Agric. Food Chem.* 2019, *67*, 9705-9718, doi:10.1021/acs.jafc.9b02861.
33. Ghanbari, R.; Anwar, F.; Alkharfy, K.M.; Gilani, A.-H.; Saari, N. Valuable nutrients and functional bioactives in different parts of olive (Olea europaea L.)—A Review. *Int. J. Mol. Sci.* 2012, *13*, 3291-3340, doi:10.3390/ijms13033291.
34. Gerasopoulos, K.; Stagos, D.; Krouezas, A.; Karaveli, C.; Barda, C.; Gkika, H.; Mitsiou, D.; Petrotos, K.; Goulas, P.; Kouretas, D. Assessment of fatty acid allocation in plasma and tissues in piglets, using feed supplemented with byproducts from processed olive mill wastewater. *In Vivo* 2016, *30*, 291-301.
35. Liehr, M.; Mereu, A.; Pastor, J.J.; Quintela, J.C.; Staats, S.; Rimbach, G.; Ipharraguerre, I.R. Olive oil bioactives protect pigs against experimentally-induced chronic inflammation independently of alterations in gut microbiota. *PLOS ONE* 2017, *12*, e0174239, doi:10.1371/journal.pone.0174239.
36. Maswaure, S.M.; Mandisodza, K.T. An evaluation of the performance of weaner pigs fed diets incorporating fresh sweet liquid whey. *Anim. Feed Sci. Technol.* 1995, *54*, 193-201, doi:10.1016/0377-8401(95)00775-I.
37. Formigoni, A.; Piva, A.; Pezzi, P.; Castellani, G.; Biagi, G. The influence of feeding fresh liquid whey on some blood metabolites, insulin, and cecal fermentations of growing pigs. *Anim. Feed Sci. Technol.* 2006, *131*, 53-66, doi:10.1016/j.anifeedsci.2006.02.007.
38. Martins, T.D.D.; Pimenta Filho, E.; Costa, R.; Souza, J.H.M. Liquid serum from cheese as feeding supplement for growing pigs. *Rev. Cienc. Agron.* 2008, *39*, 301-307.

39. Kobayashi, Y.; Itoh, A.; Miyawaki, K.; Koike, S.; Iwabuchi, O.; Iimura, Y.; Kobashi, Y.; Kawashima, T.; Wakamatsu, J.; Hattori, A.; et al. Effect of liquid whey feeding on fecal microbiota of mature and growing pigs. *Anim. Sci. J.* 2011, *82*, 607-615, doi:10.1111/j.1740-0929.2011.00876.x.
40. Patil, Y.; Gooneratne, R.; Ju, X.-H. Interactions between host and gut microbiota in domestic pigs: a review. *Gut Microbes* 2020, *11*, 310-334, doi:10.1080/19490976.2019.1690363.
41. Williams, A.R.; Krych, L.; Fauzan Ahmad, H.; Nejsum, P.; Skovgaard, K.; Nielsen, D.S.; Thamsborg, S.M. A polyphenol-enriched diet and Ascaris suum infection modulate mucosal immune responses and gut microbiota composition in pigs. *PLOS ONE* 2017, *12*, e0186546, doi:10.1371/journal.pone.0186546.
42. Sehm, J.; Lindermayer, H.; Dummer, C.; Treutter, D.; Pfaffl, M.W. The influence of polyphenol rich apple pomace or red-wine pomace diet on the gut morphology in weaning piglets. *J. Anim. Physiol. Anim. Nutr.* 2007, *91*, 289-296, doi:10.1111/j.1439-0396.2006.00650.x.
43. Sehm, J.; Treutter, D.; Lindermayer, H.; Meyer, H. The influence of apple- or red-grape pomace enriched piglet diet on blood parameters, bacterial colonisation, and marker gene expression in piglet white blood cells. *Food Nut. Scien.* 2011, *2*, 366-376, doi:10.4236/fns.2011.24052.
44. Marušić, N.; Petrović, M.; Vidaček, S.; Petrak, T.; Medić, H. Characterization of traditional Istrian dry-cured ham by means of physical and chemical analyses and volatile compounds. *Meat Sci.* 2011, *88*, 786-790, doi:10.1016/j.meatsci.2011.02.033.
45. Okrouhla, M.; Stupka, R.; Citek, J.; Sprysl, M.; Trnka, M.; Kluzakova, E. Effect of lean meat proportion on the chemical composition of pork. *Czech J. Food Scien.* 2008, *26*, 464-469.
46. Bertol, T.M.; Ludke, J.V.; Campos, R.M.L.d.; Kawski, V.L.; Cunha Junior, A.; Figueiredo, E.A.P.d. Inclusion of grape pomace in the diet of pigs on pork quality and oxidative stability of omega-3 enriched fat. *Cienc. Rural* 2017, *47*.
47. Tian, X.; Li, D.; Zhao, X.; Xiao, Z.; Sun, J.; Yuan, T.; Wang, Y.; Zuo, X.; Yang, G.; Yu, T. Dietary grape pomace extract supplementation improved meat quality, antioxidant capacity, and immune performance in finishing pigs. *Front. Microbiol.* 2023, *14*, 1116022, doi:10.3389/fmicb.2023.1116022.

48. Alfaia, C.M.; Costa, M.M.; Lopes, P.A.; Pestana, J.M.; Prates, J.A.M. Use of grape by-products to enhance meat quality and nutritional value in monogastrics. *Foods* 2022, *11*, doi:10.3390/foods11182754.
49. Katalinić, V.; Možina, S.S.; Skroza, D.; Generalić, I.; Abramovič, H.; Miloš, M.; Ljubenkov, I.; Piskernik, S.; Pezo, I.; Terpinc, P.; et al. Polyphenolic profile, antioxidant properties and antimicrobial activity of grape skin extracts of 14 Vitis vinifera varieties grown in Dalmatia (Croatia). *Food Chem.* 2010, *119*, 715-723, doi:10.1016/j.foodchem.2009.07.019.
50. Friedman, M. Antibacterial, antiviral, and antifungal properties of wines and winery byproducts in relation to their flavonoid content. *J. Agric. Food Chem.* 2014, *62*, 6025-6042, doi:10.1021/jf501266s.
51. Qi, R.; Sun, J.; Qiu, X.; Zhang, Y.; Wang, J.; Wang, Q.; Huang, J.; Ge, L.; Liu, Z. The intestinal microbiota contributes to the growth and physiological state of muscle tissue in piglets. *Scientific Reports* 2021, *11*, 11237, doi:10.1038/s41598-021-90881-5.
52. Chen, B.; Li, D.; Leng, D.; Kui, H.; Bai, X.; Wang, T. Gut microbiota and meat quality. *Front. Microbiol.* 2022, *13*, 951726, doi:10.3389/fmicb.2022.951726.
53. Khanal, P.; Maltecca, C.; Schwab, C.; Fix, J.; Tiezzi, F. Microbiability of meat quality and carcass composition traits in swine. *J. Anim. Breed. Genet.* 2021, *138*, 223-236, doi:10.1111/jbg.12504.
54. Campbell, J.M.; Crenshaw, J.D.; Polo, J. The biological stress of early weaned piglets. *J. Anim. Sci. Biotechnol.* 2013, *4*, doi:10.1186/2049-1891-4-19.
55. Tang, X.; Xiong, K.; Fang, R.; Li, M. Weaning stress and intestinal health of piglets: A review. *Front. Immunol.* 2022, *13*, 1042778, doi:10.3389/fimmu.2022.1042778.
56. Varricchio, E.; Coccia, E.; Orso, G.; Lombardi, V.; Imperatore, R.; Vito, P.; Paolucci, M. Influence of polyphenols from olive mill wastewater on the gastrointestinal tract, alveolar macrophages and blood leukocytes of pigs. *Ital. J. Anim. Sci.* 2019, *18*, 574-586, doi:10.1080/1828051X.2018.1548911.
57. Skiba, G.; Poławska, E.; Sobol, M.; Raj, S.; Weremko, D. Omega-6 and omega-3 fatty acids metabolism pathways in the body of pigs fed diets with different sources of fatty acids. *Arch. Anim. Nutr.* 2015, *69*, 1-16, doi:10.1080/1745039X.2014.992173.
58. Madeira, M.S.; Alfaia, C.M.; Lopes, P.A.; Pestana, J.; Coelho, D.; Fontes, C.M.G.A.; Prates, J.A.M. Ameliorating pork marbling and quality with novel feeding approaches. In

Proceedings of the Advances in Animal Health, Medicine and Production, Cham, 2020//, 2020; pp. 161-177.

59. Tsala, A.; Mpekelis, V.; Karvelis, G.; Tsikakis, P.; Goliomytis, M.; Simitzis, P. Effects of dried olive pulp dietary supplementation on quality characteristics and antioxidant capacity of pig meat. *Foods* 2020, *9*, doi:10.3390/foods9010081.
60. Joven, M.; Pintos, E.; Latorre, M.A.; Suárez-Belloch, J.; Guada, J.A.; Fondevila, M. Effect of replacing barley by increasing levels of olive cake in the diet of finishing pigs: Growth performances, digestibility, carcass, meat and fat quality. *Anim. Feed Sci. Technol.* 2014, *197*, 185-193, doi:10.1016/j.anifeedsci.2014.08.007.
61. Ferrer, P.; Calvet, S.; García-Rebollar, P.; de Blas, C.; Jiménez-Belenguer, A.I.; Hernández, P.; Piquer, O.; Cerisuelo, A. Partially defatted olive cake in finishing pig diets: implications on performance, faecal microbiota, carcass quality, slurry composition and gas emission. *Animal* 2020, *14*, 426-434, doi:10.1017/S1751731119002040.
62. Vlaicu, P.A.; Panaite, T.D.; Cornescu, M.G.; Ropota, M.; Olteanu, M.; Drăgotoiu, D. The influence of by-products on the production parameters and nutrient digestibility in fattening pigs diet (60-100kg). *AgroLife Sci. J.* 2019, *8*.

Chapter 3 : Effect of a novel dietary silage on health parameters and meat quality characteristics of finishing pigs

Sections of this chapter have been published in:

Sustainable use of agro-industrial by-products as feed in finishing pigs. **Magklaras G**, Skoufos I, Bonos E, Zacharis C, Nikolaou K, Gouva E, Giannenas I, Giavasis I, Tzora A. Sustainable Use of Agro-Industrial By-Products as Feed in Finishing Pigs. Veterinary Sciences. 2026; 13(1):39. https://doi.org/10.3390/vetsci13010039

3.1 Introduction

In the modern era, livestock production has operated under escalating input costs and stringent sustainability constraints. Volatility in feed prices has intensified as climate change, competing land uses, and deforestation perturb global supplies of energy- and protein-rich feedstuffs. At the same time, European policy and consumer preferences are steering production toward healthier, more "sustainably produced" animal foods and toward reduced on-farm antimicrobial inputs. The EU Farm-to-Fork agenda explicitly targets a 50% cut in sales of antimicrobials for farmed animals and aquaculture by 2030, while recent Eurobarometer work and allied surveys indicate that many consumers increasingly associate "healthy and sustainable" with low-pesticide, welfare-friendly, and lower-impact food chains [1–3].

In this context, researchers have intensified the search for the development and utilization of unconventional feed, primarily affordable and locally available feed resources, that can partially replace imported soybean meal and cereals, while also conferring benefits for animals and consumers. Southern Europe—and Greece in particular—produces large volumes of agro-industrial by-products (AIBPs) from three emblematic sectors: olive oil production, winemaking, and cheese manufacture. The Mediterranean basin generates approximately 30 million m^3 of olive mill wastewater (OMW) annually. This effluent is rich in organic load and phenolics and, if mismanaged, is a significant pollutant but also a potential source of bioactive compounds [4,5]. Greece is among the world's top olive oil producers, with around 430 thousand tons of olive oil per year processed in approximately 2,800 mills—an industrial structure that underscores both the OMW burden and the valorization opportunity at the national scale [6]. Depending on extraction technology, one ton of olives can yield up to approximately 1.6 m^3 of OMW, again highlighting the magnitude of the stream that must be managed or valorized [7]. Wine by-products represent a second major AIBP resource. Across Europe, grape pomace (skins, seeds, residual pulp) typically accounts for roughly 20–30% of processed grape mass, contributing millions of tons of fibrous, phenolic-rich biomass annually. Greek data and case studies are consistent with this range and document substantial volumes from national wine regions [8–10]. These lignocellulosic residues carry tannins and other polyphenols with antimicrobial and antioxidant properties but pose storage and handling challenges when used raw. Cheese processing, finally, produces large quantities of whey—the liquid fraction remaining after curd

formation. Global whey output is commonly estimated at 145–200 million tons per year; unmanaged whey has very high biodegradability/total organic load numbers [high Biochemical Oxygen Demand/Chemical Oxygen Demand (BOD/COD)], making it an environmental liability but also a fermentable substrate in biorefineries and feed applications [11,12]. In Greece, where Feta, Protected Designation of Origin (PDO), dominates the cheese sector, 2021 production exceeded 130 thousand tons; given that about 9–10 L of whey are generated per kg of cheese, this single PDO category implies a production of more than 1.2–1.3 billion liters of whey annually—before accounting for other Greek cheeses—pointing to a substantial local feedstock for valorization (and, when deproteinized, a source of solids useful in mixed silages) [13,14]. Despite their promise, the direct inclusion of these by-products in monogastric rations is technically challenging: high moisture, rapid spoilage, variable composition, anti-nutritional phenolics, and limited shelf life can compromise digestibility, palatability, and safety. Consequently, stabilization and "upgrading" via bioprocessing—ensiling, solid-state fermentation, enzyme or microbial treatments—has become central to feed applications. Recent EU-Mediterranean work has shown that mixed-by-product silages (e.g., wheat straw, grape pomace, OMW, and cheese whey) can achieve desirable pH, lactic fermentation profiles, and preserved phenolics over 90 days, supporting their practicality as functional feed ingredients if well-formulated [15-18].

Within this trajectory, a Greek research scientific group designed and optimized a novel mixed silage using locally abundant AIBPs—olive mill wastewater solids, grape pomace solids, and de-proteinized feta cheese whey solids—arranged at approximately 60:20:20 (w/w) and supported by a cereal co-substrate to guide lactic fermentation [19]. In broiler chickens, dietary inclusion (5–10%) of this silage-maintained performance and improved meat oxidative stability and lipid profile, indicating that the phenolic-fiber matrix can beneficially modulate product quality without penalizing growth [20]. Building on the poultry results, the same innovative silage was subsequently tested in pigs. In a pilot study, pigs received 0%, 5%, or 10% inclusion of the tested silage. Growth performance was maintained, while gut microbiota profiling revealed increases in beneficial taxa (e.g., Bifidobacterium pseudolongum) and reductions in potentially problematic Streptococcus spp., suggesting that the silage's fermentable fiber and polyphenols reshaped the intestinal ecosystem in a potentially favorable way; carcass and meat-quality indices remained largely unaffected under the tested conditions [21,22]. Collectively, the

evidence supports the hypothesis that silages formulated from agro-industrial by-products can partially substitute imported feed inputs while delivering antioxidant and microbiota-modulatory bioactives that promote host health, in alignment with EU antimicrobial-use reduction and sustainability directives. European and Greek data indicate that (i) OMW streams in olive-producing regions (notably Greece) are large enough to justify feed-grade valorization pathways; (ii) grape pomace provides a seasonally abundant, phenolic-rich, fiber matrix compatible with ensiling; and (iii) cheese whey (or its de-proteinized solids) is both an environmental pressure point and a useful fermentable component in mixed silages [1,23,24]. When properly stabilized—most practically via ensiling and/or fermentation—these by-products can be blended into functional feeds for monogastrics such as pigs and poultry, with encouraging evidence for maintained performance, higher composition of antioxidant polyphenols, which may lead to improved oxidative stability of products during storage or further processing, and microbiome modulation [4,5,8,11,15,20-22]. Future work should focus on the finishing pig production stage, refine inclusion levels beyond 10% where feasible, and incorporate techno-economic and life-cycle assessments to quantify cost savings and environmental co-benefits alongside animal health and product-quality outcomes [20-22].

The present study evaluated a new type of silage aimed at overcoming limitations in the utilization of locally generated agro-industrial by-products, specifically olive mill wastewater solids, grape pomace solids, and deproteinized feta cheese waste solids. The silage formulation was established following optimization trials of the three components [19] and has been previously investigated in broiler chickens [20] and weaned pigs [22], yielding promising results in terms of health, productivity, and meat quality characteristics. To our knowledge, this is the first study evaluating this silage in finishing pigs. In doing so, we extend previous research conducted in weaned pigs by examining its effects on growth performance, health indicators, and meat quality at the finishing stage, when feed consumption is highest and the valorization of agro-industrial by-products may provide considerable environmental and economic advantages. (Fig.3.1.)

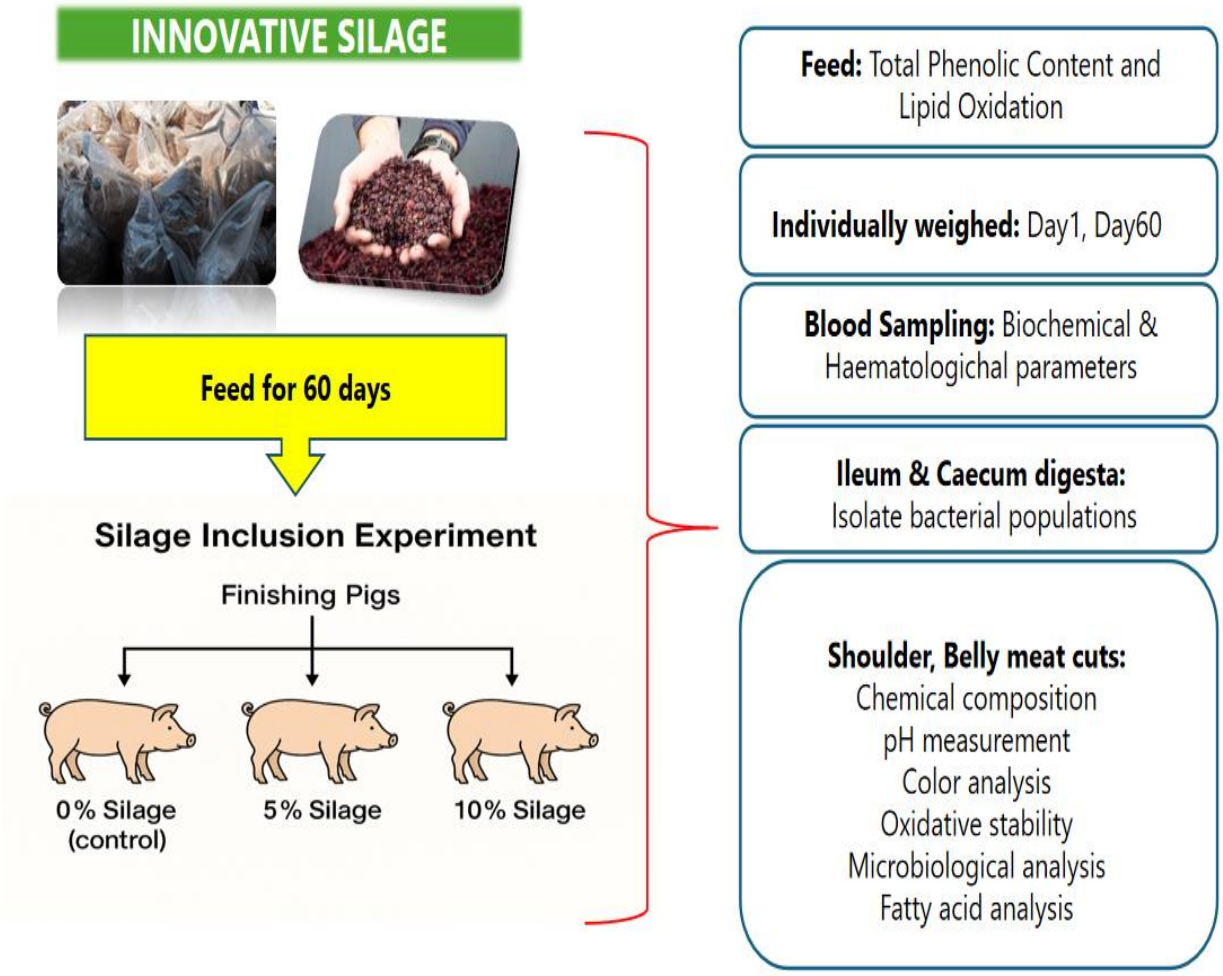


Figure 3.1. Experimental summary schematic illustration of Chapter 3 (exp.No2).

3.2 Materials and methods

3.2.1 Experimental design, animals and diets

All experimental procedures strictly followed the National Guidelines for Animal Trials (PD, 2013) [25] and received approval from the Department of Agriculture, University of Ioannina, Greece, through the University Research Committee (approval no. 61291/135/10.06.2020). The animal phase of the experiment was designed in compliance with the welfare considerations outlined in the Good Farming Practice Guidelines (Directive 2010/63/EC; Commission Recommendation 2007/526/EC). Throughout the experimental period,

farming conditions and animal health were continuously monitored under veterinary and animal scientist supervision; all members of the Department of Agriculture, University of Ioannina.

Animals were eighteen terminal crossbred pigs derived from Large White–Landrace maternal lines and Duroc paternal genetics, aged 120 days obtained from an intensive pig production facility, located near the University campus. Pigs were individually ear-tagged and randomly assigned to one of three dietary treatments: Control (0% silage), 5% silage, or 10% silage inclusion. The bioactive innovative silage tested as a feed additive in this trial was previously created following a novel multi-criteria mathematical optimization of the composition of 67 different recipes, as described by Petrotos et al. [19]. After fermentation of olive mill wastewater solids, grape pomace solids and de-proteinized feta cheese way solids, the optimal mixing ratio was chosen, and a novel silage was produced, by establishing specific characteristics: a low pH value (pH=4.37), a higher lactic acid content (total acidity=2.52 expressed as lactic acid), a higher lactic acid bacteria count (total Lactic acid bacteria=6.9 cfu/g) and simultaneously yeast and mold count at lower levels (total yeast and mold count=0.1 cfu/g). Table 3.1 presents the chemical analysis of the tested silage.

Table 3.1. Chemical composition of the evaluated silage.

Silage Chemical Analysis (As-Fed Basis)	
Moisture (%)	42.89
Dry matter (%)	57.11
Ash (%)	1.15
Crude fat (%)	3.21
Crude fiber (%)	2.63
Crude protein (%)	5.51
Total Ca (%)	0.05
Total P (%)	0.18
Mn (mg/kg)	16.95
Fe (mg/kg)	82.48
Cu (mg/kg)	3.21
Zn (mg/kg)	30.43

At the beginning of the trial, pigs across all three groups had comparable body weights (average initial mean body weight: 59.47 ± 0.85 kg). Each group comprised six animals (three gilts and three barrows). From a husbandry perspective, pigs were housed in semi-slatted concrete–floor pens within a controlled-environment facility. During the finishing period, the temperature was maintained between 18–23 °C, relative humidity between 50–70%, and ventilation rates adjusted to avoid draughts, while maintaining air speed below 0.3–0.5 m/s. Stocking density was 1.1 m^2/pig, in accordance with EU legislation (Council Directive 2008/120/EC). Standard farm management procedures were followed, including vaccination. Feed and water were provided ad libitum. Pigs in the control group (Silage-0%) were offered a commercial diet formulated according to National Research Council guidelines [26] and the Premier Nutrition database [27], whereas the experimental diets included the tested silage at 5% (Silage-5%) or 10% (Silage-10%) inclusion rates. All diets were balanced to be isonitrogenous and isocaloric. The ingredient composition and chemical analysis of diets are presented in Table 3.2. The trial lasted 60 days (from 120 to 180 days of age). Pigs were individually weighed at day 1 and 60 days later, when the experiment ended, using a Mini–L 3510 animal scale (Zygisis, Chalkidiki, Greece). Daily records were kept of feed intake, water consumption, and mortality rates. Zootechnical performance indices were calculated, including average weight gain (AG, kg/period), average feed intake (AFI, kg feed/period), and feed conversion ratio (FCR, kg feed intake/kg live weight gain). On day 60, blood samples were collected from six pigs per group before slaughter. Animals were humanely slaughtered in an abattoir near the experimental premises. Carcass samples of shoulder (triceps brachii) and belly (external abdominal muscle) were collected, along with intestinal samples (ileum and caecum), which were obtained aseptically during evisceration.

Table 3.2. Composition and calculated proximate analysis of experimental diets.

	Diets		
Ingredients (%)	**Silage-0%**	**Silage-5%**	**Silage-10%**
Wheat	45.00	37.49	29.98
Silage	0.00	5.00	10.00
Soybean meal (47% CP)	14.00	15.57	17.14
Barley	27.60	27.60	27.60
Wheat middlings	9.50	9.50	9.50
Soybean oil	1.15	2.09	3.03
Commercial premix[1]	1.00	1.00	1.00
Amino-acid Premix[2]	0.25	0.25	0.25
Salt	0.50	0.50	0.50
Limestone	1.00	1.00	1.00
Total	**100.00**	**100.00**	**100.00**
Calculated chemical analysis			
Digestible energy, MJ/kg	13.339	13.339	13.339
Crude protein, %	16.553	16.750	16.946
Dry matter, %	87.684	86.331	84.977
Ash, %	4.557	4.571	4.584
Crude fat, %	2.425	3.377	4.329
Crude fiber, %	3.878	3.861	3.844
ADF, %	4.620	4.588	4.555
NDF, %	13.653	13.448	13.243
Ca, %	0.568	0.571	0.574
Total P, %	0.385	0.386	0.386
Lysine, %	1.072	1.102	1.131
Methionine+Cystine, %	0.615	0.615	0.615
Threonine, %	0.747	0.762	0.776
Tryptophan, %	0.232	0.234	0.235

[1] Provided per kg complete diet: 6,500 IU retinyl acetate; 1,200 IU cholecalciferol; 12.5 mcg 25-hydroxycholecalciferol; 60 mg alpha-tocopherol acetate; 2 mg menadione nicotinamide bisul-phite; 2 mg thiamine mononitrate; 7 mg riboflavin; 25 mg pantothenic acid; 3 mg pyridoxine hydrochloride; 25 mcg cyanocobalamin; 25 mg nicotinic acid; 1 mg folic acid; 0.15 mg biotin; 300 mg choline chloride; 108 mg Fe from ferrous sulphate monohydrate; 25 mg Cu from copper sulphate; 48 mg Mn from manganese oxide; 84 mg Zn from zinc oxide; 1.2 mg I from calcium iodate; 0.24 mg Se from sodium selenite; 700 mg methionine; 100 mg L-tryptophan; 2730 L-Lysine mg HCl; 1,182.02 mg L-threonine; 1,500 FYT 6-fytase; 200 FXU endo-1,4-β-xylanase.
[2] Provided per kg complete diet in group A: 871.88 mg L-lysine HCl; 824.74 mg L-threonine; 98.87 mg L-tryptophan; 44 mg DL-methionine;

3.2.2 Phenolic Content and Thiobarbituric Acid Reactive Substances (TBARs) Determination in Animal Feed

The total phenolic content of the diets was determined using the Folin–Ciocalteu assay, following the procedure of Vasilopoulos et al. [28]. Lipid oxidation was assessed by a thiobarbituric acid–reactive substances (TBARS) method modified from Botsoglou et al. [29]. In brief, 1.0 g of feed was homogenized with 8 mL of 5% (w/v) trichloroacetic acid (TCA) and 5 mL of 0.8% (w/v) butylated hydroxytoluene (BHT) prepared in hexane. The homogenate was centrifuged at 3,000 × g for 5 min, and 1.5 mL of the resulting aqueous phase was collected. This fraction was mixed with 2.5 mL of 0.8% (w/v) thiobarbituric acid (TBA) and incubated in a water bath at 70 °C for 30 min. For the estimation of TBARs values a standard curve was prepared using standard solutions of 1,1,3,3-tetraethoxypropane, a precursor of malondialdehyde. Absorbance was measured at 532 nm, and lipid oxidation was expressed as TBARs, reported as mg malondialdehyde (MDA) per Kg of feed.

3.2.3 Isolation, enumeration and identification of bacterial populations

Fresh ileal and caecal digesta were obtained immediately post-slaughter from six pigs per dietary treatment. For primary processing, 1 g of intestinal content was homog- enized in 9 mL of sterile 0.1% (w/v) peptone water. Quantitative bacteriology followed the Miles–Misra surface-drop technique: twelve-step, tenfold serial dilutions (10−1–10−12) were prepared in 96-well microplates, and 10 µL aliquots from each dilution were spotted onto the appropriate media. MacConkey agar and Kanamycin Aesculin Azide (KAA) agar (Merck, Darmstadt, Germany) were used to recover *Enterobacteriaceae* and Enterococci, respectively; plates were incubated aerobically at 37 ◦C for 24–48 h. Lactic acid bacteria were enumerated on De Man, Rogosa and Sharpe (MRS) agar (Oxoid, Basingstoke, UK) and on M17 agar (Lab M Limited, Lancashire, UK), with incubation at 37 ◦C for 48 h under anaerobiosis. *Bifidobacteriaceae* were quantified

on Transgalactooligosaccharide Propionate (TOS) agar (Merck, Darmstadt, Germany) supplemented with glacial acetic acid (1%, v/v) and mupirocin (100 µL/mL), incubated anaerobically at 37 ◦C for 72 h. Total aerobic and anaerobic counts were estimated on plate count agar (Oxoid) after incubation at 30 ◦C for 48 h (aerobic) and at 37 ◦C for 48–72 h (anaerobic), respectively. Characteristic colonies from countable spots were recorded using a colony counter, expressed as log10 CFU per g of wet digesta, then described and subcultured. Taxonomic assignment at the family level was performed with the VITEK® 2 compact system (bioMérieux, Marcy l'Etoile, France), which provides reliable identification across a broad range of Gram-positive and Gram-negative bacteria [30]. For *Enterobacteriaceae, Enterococcaceae, Lactobacillaceae, and Bifidobacteriaceae*, the VITEK® 2 ID-GN, ID-GP, CBC/ANC, and ANC ID cards (bioMérieux) were used, respectively.

3.2.4. Hematological and Biochemical Analysis of the Blood

On day 60 (final sampling, pre-slaughter), blood was obtained from six pigs per dietary group following a 4 h feed withdrawal. Approximately 4 mL was collected by jugular venipuncture into EDTA-treated vacutainer tubes for hematology. Hematological indices—hemoglobin concentration, erythrocyte count, hematocrit (HCT), total leukocytes, and lymphocytes—were quantified using the MS4 (Melet Schloesing Lab, Osny, France) automated analyzer. Serum biochemical variables—albumin (ALB), alanine aminotrans- ferase (ALT), aspartate aminotransferase (AST), cholesterol (CHOL), creatine kinase (CK), glucose (GLU), total bilirubin (TBIL), and triglycerides (TRIG)—were measured with a VetTest 8008 analyzer (IDEXX Laboratories, Westbrook, ME, USA).

3.2.5. Meat Chemical and Color Analysis

Triceps brachii and external abdominal oblique muscle samples were excised and immediately frozen at −20 °C for subsequent analyses. Portions of 200 g were comminuted in a heavy-duty meat grinder (Bosch, Gerlingen, Germany). Near-infrared spectroscopy (FoodScan™ Lab, FOSS, Hillerød, Denmark) was used to quantify moisture, crude protein, fat, collagen, and ash, following AOAC 2007.04 for meat and meat products [31].

Muscle pH (triceps brachii and external abdominal oblique meat cuts) was measured with a portable pH meter designed for solid matrices (HI981036; Hanna Instruments, Woonsocket, RI). For each dietary group, six pork samples were assessed by inserting the stainless-steel probe into the geometric center of the tissue; group means were then calculated.

Color measurements of the triceps brachii and external abdominal oblique muscles were conducted using a Hunter L*, a*, b* color system with a CAM-System 500 chromameter (Lovibond, Amesbury, UK) [22]. Meat samples, cut to a thickness of 1.2 cm, were placed on polystyrene trays and allowed to bloom for 30 min at 4°C under aerobic conditions prior to analysis. Color parameters were recorded in the CIE Lab* space, where L* corresponds to lightness, a* to red–green intensity, and b* to yellow–blue intensity.

3.2.6. Oxidative stability analysis of the meat

A modified Folin–Ciocalteu assay was used to quantify total polyphenols in fresh pork tissues (3rd day after slaughter). A 0.2 g/L gallic acid stock (Merck, Germany) was prepared in 100 mL distilled water and serially diluted to generate standards of 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, and 1.0 g/L. For each calibrant, 0.2 mL was dispensed into a 50 mL polypropylene tube and combined with 10.8 mL distilled water, 8.0 mL sodium carbon- ate solution (75 g Na2CO3 per L; Penta Chemicals, Prague, Czech Republic), and 1.0 mL Folin–Ciocalteu reagent (PanReac AppliChem, Darmstadt, Germany). A reagent blank contained 0.2 mL distilled water in place of standard. After vortex mixing, tubes were incubated for 1 h at room temperature in the dark. Absorbance was recorded at 750 nm on a DR 5000 UV–Vis spectrophotometer (Hach Lange, Düsseldorf, Germany), using the blank for instrument zeroing. The calibration curve (absorbance vs. gallic acid concentration) was linear with R2 = 0.9989 (constructed in Microsoft Excel). Meat extracts were prepared by homogenizing 5 g of shoulder, belly, or ham with 10 mL distilled water, followed by filtration through paper. Aliquots (0.2 mL) of filtrate were reacted identically to the stan- dards, with a matrix blank (0.2 mL water) used for spectrophotometer calibration. Results were expressed as gallic acid equivalents.

Lipid oxidation was quantified using a thiobarbituric acid reactive substances (TBARS) assay, as modified based on Dias et al. [32], on the 3rd day after slaughter. For this procedure, 5 g samples of triceps brachii or external abdominal oblique muscle were homogenized with 25

mL of trichloroacetic acid (TCA), transferred to glass containers, and allowed to stand for 20 min. The homogenate was then filtered, and 5 mL of the resulting filtrate was mixed with 5 mL of 2-thiobarbituric acid (TBA) solution in glass tubes. A reagent blank was prepared by replacing the filtrate with 5 mL of TCA. After vortexing, tubes were incubated in a water bath at 60 ◦C for 15 min and cooled to room temperature, and the absorbance was read at 532 nm on a UV–Vis spectrophotometer using the blank for baseline correction. For the estimation of TBARs values, a standard curve was prepared using standard solutions of 1,1,3,3-tetraethoxypropane.

3.2.7. Microbiological analysis of meat cuts

Following 48 h of refrigerated storage at 4 °C (to accommodate transport and handling), microbiological analyses were conducted on meat from six pigs per treatment. For each sample, 10 g of shoulder or belly meat was homogenized in a BagMixer 400 (Interscience, France) with 90 mL of sterile Maximum Recovery Diluent (MRD; Oxoid, Basingstoke, UK) to obtain a 10^{-1} suspension. Tenfold serial dilutions were then prepared in 9 mL MRD, and appropriate dilutions (1.0 or 0.1 mL) were plated for enumeration as follows: *Escherichia coli* on Tryptone Bile X-Glucuronide (TBX) agar at 37 °C for 24 h (aerobic); sulfite-reducing clostridia on Perfringens Agar Base at 37 °C for 48 h under anaerobiosis generated with Anaerocult A (all Oxoid); *Staphylococcus aureus* and other *Staphylococcus* spp. on Baird–Parker agar supplemented with egg-yolk tellurite (50 mL L^{-1}) at 37 °C for 48 h (aerobic); total mesophilic counts on Plate Count Agar at 30 °C for 48 h (aerobic); and *Campylobacter jejuni* on Campy Blood-Free Selective Medium (CCDA; Acumedia–Lab M, Lansing, MI, USA) with selective supplement under microaerophilic conditions (10% CO_2) at 37 °C for 72 h. Detection of *Salmonella* spp. and *Listeria monocytogenes* was performed on 25 g portions in accordance with ISO 6579:2002 [33] and ISO 4833:2001 [34], respectively. All plates were incubated in thermostatic cabinets (BD 115; Binder, Germany).

3.2.8. Fatty acid analysis of the meat

Fatty acids in triceps brachii and external abdominal oblique muscles were analyzed after in situ transesterification according to the method described by O'Fallon et al. [35]. Fatty acid methyl esters (FAME) were separated and quantified following the procedure of Giannenas et al.

[36] on a TraceGC gas chromatograph (Model K07332; ThermoFinni- gan/Thermoquest, Milan, Italy) equipped with a flame ionization detector. Retention times and elution order were assigned using the Supelco “37 Component FAME Mix” reference standard (Sigma-Aldrich, Darmstadt, Germany). Individual fatty acids were expressed as a percentage of total identified FAME (peak area of the analyte divided by the sum of peak areas for all identified fatty acids). From these data, the PUFA/SFA ratio, the n-6/n-3 PUFA ratio, and the hypocholesterolemic-to-hypercholesterolemic fatty acid index (h/H) were calculated, where h/H = (C18:1n-9 + ΣPUFA)/(C12:0 + C14:0 + C16:0). The h/H index was used as an indicator of the predicted cholesterolemic impact of the lipid fraction [37].

3.2.9. Statistical analysis

A randomized complete block design (RCB) was employed, considering each ear- tagged pig to be an experimental unit. Microbiological data were log10-transformed prior to statistical analysis. Homogeneity of variances was assessed using Levene’s test. Depending on data distribution, either one-way ANOVA (parametric) or the Kruskal–Wallis test (non- parametric) was applied in SPSS v20 [38]. When ANOVA revealed a significant treatment effect ($p \leq 0.05$), mean separation among the three dietary treatments was conducted using Tukey’s HSD test. Statistical significance was set at $\alpha = 0.05$ for all analyses.

3.3. Results

3.3.1. Total Phenolic Content and Lipid Oxidation of the Control and Experimental Diets

Total phenolic content value in the control group (Silage-0%) feed was significantly lower ($p = 0.027$) than in both treatment groups (Silage-5% and Silage-10%). A similar positive pattern was noticed for Malondialdehyde (MDA), which was used as an indicator in evaluating the degree of feed lipid oxidation. MDA levels significantly ($p = 0.001$) deteriorated in all the silage-enriched diets compared to the control Silage-0% group (Table 3.3).

Table 3.3. Total Phenolic (TP) content and MDA in pig diets

	Diets			SEM*	p-value
	Silage-0%	**Silage-5%**	**Silage-10%**		
TP (mg GAE/L Feed extract)	139.41[a]	153.87[b]	172.44[c]	5.130	0.027
mgMDA/Kg Feed	0.091[c]	0.066[b]	0.046[a]	0.006	<0.001

[a,b] Values with no common superscript differ significantly ($P \leq 0.05$); n = 18 (6 pigs per diet);
*Standard Error of the Mean

3.3.2. Performance and carcass parameters

The effects of silage supplement on pig performance and carcass parameters are presented in Table 3.4. The final live weight and total weight gain of the finishing pigs did not differ ($p = 0.281$ and $p = 0.659$, respectively) between the three treatments. Feed intake (FI) and feed conversion efficiency (FCR) remained within the normal production ranges reported for the intensive pig production unit. Concerning the carcass parameters, carcass weight was similar for all groups, though arithmetically better for the Silage-10% animals, but the dressing percentage was significantly ($p = 0.019$) increased in Silage-5% and Silage-10% groups.

Table 3.4. Effect of silage supplementation on finishing pigs' performance and carcass parameters.

	Diets			SEM	p-value
Performance parameters	**Silage-0%**	**Silage-5%**	**Silage-10%**		
Initial bodyweight (kg)	57.75	59.48	61.18	0.850	0.272
Final bodyweight (kg)	122.08	123.6	124.03	1.510	0.281
Weight gain (kg)	64.33	64.11	66.76	1.250	0.659
FI* / per pig (kg)	187.72	189.15	191.15	NA	NA
FCR* (kg feed / kg weight gain)	2.92	2.95	2.86	NA	NA
Carcass parameters					
Carcass weight (kg)	72.08	73.47	73.94	0.876	0.138
Dressing percentage (%)	59.04[a]	59.44[b]	59.61[b]	0.002	0.019

n = 18 (6 pigs per diet); [a,b] Values with no common superscript differ significantly (P≤0.05);
*FI=Feed Intake; FCR=Feed Conversion Ratio; NA = Not applicable.

3.3.3. Intestinal microflora

The dietary use of the tested silage affected intestinal microflora populations (Table 5). In the ileum digesta, it was noted that Enterobacteriaceae were significantly reduced (p = 0.001) in Silage-10% and Enterococci were reduced in both Silage-5% and Silage-10%. Furthermore, Lactobacilli were significantly increased (p = 0.001) in treatments Silage-5% and Silage-10% compared to the control. Total anaerobes were at the lowest level (p = 0.038) in the caecum digesta, in the experimental diet (Silage-5% treatments), compared to Silage-10% treatment. Enterococci levels were significantly (p = 0.001) lowered and Lactobacilli tended to increase, but only numerically, in both of the silage-enriched diets. Bifidobacterium was not affected (p = 0.632) by the treatments in any of the intestinal samples.

Table 3.5. Silage effects on finishing pigs' intestinal microbial populations.

	Diets			SEM	p-value
Ileum microbes (Log_{10} CFU/g)	**Silage-0%**	**Silage-5%**	**Silage-10%**		
Total aerobes	9.10	8.77	8.16	0.168	0.060
Total anaerobes	8.80	8.88	8.55	0.111	0.484
Enterobacteriaceae	4.96^{b}	5.14^{b}	3.86^{a}	0.171	0.001
Enterococci	5.96^{c}	4.58^{b}	3.46^{a}	0.273	0.001
Lactobacilli	6.67^{a}	8.16^{b}	9.00^{b}	0.269	0.001
Bifidobacterium	6.05	5.82	5.92	0.105	0.703
Caecum microbes (Log_{10} CFU/g)					
Total aerobes	9.19^{ab}	8.96^{a}	9.48^{b}	0.086	0.038
Total anaerobes	9.25	8.90	9.02	0.095	0.344
Enterobacteriaceae	5.38	5.46	4.87	0.126	0.112
Enterococci	7.00^{a}	3.75^{b}	4.74^{c}	0.341	0.001
Lactobacilli	8.75	9.64	9.46	0.178	0.090
Bifidobacterium	6.02	6.28	6.09	0.107	0.632

n = 18 (6 pigs per treatment); SEM: standard error of the mean;
^{a,b} Values with no common superscript differ significantly ($P \leq 0.05$)

3.3.4. Hematological and Biochemical Parameters

Concerning hematological values, the pigs in treatment groups Silage-5% and Silage-10% tended ($p = 0.060$) to have higher monocyte levels compared to the control Silage-0% group. Regarding the biochemical parameters, blood alanine aminotransferase (ALT) was diminished in both silage-enriched treatments, but this reduction was only significant in group Silage-10% ($p = 0.030$) compared to control. Blood hematological and biochemical parameters generally did not differ ($p > 0.05$) between the three treatments.Table 3.6 presents the effect of the silage on the pigs' blood hematological and biochemical parameters.

Table 3.6. Silage effects on finishing pigs' blood hematological and biochemical parameters.

	Diets			SEM	p-value
Hematological parameters	**Silage-0%**	**Silage-5%**	**Silage-10%**		
WBC (10^3/μL)	20.71	18.89	20.31	0.720	0.590
Lymphocytes (%)	39.73	43.33	38.25	1.060	0.130
Monocytes (%)	7.28^{x}	8.65^{x}	9.18^{y}	0.350	0.060
Granulocytes (%)	52.98	49.01	52.56	1.000	0.220
RBC (10^6/μL)	6.85	6.81	6.77	0.320	1.000
Hct (%)	38.4	36.15	35.13	1.740	0.760
Hb (g/dL)	11.66	12.2	11.88	0.380	0.870
THR (m/mm^3)	237.66	224.33	240	14.660	0.910
Blood biochemical parameters					
ALP (u/L)	98.66	136.5	102,.25	8.820	0.150
ALT (u/L)	70.33^{b}	69.16^{ab}	56.75^{a}	2.460	0.030
AST (u/L)	32.66	43.83	42.16	3.790	0.450
CHOL (mg/dL)	87.5	98.83	88.41	2.530	0.170
GLU (mg/dL)	78.16	76.5	78.25	1.920	0.920
TRIG (mg/dL)	41.83	50	42.58	3.450	0.590
CK (u/l)	454.33	505.66	515.08	28.960	0.680

n = 18 (6 pigs per treatment); SEM: standard error of the mean; ^{a,b} Values with no common superscript differ significantly ($P \leq 0.05$); ^{x,y} Values with no common superscript tend to ($0.05 < p \leq 0.10$). WBC: white blood cells; RBC: Red blood cells; HCT: hematocrit; HB: hemoglobin; ALB: Albumin; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; CHOL: Cholesterol; GLU: Glucose; TRIG: Triglycerides; CK: Creatine Kinase,

3.3.5. Meat Analysis

As shown in Table 3.7, no differences were noted in the chemical composition of the triceps brachii muscle samples for all silage treatments. Regarding the external abdominal oblique samples, moisture content was significantly decreased ($p = 0.020$) in treatment Silage-10%, but only when compared to Silage-5% group, where a slight elevation was noticed. All other parameters examined (fat, protein, collagen, and ash) were not affected (p

= 0.930, p = 0.450, p = 0.740 and p = 0.150, respectively) by the treatments. The pH values of all samples for both shoulder and belly meat did not differ between the treatments (p = 0.590).

Table 3.7. Effect of silage supplementation on pig triceps brachii and external abdominal oblique meat chemical composition and pH

Triceps brachii muscle chemical composition (%)	**Diets**			**SEM**	**p-value**
	Silage-0%	**Silage-5%**	**Silage-10%**		
Fat	9.77	10.08	9.31	0.460	0.930
Moisture	69.74	69.69	69,.6	0.350	0.990
Protein	19.97	19.92	20.24	0.320	0.450
Collagen	1.7	1.64	1.56	0.070	0.740
Ash	0.99	0.95	0.83	0.340	0.150
pH	5.52	5.56	5.54	0.220	0.820
External abdominal oblique muscle chemical composition (%)					
Fat	20.02	21.48	23.42	0.790	0.220
Moisture	60.54^{ab}	60.91^{a}	57.24^{b}	0.640	0.020
Protein	18.93	17.4	18.44	0.410	0.30
Collagen	2.13	2.06	2.31	0.070	0.340
Ash	0.9	0.81	0.78	0.410	0.430
pH	5.55	5.51	5.53	0.020	0.590

n = 18 (6 pigs per treatment); SEM: standard error of the mean
^{a,b} Values with no common superscript differ significantly (P≤0.05)

As shown in Table 3.8, colorimetric analysis revealed a significant increase (p = 0.001) in redness (a * value) of the triceps brachii muscle in both silage-supplemented groups (5% and 10%), while simultaneously, there was a significant (p = 0.049) L * value reduction in the experimental groups. No statistical differences (p > 0.05) were noticed in the external abdominal oblique meat samples.

Table 3.8. Effect of silage addition on pigs' triceps brachii and external abdominal oblique meat color

	Diets			SEM	p-value
Triceps brachii meat color	**Silage-0%**	**Silage-5%**	**Silage-10%**		
L*	61.65[b]	55.3[a]	55.32[a]	1.259	0.049
a*	10.08[a]	16.78[b]	16.2[b]	0.947	0.001
b*	13.07	12.97	14.37	0.436	0.366
External abdominal oblique meat color					
L*	56.26	56.44	57.00	0.738	0.767
a*	13.40	15.46	16.56	0.841	0,.319
b*	9.96	10.88	11.84	0.804	0.668

n = 18 (6 pigs per treatment); SEM: standard error of the mean; L*: Lightness; *a**: redness; *b**: yellowness.
[a,b] Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)

The results of the meat microbiological analysis are presented in Table 3.9. In the triceps brachii, *Campylobacter jejuni* was lower (p = 0.039) in the Silage-10% treatment compared to the control. Total Mesophilic count, *Staphylococcus aureus*, *Staphylococcus* spp., *Escherichia coli*, and Sulfite-reducing *Clostridium* did not differ (p > 0.05) between the treatments in triceps brachii and external abdominal oblique samples. E. coli counts in external abdominal oblique meat ranged between 1.04 and 1.46 log CFU/g, corresponding to approximately 10–30 CFU/g. All meat cuts tested negative for *Salmonella* spp. and *Listeria monocytogenes* (per 25 g of sample).

Table 3.9. Effects of silage on meat microbial populations.

	Diets			SEM	p-value
Triceps brachii meat microbes (Log CFU/g)	**Silage-0%**	**Silage-5%**	**Silage-10%**		
Total Mesophilic count	4.99	3.94	4.83	0.23	0.14
Campylobacter jejuni	4.05[b]	3.51[ab]	3.09[a]	0.16	0.039
Staphylococcus spp.	2.78	2.37	2.04	0.24	0.72
Staphylococcus aureus	0.92	1.43	0.70	0.21	0.38
Sulfite-reducing *Clostridium*	1.01	0.85	1.15	0.18	0.81
External abdominal oblique meat microbes (Log CFU/g)					
Total Mesophilic count	5.59	5.34	4.95	0.23	0.55
Campylobacter jejuni	0.80	0.80	0.30	0.15	0.29
Staphylococcus spp.	2.97	3.07	3.05	0.15	0.97
Staphylococcus aureus	0.88	1.10	0.76	0.17	0.72
Sulfite-reducing *Clostridium*	1.55	1.47	1.28	1.49	0.58
Escherichia coli	1.04	1.15	1.46	0.17	0.61

n = 18 (6 pigs per treatment); SEM: standard error of the mean; [a,b] Values with no common superscript differ significantly (P≤0.05)

Table 3.10 presents data on the total phenolic content and oxidative stability of the meat samples. A significantly higher phenolic content (p = 0.013) was observed in triceps brachii meat from the Silage-10% group compared to the control group. Total phenols were not significantly affected by treatment in the external abdominal oblique meat samples. Thiobarbituric acid reactive substances (TBARS) did not differ in any of the meat cuts (p = 0.201 and p = 0.171 for triceps brachii and external abdominal oblique, respectively) and showed very low values (almost an absence) of lipid oxidation, since all samples were fresh and not stored for a prolonged period of time (3rd day after slaughter).

Table 3.10. Effects of silage addition on pig meat oxidative stability.

	Diets			SEM	p-value
Triceps brachii meat	**Silage-0%**	**Silage-5%**	**Silage-10%**		
Total phenols (g / L)	1.51[a]	1.82[ab]	2.28[b]	0.116	0.013
TBARS (mg MDA / kg)	0.09	0.06	0.05	0.008	0.201
External abdominal oblique meat					
Total phenols (g / L)	1.90	2.29	2.24	0.112	0.332
TBARS (mg MDA / kg)	0.07	0.05	0.04	0.007	0.171

n = 18 (6 pigs per treatment); SEM: standard error of the mean; TBARS: Thiobarbituric acid reactive substances. MDA: Malondialdehyde; [a,b,c] Values with no common superscript differ significantly (P≤0.05)

Fatty acid profiles of the triceps brachii cuts are presented in Table 3.11. Dietary supplementation with the tested silage significantly ($p < 0.05$) altered several fatty acid indices in the 5% and 10% groups compared with the 0% control group. Specifically, total saturated fatty acids (Σ SFA) were lowest (p=0.049) in the Silage 5% group, intermediate in the Control 0% group, and highest in the Silage 10% group. Total monounsaturated fatty acids (Σ MUFA) were highest (p=0.021) in the Silage 5% group, intermediate in the Silage 10% group, and lowest in the Control 0% group. Total polyunsaturated fatty acids (Σ PUFA) did not differ between the Silage 5% and Control 0% treatments but were reduced (p=0.029) in the Silage 10% group.

Table 3.11. Effect of silage supplementation on pig triceps brachii meat fatty acid composition**.**

	Diets			SEM	p-value
Triceps brachii meat FA (%)	**Silage-0%**	**Silage-5%**	**Silage-10%**		
C8:0 (Caprylic)	0.01	0.01	0.01	-	-
C10:0 (Capric)	0.09	0.09	0.10	0.011	0.972
C12:0 (Lauric)	0.07	0.08	0.09	0.006	0.630
C14:0 (Myristic)	1.44	1.47	1.57	0.083	0.850
C14:1 (Myristoleic)	0.02	0.01	0.02	0.002	0.202
C15:0 (Pentadecanoic)	0.04	0.05	0.04	0.005	0.780
C16:0 (Palmitic)	26.59^{xy}	25.64^{x}	29.19^{y}	0.693	0.071
C16:1 cis (Palmitoleic)	2.60	2.43	2.40	0.161	0.670
C17:0 (Heptadecanoic)	0.24	0.28	0.26	0.022	0.836
C17:1 (cis-10 Heptadecenoic cis)	0.16^{x}	0.27^{y}	0.25^{xy}	0.023	0.055
C18:0 (Stearic)	11.83	11.78	12.31	0.247	0.693
C18:1n-9t (Elaidic)	0.19	0.09	0.19	0.039	0.543
C18:1 cis n-9 (Oleic)	37.65	40.05	38.20	0.821	0.522
C18:2n-6t (Linolelaidic)	0.03	0.03	0.02	0.003	0.702
C18:2 n-6c (Linoleic)	15.28	14.45	13.07	0.845	0.625
C18:3n-3 (a-Linolenic)	0.71	0.85	0.4	0.059	0.690
C18:3n-6 (γ-Linolenic)	0.08^{y}	0.08^{y}	0.03^{x}	0.009	0.085
C20:0 (Arachidic)	0.05	0.09	0.10	0.010	0.178
C20:1 cis n-9 (cis-11 Eicosenoic)	0.33^{x}	0.45^{y}	0.42^{xy}	0.026	0.097
C20:2 cis n-6 (cis-11,14-Eicosadienoic)	0.37	0.45	0.33	0.029	0.219
C20:3 cis n-3 (cis-11-14-17-Eicosatrienoate)	0.22	0.16	0.07	0.033	0.245
C20:4 cis n-6 (Arachidonic)	1.65^{y}	0.93^{xy}	0.46^{x}	0.314	0.079
C20:5 cis n-3 (Cis-5,8,11,14,17-Eicosapentaenoic)	0.05^{y}	0.03^{xy}	0.01^{x}	0.010	0.069
C21:0 (Henicosanoic)	0.03	0.04	0.02	0.004	0.138
C22:6 cis n-3 (cis-4,7,10,13,16,19-Docosahexaenoic)	0.08^{y}	0.04^{xy}	0.02^{x}	0.120	0.057
C23:0 (Tricosanoic)	0.01	0.01	0.01	0.003	0.498

C24:1n-9 (Nervonic)	0.21^{y}	0.13^{xy}	0.05^{x}	0.035	0.069
Σ SFA (Total Saturated FA)	40.40^{a}	39.54^{a}	43.60^{b}	0.614	0.049
Σ MUFA (Total Monounsaturated FA)	40.95^{a}	43.30^{b}	41.48^{a}	0.518	0.021
Σ PUFA (Total Polyunsaturated FA)	18.47^{b}	17.02^{b}	14.75^{a}	0.535	0.023
Σ n-6 (Total omega-6 FA)	17.41	15.94	13.91	1.089	0.497
Σ n-3 (Total omega-3 FA)	1.06	1.07	0.84	0.071	0.405
Ratio n-6/n-3 FA	16.42	14.61	16.55	0.565	0.532
PUFA/SFA	0.45^{y}	0.43^{xy}	0.34^{x}	0.029	0.06
h/H^{c}	1.99^{xy}	2.09^{y}	1.71^{x}	0.093	0.097

n = 6 pigs per group.
FA: Fatty acids; ΣSFA = (C10:0) + (C12:0) + (C14:0) + (C16:0) + (C17:0) + (C18:0); ΣMUFA = (C16:1 cis) + (C17:1 cis-10) + (C18:1 cis n9) + (C18:1 n7) + (C20:1 cis n9); ΣPUFA = (C18:2 n-6c) + (C18:4n-3) + (C20:2 cis n-6) + C20:3 cis n-6) + (C22:5 cis n-3) + (C20:3 cis n-3) + (C20:4 cis n-6) + (C20:5 cis n-3) + (C21:5 n-3) + (C22 :6 cis n-3)
[a,b] Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)
[x,y] Values with no common superscript tend to (0.05 < p ≤ 0.10).
[c] hypocholesterolemic/Hypercholesterolemic ratio = (cis-C18:1+ΣPUFA)/(C12:0+C14:0+C16:0

Fatty acid results for the external abdominal oblique cuts are shown in Table 3.12. In this case, dietary silage supplementation affected only a limited number of fatty acids and indices. In particular, total MUFA were reduced (p=0.043) in the Silage 10% group, and the n-6/n-3 fatty acid ratio was significantly decreased (p=0.005) in the same treatment.

Table 3.12. Effect of silage supplementation on pig external abdominal oblique meat fatty acid composition.

	Diets			**SEM**	**p-value**
External abdominal oblique meat FA (%)	**Silage-0%**	**Silage-5%**	**Silage-10%**		
C8:0 (Caprylic)	0.01	0.01	0.01	0.001	0.368
C10:0 (Capric)	0.13	0.10	0.12	0.016	0.394
C12:0 (Lauric)	0.10	0.09	0.11	0.005	0.422
C14:0 (Myristic)	1.75	1.81	1.97	0.060	0.327
C14:1 (Myristoleic)	0.02	0.02	0.02	0.002	0.264
C15:0 (Pentadecanoic)	0.04	0.04	0.04	0.003	0.702
C16:0 (Palmitic)	2.77	29.09	30.61	0.559	0.603

C16:1 cis (Palmitoleic)	2.95	2.27	2.72	0.167	0.301
C17:0 (Heptadecanoic)	0.19	0.25	0.23	0.013	0.241
C17:1 cis-10 (Heptadecenoic cis)	0.17^{x}	0.24^{y}	0.25^{y}	0.018	0.059
C18:0 (Stearic)	11..73	12.85	11.79	0.516	0.676
C18:1n-9t (Elaidic)	0.07	0.21	0.23	0.032	0.066
C18:1 cis n-9 (Oleic)	41.09	38.91	38.24	1.222	0.673
C18:2n-6t (Linolelaidic)	0.01	0.03	0.02	0.002	0.125
C18:2 n-6c (Linoleic)	10.04	11.83	11.49	0.521	0.372
C18:3n-3 (a-Linolenic)	0.52	0.65	0.72	0.041	0.133
C18:3n-6 (γ-Linolenic)	0.02	0.04	0.03	0.003	0.296
C20:0 (Arachidic)	0.10	0.11	0.11	0.005	0.651
C20:1 cis n-9 (cis-11-Eicosenoic)	0.48	0.41	0.40	0.033	0.541
C20:2 cis n-6 (cis-11,14-Eicosadienoic)	0.32	0.34	0.33	0.009	0.637
C20:3 cis n-3 (cis-11-14-17-Eicosatrienoate)	0.06	0.07	0.06	0.033	0.202
C20:4 cis n-6 (Arachidonic)	0.31^{x}	0.50^{y}	0.36^{xy}	0.038	0.097
C20:5 cis n-3 (Cis-5,8,11,14,17-Eicosapentaenoic)	0.01	0.01	0.01	-	-
C21:0 (Henicosanoic)	0.03	0.03	0.03	0.002	0.579
C22:6 cis n-3 (cis-4,7,10,13,16,19-Docosahexaenoic)	0.02	0.02	0.02	0.001	0.565
C24:1n-9 (Nervonic)	0.06	0.07	0.05	0.005	0.441
Σ SFA (Total Saturated FA)	43.85	44.38	45.02	0.490	0.653
Σ MUFA (Total Monounsaturated FA)	44.84^{b}	42.13^{ab}	41.91^{a}	0.659	0.043
Σ PUFA (Total Polyunsaturated FA)	11.31	13.49	13.04	0.397	0.113
Σ n-6 (Total omega-6 FA)	10.70	12.74	12.23	0.544	0.328
Σ n-3 (Total omega-3 FA)	0.61	0.76	0.81	0.044	0.127
Ratio n-6/n-3 FA	17.54^{b}	16.76^{b}	15.09^{a}	0.416	0.005
PUFA/SFA	0.25	0.30	0.29	0.021	0.944
h/H^{c}	1.65	1.69	1.56	0.062	0.838

n = 6 pigs per group.
FA: Fatty acids; ΣSFA = (C10:0) + (C12:0) + (C14:0) + (C16:0) + (C17:0) + (C18:0); ΣMUFA = (C16:1 cis) + (C17:1 cis-10) + (C18:1 cis n9) + (C18:1 n7) + (C20:1 cis n9); ΣPUFA = (C18:2 n-6c) + (C18:4n-3) + (C20:2 cis n-6) + C20:3 cis n-6) + (C22:5 cis n-3) + (C20:3 cis n-3) + (C20:4 cis n-6) + (C20:5 cis n-3) + (C21:5 n-3) + (C22 :6 cis n-3)
[a,b] Values (n = 6 per treatment) with no common superscript differ significantly (P≤0.05)
[x,y] Values with no common superscript tend to (0.05 < p ≤ 0.10).
[c] hypocholesterolemic/Hypercholesterolemic ratio = (cis-C18:1+ΣPUFA)/(C12:0+C14:0+C16:0

3.4 Discussion

Greece generates substantial quantities of agro-industrial by-products due to its strong and regionally concentrated production of wine, olive oil, and dairy products—three pillars of the Mediterranean agri-food sector. The country ranks among the leading EU producers of table olives, olive oil, and feta cheese, and maintains a long tradition of small- to medium-scale wineries. As a result, large seasonal volumes of grape pomace, olive-mill wastewater, and cheese whey are produced annually. Although often treated as waste, these streams contain valuable bioactive components such as polyphenols, organic acids, fibers, residual sugars, and antioxidant compounds. Their biological significance lies in their potential to enhance gut health, modulate oxidative balance, and improve meat quality when appropriately processed and included in animal feed [1,10,11,24]. Consequently, valorizing these abundant by-products through controlled fermentation or silage production offers both an environmental management strategy and an opportunity for functional feed innovation in Mediterranean livestock systems. It is estimated that the annual production of fresh grape pomace (GP), olive-mill wastewater (OMWW), and cheese whey (CW) in Greece is about 200,000 tons, 0.8–1.2 × 10^6 tons and 1.0–1.3 × 10^6 tons, respectively [39]. Thus, it becomes evident that there are considerable amounts of agri-food waste that are potential pollutants and could be recycled and reused in modern livestock production. This trial evaluated for the first time a novel silage manufactured from three Greek agro-industrial by-products—olive-mill wastewater solids, grape pomace solids, and de-proteinized feta whey—fed at 0%, 5%, and 10% inclusion levels in finishing-pig diets. The concept aligns with recent circular-bioeconomy approaches that have formulated silages or feeds from exactly these streams and tested them in poultry and swine, demonstrating technical feasibility and safety while aiming to reduce dependence on conventional grains and soybean meal [15,19-22]. Within this framework, our data show that (i) productive performance, final weight, feed conversion efficiency and carcass weight were preserved across treatments, while dressing percentage increased at 5–10%; (ii) ileal eubiosis indicators improved (↓Enterobacteriaceae, ↓Enterococci; ↑Lactobacilli), with more modest changes in the caecum; (iii) serum biochemistry remained largely within reference limits with a modest reduction in ALT at 10%; (iv) meat proximate composition and pH were unaffected, triceps brachii became redder (↑a*) and slightly darker (↓L*), *Campylobacter jejuni* counts decreased in shoulder cuts, and (v)

the fatty acid contents remained generally unchangeable and the n-6/n-3 ratio in muscle fat showed a slightly improvement in the 10% Silage group.

All of these parameters evaluate the usage of agricultural by products in monogastric animals, specifically pigs, without adverse effects in all zootechnical, health and meat quality parameters. This allows for significant improvements in the silage or by-products characteristics in order to achieve beneficial results in the fatty acid profile and the antioxidant capacity of meat, as well as in the microbial populations in the gut of fattening pigs, with the addition of higher percentage of agro-industrial by-products.

Feed Antioxidant Metrics and Lipid Peroxidation

Feed MDA was lower in both silage-enriched diets (5% and 10%) than in the control, with higher dietary total phenolic content also recorded in the former. This pattern is consistent with the in-matrix antioxidant activity of grape- and olive-derived polyphenols, which can quench lipid radicals and chelate pro-oxidant metals, thereby interrupting propagation steps of lipid peroxidation in the feed itself. Comparable outcomes—reduced oxidative in- dices and/or enhanced phenolic density of the diet—have been reported when sole winery or olive coproducts (grape pomace or olive leaves, or OMWW extracts) were incorporated into monogastric rations or used to fortify food/feed matrices. These interventions frequently lower MDA or peroxide values in the matrix and/or downstream animal products, or at minimum, maintain oxidative status despite higher unsaturation loads [15,40,41]. In pigs, phytogenic/phenolic preparations from olive mill wastewater have repeatedly reduced TBARS (and protein carbonyls) in vivo, supporting the notion that OMWW phenolics exert meaningful antioxidant effects within swine systems [42]. Physiologically, a less oxidized diet can reduce the oxidative challenge entering the gastrointestinal tract, which may help explain the favorable ileal shifts we observed (↓*Enterobacteriaceae*/Enterococci; ↑Lactobacilli), showing absence of inflammatory indices; this was also observed in the biochemical and hematological results, in addition to the absence of pro-oxidative signals in meat despite modest fatty-acid changes. While much of the oxidized-feed literature is poultry-centric, it consistently shows that controlling feed oxidation improves redox balance and product quality—principles that plausibly extend to pigs as monogastrics [43]. In our case, the combination of lower feed TBARS, unchanged meat TBARS, and redder shoulder color (↑a*) suggests that the silage's phenolic fraction chiefly protected the diet and

may have contributed to a stable post-absorptive oxidative environment, in line with prior reports on grape/olive polyphenols in monogastric nutrition [40-42,44]. In finishing pigs, substituting part of the fat with olive-pomace acid oil elevated product oxidation at certain inclusion levels compared with refined blends, underscoring that the source and handling of ingredients modulate oxidative outcomes [45]. At the same time, polyphenol supplementation in pigs yields heterogeneous antioxidant results—some studies show reduced TBARS in plasma or meat, others show no change—depending on extract type, dose, basal diet, and sampling matrix [46,47]. For instance, Gessner et al. reported no dif- ferences in plasma or liver TBARS in pigs fed grape seed/grape marc meal extract, despite anti-inflammatory intestinal effects [48]. Conversely, classical α-tocopherol trials consis- tently lowered pork TBARS during storage, illustrating that not all antioxidants behave identically in vivo or in feed [49]. In our study, the lack of treatment differences in meat TBARS—despite lower feed MDA—suggests that (a) dietary phenolics and/or improved gut ecology may have protected muscle lipids post-absorption, (b) the freezing/holding regime truncated oxidative divergence, or (c) the sampling window was too narrow to detect downstream effects. Similar null effects in pork TBARS with botanical phenolics have been reported when storage conditions were not strongly pro-oxidant [50–52].

Performance and Carcass Traits

Final live weight, ADG, and FCR did not differ among A, B, and C and were within the farm's expected range, matching numerous finishing-pig studies where grape or olive by-products at modest inclusion preserved growth [3–6]. For example, replacing wheat bran with grape pomace improved water-holding traits but not growth in finishing pigs [53]. Additionally, dehydrated grape pomace at 5–10% levels maintained performance while altering some carcass and fat traits in a local genotype [51]. For olive coproducts, both neutral and beneficial effects on growth and carcass have been observed depending on inclusion level and processing (e.g., partially de-stoned or ensiled olive cake) [54–58]. We observed a higher dressing percentage at 5–10% silage. While not universal, increased dressing has been reported with phenolic-rich additives (e.g., grape seed proanthocyanidins or ferulic acid mixtures) in finishing pigs, potentially via lean-to-fat repartitioning and water losses at slaughter [59,60]. Conversely, some olive-cake programs reported unchanged dressing or minimal at the highest inclusions, indicating that matrix, dose, and genotype interact [54,56]. Within that landscape, our response—an

improved dressing percentage without weight penalty—fits within the "no-harm to growth, occasional carcass benefit" envelope reported for moderate inclusions of winery/olive by-products [55,56].

Intestinal Microbiology

The ileal shift toward eubiosis (↓ *Enterobacteriaceae* and Enterococci; ↑ Lactobacilli) in B and C is consistent with the prebiotic and antimicrobial profile of plant polyphenols and fermentable fiber [61]. Grape pomace in pigs increased *Lactobacillus*/*Bifidobacterium* and modulated performance and antioxidant status, and broader reviews conclude that grape phenolics in pigs enhance antioxidant capacity, immune tone, and gut biodiversity [62]. Mechanistically, polyphenols can reduce pathobiont abundance through direct bacterio- static or bactericidal action and by shifting substrates toward commensal fermenters; several pig studies and reviews support these pathways [61,62]. Our silage also contributed whey solids to the diet; independent studies show improved growth and beneficial fecal microbiota when liquid whey is included in pig diets [63,64]. Beyond single ingredients finishing pigs fed non-conventional mulberry silages exhibited improved meat quality via gut-microbiota modulation and barrier integrity, supporting a "silage-to-gut-to-meat" axis [65]. Finally, closely related work with exactly this innovative silage in pigs demon- strated microbiota compositional shifts without performance penalties, reinforcing our enteric findings [21].

Serum Biochemistry

The modest reduction in ALT in the Silage-10% group is directionally compatible with hepatoprotective effects of certain plant polyphenols under oxidative or inflammatory challenge in piglets (e.g., holly leaf Ilex latifolia polyphenols attenuating LPS/diquat- induced liver injury with lower ALT/AST/GGT). However, in non-challenged settings, olive or grape polyphenols often leave transaminases unchanged [66]. Thus, our small ALT decrease should be interpreted as biologically modest but not inconsistent with a mild improvement in hepatic redox/immune milieu.

Meat Composition, pH, and Color

The proximate composition and pH of triceps brachii and external abdominal mus- cles were unaffected, as commonly observed with moderate botanical inclusions. Triceps brachii became redder (↑ a *) and slightly darker (↓ L *) in B and C. Although color can be largely affected by parameters like pH, water content and water holding capacity, and myglobin concentration and redo/oxidative state, these characteristics was not altered after the applied treatments. It seems that the increased redness resulted from the addition of antioxidant compounds in the bioactive feed supplement (silage). Antioxidants can stabilize oxymyoglobin and mitigate metmyoglobin formation, thereby sustaining redness. Phenolics may also interact with muscle iron chemistry and endogenous enzymes, contributing to color stability, while darker appearance (lower L *) can arise from subtle differences in water distribution or myoglobin state. Reports with grape products or antioxidant mixtures in pigs similarly describe improved early post-mortem redness and/or water holding without large pH shifts [53,59]. The observed decrease in moisture percentage in the pork belly meat cuts can be attributed to the elongation of fatty acids towards monosaturated and unsaturated, with the simultaneous increase in fat content functioning to advertise possible flavor and texture shifts; it is possible to differentiate these cuts according to consumers' selection criteria.

Meat Microbiology

The lower *Campylobacter jejuni* counts in the shoulder in the 10% group dovetail with extensive in vitro evidence that phenolic extracts—including grape seed and sorghum phenolics—and even olive-mill wastewater polyphenols inhibit *Campylobacter* growth, adhesion, and biofilm formation [67]. While most intervention data are post-harvest or in poultry systems, the directionality supports a plausible diet-to-muscle reduction mediated either by (i) lower intestinal/lymphatic carriage at slaughter or (ii) carry-over of phenolics to the muscle microenvironment where they can exert antimicrobial effects. The absence of *Salmonella* and *L. monocytogenes* across treatments is foremost an indicator of sound hygiene; polyphenols can suppress these pathogens in meat systems, but our experimental design cannot attribute this absence to the diet. The sporadic *E. coli* in the belly (but not the shoulder) is consistent with anatomical contamination risk rather than a treatment effect.

Phenolic Content in Meat and Oxidative Stability

The detected higher total phenolics in triceps brachii at 10% silage are consistent with reports that dietary plant phenolics (especially from grape) can increase meat total phenolics and antioxidant capacity in monogastrics, though the magnitude varies with extractability and conjugation [66]. Meat TBARS did not differ among groups, despite higher feed MDA; this outcome is congruent with several pig studies in which grape-derived phenolics either reduced systemic lipid peroxidation or left TBARS unchanged at slaughter, depending on dose and storage protocol [50,51]. Together with improved redness, unchanged TBARS points to adequate oxidative protection of muscle lipids at the tested inclusion levels and under our storage conditions.

Fatty-Acid Profile

It is well-established that the fatty acid profiles of meat lipids in pigs and poultry closely reflect the composition of fats in their diets [68,69]. Dietary enrichment with polyunsaturated fatty acids is often linked to elevated levels of these acids in the muscle and adipose tissues, both through direct incorporation and modification of unsaturated fatty acids synthesis, by modulating lipid metabolism through the suppression of key lipogenic enzymes involved in de novo fatty-acid synthesis [70]. Thus, the formulation of the silage containing a high proportion of OMWW [19] may explain the improved ($p = 0.005$) n-6/n-3 ratio observed in the Silage-10% group (17.54 → 15.09), reflecting a modest yet favorable shift in muscle lipid quality in the external abdominal oblique cuts, despite the concurrent reduction in total MUFA ($p = 0.049$). Reviews and trials document that grape by-products can beneficially influence pork FA profile (including modest n-3 increases) and that certain olive-cake programs shift MUFA upward and, in some cases, lower dietary n-6/n-3 [48,51,53]. Moreover, controlled manipulations of the dietary n-6/n-3 ratio in pigs produce corresponding shifts in tissue FA and can improve meat-quality indices without compromising oxidative stability when properly balanced. Another study reported that inclusion of grape pomace solids at a 5% rate in finishing-pig diets did not affect the SFA, MUFA, PUFA, n-6 or n-3 PUFA percentages in the meat [51,71,72]. Although the innovative silage used was not a n-3 supplement per se, the aggregate of olive- and grape-derived lipids plus secondary metabolic effects may explain the modest reduction observed. If a larger decrease is targeted, co-inclusion of known n-3 sources (e.g., linseed oil) with grape/olive by-products could

be effective and widely reported [73]. Finally, ensiled olive cake at high inclusion has been shown to improve pork nutritional indices while valorizing a key Mediterranean by-product [53].

Overall, the data support that a 5–10% inclusion of this Greek mixed silage can (i) maintain finishing performance; (ii) modestly improve carcass dressing; (iii) promote ileal eubiosis with lower *Enterobacteriaceae*/Enterococci and higher Lactobacilli; (iv) produce redder triceps brachii meat without compromising pH, proximate composition, or TBARS; (v) reduce *C. jejuni* recovery in triceps brachii meat; and (vi) slightly improve the n-6/n-3 ratio in muscle lipids. These outcomes are consistent with prior work on weaned pigs and microbiomes using the same silage concept and with broader evidence on grape/olive phenolics in pigs [18,19]. The study scope and boundary conditions included a modest sample size, assessment within a single finishing cycle and genotype, and potential matrix effects specific to the silage (e.g., phenolic spectrum, degree of fatty-acid unsaturation, and mineral load) that may influence feed oxidation indices without necessarily manifesting in meat outcomes. The results are also expected to depend on ingredient composition, inclusion level, and processing conditions; any scale-up should additionally consider regulatory context, cost, and palatability. Future work could incorporate extended storage simulations (e.g., refrigerated retail display) and targeted lipidomics to characterize oxidative resilience, while metagenomic and metabolomic profiling would clarify microbe–phenolic interactions along the gut–muscle axis. Finally, evaluating defined n-3 co-supplementation strategies to enhance the favorable shift in the n-6/n-3 ratio—concurrent with sensory assessment—would help optimize nutritional and product-quality endpoints.

3.5 Conclusion

In contemporary animal production, developing alternative feed sources is increasingly important for reducing reliance on conventional ingredients, lowering costs, enhancing sustainability, and recycling agro-industrial residues. The present study evaluated, for the first time in finishing pigs, a silage produced from olive, winery, and cheese by-products incorporated at 0%, 5%, and 10% of the diet. The inclusion of this silage did not negatively affect growth performance, carcass traits, hematological indices, or general meat quality. In- stead, beneficial effects were observed on intestinal microbiota composition, with increased Lactobacilli and decreased *Enterobacteriaceae* and Enterococci, together with elevated muscle phenolic content and an improved n-6/n-3 fatty acid ratio at higher inclusion levels. Although lipid oxidation

(TBARS) was not significantly altered, meat color and some microbial parameters, such as *Campylobacter jejuni* prevalence, were favorably influenced. Overall, these findings indicate that the investigated silage supports pig health and meat functionality while simultaneously valorizing significant Greek agro-industrial by-products, thereby contributing to more circular and environmentally sustainable livestock production.

3.6 References

1. Georganas, A.; Kyriakaki, P.; Giamouri, E.; Mavrommatis, A.; Tsiplakou, E.; Pappas, A.C. Mediterranean Agro-Industrial By-Products and Food Waste in Pig and Chicken Diets: Which Way Forward? Livestock Science 2024, 105584. https://doi.org/10.1016/j.livsci.2024.105584
2. Godde, C.M.; Mason-D'Croz, D.; Mayberry, D.E.; Thornton, P.K.; Herrero, M. Impacts of Climate Change on the Livestock Food Supply Chain: A Review of the Evidence. Global Food Security 2021, 28, 100488. https://doi.org/10.1016/j.gfs.2020.100488
3. European Commission. Farm to Fork Strategy—Action Plan (2020). Available online: https://food.ec.europa.eu/ (accessed on 2 October 2025).
4. Mattioli, L.B.; Corazza, I.; Budriesi, R.; Hrelia, S.; Malaguti, M.; Caliceti, C.; Amoroso, R.; Maccallini, C.; Crupi, P.; Clodoveo, M.L.; et al. From Waste to Health: Olive Mill Wastewater for Cardiovascular Disease Prevention. Nutrients 2024, 16, 2986. https://doi.org/10.3390/nu16172986
5. Cuffaro, D.; Bertolini, A.; Silva, A.M.; Rodrigues, F.; Gabbia, D.; De Martin, S.; Saba, A.; Bertini, S.; Digiacomo, M.; Macchia, M. Comparative Analysis on Polyphenolic Composition of Different Olive Mill Wastewater and Related Extra Virgin Olive Oil Extracts and Evaluation of Nutraceutical Properties by Cell-Based Studies. Foods 2024, 13, 3312. https://doi.org/10.3390/foods13203312
6. Kapellakis IE, Tzanakakis VA and Kabourakis EM (2025) Circular economy and olive mill wastewater management—from production to direct land application and short-term effects on soil properties. Front. Sustain. 6:1545806. doi: 10.3389/frsus.2025.1545806
7. Benalia S, Falcone G, Stillitano T, De Luca AI, Strano A, Gulisano G, Zimbalatti G, Bernardi B. Increasing the Content of Olive Mill Wastewater in Biogas Reactors for a Sustainable Recovery: Methane Productivity and Life Cycle Analyses of the Process. Foods. 2021 May 10;10(5):1029. doi: 10.3390/foods10051029. PMID: 34068520; PMCID: PMC8150611.
8. Karastergiou A, Gancel AL, Jourdes M, Teissedre PL. Valorization of Grape Pomace: A Review of Phenolic Composition, Bioactivity, and Therapeutic Potential. Antioxidants

(Basel). 2024 Sep 19;13(9):1131. doi: 10.3390/antiox13091131. PMID: 39334790; PMCID: PMC11428247.

9. Karastergiou, A.; Gancel, A.-L.; Jourdes, M.; Teissedre, P.-L. Transforming winemaking waste: Grape pomace as a sustainable source of bioactive compounds. OENO One 2025, 59(2), 9202. https://doi.org/10.20870/oeno-one.2025.59.2.9202.
10. Xagoraris, M.; Oikonomou, I.; Daferera, D.; Kanakis, C.; Lappa, I.K.; Giotis, C.; Pappas, C.S.; Tarantilis, P.A.; Skotti, E. Quality Evaluation of Winery By-Products from Ionian Islands Grape Varieties in the Concept of Circular Bioeconomy. Sustainability 2021, 13, 5454. https://doi.org/10.3390/su13105454
11. Bintsis, T.; Papademas, P. Sustainable Approaches in Whey Cheese Production: A Review. Dairy 2023, 4, 249-270. https://doi.org/10.3390/dairy4020018
12. Lappa, I.K.; Papadaki, A.; Kachrimanidou, V.; Terpou, A.; Koulougliotis, D.; Eriotou, E.; Kopsahelis, N. Cheese Whey Processing: Integrated Biorefinery Concepts and Emerging Food Applications. Foods 2019, 8, 347. https://doi.org/10.3390/foods8080347
13. Pappa, E.C.; Kondyli, E.; Pappas, A.C.; Giamouri, E.; Sarri, A.; Mavrommatis, A.; Zoidis, E.; Papalamprou, L.; Simitzis, P.; Goliomytis, M.; et al. Compositional Differences of Greek Cheeses of Limited Production. Foods 2023, 12, 2426. https://doi.org/10.3390/foods12122426
14. Pires, A.F.; Marnotes, N.G.; Rubio, O.D.; Garcia, A.C.; Pereira, C.D. Dairy By-Products: A Review on the Valorization of Whey and Second Cheese Whey. Foods 2021, 10, 1067. https://doi.org/10.3390/foods10051067
15. D'Alessandro, A.G.; Dibenedetto, R.S.; Skoufos, I.; Martemucci, G. Potential Use of Wheat Straw, Grape Pomace, Olive Mill Wastewater and Cheese Whey in Mixed Formulations for Silage Production. Agronomy 2023, 13, 2323. https://doi.org/10.3390/agronomy13092323
16. Kazemi M. Recycling Agricultural Waste: Sustainable Solutions for Enhancing Livestock Nutrition. Vet Med Sci. 2025;11(3):e70321. doi:10.1002/vms3.70321
17. Ressa, A.; Calasso, M.; De Angelis, M.; Martemucci, G.; D'Alessandro, G. (2023). Ensiling of Agro-Industrial By-Products Mixtures (Olive Leaves, Olive Mill Wastewater, Olive Pomace, Wheat Straw, Grape Pomace, Cheese Whey) with Lactiplantibacillus plantarum: Chemical and Microbiological Characteristics. IRIS Institutional Repository. Available online: https://ricerca.uniba.it/handle/11586/493680.

18. Ogwu MC, Hills CE, Pietrosemoli S. The Piggy Solution: Harnessing Food Waste for Sustainable Hog Farming. Glob Chall. 2025;9(8):e00073. Published 2025 Jul 16. doi:10.1002/gch2.202500073.
19. Petrotos, K.; Papaioannou, C.; Kokkas, S.; Gkoutsidis, P.; Skoufos, I.; Tzora, A.; Bonos, E.; Tsinas, A.; Giavasis, I.; Mitsagga, C. Optimization of the Composition of a Novel Bioactive Silage Produced by Mixing Ground Maize with Olive Mill Wastewaters, Grape Pomace and Feta Whey. AgriEngineering 2021, 3, 868–893. https://doi.org/10.3390/agriengineering3040055
20. Bonos, E.; Skoufos, I.; Petrotos, K.; Giavasis, I.; Mitsagga, C.; Fotou, K.; Vasilopoulou, K.; Giannenas, I.; Gouva, E.; Tsinas, A.; et al. Innovative Use of Olive, Winery and Cheese Waste By-Products as Functional Ingredients in Broiler Nutrition. Veterinary Sciences 2022, 9, 290. https://doi.org/10.3390/vetsci9060290
21. Skoufos, I.; Nelli, A.; Venardou, B.; Lagkouvardos, I.; Giannenas, I.; Magklaras, G.; Zacharis, C.; Jin, L.; Wang, J.; Gouva, E.; et al. Use of an Innovative Silage of Agro-Industrial Waste By-Products in Pig Nutrition: Effects on the Gastrointestinal Microbiota. Microorganisms 2023, 11, 1723. https://doi.org/10.3390/microorganisms11071723
22. Magklaras, G.; Skoufos, I.; Bonos, E.; Tsinas, A.; Zacharis, C.; Giavasis, I.; Petrotos, K.; Fotou, K.; Nikolaou, K.; Vasilopoulou, K.; et al. Innovative Use of Olive, Winery and Cheese Waste By-Products in Weaned Pigs' Nutrition. Veterinary Sciences 2023, 10, 397. https://doi.org/10.3390/vetsci10060397
23. Saini, R.K.; Khan, M.I.; Kumar, V.; Shang, X.; Lee, J.-H.; Ko, E.-Y. Bioactive Compounds of Agro-Industrial By-Products: Current Trends, Recovery, and Possible Utilization. Antioxidants 2025, 14(6), 650. https://doi.org/10.3390/antiox14060650.
24. Valta, K.; Damala, P.; Angeli, E.; Antonopoulou, G.; Malamis, D.; Haralambous, K.J. Current treatment technologies of cheese whey and wastewater by Greek cheese manufacturing units and potential valorisation opportunities. Waste Biomass Valor. 2017, 8, 1649-1663. https://doi.org/10.1007/s12649-017-9862-8.
25. Presidential Decree 56/2013 (Government Gazette A' 106). Protection of Animals Used for Scientific Purposes (Transposition of Directive 2010/63/EU into Greek Legislation). Hellenic Republic: Athens, Greece, 2013. Available online: https://anilab.decentral.minagric.gr/index.php/en/legal-framework-en/presidential-

decrees-en/178- pd56_2013_fek106_en?utm_source=chatgpt.com (accessed on 17 December 2025).

26. National Research Council. Nutrient Requirements of Swine, 11th ed.; National Academies Press: Washington, DC, 2012.
27. Premier Nutrition. Premier Atlas; Premier Nutrition: Brereton, UK, 2014.
28. Vasilopoulos, S.; Dokou, S.; Papadopoulos, G.A.; Savvidou, S.; Christaki, S.; Kyriakoudi, A.; Dotas, V.; Tsiouris, V.; Bonos, E.; Skoufos, I.; et al. Dietary Supplementation with Pomegranate and Onion Aqueous and Cyclodextrin Encapsulated Extracts Affects Broiler Performance Parameters, Welfare and Meat Characteristics. Poultry 2022, 1, 74-93. https://doi.org/10.3390/poultry1020008
29. Botsoglou NA, Fletouris DJ, Papageorgiou G, Vassilopoulos VN, Mantis AJ, Trakatellis AG. Rapid, sensitive, and specific thiobarbituric acid method for measuring lipid peroxidation in animal tissue, food, and feedstuff samples. J Agric Food Chem. 1994;42(9):1931–1937. doi:10.1021/jf00045a019.
30. Hernández-Durán, M.; López-Jácome, L.E.; Colín-Castro, C.A.; Cerón-González, G.; Ortega-Peña, S.; Vanegas-Rodríguez, E.S.; Mondragón-Eguiluz, J.A.; Franco-Cendejas, R. Comparison of the MicroScan WalkAway and VITEK 2 Compact Systems for the Identification and Susceptibility of Clinical Gram-Positive and Gram-Negative Bacteria. Investigación en Discapacidad 2017, 6(3), 105–114.
31. Anderson, S. Determination of Fat, Moisture, and Protein in Meat and Meat Products by FOSS FoodScan: Collaborative Study. J. AOAC Int. 2007, 90, 1073–1083. https://doi.org/10.1093/jaoac/90.4.1073
32. Dias, F.F.G.; Augusto-Obara, T.R.; Hennebelle, M.; Chantieng, S.; Ozturk, G.; Taha, A.Y.; Vieira, T.; de Moura Bell, J.M.L.N. Effects of Industrial Heat Treatments on Bovine Milk Oxylipins and Lipid Oxidation Markers. Prostaglandins Leukot. Essent. Fatty Acids 2020, 152, 102040. https://doi.org/10.1016/j.plefa.2019.102040
33. ISO 6579:2002; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Detection of Salmonella spp. International Organization for Standardization (ISO): Geneva, Switzerland, 2002.

34. ISO 4833:2001; Microbiology of Food and Animal Feeding Stuffs—Horizontal Method for the Enumeration of Microorganisms—Colony-Count Technique at 30 ◦C. International Organization for Standardization (ISO): Geneva, Switzerland, 2001.
35. O'Fallon, J.V.; Busboom, J.R.; Nelson, M.L.; Gaskins, C.T. A Direct Method for Fatty Acid Methyl Ester Synthesis:Application to wet meat tissues, oils, and feedstuffs. J. Anim. Sci. 2007, 85, 1511–1521. https://doi.org/10.2527/jas.2006-491
36. Giannenas I, Tzora A, Sarakatsianos I, Karamoutsios A, Skoufos S, Papaioannou N, Anastasiou I, Skoufos I. The Effectiveness of the Use of Oregano and Laurel Essential Oils in Chicken Feeding. Annals of Animal Science. National Research Institute of Animal Production, 2016;16(3): 779-796. https://doi.org/10.1515/aoas-2015-0099
37. Minelli, G.; D'Ambra, K.; Macchioni, P.; Lo Fiego, D.P. Effects of Pig Dietary n-6/n-3 Polyunsaturated Fatty Acids Ratio and Gender on Carcass Traits, Fatty Acid Profiles, Nutritional Indices of Lipid Depots and Oxidative Stability of Meat in Medium–Heavy Pigs. Foods 2023, 12, 4106. https://doi.org/10.3390/foods12224106
38. IBM Corp. IBM SPSS Statistics for Windows, Version 25.0; IBM Corp.: Armonk, NY, USA, 2017.
39. Hellenic Statistical Authority (ELSTAT). Annual Agricultural Statistical Survey 2022 & 2023: Grapes and Must Production in Greece. Hellenic Statistical Authority: Piraeus, Greece, 2024. Available online: https://www.statistics.gr/en/statistics/AGR_PRODC (accessed on 4 November 2025)
40. Proca, A.C.; Horodincu, L.; Solcan, C.; Solcan, G. The Potential of Grape Polyphenols Additive in Pig Nutrition: Chemical Structure, Bioavailability and Their Effect on Intestinal Health of Pigs. Agriculture 2024, 14, 1142. https://doi.org/10.3390/agriculture14071142
41. Scerra M, Foti F, Caparra P, Cilione C, Bognanno M, Paolo F, Paolo C, Natalello A, Musati M, Chies L. Effects of feeding bergamot pulp and olive leaves on performance and meat quality in Apulo-Calabrese pigs. Vet Anim Sci. 2024 Jan 21;23:100336. doi: 10.1016/j.vas.2024.100336. PMID: 38283331; PMCID: PMC10818079.
42. Papakonstantinou, G.I.; Meletis, E.; Petrotos, K.; Kostoulas, P.; Tsekouras, N.; Kantere, M.C.; Voulgarakis, N.; Gougoulis, D.; Filippopoulos, L.; Christodoulopoulos, G.; et al. Effects of a Natural Polyphenolic Product from Olive Mill Wastewater on Oxidative Stress

and Post-Weaning Diarrhea in Piglets. Agriculture 2023, 13, 1356. https://doi.org/10.3390/agriculture13071356

43. Gerasopoulos K, Stagos D, Kokkas S, Petrotos K, Kantas D, Goulas P, Kouretas D. Feed supplemented with byproducts from olive oil mill wastewater processing increases antioxidant capacity in broiler chickens. Food Chem Toxicol. 2015 Aug;82:42-9. doi: 10.1016/j.fct.2015.04.021. Epub 2015 Apr 24. PMID: 25916917.
44. Desbruslais, A.; Wealleans, A.L. Oxidation in Poultry Feed: Impact on the Bird and the Efficacy of Dietary Antioxidant Mitigation Strategies. Poultry 2022, 1, 246-277. https://doi.org/10.3390/poultry1040022
45. Verge-Mèrida, G.; Barroeta, A.C.; Guardiola, F.; Verdú, M.; Balart, M.; Font-i-Furnols, M.; Solà-Oriol, D. Crude and Acid Oils from Olive Pomace as Alternative Fat Sources in Growing–Finishing Pigs. Animal 2021, 15(12), 100389. https://doi.org/10.1016/j.animal.2021.100389
46. Albendea, P.; Tres, A.; Rafecas, M.; Vichi, S.; Solà-Oriol, D.; Verdú, M.; Guardiola, F. Effect of Feeding Olive Pomace Acid Oil on Pork Lipid Composition, Oxidative Stability, Colour, and Sensory Acceptance. Animal 2023, 17(8), 100879. https://doi.org/10.1016/j.animal.2023.100879
47. González, E.; Hernández-Matamoros, A.; Tejeda, J.F. Two By-Products of the Olive Oil Extraction Industry as Oleic Acid Supplement Source for Iberian Pigs: Effects on Meat Composition and Lipid Peroxidation. Journal of the Science of Food and Agriculture 2012, 92(12), 2543–2551. https://doi.org/10.1002/jsfa.5669
48. Gessner DK, Fiesel A, Most E, Dinges J, Wen G, Ringseis R, Eder K. Supplementation of a grape seed and grape marc meal extract decreases activities of the oxidative stress-responsive transcription factors NF-κB and Nrf2 in the duodenal mucosa of pigs. Acta Vet Scand. 2013 Mar 2;55(1):18. doi: 10.1186/1751-0147-55-18. PMID: 23453040; PMCID: PMC3599961.
49. Buckley DJ, Morrissey PA, Gray JI. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. J Anim Sci. 1995;73(10):3122-3130. doi:10.2527/1995.73103122x
50. Bertol, T.M.; Silva, F.; Poleti, M.D.; et al. Inclusion of grape pomace in the diet of pigs on pork quality and oxidative stability of omega-3 enriched fat. Ciência Rural 2017, 47(11), e20160630. https://doi.org/10.1590/0103-8478cr20150358

51. da Silveira Almeida, B.C.; Ludke, M.d.C.M.M.; Bertol, T.M.; Ludke, J.V.; Bernardi, D.M.; Cunha, A., Jr.; Coldebella, A. Growth Performance, Meat Quality, and Lipid Oxidation in Pigs' Fed Diets Containing Grape Pomace. Appl. Biosci. 2024, 3, 378–391. https://doi.org/10.3390/applbiosci3030025
52. Skoufos, I.; Bonos, E.; Anastasiou, I.; Tsinas, A.; Tzora, A. Effects of Phytobiotics in Healthy or Disease-Challenged Animals. In Feed Additives: Aromatic Plants and Herbs in Animal Nutrition and Health; Florou-Paneri, P., Christaki, E., Giannenas, I., Eds.; Academic Press: London, UK, 2019; pp. XXX–XXX. https://doi.org/10.1016/B978-0-12-814700-9.00018-2
53. Tian, X.; Li, Z.; Zhao, Y.; Wang, C.; Han, Y.; Wang, Y.; Zhu, Y.; Xu, Y. Dietary grape pomace extract supplementation improved meat quality, antioxidant capacity, and immune performance in finishing pigs. Front. Microbiol. 2023, 14, 1116022. https://doi.org/10.3389/fmicb.2023.1116022
54. Liotta, L.; Chiofalo, V.; Lo Presti, V.; Chiofalo, B. In vivo performances, carcass traits and meat quality of pigs fed olive cake processing waste. Animals 2019, 9(11), 1155. https://doi.org/10.3390/ani9121155
55. Ferlisi F, Tang J, Cappelli K, Trabalza-Marinucci M. Dietary supplementation with olive oil co-products rich in polyphenols: a novel nutraceutical approach in monogastric animal nutrition. Front Vet Sci. 2023 Oct 13;10:1272274. doi: 10.3389/fvets.2023.1272274. PMID: 37901105; PMCID: PMC10611480.
56. Joven, M.; Pintos, E.; Latorre, M.A.; Suárez-Belloch, J.; Guada, J.A.; Fondevila, M. Effect of replacing barley by increasing levels of olive cake in the diet of finishing pigs: Growth performances, digestibility, carcass, meat and fat quality. Anim. Feed Sci. Technol. 2014, 197, 185–193. https://doi.org/10.1016/j.anifeedsci.2014.08.007
57. Ferrer P, Calvet S, García-Rebollar P, de Blas C, Jiménez-Belenguer AI, Hernández P, Piquer O, Cerisuelo A. Partially defatted olive cake in finishing pig diets: implications on performance, faecal microbiota, carcass quality, slurry composition and gas emission. Animal. 2020 Feb;14(2):426-434. doi: 10.1017/S1751731119002040. Epub 2019 Sep 30. PMID: 31566173.
58. Leite, A.; Vasconcelos, L.; Rodrigues, S.; Pereira, E.; Domínguez-Valencia, R.; Lorenzo, J.M.; Teixeira, A. Effect of olive cake in Bísaro pig feed on physicochemical composition

and fatty acid profile of three different muscles of dry-cured shoulder. Animals 2024, 14(11), 1697. https://doi.org/10.3390/ani14111697

59. Zheng, Y.; Yu, B.; Luo, Y.; Huang, Z.; Zheng, P.; Mao, X.; Dai, Z.; Yu, J.; Yan, H.; Luo, J.; He, J. Dietary supplementation of grape seed proanthocyanidins improves growth performance, carcass traits, and meat quality in growing-finishing pigs. Anim. Nutr. 2024, 20, xxx–xxx. https://doi.org/10.1016/j.aninu.2024.10.006
60. Valenzuela-Grijalva, N.; Jiménez-Estrada, I.; Mariscal-Tovar, S.; López-García, K.; Pinelli-Saavedra, A.; Peña-Ramos, E.A.; Muhlia-Almazán, A.; Zamorano-García, L.; Valenzuela-Melendres, M.; González-Ríos, H. Effects of Ferulic Acid Supplementation on Growth Performance, Carcass Traits and Histochemical Characteristics of Muscle Fibers in Finishing Pigs. Animals 2021, 11, 2455. https://doi.org/10.3390/ani11082455
61. Eder, K.; Ringseis, R.; Gessner, D.K. Effects of grape by-products on oxidative stress and inflammation in farm animals: An overview of studies performed in pigs, chickens, and cattle. Animals 2025, 15(11), 1536. https://doi.org/10.3390/ani15111536
62. Horodincu, L.; Proca, A.C.; Șlencu, B.G.; Trifan, A.; Pavel, G.; Solcan, G.; Solcan, C. Modulating Effects of Grape Pomace on the Intestinal Antioxidative and Inflammatory Status in Fattening Pigs. Agriculture 2025, 15, 740. https://doi.org/10.3390/agriculture15070740
63. Sutera, A.M.; Arfuso, F.; Tardiolo, G.; Riggio, V.; Fazio, F.; Aiese Cigliano, R.; Paytuví, A.; Piccione, G.; Zumbo, A. Effect of a Co-Feed Liquid Whey-Integrated Diet on Crossbred Pigs' Fecal Microbiota. Animals 2023, 13, 1750. https://doi.org/10.3390/ani13111750
64. Kobayashi, Y., Itoh, A., Miyawaki, K., Koike, S., Iwabuchi, O., Iimura, Y., Kobashi, Y., Kawashima, T., Wakamatsu, J., Hattori, A., Murakami, H., Morimatsu, F., Nakaebisu, T., & Hishinuma, T. (2011). Effect of liquid whey feeding on fecal microbiota of mature and growing pigs. Animal science journal = Nihon chikusan Gakkaiho, 82(4), 607–615. https://doi.org/10.1111/j.1740-0929.2011.00876.x
65. Niu, J.; Yu, J.; Zhang, H.; et al. Effects of silage diet on meat quality through shaping gut microbiota in finishing pigs. Microbiol. Spectr. 2023, 11(1), e02416–22. https://doi.org/10.1128/spectrum.02416-22

66. Chedea, V.S.; Palade, L.M.; Pelmus, R.S.; Dragomir, C.; Taranu, I. Red Grape Pomace Rich in Polyphenols Diet Increases the Antioxidant Status in Key Organs—Kidneys, Liver, and Spleen of Piglets. Animals 2019, 9, 149. https://doi.org/10.3390/ani9040149
67. Roila, R.; Ranucci, D.; Valiani, A.; Galarini, R.; Servili, M.; Branciari, R. Antimicrobial and Anti-Biofilm Activity of Olive Oil By-Products against Campylobacter spp. Isolated from Chicken Meat. Acta Sci. Pol. Technol. Aliment. 2019, 18, 43–52. https://doi.org/10.17306/J.AFS.0629
68. Skiba G, Poławska E, Sobol M, Raj S, Weremko D. Omega-6 and omega-3 fatty acids metabolism pathways in the body of pigs fed diets with different sources of fatty acids. Arch Anim Nutr. 2015;69(1):1-16. doi:10.1080/1745039X.2014.992173
69. Madeira, M.S.; Alfaia, C.M.; Lopes, P.A.; Pestana, J.; Coelho, D.; Fontes, C.M.G.A.; Prates, J.A.M. Ameliorating Pork Marbling and Quality with Novel Feeding Approaches. In Advances in Animal Health, Medicine and Production; Freitas Duarte, A., Lopes da Costa, L., Eds.; Springer: Berlin/Heidelberg, Germany, 2020; pp. 161–177. https://doi.org/10.1007/978-3-030-61981-7_8
70. Schumacher, M.; DelCurto-Wyffels, H.; Thomson, J.; Boles, J. Fat Deposition and Fat Effects on Meat Quality—A Review. Animals 2022, 12, 1550. https://doi.org/10.3390/ani12121550
71. Habeanu, M.; Lefter, N.A.; Ropota, M.; Chedea, V.S.; Gheorghe, A.; Toma, S.M.; Ciurescu, G.; Dragomir, C. Dried Grape Pomace Influenced Fatty Acid Composition of Longissimus dorsi Muscle and Plasma Polyphenol Spectrum in Finishing Pigs. Indian J. Anim. Sci. 2015, 85, 786–789.
72. Vlaicu, P.A.; Panaite, T.D.; Cornescu, M.G.; Ropota, M.; Olteanu, M.; Dragotoiu, D. The influence of by-products on the production parameters and nutrient digestibility in fattening pigs diet (60–100 kg). AgroLife Sci. J. 2019, 8, 261–269.
73. Nuernberg, K.; Fischer, K.; Nuernberg, G.; Kuechenmeister, U.; Klosowska, D.; Eliminowska-Wenda, G.; Fiedler, I.; Ender, K.
Effects of Dietary Olive and Linseed Oil on Lipid Composition, Meat Quality, Sensory Characteristics and Muscle Structure in Pigs. Meat Sci. 2005, 70, 63–74. https://doi.org/10.1016/j.meatsci.2004.12.001

Chapter 4 : Dietary Use of a Mixture of Greek Aromatic/Medicinal Plants in Diets of Weaned Pigs

Sections of this chapter have been published in:

Nutritional Use of Greek Medicinal Plants as Diet Mixtures for Weaned Pigs and Their Effects on Production, Health and Meat Quality. **Magklaras G**, Tzora A, Bonos E, Zacharis C, Fotou K, Wang J, Grigoriadou K, Giannenas I, Jin L, Skoufos I. Applied Sciences. 2025; 15(17):9696. https://doi.org/10.3390/app15179696

4.1. Introduction

There is a global need to identify new production systems in agriculture that will increase the efficiency of food produced from the animal production sector [1] and satisfy the increasing demand for meat and meat products. In addition to sustainability in production, limitations in global natural resources and nutritional quality have become decisive factors for consumer choice [2]. Furthermore, the growing movement to reduce antibiotic usage in livestock has underlined the need for efficient substitutes that improve growth and facilitate disease prevention in pigs while ensuring food security [3–6]. The reliance on antibiotics has raised worries about the emergence of antimicrobial resistance, prompting a trend towards the inclusion of natural additives, such as extracts from aromatic and medicinal plants (phytobiotics), in animal diets [7,8]. Phytobiotics are a type of nutraceuticals that consist of non-nutritive, plant-derived bioactive chemicals used as feed additives in swine diets [9–11]. These can be divided into four categories based on their origin and processing characteristics: herbs (blooming, non-woody, and non-persistent plants), spices (plants with a strong odor or flavor), essential oils (volatile lipophilic compounds) and oleoresins (extracts obtained from non-aqueous solutions) [12]. This new generation of feed additives encompasses a diverse array of substances, including essential oils, herbal extracts and functional components derived from various plant species, which are acknowledged for their antimicrobial, antioxidant and anti-inflammatory properties [13–17], as well as their capacity to enhance growth [18–20] and positively influence the gut microbiome [21–23]. The favorable effects may be linked to improvements in intestinal architecture, enhanced antioxidant capacity and modification of microbial communities. Phytobiotics are rich in various bioactive chemicals responsible for their functional actions. Flavonoids, alkaloids (containing alcohols, aldehydes, ketones, esters, and lactones), phenols (tannins), glycosides, terpenoids and glucosinolates are the components that make up the bioactive chemicals. Although phytobiotics represent a wide spectrum of bioactive chemicals, their precise mechanisms of action, inclusion dosage and possible interactions with other feed components are yet unknown. Several factors can affect the quantity and chemical composition of active compounds present in phytobiotics. These factors encompass the plant component utilized (e.g., seeds or leaves), the geographical region, the harvesting season and the time of year. An understanding of the specific processes through which phytobiotics exert their effects is quite

complicated due to the factors mentioned [12,24–26]. The use of a blend of phytobiotic sources in pig diets is increasingly justified by the diverse bioactive properties these compounds offer. Individual phytobiotics—such as essential oils, plant extracts and polyphenols—possess antimicrobial, antioxidant, anti-inflammatory and gut-modulating effects. However, their efficacy can be limited when used alone due to variability in absorption, stability or target specificity [27,28]. Combining multiple phytobiotics can harness synergistic effects, where the combined action exceeds the sum of individual effects enhancing gut health, immune function and overall performance more effectively than single compounds [29,30]. Such blends may also reduce the risk of microbial resistance, offering a more robust and consistent alternative to antibiotics in sustainable pig production.

The present study evaluated, for the first time, a phytobiotic mixture of four different aromatic/medicinal plants as ingredients in weaned pig diets, in an effort to evaluate their potential benefits on production indices, animal health and meat quality attributes. The two phytobiotic mixtures (Table 4.1) were comprised of oregano (*Origanum vulgare* subsp. *hirtum*) essential oil, rock samphire (Crithmum maritimum L.) essential oil, camelina (*Camelina sativa* L. Crantz) and garlic (*Allium sativum* L.) flour, having been incorporated into pig feed at a concentration of 0.2%. Oregano essential oil, derived from the Greek aromatic plant *Origanum vulgare* subsp. *hirtum*, comprises a minimum of 24 chemical compounds, including carvacrol, thymol and β-caryophyllene [31–33]. Numerous studies indicate that the dietary inclusion of oregano essential oil enhances growth performance, immune function and antioxidative responses in weaned and finishing pigs [34–37]. It has also been utilized to improve meat quality characteristics in pigs and other animals [38–42]; however, the results were often inconsistent. Rock samphire (*Crithmum maritimum* L.), also known as sea fennel, is an edible halophyte and xerophyte that grows wildly on the Mediterranean and Atlantic coasts of Europe [43]. The leaves, flowers and schizocarps of *C. maritimum* L. consists predominantly of carbohydrates (>65%), with lesser amounts of ash, proteins and lipids. Sea fennel's briny, tender leaves provide omega-6 and omega-3 polyunsaturated fatty acids, particularly linoleic acid. Extracts derived from flowers and fruits or schizocarps are abundant in antioxidants, polyphenols, vitamins and carotenoids, exhibiting antibacterial efficacy against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Candida albicans* and *Candida parapsilosis* [44,45]. Camelina (*Camelina sativa* L. Crantz), also known as false flax, is an oilseed crop belonging to the Brassicaceae family that can grow in

different climatic and soil conditions. It has been evaluated in pig diets due to the fatty acid (FA) profile of its oil extract, which is exceptionally high in polyunsaturated FA and particularly alpha-linolenic acid (ALA), an essential omega-3 fatty acid that is beneficial for enhancing cardiovascular health [46,47], as well as its much higher protein and lysine contents than corn or other cereal grains. Blood plasma omega-3 fatty acid levels of pigs can rise when camelina is added to their food, and their serum triglyceride levels can drop [48]. Some types of camelina have compounds (glucosinolates, condensed tannins and sinapine) that make the feed taste bitter, which can potentially cause pigs to consume less [49,50]. Garlic (*Allium sativum* L.) has been used in pig diets due to its antifungal, antimicrobial and antioxidant properties [51,52]. Garlic extract contains compounds such as allicin, alliin, phenols and flavonoids, which have been demonstrated to exhibit antioxidant action through free radical scavenging mechanisms. Allium spp. derivatives, such as organosulfurate compounds, alter the gut microbiota by affecting both beneficial bacteria (*Bifidobacterium* spp. or *Lactobacillus* spp.) and pathogenic bacteria (*Escherichia coli*, *Salmonella typhimurium* or *Clostridium* spp.) [53,54]. To the best of our knowledge, it is the first time that such a combination of the previously mentioned four plant extracts and essential oils has been evaluated in weaned pig diets (Fig 4.1.)

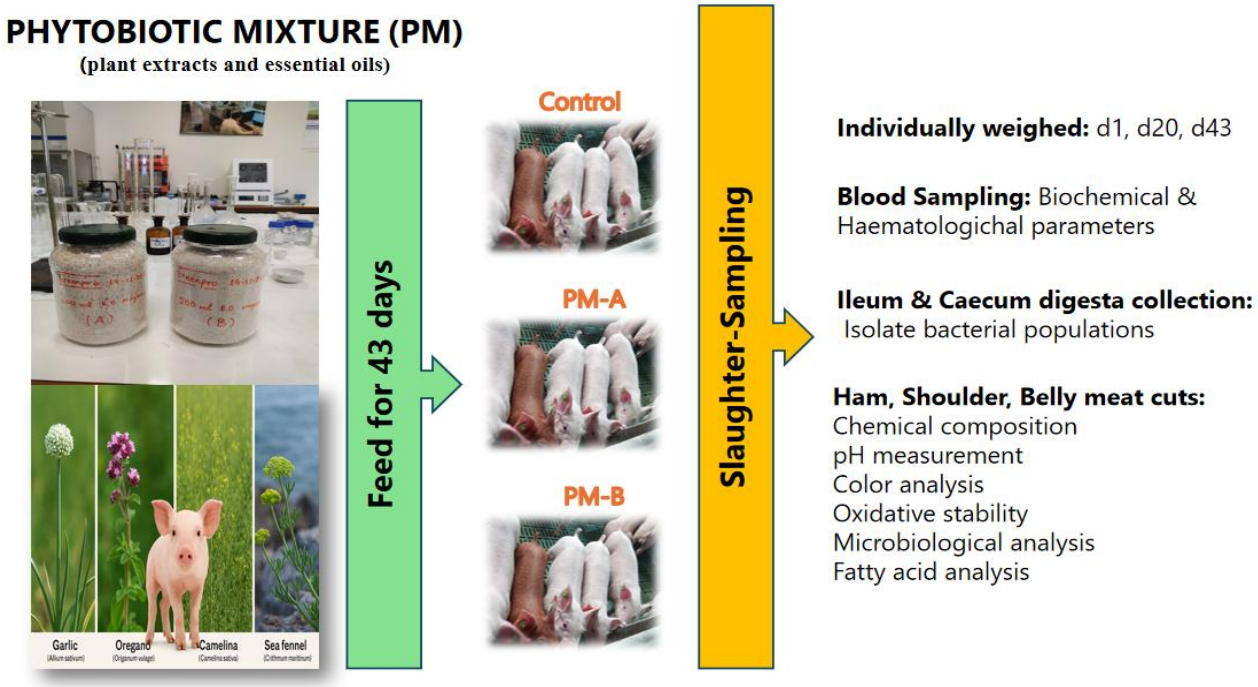


Figure 4.1. Experimental summary schematic illustration of Chapter 4 (exp. No3).

Table 4.1. Composition of the evaluated phytobiotic mixture (PM).

Plant Material/Feed Additive	PM-A	PM-B
Oregano (*Origanum vulgare* subsp. *hirtum*) (Essential oil) *	100 mL (25 mL methylcellulose/75 mL oil)	200 mL (50 mL methylcellulose/150 mL oil)
Rock samphire *(Crithmum maritimum* L.) (Essential oil)	25 mL	25mL
Camelina *(Camelina sativa* L. Crantz) (Dried and flour form)	0.5 kg	0.5 kg
Garlic *(Allium sativum* L.) (Dried and flour form)	0.5 kg	0.5 kg

PM-A: phytobiotic mixture in diet of pig group A; PM-B: phytobiotic mixture in diet of pig group B.
* Microencapsulated form.

4.2. Materials and Methods

4.2.1. Experimental Design, Animals and Diets

All experimental protocols adhered to the National Guidelines for Animal Trials (PD, 2013) and received approval from the authorities of the School of Agriculture at the University of Ioannina, Greece (UOI University Research Committee research registration: 61291/135/10.06.2020. A veterinary surgeon and an animal scientist from the Department of Agriculture at the University of Ioannina oversaw the farm environment and the piglets throughout the entire experimental period. The collaborating commercial pig farm was situated in the region of Epirus, Greece, and fulfilled the national regulations and the European directive for the protection of animal welfare in research (Directive 2010/63/EU, European Commission, 2010). According to Eurostat data, the pig population in Greece was estimated at approximately 786,200 heads in 2024 [55]. Domestic pork production remains insufficient to meet national demand, covering only 30–35% of consumption, which necessitates substantial imports. Intensive production systems constitute the dominant farming model, particularly farrow-to-finish units, with the average farm maintaining around 200 sows. In these systems, pigs are generally provided with ad libitum dry feed composed primarily of cereal-based diets supplemented with essential amino acids, vitamins and minerals. Feed expenses represent a major cost component, accounting for approximately

55–70% of total production costs. Nevertheless, organic and pasture-based systems have been gaining momentum since the early 2000s, reflecting increasing interest in alternative and potentially more sustainable production practices.

Table 4.1 presents the composition of the phytobiotic mixture (PM) tested in the present trial, with the oregano oil added in a micro-encapsulated form, produced by the methodology described by Partheniadis et al. [56]. The liquid forms of the essential oils of oregano and rock samphire were mixed thoroughly with the powder form of camelina and garlic, which were previously ground to provide a fine flour (Table 4.2).

The Institute of Plant Breeding and Genetic Resources (IPB&GR) in Thessaloniki, Greece, provided plant samples of *Origanum vulgare* subsp. *hirtum* and *Crithmum maritimum* L. The biomass was dried at room temperature in the shade using a 50 L pilot-scale steam distillatory apparatus with a steam pressure of 1.2 atm. It was then distillated for 1.5 h for Origanum vulgare subsp. hirtum and 1 h for the other three species. The dried plant components were hydro-distilled for two hours with a Clevenger-style apparatus coupled to a specially refrigerated EO container. Essential oils (EOs) for the trial were extracted from *Origanum vulgare* subsp. *hirtum* (IPEN: GR-1-BBGK-03,2107) and *Crithmum maritimum* L. (IPEN: GR-1-BBGK-97,719), provided by ELGO-DIMITRA's Institute of Plant Breeding and Genetic Resources. Additionally, *Camelina sativa* L. Crantz seeds and *Allium sativum* L. bulbs were supplied by a Union of Agricultural Cooperation (Vyssa, Greece). The methodology used to process the samples was based on that described by O'Fallon et al. [57]. The fatty acid composition was determined by gas chromatography; fatty acids methyl esters were obtained from the samples. Then, the separation and quantification of the methyl esters was carried out with a gas chromatographic system (TraceGC model K07332, ThermoFinnigan, ThermoQuest, Milan, Italy) equipped with a flame ionization detector and a fused silica capillary column (phase type SP-2380, Supelco, Bellefonte, PA, USA). Individually identified fatty acids were reported as percentages (%) of the total identified acids. The herbal mixture was formulated to provide in the feed: 100 or 200 mL/kg *Origanum vulgare* subsp. *hirtum* essential oil; 25 mL/kg *Crithmum maritimum* L. essential oil; 0.5 g/kg dried *Camelina sativa* L. Crantz; and 0.5 g/kg dried *Allium sativum* L. (Table 4.1).

A total of 45 crossbred weaned pigs (1/4 Large White, 1/4 Landrace, 1/2 Duroc) at 34 days old were randomly assigned to one of three treatment groups—CONT, PM- A or PM-B. The control

treatment group (CONT) was fed a commercially formulated maize-based diet suitable for weaned pigs, adhering to the guidelines established by the National Research Council [58] and the Premier Nutrition database [59]. The remaining two treatments received diets containing 0.2% of the evaluated quadruple phytobiotic mixture (PM), PM-A with 200mL/t feed oregano oil or PM-B with 400mL/t feed oregano oil. All three diets were designed to be isonitrogenous and isocaloric. The ingredient composition and proximate analysis of the experimental diets are presented in Table 4.3.

The initial body weights of the pigs were comparable throughout the three groups, with an average mean body weight of 8.31 ± 0.94 kg. Each group had equal numbers of females (7) and males (8). Each pig was distinctly identified with numbered plastic ear tags. The pigs in each treatment group (n = 15) were accommodated in pens with slated plastic flooring, and maintained under regulated environmental conditions (ambient temperature, average humidity, ventilation rate and animal density) appropriate for their production stage. All pigs were vaccinated against porcine circovirus type 2 (PCV2), Mycoplasma hyopneumoniae, Actinobacillus pleuropneumoniae and Aujeszky's disease, under the farm's regular management protocols. Access to feed and water was provided ad libitum. The trial lasted for 43 days (from 34 to 77 days of age), during which pigs were weighed individually on the mornings of the 1st, 22nd and 43rd days using a Mini–L 3510 scale for animals (Zigisis S.A., Chalkidiki, Greece), while feed intake and mortality data were documented daily. To evaluate the impact of dietary interventions on the performance metrics of pigs, the average gain (AG, kg per period), average feed intake (AFI, kg feed intake per period) and feed conversion ratio (FCR, kg feed intake/kg live weight gain) were recorded. On the final day of the trial, blood samples were collected from eight pigs (four males and four females) in each group, after which these pigs were humanely slaughtered in a modern abattoir near the experimental farm.

Table 4.2. Chemical composition of phytobiotic mixture (plant extracts and essential oils).

Garlic (*Allium sativum* L.) [1]		**Oregano (*Origanum vulgare* subsp. *hirtum*)** [1]		**Rock Samphire (*Crithmum maritimum* L.)** [1]		**Camelina (*Camelina sativa* L. Crantz)** [2]	
Compound	**%**	**Compound**	**%**	**Compound**	**%**	**FA**	**%**
Diallyl trisulfide	58.46	Carvacrol	78.72	β-phellandrene	28.01	C11:0 (Undecanoic)	0.01
Diallyl disulphide	24.54	p-cymene	8.19	Sabinene	20.96	C12:0 (Lauric)	0.01
Diallyl tetrasulphide	4.73	γ-terpinene	2.11	γ-terpinene	18.69	C14:0 (Myristic)	0.11
3-Vinyl-1,2-dithiocyclohex-5-ene	0.64	Myrcene	1.64	1,8-cineol	9.53	C15:0 (Pentadecanoic)	0.03
N,N-dimethyl-Ethanethioamide	0.63	β-caryophyllene	1.27	Thymol methyl ether	4.07	C16:0 (Palmitic)	8.29
Allyl methyl trisulphide	4.42	α-terpinene	1.01	cis-β-ocimene	3.68	C16:1 (Palmitoleic)	0.14
Dimethyl trisulphide	1.25	α-pinene	0.98	p-cymene	3.55	C17:0 (Heptadecanoic)	0.05
Apiol	0.26	cis-sabinene hydrate	0.62	Terpinen-4-ol	2.66	C17:1 (cis-10-Heptadecenoic)	0.05
(methylsulfinyl)(methylthio)-Methane	0.24	Terpinen-4-ol	0.55	α-pinene	2.42	C18:0 (Stearic)	2.24
Carvacrol	1.22	α-thujene	0.48	α-terpinene	1.64	C18:1n9t (Elaidic)	0.03
Epiglobulol	0.18	Borneol	0.42	Myrcene	1.44	C18:1n9c (Oleic)	15.36
3-Vinyl-1,2-dithiocyclohex-4-ene	0.17	1-octen-3-ol	0.38	α-terpinolene	0.91	C18:2n6t (Linolelaidic)	0.01
Hinesol	0.16	α-humulene	0.30	α-thujene	0.48	C18:2n6c (Linoleic)	22.31
Patchoulane	0.15	Thymol	0.28	α-phenalldrene	0.44	C18:3n6 (γ-Linolenic)	0.00
p-Cymene	0.14	Limonene	0.27	trans-β-ocimene	0.24	C20:0 (Arachidic)	1.11
1-Docosanol	0.12	Camphene	0.25	Allo-ocimene	0.23	C18:3n3 (a-Linolenic)	34.54
3-(Methylthio)pent-4-yn-1-ol	0.11	Caryophyllene oxide	0.24	β-pinene	0.20	C20:1n9c (cis-11-Eicosenoic)	11.14
D-Limonene	0.09	β-phellandrene	0.23	Bicyclogermacrene	0.14	C20:2 (cis-11,14-Eicossadienoic)	1.59
Isobutyl isothiocyanate	0.08	α-phellandrene	0.18	cis-2-p-menthen-1-ol	0.11	C22:0 (Behenic)	0.13
Linalool	0.07	β-pinene	0.16	α-terpineol	0.08	C20:4n6 (Arachidonic)	1.00
cis-2-Thiabicyclo [3.3.0]Octane	0.06	α-terpinolene	0.15	β-caryophyllene	0.08	C22:1n9 (Erucic)	1.50
Eucalyptol	0.05	δ-cadinene	0.13	Camphene	0.07	C22:2 (cis-13,16-Docosadienoic)	0.09
Camphor	0.05	δ-3-carene	0.10	cis-sabinene hydrate	0.07	C20:5n3 (cis-5,8,11,14,17-Eicosapentaenoic)	0.02
p-Cymen-7-ol	0.05	trans-β-farnesene	0.10	Caryophyllene oxide	0.02	C24:0 (Lignoceric)	0.04
Linalyl butyrate	0.04	β-bisabolene	0.10			C24:1n9 (Nervonic)	0.15

Butyl isothiocyanate	0.02	Germacrene D	0.08	Σ SFA (Total Saturated FA)	12.07
		1,8-cineol	0.07	Σ MUFA (Total Monounsaturated FA)	28.38
				Σ PUFA (Total Polyunsaturated FA)	56.83

[1] Bioactive compounds; [2] Fatty acids (FAs).

Table 4.3. Compositions and the calculated proximate analysis of experimental diets.

	Diets		
Ingredients, %	**CONT**	**PM-A**	**PM-B**
Maize	33.48	33.28	33.28
Barley	34.80	34.80	34.80
Phytobiotic Mixture (PM)	0.00	0.20	0.20
Soybean meal (47% CP)	16.81	16.81	16.81
Fishmeal 62% CP	3.00	3.00	3.00
Wheat middlings	3.00	3.00	3.00
Soybean oil	1.91	1.91	1.91
Vitamin and mineral premix 6% *	6.00	6.00	6.00
Zinc oxide	0.30	0.30	0.30
Benzoic acid	0.30	0.30	0.30
Monocalcium phosphate	0.20	0.20	0.20
Salt	0.20	0.20	0.20
Total	**100.00**	**100.00**	**100.00**
Calculated proximate analysis			
Digestible energy, MJ/kg	14.18	14.18	14.18
Crude protein, %	18.78	18.78	18.78
Dry matter, %	88.31	88.31	88.31
Ash, %	5.26	5.26	5.26
Crude fat, %	5.00	5.00	5.00
Crude fiber, %	3.41	3.41	3.41
ADF, %	3.94	3.94	3.94
NDF, %	11.25	11.25	11.25
Ca, %	0.20	0.20	0.20
Total P, %	0.43	0.43	0.43
Lysine, %	1.11	1.11	1.11
Methionine + Cystine, %	0.49	0.49	0.49

CONT: control pig diet group; PM-A: phytobiotic mixture in diet of pig group A; PM-B: phytobiotic mixture in diet of pig group B. * Provided per kg of diet: 15,000 IU vitamin A, 50 mcg 25-hydroxycholecalciferol, 9.96 mg vitamin E, 10.02 mg vitamin K3, 3 mg vitamin B1, 10.02 mg vitamin B2, 6 mg pantothenic acid, 6 mg vitamin B6, 40.02 mcg vitamin B12, 100 mg vitamin C, 35 mg niacin, 300 mcg biotin, 1.5 mg folic acid, 375 mg choline chloride, 200 mg ferrous sulfate monohydrate, 90 mg copper sulfate pentahydrate, 60 mg manganese sulfate monohydrate, 100 mg zinc sulfate monohydrate, 2 mg calcium iodate, 300 mg sodium selenide, 150 mg L-selenomethionine–selenium, 1500 FYT 6-phytase, 80 U β-1,4-endoglucanase, 70 U β-1,3 (4)-endoglucanase, 270 U β-1,4-endoxylanase, 5000 mg benzoic acid, 40,8 mg butylated hydroxytoluene, 3.5 mg propyl gallate.

4.2.2. Microbial Analysis

Digesta samples were taken aseptically from the ileum and caecum of eight animals (four males and four females) per treatment immediately after slaughter on day 43 of the trial. Regarding isolation, enumeration and identification of bacterial populations, 1 g of intestinal material was homogenized with 9 mL of a 0.1% sterile peptone water solution. Bacterial enumeration was based on the Miles and Misra plate method (surface drop). Each sample underwent repeated dilution through 12-fold dilutions (ranging from 10-1 to 10-12) utilizing typical 96-well plates for microdilution procedures. A total of 10 μL of each dilution was injected into the media and incubated properly. MacConkey agar and Kanamycin aesculin azide (KAA) agar (Merck, Darmstadt, Germany) were utilized for the isolation and enumeration of Enterobacteriaceae and Enterococci, respectively, while plates were incubated aerobically at 37 °C for 24 to 48 h. De Man, Rogosa and Sharpe (MRS) agar (Oxoid, Basingstoke, UK) and M17 agar (Lab M Limited, Lancashire, UK) were employed for the isolation and enumeration of Lactobacillaceae, while media were incubated at 37 °C for 48 h under anaerobic conditions. Total aerobic and anaerobic bacterial counts were assessed using plate count agar medium (Oxoid, Basingstoke, UK). Plates were incubated aerobically at 30 °C for 48 h and anaerobically at 37 °C for 48 to 72 h, respectively. Bacterial counts were determined by enumerating representative colonies from a suitable dilution using a microbial colony counter, with results represented as colony-forming units (CFU) x log per 1 g of wet weight material. Typical colonies grown on different media were then described and subcultured. All bacterial populations were classified using the automated Vitek 2 compact system (bioMérieux, Marcy l'Etoile, France), which yielded dependable and precise findings for a broad spectrum of Gram-positive and Gram-negative bacteria. The identification of Enterobacteriaceae, Enterococcaceae, Lactobacillaceae and Bifidobacteriaceae was conducted using the Vitek 2 compact system (bioMérieux, Marcy l'Etoile, France), as well as Gram-negative identification card (ID-GN), Gram-positive identification card (ID-GP) and CBC and ANC identification cards (bioMérieux, Marcy l'Etoile, France), [60].

4.2.3. Hematological and Biochemical Analysis of the Blood

Blood samples were obtained from eight pigs (four males and four females) per treatment before slaughter, for the assessment of hematological and biochemical parameters, on the final day of

the trial (day 43). Feed was withdrawn from the feeders four hours prior to blood sampling. Initially, a sample of 4 mL blood was obtained from the pigs' jugular vein and then transferred into vacutainer tubes containing ethylenediamine tetra acetic acid (EDTA). Hematological parameters (hemoglobin, erythrocytes, hematocrit, leucocytes, lymphocytes) were assessed using an MS4 automated analyzer (Melet Schloesing Lab, Osny, France), while biochemical parameters (albumin, alanine aminotransferase, aspartate aminotransferase, cholesterol, creatine kinase, glucose, total bilirubin, triglycerides) in serum were evaluated using the IDEXX VETTEST 8008 instrument (IDEXX LAB, Westbrook, ME, USA).

4.2.4. Chemical Analysis, pH Measurement and Color Analysis of the Meat

Meat samples from eight pigs per group were collected post-slaughter from the ham (biceps femoris), shoulder (triceps brachii) and belly (external abdominal muscles) and stored at –20 °C. At the same day, 200 g subsamples were homogenized using an industrial grinder (Bosch, Gerlingen, Germany). Moisture, crude protein, fat, collagen and ash contents were determined by near-infrared spectroscopy (FoodScan™ Lab, FOSS, Hillerød, Denmark) following AOAC 2007.04 [61]. The pH was measured in each muscle using a portable pH meter with a stainless-steel probe (HI981036, Hanna Instruments, Woonsocket, RI, USA). Meat color (L*, a*, b* values) was assessed with a CAM-System 500 Chromatometer (Lovibond, Amesbury, UK) according to the Hunter scale [62].

4.2.5. Oxidative Stability Analysis of the Meat

The lipid oxidation (malondialdehyde levels) of the meat samples was performed according to the method described by Florou-Paneri et al. [63] and Giannenas et al. [64]. In brief, the frozen specimens were thawed overnight at 4 °C, minced with a commercial food processor, wrapped in oxygen-permeable film and stored in a non-illuminated refrigerated cabinet at 4 °C for 7 days. On the 4th and 7th refrigeration days, subsamples were extracted from each sample and processed according to the methodology. Absorbance was measured at 532 nm relative to a blank sample utilizing a UV–Vis spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Japan). Tetraethoxypropane at a concentration of 1.1 to 3.3 was utilized as a reference, and the results were quantified as nanograms of malondialdehyde (MDA) per gram of meat (ng/g).

4.2.6. Fatty Acid Analysis of the Meat

Samples for the fatty acid analysis of shoulder, ham and belly meat cuts were processed according to the guidelines established by O'Fallon et al. [57]. The separation and quantification of methyl esters were conducted using the methodology outlined by Skoufos et al. [25] utilizing a TraceGC (Model K07332, Thermofinigan, Thermoquest, Milan, Italy) fitted with a flame ionization detector. The fatty acid methylester retention time and elusion order were identified using as the reference standard the Supelco '37 Component FAME Mix' (Sigma-Aldrich, Darmstadt, Germany). The percentages (%) of individually identified fatty acids were calculated as their peak areas divided by the total peak areas of all identified fatty acids. Additionally, the total polyunsaturated fatty acid-to-saturated fatty acid (PUFA/SFA) ratio, the PUFA n-6/n-3 ratio and the hypocholesterolemic/hypercholesterolemic fatty acid ratio (h/H) were calculated. The h/H index illustrates the connection between low-cholesterol fatty acids (C18:1n-9 + PUFA) and high-cholesterol fatty acids (C12:0, C14:0 and C16:0). This index can be utilized to assess the cholesterolemic effect of dietary lipids [65].

4.2.7. Statistical Analysis

The fundamental study design employed was a random complete block design (RCB), with each ear-tagged pig serving as the experimental unit. Microbiology data underwent log transformation (Log_{10}) prior to the statistical analysis. Levene's test was employed to assess data homogeneity. Experimental data were analyzed using a one-way analysis of variance (one-way ANOVA) or the Kruskal–Wallis test, contingent upon the data format, employing the SPSS v20 statistical program [66]. Tukey's test was conducted for post hoc comparisons among the three treatments when significant effects were identified by the one-way ANOVA. The significance threshold for all tests was established at 5% ($p \leq 0.05$).

4.3. Results

4.3.1. Performance Parameters

The performance parameters of pigs were influenced by the dietary use of the phytobiotic mixture, as illustrated in Table 4.4. The final body weight and average daily weight gain of the pigs exhibited no significant differences ($p > 0.05$) between the three treatments. The feed intake and feed conversion ratio were consistent with the anticipated values for the commercial pig farm conducting the experimental trial and exhibited no significant differences between treatments ($p > 0.05$), even though the piglets were in the critical post-weaning phase of their development. Concerning the carcass parameters, carcass weights and dressing percentages exhibited no significant differences ($p > 0.05$) among the three treatments.

Table 4.4. Effects of the phytobiotic mixture supplementation on performance parameters of pigs.

	Diets				
	CONT	**PM-A**	**PM-B**	**SEM**	**p-value**
Body weight on day (kg)					
1	8.38	8.40	8.16	0.943	0.571
20	14.56	14.19	14.28	0.185	0.752
43	26.89	26.55	27.15	0.330	0.754
Weight gain for days (kg)					
1–20	6.19	5.79	6.12	0.176	0.632
20–43	12.33	12.37	12.87	0.276	0.712
1–43	18.51	18.15	18.98	0.331	0.573
Feed intake per group for days (kg)					
1–20	169.7	161.6	166.9	NA	NA
20–43	283.18	290.36	283.44	NA	NA
1–43	452.88	451.96	450.34	NA	NA
FCR for days (g feed/g weight gain)					
1–20	1.83	1.86	1.82	NA	NA
20–43	1.53	1.56	1.47	NA	NA
1–43	1.63	1.66	1.58	NA	NA
Carcass parameters					
Carcass weight (kg)	19.30	18.92	19.92	0.283	0.368
Carcass dressing percentage (%)	67.67	66.67	67.50	0.004	0.505

N = 15 pigs per group. NA = not applicable.

4.3.2. Intestinal Microflora

The dietary inclusion of the phytobiotic mixture induced significant alterations in the intestinal microflora populations (Table 4.5). In the ileum digesta, total aerobic bacterial counts (TABCs)

were significantly increased ($p \leq 0.05$) in the PM-A group compared to the control. Although a similar trend was observed in the PM-B group, the increase did not reach statistical significance relative to either the control or PM-A groups. Furthermore, the *Enterobacteriaceae* population—composed of *E. coli* and *E. fergusonii* (Table 4.6)—was lower in the PM-B group, showing a significant reduction when compared to PM-A and control groups. *Lactobacillaceae* counts, predominantly consisting of *Limosilactobacillus reuteri, Streptococcus alactolyticus, Streptococcus hyointestinalis* and *Lactobacillus johnsonii* (Table 4.6), were not affected by the treatments in the ileum. In the caecum, both treatment groups exhibited reduced *Enterobacteriaceae* levels, with the most abundant species being *E. coli* and *E. fergusonii* (Table 6.); however, statistical significance ($p \leq 0.05$) was noted only for PM-B relative to the control. *Lactobacillaceae* counts were significantly reduced in both PM-A and PM-B groups ($p \leq 0.05$), whilst the population of total anaerobe counts remained consistent ($p > 0.05$) across all treatments in both the ileum and caecum. The most abundant species *Lactobacillaceae* were *Limosilactobacillus reuteri* and *Streptococcus alactolyticus*, with the highest occurrence percentages in all experimental groups, followed by *Lactobacillus johnsonii*, *Limosilactobacillus mucosae*, *Lactobacillus gasseri*, *Lactobacillus delbrueckii* and *Streptococcus hyointestinalis* (Table 6). Finally, no statistically significant differences were observed in *Enterococcaceae* counts among the groups in either the ileum or caecum. The most abundant species was *E. faecium*, present in all samples, with only single isolates of *E. durans* and *E. hirae* detected in the PM-B and control groups of the caecum and ileum samples, respectively (Table 4.6).

Table 4.5. Effects of phytobiotic mixture supplementation on intestinal (ileum and caecum) microflora populations of pigs.

	CONT	PM-A	PM-B	SEM	p-value
Ileum microbes (Log_{10} CFU/g)					
Total Aerobic Bacterial Count (TABC)	6.99 [a]	8.08 [b]	7.21 [ab]	0.201	0.044
Total Anaerobes	7.146	7.795	7.265	0.190	0.374
Enterobacteriaceae	5.24 [b]	5.78 [b]	4.69 [a]	0.352	0.020
Enterococcaceae	4.758	5.775	4.556	0.295	0.218
Lactobacillaceae	6.795	6.684	6.660	0.183	0.959
Caecum microbes (Log_{10} CFU/g)					
Total Aerobic Bacterial Count (TABC)	8.545	7.882	8.644	0.189	0.072
Total Anaerobes	8.394	8.071	8.209	0.148	0.689
Enterobacteriaceae	7.27 [b]	5.937 [ab]	4.235 [a]	0.446	0.004
Enterococcaceae	5.071	5.146	4.801	0.257	0.872
Lactobacillaceae	7.715[b]	6.88 [a]	6.793 [a]	0.138	0.013

[a,b] Values with no common superscript differ significantly ($p \leq 0.05$).

Table 4.6. Isolated bacteria and their distributions (log cfu/g) and mean counts in the ileum and caecum (8 in total for each group).

	CONT		PM-A		PM-B	
Isolated Bacteria/Ileum	**Samples (%)**	**CountLog$_{10}$**	**Samples (%)**	**CountLog$_{10}$**	**Samples (%)**	**CountLog$_{10}$**
Enterococcus faecium	8 (100%)	5.44	8 (100%)	6.53	8 (100%)	4.65
Enterococcus hirae	2 (25%)	6.00	-	-	-	-
Escherichia coli	8 (100%)	6.06	8 (100%)	7.10	8 (100%))	5.52
Escherichia fergusonii	4 (50%)	4.71	6 (75%)	7.02	2 (25%)	4.38
Lactobacillus amylovorus	-	-	2 (25%)	7.00	2 (25%)	7.85
Lactobacillus crispatus	-	-	2 (25%)	7.30	-	-
Lactobacillus gasseri	4 (50%)	7.18	-	-	-	-
Lactobacillus johnsonii	2 (25%)	5.48	2 (25%)	7.08	-	-
Lactobacillus kitasatonis	-	-	2 (25%)	6.08	2 (25%)	7.30
Lactobacillus ultunensis	-	-	2 (25%)	6.90	-	-
Ligilactobacillus murinus	2 (25%)	5.00	-	-	-	-
Ligilactobacillus salivarius	-	-	-	-	2 (25%)	6.95
Limosilactobacillus mucosae	2 (25%)	7.70	-	-	4 (50%)	7.58
Limosilactobacillus reuteri	8 (100%)	7.14	8 (100%)	7.55	6 (75%)	6.80
Streptococcus alactolyticus	4 (50%)	8.22	4 (50%)	7.70	2 (25%)	5.70
Streptococcus hyointestinalis	2 (25%)	8.48	4 (50%)	7.70	2 (25%)	5.30
Streptococcus infantarius	-	-	2 (25%)	7.95	-	-
Streptococcus oralis	-	-	-	-	2 (25%)	7.85
Streptococcus pneumoniae	-	-	-	-	2 (25%)	3.70
	CONT		**PM-A**		**PM-B**	
Isolated bacteria/Caecum	**Samples (%)**	**CountLog$_{10}$**	**Samples (%)**	**CountLog$_{10}$**	**Samples (%)**	**CountLog$_{10}$**
Enterococcus durans	-	-	-		2 (25%)	5.00
Enterococcus faecium	8 (100%)	5.29	8 (100%)	6.25	8 (100%)	5.25
Escherichia coli	8 (100%)	7.58	8 (100%)	6.14	8 (100%)	4.79
Escherichia fergusonii	-	-	4 (50%)	5.01	2 (25%)	4.30
Lactobacillus delbrueckii	-	-	2 (25%)	6.48	-	
Lactobacillus gasseri	-	-	-		2 (25%)	5.95
Lactobacillus johnsonii	2 (25%)	7.00	4 (50%)	6.93	2 (25%)	6.00
Ligilactobacillus salivarius	-	-	-		2 (25%)	6.30
Limosilactobacillus mucosae	2 (25%)	7.90	2 (25%)	7.00	4 (50%)	8.19
Limosilactobacillus reuteri	8 (100%)	7.65	8 (100%)	6.74	8 (100%)	7.01
Streptococcus alactolyticus	8 (100%)	8.62	8 (100%)	7.99	8 (100%)	7.72
Streptococcus hyointestinalis	-	-	2 (25%)	5.30	-	

4.3.3. Blood Parameters

The effects of the phytobiotic mixture on pig blood hematological and biochemical parameters are presented in Table 4.7. No significant differences ($p > 0.05$) were observed in hematological and biochemical parameters among the three groups.

Table 4.7. Effects of phytobiotic mixture supplementation on blood hematological and biochemical parameters of pigs.

Hematological Parameters	**CONT**	**PM-A**	**PM-B**	**SEM**	**p-value**
WBC (10^3/μL)	23.47	22.03	21.70	1.338	0.87
Lymphocytes (%)	33.65	37.22	34.47	1.246	0.50
Monocytes (%)	6.27	6.53	7.55	0.500	0.58
Granulocytes (%)	61.12	55.62	56.63	1.431	0.27
RBC (10^6/μL)	6.06	6.03	6.93	0.433	0.70
HCT (%)	33.30	32.83	33.30	1.910	1.00
HB (g/dL)	11.77	10.95	11.52	0.693	0.89
THR (m/mm^3)	271.17	277.67	287.17	10.007	0.811
Blood biochemical parameters	**CONT**	**PM-A**	**PM-B**	**SEM**	**p-value**
ALB (g/dL)	3.33	3.08	3.28	0.150	0.796
ALP (U/L)	285.17	278.33	310.33	13.621	0.631
ALT (U/L)	165.00	168.00	175.50	7.566	0.854
AST (U/L)	129.00	125.50	133.33	5.775	0.86
CHOL (mg/dL)	117.17	113.50	116.67	3.029	0.887
GLU (mg/dL)	130.00	128.00	131.00	4.274	0.961
TBIL (mg/dL)	2.12	1.90	2.35	1.332	0.378
TRIG (mg/dL)	59.67	57.83	58.17	2.155	0.943

N = 8 pigs per group. WBCs: white blood cells; RBCs: red blood cells; HCT: hematocrit; HB: hemoglobin; THR: transient hyperemic response; ALB: albumin; ALP: alkaline phosphatase; ALT: alanin aminotransferase; AST: aspartate aminotransferase; CHOL: cholesterol; GLU: glucose; TBIL: total bilirubin; TRIG: triglycerides.

4.3.4. Chemical Analysis of the Meat

Table 4.8 indicates that there were no significant differences ($p > 0.05$) in any of the assessed parameters (collagen, fat, moisture, protein, and ash) in shoulder meat (triceps brachii) cuts. In the case of ham meat (biceps femoris), the protein content was significantly lower ($p \leq 0.05$) in group PM-A compared to the control group, while group PM-B exhibited protein levels comparable to both the control and PM-A groups. No significant differences ($p > 0.05$) were observed in any of the parameters examined for the belly meat cuts (external abdominal).

The pH measurements (Table 8) for shoulder (triceps brachii) and ham (biceps femoris) meat showed no significant differences between the treatments ($p > 0.05$). The PM-A group tended to have a lower pH level ($0.05 < p \leq 0.10$) in the external abdominal belly meat, in comparison to the other two groups.

Table 4.8. Effects of phytobiotic mixture supplementation on shoulder, ham and belly meat chemical compositions and pH.

	CONT	PM-A	PM-B	SEM	p-value
Shoulder meat (triceps brachii) chemical composition (%)					
Collagen	1.63	1.60	1.65	0.517	0.912
Fat	6.09	6.27	6.23	0.229	0.953
Moisture	74.16	75.66	74.62	0.443	0.379
Protein	17.56	18.04	17.54	0.113	0.163
Ash	1.10	1.06	1.11	0.304	0.776
pH	5.82	5.85	5.85	0.231	0.841
Ham meat (biceps femoris) chemical composition (%)					
Collagen	1.34	1.43	1.24	0.3711	0.108
Fat	6.04	6.14	6.14	0.1659	0.970
Moisture	74.34	75.43	75.42	0.4642	0.628
Protein	18.88 [b]	18.15 [a]	18.56 [ab]	0.1189	0.028
Ash	1.08	1.02	1.11	0.0247	0.361
pH	5.68	5.78	5.61	0.0338	0.109
Belly meat (external abdominal) chemical composition (%)					
Collagen	1.86	1.85	1.81	0.445	0.920
Fat	8.97	8.19	8.29	0.385	0.708
Moisture	71.49	71.49	71.62	0.570	0.955
Protein	17.11	17.06	17.16	0.141	0.954
Ash	1.05	1.09	1.10	0.160	0.409
pH	5.67	5.78	5.77	0.230	0.085

[a,b] Values with no common superscript differ significantly ($p \leq 0.05$).

Regarding the color measurements (Table 4.9), the color of the shoulder meat (triceps brachii) samples was significantly ($p \leq 0.05$) redder (increased a* value) in group PM-B, while L* and b* values did not differ between all groups. Ham meat (biceps femoris) color exhibited significantly ($p \leq 0.05$) higher a* values and lower b* values for both treatment groups PM-A and PM-B, respectively. A similar pattern was observed for lightness (L* value), where the supplemented groups PM-A and PM-B were significantly darker ($p \leq 0.05$), with group PM-B displaying the lowest (61.18) L* value. The belly meat (external abdominal) color analysis revealed significantly ($p \leq 0.05$) lower a* values for both groups PM-A and PM-B and a higher L* value for group PM-A but only compared to the control group. Yellowness (b* value) did not differ ($p > 0.05$) between treatments.

Table 4.9. Effects of phytobiotic mixture supplementation on shoulder, ham and belly meat colors.

	CONT	PM-A	PM-B	SEM	p-value
Shoulder meat (triceps brachii) color					
L*	62.74	62.06	61.10	0.322	0.105
a*	10.30 [a]	11.10 [ab]	11.77 [b]	0.228	0.020
b*	9.34	9.87	9.60	0.169	0.464
Ham meat (biceps femoris) color					
L*	65.85 [b]	62.66 [a]	61.18 [a]	0.689	0.008
a*	9.01 [a]	11.67 [b]	12.09 [b]	0.435	0.002
b*	11.07 [b]	9.88 [a]	9.68 [a]	0.220	0.011
Belly meat (external abdominal) color					
L*	64.41 [a]	68.63 [b]	66.38 [ab]	0.674	0.026
a*	11.42 [b]	8.4 [a]	9.3 [a]	0.443	0.007
b*	9.88	10.45	10.52	0.297	0.654

L*: lightness; a*: redness; b*: yellowness. [a,b] Values with no common superscript differ significantly ($p \leq 0.05$).

Data on oxidative stability of meat samples is presented in Table 4.10. In the ham meat (biceps femoris), the malondialdehyde (MDA) level on the 4th day of refrigerating was the lowest ($p \leq 0.05$) in the PM-B treatment (3.70 ng/g), intermediate in the PM-A treatment (4.35 ng/g) and highest in the control treatment (5.55 ng/g). Furthermore, the MDA level was significantly lower ($p \leq 0.05$) in the shoulder meat (triceps brachii) of group PM-B (5.27 ng/g) compared to both group PM-A (7.36 ng/g) and control (9.94 ng/g). In the belly meat (external abdominal), the control group tended to have higher MDA levels ($0.05 < p \leq 0.10$) compared to the phytobiotic-enriched groups (PM-A and PM-B). Malondialdehyde (MDA) levels on the 7th day of refrigerating shoulder meat (triceps brachii) and belly meat (external abdominal) samples were significantly ($p \leq 0.05$) lower for treatment groups PM-B and PM-A (17.56 ng/g and 22.51 ng/g, respectively). In the ham meat (biceps femoris), the PM-B group tended to ($0.05 < p \leq 0.10$) have the lowest MDA level (18.91 ng/g) among all groups.

Table 4.10. Effects of phytobiotic mixture supplementation on ham, shoulder and belly meat oxidative stability levels

Day 4, MDA (ng/g)	**CONT**	**PM-A**	**PM-B**	**SEM**	**p-value**
Ham meat (biceps femoris)	5.55 ^{b}	4.35 ^{a}	3.70 ^{a}	0.25	0.003
Shoulder meat (triceps brachii)	9.94 ^{b}	7.365^{ab}	5.27 ^{c}	0.72	0.020
Belly meat (external abdominal)	6.31 ^{x}	4.71 ^{y}	4.33 ^{y}	0.39	0.085
Day 7, MDA (ng/g)	**CONT**	**PM-A**	**PM-B**	**SEM**	**p-value**
Ham meat (biceps femoris)	22.69 ^{y}	20.45 ^{xy}	18.91 ^{x}	0.69	0.073
Shoulder meat (triceps brachii)	24.28 ^{b}	19.50 ^{a}	17.56 ^{a}	1.14	0.036
Belly meat (external abdominal)	28.88 ^{b}	23.25 ^{a}	22.51 ^{a}	0.97	0.006

MDA: malondialdehyde. $^{a–c}$ Values with no common superscript differ significantly ($p \leq 0.05$). ^{x,y} Means (n = 8 per treatment) with no common superscript tend to ($0.05 < p \leq 0.10$).

The fatty acid analysis of shoulder meat cuts is presented in Table 4.11. The dietary supplementation of the examined phytobiotic mixture modified ($p < 0.05$) the fatty acid compositions of most examined fatty acids compared to the control treatment. Overall, the total saturated fatty acids (SFAs) were lowest ($p \leq 0.05$) in the control treatment, intermediate in the PM-A treatment and highest in the PM-B treatment. The total monounsaturated fatty acids (MUFAs) were highest ($p \leq 0.001$) in the PM-A treatment, intermediate in the control treatment and lowest in the PM-B treatment. The total polyunsaturated fatty acids (PUFAs) were highest ($p \leq 0.001$) in the PM-B treatment, intermediate in the control treatment and lowest in the PM-A treatment. The total omega-6 fatty acids were highest ($p \leq 0.001$) in the PM-B treatment, intermediate in the control treatment and lowest in the silage PM-A treatment. The polyunsaturated fatty acid (PUFA)-to-saturated fatty acid (SFA) ratio (PUFA/SFA) was highest ($p \leq 0.001$) in the control treatment, intermediate in the PM-B treatment and lowest in the PM-A treatment. Finally, the hypocholesterolemic/hypercholesterolemic fatty acid ratio (h/H) tended to be higher ($0.05 < p \leq 0.10$) in treatment PM-B compared to the other two treatments.

Table 4.11. Effect of phytobiotic mixture supplementation on shoulder meat fatty acid composition.

Shoulder Meat (triceps brachii)	CONT	PM-A	PM-B	SEM	p-value
FA (%)					
C8:0 (Caprylic	0.01	0.01	0.01	0.002	0.808
C10:0 (Capric)	0.04 ^{a}	0.10 ^{b}	0.07 ^{ab}	0.008	0.005
C12:0 (Lauric)	0.07 ^{a}	0.10 ^{b}	0.07 ^{ab}	0.004	<0.001
C14:0 (Myristic)	1.72 ^{b}	1.76 ^{b}	1.53 ^{a}	0.025	0.001
C14:1 (Myristoleic)	0.02	0.02	0.01	0.002	0.321
C15:0 (Pentadecanoic)	0.05 ^{b}	0.03 ^{a}	0.05 ^{b}	0.003	0.003
C16:0 (Palmitic)	27.46 ^{xy}	27.69 ^{y}	26.95 ^{x}	0.144	0.092
C16:1 cis (Palmitoleic)	2.70 ^{c}	2.46 ^{b}	1.97 ^{a}	0.083	0.002
C17:0 (Heptadecanoic)	0.37 ^{ab}	0.20 ^{a}	0.39 ^{b}	0.057	0.014
C17:1 (cis-10 Heptadecenoic cis)	0.22 ^{a}	0.25 ^{b}	0.31 ^{c}	0.010	<0.001
C18:0 (Stearic)	11.05 ^{a}	11.88 ^{b}	12.96 ^{c}	0.204	<0.001
C18:1n-9t (Elaidic)	0.12 ^{b}	0.06 ^{a}	0.07 ^{a}	0.007	<0.001
C18:1 cis n-9 (Oleic)	39.18 ^{b}	42.12 ^{c}	38.03 ^{a}	0.432	<0.001
C18:2n-6t (Linolelaidic)	0.02	0.02	0.03	0.002	0.233
C18:2 n-6c (Linoleic)	14.85 ^{b}	10.86 ^{a}	15.17 ^{b}	0.484	<0.001
C18:3n-6 (γ-Linolenic)	0.09 ^{b}	0.08 ^{b}	0.04 ^{a}	0.005	<0.001
C20:0 (Arachidic)	0.04 ^{a}	0.07 ^{b}	0.11 ^{c}	0.008	<0.001
C18:3n-3 (a-Linolenic)	0.78 ^{b}	0.65 ^{a}	0.93 ^{c}	0.029	<0.001
C20:1 cis n-9 (cis-11 Eicosenoic)	0.22 ^{a}	0.55 ^{c}	0.39 ^{b}	0.033	<0.001
C21:0 (Henicosanoic)	0.02	0.02	0.02	0.002	0.918
C20:2 cis n-6 (cis-11,14-Eicosadienoic)	0.30 ^{a}	0.37 ^{b}	0.45 ^{c}	0.016	<0.001
C20:3 cis n-3 (cis-11-14-17-Eicosatrienoate)	0.19	0.43	0.25	0.091	0.154
C20:4 cis n-6 (Arachidonic)	0.73 ^{c}	0.54 ^{b}	0.36 ^{a}	0.037	<0.001
C23:0 (Tricosanoic)	0.02	0.02	0.01	0.002	0.905
C20:5 cis n-3 (Cis-5,8,11,14,17-Eicosapentaenoic)	0.02	0.02	0.02	0.002	0.926
C24:1n-9 (Nervonic)	0.07 ^{xy}	0.25 ^{y}	0.06 ^{x}	0.057	0.080
C22:6 cis n-3 (cis-4,7,10,13,16,19-Docosahexaenoic)	0.03	0.04	0.03	0.003	0.419
Σ SFA (Total Saturated FA)	40.84 ^{a}	41.86 ^{ab}	42.17 ^{b}	0.227	0.033
Σ MUFA (Total Monounsaturated FA)	42.53 ^{b}	45.71 ^{c}	40.84 ^{a}	0.508	<0.001
Σ PUFA (Total Polyunsaturated FA)	17.00 ^{b}	13.00 ^{a}	17.29 ^{b}	0.487	<0.001
Σ n-3 (Total omega-3 FA)	1.02	1.13	1.23	0.089	0.208
Σ n-6 (Total omega-6 FA)	15.98 ^{b}	11.87 ^{a}	16.06 ^{b}	0.483	<0.001
Ratio n-6/n-3 FA	15.82	12.25	13.91	0.822	0.236
PUFA/SFA	0.42 ^{b}	0.31 ^{a}	0.41 ^{b}	0.012	<0.001
h/H^{c}	1.92 ^{xy}	1.87 ^{x}	1.94 ^{y}	0.014	0.066

FA: fatty acids; ΣSFA = (C10:0) + (C12:0) + (C14:0) + (C16:0) + (C17:0) + (C18:0); ΣMUFA = (C16:1 cis) + (C17:1 cis-10) + (C18:1 cis n-9) + (C18:1 n-7) + (C20:1 cis n-9); ΣPUFA = (C18:2 n-6c) + (C18:4n-3) + (C20:2 cis n-6) + C20:3 cis n-6) + (C22:5 cis n-3) + (C20:3 cis n-3) + (C20:4 cis n-6) + (C20:5 cis n-3) + (C21:5 n-3) + (C22:6 cis n-3). ^{a,b} Values with no common superscript differ significantly ($p \leq 0.05$). ^{x,y} Values with no common superscript tend to ($0.05 < p \leq 0.10$). ^{c} Hypocholesterolemic/hypercholesterolemic ratio = (cis-C18:1 + ΣPUFA)/(C12:0 + C14:0 + C16:0).

The fatty acid composition of the belly meat cuts is presented in Table 4.12. The phytobiotic mixture added in the pig diets significantly altered ($p < 0.05$) the fatty acid compositions of most of the analyzed fatty acids when compared to the control treatment. Concerning the saturated fatty acids (SFAs), the PM-A treatment exhibited the lowest levels ($p \leq 0.001$), followed by the

PM-B treatment, while the control treatment had the highest concentrations. In terms of total monounsaturated fatty acids (MUFAs), they were lower ($p \leq 0.05$) in the control treatment compared to both PM-A and PM-B treatments. The total polyunsaturated fatty acids (PUFAs) were highest ($p \leq 0.001$) in the PM-A treatment, intermediate in the control treatment and lowest in the PM-B treatment. Specifically, the levels of total omega-3 fatty acids were significantly greater ($p \leq 0.05$) in the PM-A treatment compared to the control treatment. Conversely, total omega-6 fatty acids were lowest ($p \leq 0.001$) in the PM-B treatment, intermediate in the control treatment and highest in the PM-A treatment. The omega-6-to-omega-3 fatty acid ratio was lowest ($p \leq 0.001$) in the PM-B treatment, intermediate in the PM-A treatment and highest in the control treatment. The ratio of polyunsaturated fatty acid (PUFA)-to-saturated fatty acid (SFA) (PUFA/SFA) was highest ($p \leq 0.001$) in the PM-A treatment, intermediate in the control treatment and lowest in the PM-B treatment. Lastly, the hypocholesterolemic-to-hypercholesterolemic fatty acid ratio (h/H) was highest ($p \leq 0.001$) in the PM-A treatment, intermediate in the control treatment and lowest in the PM-B treatment.

Table 4.12. Effect of phytobiotic mixture supplementation on belly meat fatty acid composition.

Belly Meat (external abdominal) FA (%)	**CONT**	**PM-A**	**PM-B**	**SEM**	**p-value**
C8:0 (Caprylic)	0.02	0.02	0.02	0.003	0.649
C10:0 (Capric)	0.14 ^{b}	0.11 ^{a}	0.12 ^{a}	0.005	0.001
C12:0 (Lauric)	0.12	0.10	0.10	0.003	0.220
C14:0 (Myristic)	1.87 ^{a}	1.91 ^{a}	2.06 ^{b}	0.025	<0.001
C14:1 (Myristoleic)	0.02	0.02	0.02	0.003	0.901
C15:0 (Pentadecanoic)	0.05	0.04	0.04	0.003	0.191
C16:0 (Palmitic)	30.88 ^{a}	30.35 ^{a}	32.51 ^{b}	0.272	<0.001
C16:1 cis (Palmitoleic)	2.06 ^{a}	2.24 ^{b}	2.67 ^{c}	0.067	<0.001
C17:0 (Heptadecanoic)	0.23 ^{a}	0.29 ^{b}	0.22 ^{a}	0.009	<0.001
C17:1 cis-10 (Heptadecenoic cis)	0.15 ^{a}	0.26 ^{ab}	0.37 ^{b}	0.055	0.001
C18:0 (Stearic)	14.91 ^{b}	11.85 ^{a}	12.10 ^{a}	0.357	0.002
C18:1n-9t (Elaidic)	0.09 ^{a}	0.21 ^{b}	0.22 ^{b}	0.015	<0.001
C18:1 cis n-9 (Oleic)	35.07 ^{a}	35.98 ^{b}	35.91 ^{b}	0.161	0.024
C18:2n-6t (Linolelaidic)	0.02	0.02	0.02	0.002	0.869
C18:2 n-6c (Linoleic)	12.01 ^{b}	13.40 ^{c}	10.55 ^{a}	0.290	0.001
C18:3n-6 (γ-Linolenic)	0.04 ^{b}	0.05 ^{b}	0.03 ^{a}	0.003	0.008
C20:0 (Arachidic)	0.09	0.11	0.10	0.004	0.140
C18:3n-3 (a-Linolenic)	0.70	0.77	0.75	0.014	0.175
C20:1 cis n-9 (cis-11-Eicosenoic)	0.31	0.31	0.32	0.005	0.384
C21:0 (Henicosanoic)	0.02	0.02	0.02	0.002	0.580
C20:2 cis n-6 (cis-11,14-Eicosadienoic)	0.32 ^{b}	0.32 ^{b}	0.30 ^{a}	0.005	0.015
C20:3 cis n-3 (cis-11-14-17-Eicosatrienoate)	0.05 ^{a}	0.07 ^{b}	0.07 ^{b}	0.004	0.023
C20:4 cis n-6 (Arachidonic)	0.31 ^{b}	0.50 ^{c}	0.25 ^{a}	0.026	<0.001

C20:5 cis n-3 (Cis-5,8,11,14,17-Eicosapentaenoic)	0.03	0.04	0.02	0.005	0.443
C24:1n-9 (Nervonic)	0.05 [b]	0.06 [b]	0.04 [a]	0.003	0.007
C22:6 cis n-3 (cis-4,7,10,13,16,19-Docosahexaenoic)	0.02	0.04	0.03	0.004	0.428
Σ SFA (Total Saturated FA)	48.47 [c]	44.79 [a]	47.27 [b]	0.422	<0.001
Σ MUFA (Total Monounsaturated FA)	37.76 [a]	39.08 [b]	39.55b	0.242	0.003
Σ PUFA (Total Polyunsaturated FA)	13.50 [b]	15.19 [c]	12.00 [a]	0.324	0.001
Σ n-3 (Total omega-3 FA)	0.80 [a]	0.91 [b]	0.87 [ab]	0.020	0.043
Σ n-6 (Total omega-6 FA)	12.70 [b]	14.28 [c]	11.13 [a]	0.319	<0.001
Ratio n-6/n-3 FA	16.13 [b]	15.76 [b]	12.87 [a]	0.462	0.001
PUFA/SFA	0.28 [b]	0.34 [c]	0.25 [a]	0.009	<0.001
h/H[c]	1.48 [b]	1.58 [c]	1.38 [a]	0.021	<0.001

FA: fatty acids; ΣSFA = (C10:0) + (C12:0) + (C14:0) + (C16:0) + (C17:0) + (C18:0); ΣMUFA = (C16:1 cis) + (C17:1 cis-10) + (C18:1 cis n-9) + (C18:1 n-7) + (C20:1 cis n-9); ΣPUFA = (C18:2 n-6c) + (C18:4n-3) + (C20:2 cis n-6) + C20:3 cis n-6) + (C22:5 cis n-3) + (C20:3 cis n-3) + (C20:4 cis n-6) + (C20:5 cis n-3) + (C21:5 n-3) + (C22:6 cis n-3). [a,b] Values with no common superscript differ significantly ($p \leq 0.05$). [c] Hypocholesterolemic/hypercholesterolemic ratio = (cis-C18:1 + ΣPUFA)/(C12:0 + C14:0 + C16:0.

The fatty acid composition of the ham meat cuts is presented in Table 4.13. Dietary supplementation with the phytobiotic mixtures significantly altered ($p < 0.05$) the profiles of most fatty acids compared with the control group. In particular, total saturated fatty acids (SFAs) were lowest ($p \leq 0.001$) in the PM-A treatment, intermediate in the control and highest in the PM-B treatment. Conversely, total monounsaturated fatty acids (MUFAs) were highest ($p \leq 0.001$) in the PM-A group, intermediate in the control and lowest in the PM-B group. The total PUFAs were significantly higher ($p < 0.05$) in the control group but only compared to PM-A treatment. The total omega-6 fatty acids were significantly lower ($p \leq 0.05$) in the PM-A treatment compared to the control group. The ratio of omega-6 to omega-3 fatty acids tended to increase ($0.05 < p \leq 0.10$) in both treatments PM-A and PM-B. The polyunsaturated fatty acid (PUFA)-to-saturated fatty acid (SFA) ratio (PUFA/SFA) was significantly lower ($p \leq 0.05$) in the PM-B treatment compared to both the PM-A treatment and the control group. Finally, the hypocholesterolemic/hypercholesterolemic fatty acid ratio (h/H) was highest ($p \leq 0.001$) in the PM-A treatment, intermediate in the control group and lowest in the PM-B treatment.

Table 4.13. Effect of phytobiotic mixture supplementation on ham meat fatty acid composition.

Ham Meat (biceps femoris) FA (%)	CONT	PM-A	PM-B	SEM	p-value
C10:0 (Capric)	0.09 [a]	0.07 [a]	0.11 [b]	0.005	<0.001
C12:0 (Lauric)	0.12	0.11	0.11	0.003	0.338
C14:0 (Myristic)	2.01 [b]	1.69 [a]	1.94 [b]	0.036	<0.001
C14:1 (Myristoleic)	0.03 [b]	0.01 [a]	0.05 [c]	0.004	<0.001
C15:0 (Pentadecanoic)	0.06 [a]	0.04 [a]	0.10 [b]	0.007	<0.001
C16:0 (Palmitic)	28.83 [b]	26.78 [a]	28.99 [b]	0.257	0.002
C16:1 cis (Palmitoleic)	3.40 [b]	3.22 [ab]	3.13 [a]	0.043	0.022
C17:0 (Heptadecanoic)	0.27 [a]	0.30 [b]	0.30 [b]	0.005	0.018
C17:1 cis-10 (Heptadecenoic cis)	0.31 [c]	0.25 [a]	0.28 [b]	0.007	<0.001
C18:0 (Stearic)	8.63 [a]	8.69 [a]	9.85 [b]	0.160	<0.001
C18:1n-9t (Elaidic)	0.10 [b]	0.11 [b]	0.08 [a]	0.006	0.019
C18:1 cis n-9 (Oleic)	29.91 [b]	33.67 [c]	29.13 [a]	0.492	<0.001
C18:2n-6t (Linolelaidic)	0.02	0.03	0.03	0.003	0.363
C18:2 n-6c (Linoleic)	22.87 [c]	20.87 [a]	22.28 [b]	0.211	0.001
C18:3n-6 (γ-Linolenic)	0.05 [a]	0.15 [b]	0.20 [c]	0.016	<0.001
C20:0 (Arachidic)	0.20 [b]	0.06 [a]	0.05 [a]	0.017	<0.001
C18:3n-3 (a-Linolenic)	1.41 [b]	1.22 [a]	1.24 [a]	0.022	<0.001
C20:1 cis n-9 (cis-11-Eicosenoic)	0.20 [a]	0.28 [b]	0.18 [a]	0.012	<0.001
C21:0 (Henicosanoic)	0.03	0.02	0.03	0.003	0.133
C20:2 cis n-6 (cis-11,14-Eicosadienoic)	0.41 [b]	0.41 [b]	0.36 [a]	0.007	0.003
C20:3 cis n-3 (cis-11-14-17-Eicosatrienoate)	0.19 [b]	0.22 [c]	0.17 [a]	0.006	<0.001
C20:4 cis n-6 (Arachidonic)	1.01 [a]	1.41 [c]	1.12 [b]	0.044	<0.001
C20:5 cis n-3 (cis-5,8,11,14,17-Eicosapentaenoic)	0.07	0.07	0.25	0.057	0.388
C22:6 cis n-3 (cis-4,7,10,13,16,19-Docosahexaenoic)	0.17	0.17	0.17	0.003	0.962
Σ SFA (Total Saturated FA)	40.25 [b]	37.77 [a]	41.48 [c]	0.396	<0.001
Σ MUFA (Total Monounsaturated FA)	33.96 [b]	37.55 [c]	32.85 [a]	0.499	<0.001
Σ PUFA (Total Polyunsaturated FA)	26.22 [b]	24.55 [a]	25.82 [b]	0.198	0.019
Σ n-3 (Total omega-3 FA)	1.85	1.68	1.83	0.056	0.418
Σ n-6 (Total omega-6 FA)	24.37 [b]	22.87 [a]	24.00 [b]	0.168	0.002
Ratio n-6/n-3 FA	13.20 [x]	13.66 [y]	13.54 [y]	0.288	0.052
PUFA/SFA	0.65 [b]	0.65 [b]	0.62 [a]	0.005	0.006
h/H[c]	1.81 [b]	2.04 [c]	1.77 [a]	0.029	<0.001

FA: fatty acids; ΣSFA = (C10:0) + (C12:0) + (C14:0) + (C16:0) + (C17:0) + (C18:0); ΣMUFA = (C16:1 cis) + (C17:1 cis-10) + (C18:1 cis n-9) + (C18:1 n-7) + (C20:1 cis n-9); ΣPUFA = (C18:2 n-6c) + (C18:4n-3) + (C20:2 cis n-6) + C20:3 cis n-6) + (C22:5 cis n-3) + (C20:3 cis n-3) + (C20:4 cis n-6) + (C20:5 cis n-3) + (C21:5 n-3) + (C22:6 cis n-3). [a,b] Values with no common superscript differ significantly ($p \leq 0.05$). [x,y] Values with no common superscript tend to ($0.05 < p \leq 0.10$). [c] hypocholesterolemic/Hypercholesterolemic ratio = (cis-C18:1 + ΣPUFA)/(C12:0 + C14:0 + C16:0).

4.4. Discussion

The growing demand for high-quality animal protein in human nutrition necessitates advancements in modern animal production. Sustainably produced food with advanced qualitative and health promoting characteristics is gaining global interest [67–70]. The economic potential and nutritional quality of pork have established it as a significant global commodity, evidenced by rising consumption [2]. The nutritional profile of pork supports a healthy, balanced and safe diet for the expanding global population [71,72]. Nevertheless, the concerns about using antibiotics as growth enhancers in the feeds of pigs and other productive animals have prompted the exploration of superior alternatives that can deliver comparable advantages and effectiveness without significant adverse consequences. As a result, numerous chemicals have been analyzed and recognized as advantageous for improving health and overall growth of livestock. These substances are essential for maintaining normal physiological functions and protecting the animals from infectious diseases. Assessing natural and phytogenic feed additives as potential substitutes for antibiotic growth promoters in swine production has led to encouraging results. Phytogenic products offer substantial benefits compared to antibiotic medications, being economically viable and demonstrating a lower propensity for resistance emergence; hence, research on phytogenic products is increasing.

Phytobiotics typically include vital nutrients such as carbohydrates, along with secondary elements such as essential oils and phenolic compounds. A range of studies have demonstrated the advantageous effects of their use in modern animal nutrition, highlighting numerous beneficial outcomes. The observed effects include growth enhancement, improved gut microbial balance and beneficial bacteria growth, in addition to antioxidative, antimicrobial and anti-inflammatory characteristics [12,26,73]. Various aromatic and medicinal plants and their essential oils have been reported in the literature to provide beneficial outcomes in pig production. Nonetheless, researchers are investigating the prospect of boosting essential oils' effectiveness and general bioactivity through their combination, owing to identified synergistic reactions among different substances [74,75]. This synergistictic effect has been demonstrated in many studies where combining multiple essential oils and herbs led to more effective results concerning improved performance, regulation of gut microbiota and nutrient absorption, immune status and antioxidative effects [16,76–78] than their individual components [26,27], while the

mechanism of these results remain inadequately elucidated. A mixture of phytobiotics with the aforementioned quadruple synthesis has been tested as an aqueous or cyclodextrin extract in broiler chickens, with positive results on composition and oxidative status of the meat, growth performance and welfare [79].

In this part of the dissertation a novel quadraple mixture of phytobiotics consisting of oregano (*Origanum vulgare* subsp. *hirtum*) essential oil, rock samphire (*Crithmum maritimum* L.) essential oil, garlic flour (*Allium sativum* L.) and false flax flour (*Camelina sativa* L. Crantz) was used as a feed ingredient for the diet of weaned pigs, to study its effects on their performance, health and meat quality characteristics. The two tested mixtures differed in their dosages of oregano essential oil, provided in a micro-encapsulated form (Table 4.1). Essential oils are rapidly metabolized in the upper gastrointestinal tract, resulting in concentrations in the distal small intestine insufficient to enhance intestinal function [80]. Therefore, using essential oils processed with an innovative microencapsulation technology may protect against oxidation and sustained release of active ingredients in the pig gut [64,81,82] or even mask strong odors not acceptable by some animals [83]. Furthermore, we explored the potential synergistic effects of their bioactive compounds' combination. Ingredient costs for phytobiotics and essential oils can vary considerably depending on factors such as raw material quality, geographical origin and procurement scale. Locally sourced oregano and garlic, particularly in Greece where oregano is of high quality, can significantly reduce expenses compared to imported products. In contrast, sea fennel (*Crithmum maritimum* L.) essential oil remains a niche and high-cost ingredient, necessitating low inclusion rates to maintain economic viability. *Camelina sativa* meal offers potential cost offsets when used as a partial replacement for protein sources such as soybean meal. Additionally, logistical considerations, the formulation form (e.g., pure oil versus encapsulated) and compliance with European Union feed additive regulations can substantially influence the final cost of incorporating these additives into pig diets. In this trial, for pigs aged 34–77 days consuming approximately 30 kg of feed per head for the experimental period, the inclusion of oregano essential oil (200 mL or 400 mL/t), sea fennel essential oil (50 mL/t), *Camelina sativa* flour (1 kg/t) and garlic flour (1 kg/t) resulted in additive costs of about €63.30/t (PM-A) and €73.30/t (PM-B), respectively. This translated to €1.90–€2.20 per pig for the whole feeding phase (≈€0.044–€0.051/day). Sea fennel EO accounted for the largest share of the cost, while the overall expenditure remained relatively low, making targeted application in the weaner

or early grower stage a cost-effective strategy to support gut health and performance if proven by results.

Growth control and enhancement through improvements in digestion, absorption of nutrients and feed consumption are factors that decisively affect farm productivity and profitability indices. Numerous studies have revealed that phytobiotics may enhance pig growth by improving feed quality and consumption, increasing flavor and palatability and promoting anabolic activity similar to that of anabolic agents [15,84,85]. Suggested modes of action may include the improvement of nutritional digestion and absorption via stimulation of the synthesis of various digestive secretions such as saliva and bile, as well as digestive enzymes such as amylase, galactosidase, lactase and sucrase [12,23], leading to elevated digestibility of feed dry matter (DM), crude protein (CP), gross energy (GE) and ether extracts (EE) [86–88]. Further suggested mechanisms involve enhanced function of the intestinal barrier and an improved ratio of the gut villus height to crypt depth [23,89,90]. Sanchez et al. [76] tested whether an extract of *Allium* spp., which includes garlic and onion, could improve the growth of growing–finishing pigs by changing the microbiome and short-chain fatty acid metabolism in the digestive tract, with promising results for average daily gain (ADG). In another trial, weaned piglets fed an allium extract supplement were compared to control and antibiotic (colistin and zinc oxide)-treated piglets. The allium extract-fed piglets had higher body weight (BW), average daily weight gain (ADWG) and feed conversion ratio (FCR) values that were only similar to the antibiotic-treated group [91]. Several other studies have attributed elevated growth performance to mixtures of plant and herbal extracts containing mainly carvacrol, thymol, cinnamaldehyde, thyme, garlic and other medicinal plants from various countries [19,92,93]. On the contrary, there is research indicating no significant influence of phytobiotic inclusion in pig diets regarding growth performance (weight gain and average daily feed intake) [37,94,95]. Our results, with the use of the phytobiotic mixture, are partly in agreement with the above findings, since it was noted that the final pig BW, ADWG, ADFI and FCR values exhibited no significant differences ($p > 0.05$) across the three treatments (Table 4.4). Similar findings were reported for carcass weight and carcass percentage at the end of the trial period. This reveals a degree of inconsistency in digestibility or palatability improvements, as reported previously. This may be due to endogenous loss caused by stimulated secretion of mucus induced by various plant extracts [96]

or the fact that phytobiotics' bioactive compound effects are a complex combination of the species variation, plant part or essential oil, chemical composition and dosage level.

The diet is a significant determinant influencing the makeup and functionality of the gut microbiota in pigs [97]. The composition of the gut microbiome and its associated bacterial metabolic products greatly affect intestinal health, nutritional absorption, overall pig health and meat quality [60,98,99]. Weaned pigs encounter stressors related to alterations in their diet and surroundings. Such changes may impede the formation of a stable gut environment and lead to increased diarrhea scores. An optimal diet directly influences digestibility and is crucial for intestinal barrier function, immune system development and feed utilization, thereby enhancing pig growth [100,101]. Numerous trials investigating phytobiotics and essential oils have reported enhancements, neutral outcomes or even detrimental effects on the regenerative capacity of pig intestinal epithelial cells regarding villus length, the ratio of the villus height to crypt depth, gastrointestinal health and absorptive efficiency [87,102]. Phytobiotics can help in regulating and enhancing the digestive process, promote the establishment of a balanced intestinal microflora and ameliorate overall health and function of the gastrointestinal tract in pigs [103,104] through enzyme activation or intestinal microflora modification [22,105]. Several studies have suggested that gut microbes play a role in metabolizing ingested phytobiotics into simpler metabolites, thereby increasing the bioavailability of the phytobiotics in the gut of young pigs. Phytobiotic mixtures have also been shown to exhibit prebiotic and probiotic activities, promoting the growth and activity of beneficial gut bacteria [84,98]. Increased populations of *Lactobacillus* spp. and *Bifidobacterium* spp., increased total bacterial counts and decreased *Enterobacteriaceae* levels in the gastrointestinal tract [26,106,107] of pigs fed phytobiotics have been reported. Conversely, there is research that supports the incorporation of phytobiotics in pig diets, resulting in lowered total aerobe, *E. coli*, *Campylobacter jejuni*, *Clostridium* spp. and *Salmonella* spp. counts [23,76,108] or even exerting no influence on the microbial population and composition of the gastrointestinal tract [96]. In the present study, total aerobic bacterial counts (TABCs) in the ileum were significantly increased ($p \leq 0.05$) in the PM-A group, and *Enterobacteriaceae* populations—predominantly composed of E. coli—were statistically decreased in the PM-B group (Tables 5 and 6) This reduction in *Enterobacteriaceae* populations was also apparent in the caecum for the diets that contained the phytobiotic mixture, in agreement with the above-mentioned research. In contrast, although *Lactobacillaceae* counts were significantly reduced in

the caecum for both groups PM-A and PM-B, their presence in all groups in the ileum and caecum promoted gastrointestinal health, as *L. johnsonii* and *L. delbrueckii* improve growth performance, intestinal barrier function and the ability of pigs to resist against pathogens, inhibiting the growth of harmful bacteria and enhancing the immunity response. *Limosilactobacillus reuteri* increases average daily gain and nitrogen digestibility levels, particularly in post-weaning piglets and also stimulates the immune system and alters swine gut microbiota, leading to a more beneficial balance of bacteria in the gut. All three aforementioned Lactobacilli species have been proposed as future probiotics [109–111]. The combined results of lower counts of *Lactobacillaceae* and *Enterobacteriaceae* in our experiment indicate that the phytobiotic mixture used here had a direct impact against possible bacterial pathogens, as the production of lactic acid was degraded due to lower numbers of Lactobacilli present in the large intestine.

This research examined porcine hematological and biochemical markers as indicators of general health status [112]. These indices are critical markers of a pig's physiological health, offering vital information regarding its capacity to adjust to diverse physiological obstacles or emerging nutrient deficiencies [113]. Numerous research studies have been undertaken to examine the impacts of phytobiotics on hematological and biochemical parameters of swine. Elevated serum levels of IgA, IgG, red blood cells (RBCs) and white blood cells (WBCs) were seen following the administration of herbal extracts to weaning and finishing pigs [114,115]. Correspondingly, enhancements in serum IgG, IgA, IgM, albumin (ALB) and alkaline phosphatase (ALP) and a decrease in aspartate transaminase (AST) were identified [93,116]. Our trial revealed no significant differences ($p > 0.05$) in hematological and biochemical parameters among the three experimental groups; all measured values remained within the defined physiological limits for pigs (Table 7) This strongly suggests that the animals were healthy and the examined diets well balanced to meet their physiological requirements and welfare.

Meat is an important component for a healthy and balanced diet. It is a rich source of bio-available iron and zinc, selenium and various vitamins and minerals. In modern societies, consumers seek meat and meat products with specific chemical compositions and organoleptic characteristics. These involve an optimum lean-to-fat percentage, increased shelf life, color and flavor. Dietary changes can significantly modify the meat composition in monogastric animals. Many studies have reported various effects of incorporating phytobiotics in pig diets on meat

composition and characteristics. Increased crude protein (CP) and amino acid concentration, lowered drip loss, improved lean meat percentage, enhanced intramuscular fat and improved pH levels are proven [93,117,118]. In this research, adding the quadruple phytobiotic mixture to the diet did not change the chemical makeup of the meat in terms of fat, collagen, moisture or pH. The only apparent difference was a significant ($p \leq 0.05$) reduction in protein in ham cuts (biceps femoris) in group PM-A compared to the control group (Table 8) Similarly, reports of lower levels of crude protein (CP) or even critical amino acids such as lysine following the addition of phytobiotics are reported [119,120]. Color is one of the most important quality characteristics of meat, used as an indirect basis for consumer preference. The depth of meat color is primarily caused by the myoglobin (Mb) content, and since meat components that affect its color are highly susceptible to oxidation, the various levels of lightness, redness and yellowness of storage meat cuts are mainly attributed to the above oxidative process [118,121]. In this study, the phytobiotic mixture affected color to some extent; we recorded elevated a* values (redness) in group PM-A of ham (biceps femoris) meat cuts and groups PM-B of both shoulder (triceps brachii) and ham (biceps femoris) meat cuts. This may impose an advantage to these meat cuts, since the redness of pork is perceived as a desirable trait by consumers [122]. Decreased lightness (L* value) was notable in both PM-A and PM-B groups of ham (biceps femoris) meat cuts with a simultaneous reduction in yellowness (b* value). The belly (external abdominal) meat cuts had reduced a* values and increased L* values in both experimental groups PM-A and PM-B. Inclusion of oregano essential oil in animal diets can alter the color of meat by reducing hemoglobin oxidation and accelerating pigment distribution [41,123]. The yellow color is primarily influenced by the meat's pH, which affects redox processes [121]. A small drop in pH can increase oxidized oxymyoglobin, making the meat yellower and slightly lighter. Li et al. [124] reported that pig feed supplemented with oregano essential oil yielded advantages in color, brightness, yellowness and lean meat percentage. More studies revealed similar increases in redness and yellowness or reductions in L* and b* values after feeding young or finishing pigs with various herbal mixtures and essential oils [114,118,120]. Our findings are consistent with most of the available literature, keeping in mind the large variability and inconsistency of the results, an issue discussed earlier. The pig carcasses and meat cut quality parameters were within acceptable limits for commercial use.

Weaning is a critical event that can cause physiological, environmental and social stress in piglets, hence increasing their vulnerability to intestinal dysfunction and oxidative stress [125,126]. Lipid oxidation is closely associated with the control of pathogenic or spoilage bacteria in meat, as well as the quality and sensory qualities of meat products. This is regarded as a crucial indicator of quality degradation in food and meat products [127]. Essential oils can enhance the oxidative stability of tissues, leading to improved product quality [128]. Integrating natural antioxidants into swine diets is an efficacious approach to augment antioxidant stability, increase sensory qualities and prolong the shelf life of pork products [77,129]. In a trial that incorporated a mixture of eucalyptus, oregano, thyme, lemon, garlic and coconut essential oils in pig feed, a reduction in malondialdehyde (MDA) was observed in combination with elevated total antioxidant capacity (T-AOC) and total superoxide dismutase (T-SOD) levels and the well-desired 'marbling fat' trait attributed by consumers with enhanced flavor. Likewise, reduced TBARS and MDA contents; improvements in gene expression of oxidative stability; and increased T-SOD, T-AOC and GSH-Px activity levels were all reported in trials that evaluated the effects of including various phytobiotic mixtures and essential oils in pig diets on meat oxidative stability [118,130]. However, trials evaluating oregano essential oils in pig diets showed no positive effect on lipid oxidation [26,28]. In the present study, malondialdehyde (MDA) levels on the 4th and 7th day of refrigeration were significantly lower ($p \leq 0,05$) or tended to be ($0.05 < p \leq 0.10$) the lowest in group PM-B for all meat samples (ham, shoulder and belly meat cuts). This finding is significant, as lipid oxidation and rancidity directly influence meat quality and storage, particularly during refrigeration or freezing. The likely explanation is that the metabolized and assimilated phytogenics may act as an exogenous antioxidant, improving the T-SOD, T-AOC and GSH-Px activity, thereby significantly reducing lipid peroxidation, lowering MDA concentrations, maintaining cell membrane integrity and ultimately enhancing the color and water retention of pork.

Pigs are monogastric and an excellent model for the study of lipid metabolism, since their meat reflects a fatty acid (FA) deposition profile based on the fatty acid composition of their diet. Therefore, in pig production, specific dietary nutrients such as fats play a crucial role in determining the quality and nutritional profile of the meat [44,131,132]. The contemporary diet in many nations is marked by a significant consumption of fats, particularly saturated and n-6 polyunsaturated fatty acids (PUFAs) [133,134]. Saturated fatty acids are associated with an

elevated risk of cardiovascular disease (CVD), whereas a substantial consumption of monounsaturated and n-3 polyunsaturated fatty acids has demonstrated a protective impact [135–137]. Conversely, a diet abundant in n-6 polyunsaturated fatty acids is deemed unbalanced. Robust scientific evidence supports a reduction in n-6 intake and the augmentation of n-3 consumption to enhance health across the lifespan, highlighting the significance of the n-6/n-3 PUFA ratio over the absolute quantities of each fatty acid family in the diet [138,139]. The n-3/n-6 PUFA ratio is a critical determinant of cell function, impacting membrane dynamics and diverse cellular processes. Currently, the n-6/n-3 fatty acid ratios in Western diets range from about 15:1 to 16.7:1, in contrast to the recommended ideal ratio range of 1:1 to 4:1 [138,140]. The ratio of polyunsaturated fatty acids (PUFAs) to saturated fatty acids (SFAs) (PUFA/SFA) is a standard metric for assessing the impact of diet on cardiovascular disease (CVD) and is the primary index for evaluating the nutritional value of foods, such as meat (0.11–2.042), fish (0.50–1.62) and dairy products (0.02–0.175). Although a large proportion of PUFAs alone is not necessarily beneficial if the n-6/n-3 ratio is not balanced, a value for the PUFA/SFA ratio greater than 0.4 is recommended for healthy foods and diets [141]. In contrast to the PUFA/SFA ratio, the hypocholesterolemic/hypercholesterolemic fatty acid ratio (h/H) may more precisely indicate the influence of the fatty acid content on cardiovascular disease, as was utilized in this research. The recommended ranges for meat and dairy products are 1.27–2.786 and 0.32–1.29, respectively. Ratusz et al. [142] analyzed the FA contents in 29 cold-pressed camelina (Camelina sativa) oils using the h/H ratio as a nutritional quality index, reporting relatively high numbers ranging from 11.7 to 14.7, which is desirable.

In the present study, the meat fatty acid composition was modified to an extensive degree by the inclusion of the phytobiotic mixture in the piglet feed. In the shoulder meat (triceps brachii), myristic (C14:0) and palmitic (C16:0) acids, associated with coronary heart diseases [143], were significantly ($p = 0.001$) or tended to ($0.05 < p \leq 0.10$) be lower, respectively, in the experimental groups PM-A and PM-B. Oleic acid (C18:1 cis n-9) levels, consisting of the largest amounts of MUFA fatty acids, were significantly ($p \leq 0.001$) increased in group PM-A. The PUFA/SFA ratio was highest ($p \leq 0.001$) in the control treatment. In the belly meat (external abdominal), stearic (C18:0) and oleic (C18:1 cis n-9) acids were significantly ($p \leq 0.005$) lower and higher, respectively, both in group PM-B. The Σ PUFA, Σ n-3, PUFA/SFA ratio and h/H ratio values were significantly higher ($p \leq 0.05$) in the PM-A group. The n-6/n-3 FA ratio was significantly

lower ($p = 0.001$) in group PM-B. In the ham meat (biceps femoris), myristic (C14:0) and palmitic (C16:0) acids were significantly ($p \leq 0.05$) decreased, while oleic acid (C18:1 cis n-9) was significantly increased, all in group PM-A. The PUFA/SFA ratio was the lowest ($p = 0.006$) in group PM-B, while the h/H ratio was significantly ($p \leq 0.001$) the highest in group PM-A. Overall, the values of the PUFA/SFA and h/H ratios observed in our study fall within the range denoted for meat and meat products in previous studies [144], and indicate a positive effect on pig meat similar to other studies. It has been documented that the inclusion of various phytobiotic mixtures and essential oils in pig diets has positive effects on n-3 FA levels [120,145] and increases MUFA levels [118] while decreasing SFA levels [93], especially palmitic (C16:0) and lauric (C12:0) acids, in pig meat, and these effects are conducive to consumer health.

4.5. Conclusions

This study evaluated for the first time the impact of a phytobiotic mixture consisting of oregano (*Origanum vulgare* subsp. *hirtum*) essential oil in two different doses, rock samphire (*Crithmum maritimum* L.) essential oil, false flax flour (*Camelina sativa* L. Crantz) and garlic flour (*Allium sativum* L.) on the productive, health and meat quality parameters of weaned pigs. The results indicate that the tested quadruple herbal mixture, containing various bioactive compounds, had notable effects regarding meat oxidative stability and fatty acid profile and color, alongside a potentially beneficial change in intestinal microbial balance, especially reducing *Enterobacteriaceae* counts. Some results, such as *Lactobacillaceae* reductions in the caeca, are inconsistent with other similar trials; however, such discrepancies can be largely ascribed to the varied array of assessed herbal components and the differing integration rates. Given that the origin, fat content, color and welfare of pigs significantly influence consumers' preferences for pork, further research studies akin to the present are required to evaluate complicated phytogenic mixtures in swine diets, utilizing various ratios and supplementation durations or research to effectively enhance desirable traits and deepen the understanding of the absorption, distribution, metabolism and excretion of phytobiotics and essential oils. Furthermore, the major concerns for the broad application of phytobiotic mixtures and essential oils in pig diets remain the cost of application, the identification of optimal inclusion levels and possible interactions with other feed ingredients.

4.6. References

1. Giller, K.E.; Delaune, T.; Silva, J.V.; Descheemaeker, K.; van de Ven, G.; Schut, A.G.T.; van Wijk, M.; Hammond, J.; Hochman, Z.; Taulya, G.; et al. The Future of Farming: Who Will Produce Our Food? Food Secur. 2021, 13, 1073–1099.
2. OECD/FAO. OECD-FAO Agricultural Outlook 2021–2030; OECD Publishing: Paris, France, 2021.
3. Lillehoj, H.; Liu, Y.; Calsamiglia, S.; Fernandez-Miyakawa, M.E.; Chi, F.; Cravens, R.L.; Oh, S.; Gay, C.G. Phytochemicals as antibiotic alternatives to promote growth and enhance host health. Veter-Res. 2018, 49, 76.
4. Xu, B.; Fu, J.; Zhu, L.; Li, Z.; Jin, M.; Wang, Y. Overall assessment of antibiotic substitutes for pigs: A set of meta-analyses. J. Anim. Sci. Biotechnol. 2021, 12, 3.
5. Matheou, A.; Abousetta, A.; Pascoe, A.P.; Papakostopoulos, D.; Charalambous, L.; Panagi, S.; Panagiotou, S.; Yiallouris, A.; Filippou, C.; Johnson, E.O. Antibiotic Use in Livestock Farming: A Driver of Multidrug Resistance? Microorganisms 2025, 13, 779.
6. Iammarino, M. Recent Advances in Meat Products Quality & Safety Improvements and Assurance: Editorial. Int. J. Food Sci. Technol. 2020, 55, 917–918.
7. Giannenas, I.; Tzora, A.; Sarakatsianos, I.; Karamoutsios, A.; Skoufos, S.; Papaioannou, N.; Anastasiou, I.; Skoufos, I. The Effectiveness of the Use of Oregano and Laurel Essential Oils in Chicken Feeding. Ann. Anim. Sci. 2016, 16, 779–796.
8. Ersanli, C.; Tzora, A.; Skoufos, I.; Fotou, K.; Maloupa, E.; Grigoriadou, K.; Voidarou, C.; Zeugolis, D.I. The Assessment of Antimicrobial and Anti-Biofilm Activity of Essential Oils against Staphylococcus aureus Strains. Antibiotics 2023, 12, 384.
9. Li, L.; Sun, X.; Zhao, D.; Dai, H. Pharmacological Applications and Action Mechanisms of Phytochemicals as Alternatives to Antibiotics in Pig Production. Front. Immunol. 2021, 12, 798553.
10. Ndomou, S.C.; Mube, H.K. The Use of Plants as Phytobiotics: A New Challenge. In Phytochemicals in Agriculture and Food; Soto-Hernández, M., Aguirre-Hernández, E., Palma-Tenango, M., Eds.; IntechOpen: Rijeka, Croatia, 2023.

11. Skoufos, I.; Bonos, E.; Anastasiou, I.; Tsinas, A.; Tzora, A. Effects of Phytobiotics in Healthy or Disease-Challenged Animals. In Feed Additives; Academic Press: Oxford, UK, 2020; pp. 311–337.

12. Pandey, S.; Kim, E.S.; Cho, J.H.; Song, M.; Doo, H.; Kim, S.; Keum, G.B.; Kwak, J.; Ryu, S.; Choi, Y.; et al. Cutting-Edge Knowledge on the Roles of Phytobiotics and Their Proposed Modes of Action in Swine. Front. Vet. Sci. 2023, 10, 1265689.

13. Giannenas, I.; Florou-Paneri, P.; Papazahariadou, M.; Christaki, E.; Botsoglou, N.A.; Spais, A.B. Effect of dietary supplementation with oregano essential oil on performance of broilers after experimental infection with Eimeria tenella. Arch. Fur Tierernahr. 2003, 57, 99–106.

14. Ahmed, S.T.; Hossain, M.E.; Kim, G.M.; Hwang, J.A.; Ji, H.; Yang, C.J. Effects of Resveratrol and Essential Oils on Growth Performance, Immunity, Digestibility and Fecal Microbial Shedding in Challenged Piglets. Asian-Australas. J. Anim. Sci. 2013, 26, 683–690.

15. Mohammadi Gheisar, M.; Kim, I.H. Phytobiotics in Poultry and Swine Nutrition—A Review. Ital. J. Anim. Sci. 2018, 17, 92–99.

16. Wei, H.-K.; Wang, J.; Cheng, C.; Jin, L.-Z.; Peng, J. Chapter 13—Application of Plant Essential Oils in Pig Diets. In Feed Additives; Florou-Paneri, P., Christaki, E., Giannenas, I.B.T.-F.A., Eds.; Academic Press: Cambridge, MA, USA, 2020; pp. 227–237. ISBN 978-0-12-814700-9.

17. Wang, J.; Deng, L.; Chen, M.; Che, Y.; Li, L.; Zhu, L.; Chen, G.; Feng, T. Phytogenic Feed Additives as Natural Antibiotic Alternatives in Animal Health and Production: A Review of the Literature of the Last Decade. Anim. Nutr. 2024, 17, 244–264.

18. Li, S.Y.; Ru, Y.J.; Liu, M.; Xu, B.; Péron, A.; Shi, X.G. The Effect of Essential Oils on Performance, Immunity and Gut Microbial Population in Weaner Pigs. Livest. Sci. 2012, 145, 119–123.

19. Dávila-Ramírez, J.L.; Munguía-Acosta, L.L.; Morales-Coronado, J.G.; García-Salinas, A.D.; González-Ríos, H.; Celaya-Michel, H.; Sosa-Castañeda, J.; Sánchez-Villalba, E.; Anaya-Islas, J.; Barrera-Silva, M.A. Addition of a Mixture of Plant Extracts to Diets for Growing-Finishing Pigs on Growth Performance, Blood Metabolites, Carcass Traits, Organ Weight as a Percentage of Live Weight, Quality and Sensorial Analysis of Meat. Animals 2020, 10, 1229.

20. Girard, M.; Hu, D.; Pradervand, N.; Neuenschwander, S.; Bee, G. Chestnut Extract but Not Sodium Salicylate Decreases the Severity of Diarrhea and Enterotoxigenic Escherichia coli F4 Shedding in Artificially Infected Piglets. PLoS ONE 2020, 15, e0214267. [CrossRef] [PubMed]
21. Qu, H.; Huang, Y.; Shi, Y.; Liu, Y.; Wu, S.; Bao, W. Effect of Bamboo Vinegar Powder as an Antibiotic Alternative on the Digesta Bacteria Communities of Finishing Pigs. Can. J. Microbiol. 2018, 64, 732–743.
22. Li, Y.; Fu, X.; Ma, X.; Geng, S.; Jiang, X.; Huang, Q.; Hu, C.; Han, X. Intestinal Microbiome-Metabolome Responses to Essential Oils in Piglets. Front. Microbiol. 2018, 9, 1988.
23. Su, G.; Zhou, X.; Wang, Y.; Chen, D.; Chen, G.; Li, Y.; He, J. Dietary Supplementation of Plant Essential Oil Improves Growth Performance, Intestinal Morphology and Health in Weaned Pigs. J. Anim. Physiol. Anim. Nutr. 2020, 104, 579–589.
24. Zeng, Z.; Xu, X.; Zhang, Q.; Li, P.; Zhao, P.; Li, Q.; Liu, J.; Piao, X. Effects of Essential Oil Supplementation of a Low-Energy Diet on Performance, Intestinal Morphology and Microflora, Immune Properties and Antioxidant Activities in Weaned Pigs. Anim. Sci. J. 2015, 86, 279–285.
25. Skoufos, I.; Giannenas, I.; Tontis, D.; Bartzanas, T.; Kittas, C.; Panagakis, P.; Tzora, A. Effects of Oregano Essential Oil and Attapulgite on Growth Performance, Intestinal Microbiota and Morphometry in Broilers. S. Afr. J. Anim. Sci. 2016, 46, 77.
26. Madesh, M.; Yan, J.; Jinan, G.; Hu, P.; Kim, I.H.; Liu, H.-Y.; Ennab, W.; Jha, R.; Cai, D. Phytogenics in Swine Nutrition and Their Effects on Growth Performance, Nutrient Utilization, Gut Health, and Meat Quality: A Review. Stress Biol. 2025, 5, 11.
27. Zhao, B.C.; Wang, T.H.; Chen, J.; Qiu, B.H.; Xu, Y.R.; Zhang, Q.; Li, J.J.; Wang, C.J.; Nie, Q.F.; Li, J.L. Effects of Dietary Supplementation with a Carvacrol–Cinnamaldehyde–Thymol Blend on Growth Performance and Intestinal Health of Nursery Pigs. Porc. Health Manag. 2023, 9, 24.
28. Chang, S.Y.; Lee, J.H.; Oh, H.J.; An, J.W.; Song, D.C.; Cho, H.A.; Park, S.H.; Jeon, K.H.; Cho, S.Y.; Kim, D.J.; et al. Effect of Different Ratios of Phytogenic Feed Additives on Growth Performance, Nutrient Digestibility, Intestinal Barrier Integrity, and Immune Response in Weaned Pigs Challenged with a Pathogenic Escherichia coli. J. Anim. Sci. 2023, 101, skad148.
29. Pezzani, R.; Vitalini, S.; Iriti, M. Bioactivities of Origanum vulgare L.: An Update. Phytochem. Rev. 2017, 16, 1253–1268.

30. Pu, J.; Chen, D.; Tian, G.; He, J.; Zheng, P.; Mao, X.; Yu, J.; Huang, Z.; Luo, J.; Luo, Y.; et al. Effects of Benzoic Acid, Bacillus Coagulans and Oregano Oil Combined Supplementation on Growth Performance, Immune Status and Intestinal Barrier Integrity of Weaned Piglets. Anim. Nutr. 2020, 6, 152–159.

31. Kolypetri, S.; Kostoglou, D.; Nikolaou, A.; Kourkoutas, Y.; Giaouris, E. Chemical Composition, Antibacterial and Antibiofilm Actions of Oregano (Origanum vulgare subsp. hirtum) Essential Oil against Salmonella typhimurium and Listeria monocytogenes. Foods 2023, 12, 2893.

32. We˛glarz, Z.; Kosakowska, O.; Przybył, J.; Pióro-Jabrucka, E.; Baczek, K. The Quality of Greek Oregano (O. vulgare L. subsp. hirtum (Link) Ietswaart) and Common Oregano (O. vulgare L. subsp. vulgare) Cultivated in the Temperate Climate of Central Europe. Foods 2020, 9, 1671.

33. Sidiropoulou, E.; Marugán-Hernández, V.; Skoufos, I.; Giannenas, I.; Bonos, E.; Aguiar-Martins, K.; Lazari, D.; Papagrigoriou, T.; Fotou, K.; Grigoriadou, K.; et al. In Vitro Antioxidant, Antimicrobial, Anticoccidial, and Anti-Inflammatory Study of Essential Oils of Oregano, Thyme, and Sage from Epirus, Greece. Life 2022, 12, 1783.

34. Ranucci, D.; Beghelli, D.; Trabalza-Marinucci, M.; Branciari, R.; Forte, C.; Olivieri, O.; Badillo Pazmay, G.V.; Cavallucci, C.; Acuti, G. Dietary Effects of a Mix Derived from Oregano (Origanum vulgare L.) Essential Oil and Sweet Chestnut (Castanea sativa Mill.) Wood Extract on Pig Performance, Oxidative Status and Pork Quality Traits. Meat Sci. 2015, 100, 319–326.

35. Zou, Y.; Xiang, Q.; Wang, J.; Wei, H.; Peng, J. Effects of Oregano Essential Oil or Quercetin Supplementation on Body Weight Loss, Carcass Characteristics, Meat Quality and Antioxidant Status in Finishing Pigs under Transport Stress. Livest. Sci. 2016, 192, 33–38.

36. Tian, Q.; Piao, X. Essential Oil Blend Could Decrease Diarrhea Prevalence by Improving Antioxidative Capability for Weaned Pigs. Animals 2019, 9, 847.

37. Duarte, M.E.; Kim, S.W. Phytobiotics from Oregano Extracts Enhance the Intestinal Health and Growth Performance of Pigs. Antioxidants 2022, 11, 2066.

38. Janz, J.A.M.; Morel, P.C.H.; Wilkinson, B.H.P.; Purchas, R.W. Preliminary Investigation of the Effects of Low-Level Dietary Inclusion of Fragrant Essential Oils and Oleoresins on Pig Performance and Pork Quality. Meat Sci. 2007, 75, 350–355.

39. Brenes, A.; Roura, E. Essential Oils in Poultry Nutrition: Main Effects and Modes of Action. Anim. Feed. Sci. Technol. 2010, 158,

1–14.

40. Simitzis, P.E.; Symeon, G.K.; Charismiadou, M.A.; Bizelis, J.A.; Deligeorgis, S.G. The Effects of Dietary Oregano Oil Supplementa- tion on Pig Meat Characteristics. Meat Sci. 2010, 84, 670–676.

41. Garcia-Galicia, I.A.; Arras-Acosta, J.A.; Huerta-Jimenez, M.; Rentería-Monterrubio, A.L.; Loya-Olguin, J.L.; Carrillo-Lopez, L.M.; Tirado-Gallegos, J.M.; Alarcon-Rojo, A.D. Natural Oregano Essential Oil May Replace Antibiotics in Lamb Diets: Effects on Meat Quality. Antibiotics 2020, 9, 248.

42. Kołodziej-Skalska, A.; Kamyczek, M.; Pietruszka, A.; Matysiak, B. Effect of Dietary Oregano Essential Oil on the Growth, Meat Quality, Selenium Distribution, and Serum Biochemical Traits of Pigs. Anim. Sci. Genet. 2022, 18, 115–128.

43. Martins-Noguerol, R.; Matías, L.; Pérez-Ramos, I.M.; Moreira, X.; Francisco, M.; Pedroche, J.; DeAndrés-Gil, C.; Gutiérrez, E.; Salas, J.J.; Moreno-Pérez, A.J.; et al. Soil Physicochemical Properties Associated with the Yield and Phytochemical Composition of the Edible Halophyte Crithmum Maritimum. Sci. Total Environ. 2023, 869, 161806.

44. Bonos, E.; Skoufos, I.; Giannenas, I.; Sidiropoulou, E.; Fotou, K.; Stylianaki, I.; Tsiftsoglou, O.; Lazari, D.; Venardou, B.; Galamatis, D.; et al. Effect of an Herbal Mixture of Oregano, Garlic, Sage and Rock Samphire Extracts in Combination with Tributyrin on Growth Performance, Intestinal Microbiota and Morphology, and Meat Quality in Broilers. Sustainability 2022, 14, 13565.

45. Correia, I.; Antunes, M.; Tecelão, C.; Neves, M.; Pires, C.L.; Cruz, P.F.; Rodrigues, M.; Peralta, C.C.; Pereira, C.D.; Reboredo, F.; et al. Nutritive Value and Bioactivities of a Halophyte Edible Plant: Crithmum maritimum L. (Sea Fennel). Plants 2024, 13, 427. [CrossRef]

46. Zubr, J. Dietary Fatty Acids and Amino Acids of Camelina sativa Seed. J. Food Qual. 2003, 26, 451–462.

47. Waraich, E.A.; Ahmed, Z.; Ahmad, R.; Yasin Ashraf, M.; Saifullah; Naeem, M.S.; Rengel, Z. Camelina sativa, a Climate Proof Crop, Has High Nutritive Value and Multiple-Uses: A Review. Aust. J. Crop Sci. 2013, 7, 1551–1559.

48. Ni Eidhin, D.; Burke, J.; Lynch, B.; O'Beirne, D. Effects of Dietary Supplementation with Camelina Oil on Porcine Blood Lipids. J. Food Sci. 2003, 68, 671–679.

49. Smit, M.N.; Beltranena, E. Effects of Feeding Camelina Cake to Weaned Pigs on Safety, Growth Performance, and Fatty Acid Composition of Pork. J. Anim. Sci. 2017, 95, 2496–2508.
50. Delver, J.J.; Smith, Z.K. Opportunities for Camelina Meal as a Livestock Feed Ingredient. Agriculture 2024, 14, 116.
51. Liu, Y.; Espinosa, C.D.; Abelilla, J.J.; Casas, G.A.; Lagos, L.V.; Lee, S.A.; Kwon, W.B.; Mathai, J.K.; Navarro, D.M.D.L.; Jaworski, N.W.; et al. Non-Antibiotic Feed Additives in Diets for Pigs: A Review. Anim. Nutr. 2018, 4, 113–125.
52. Ogbuewu, I.P.; Okoro, V.M.; Mbajiorgu, E.F.; Mbajiorgu, C.A. Beneficial Effects of Garlic in Livestock and Poultry Nutrition: A Review. Agric. Res. 2019, 8, 411–426.
53. Ruiz, R.; García, M.P.; Lara, A.; Rubio, L.A. Garlic Derivatives (PTS and PTS-O) Differently Affect the Ecology of Swine Faecal Microbiota In Vitro. Vet. Microbiol. 2010, 144, 110–117.
54. Filocamo, A.; Nueno-Palop, C.; Bisignano, C.; Mandalari, G.; Narbad, A. Effect of Garlic Powder on the Growth of Commensal Bacteria from the Gastrointestinal Tract. Phytomedicine 2012, 19, 707–711.
55. Eurostat. Pigs—Number by Country [Data Set]. 2024. Available online: https://ec.europa.eu/eurostat/databrowser/bookmark/036f5b39-6e43-4428-bcd2-d51feba8c58d?lang=en (accessed on 11 August 2025).
56. Partheniadis, I.; Karakasidou, P.; Vergkizi, S.; Nikolakakis, I. Spectroscopic Examination and Release of Microencapsulated Oregano Essential Oil. ADMET and DMPK 2017, 5, 224–233.
57. O'Fallon, J.V.; Busboom, J.R.; Nelson, M.L.; Gaskins, C.T. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. J. Anim. Sci. 2007, 85, 1511–1521.
58. NRC National Research Council. Nutrient Requirements of Swine; The National Academies Press: Washington, DC, USA, 2012.
59. Premier Nutrition. Premier Atlas 2014; Premier Nutrition: Brereton, UK, 2014.
60. Skoufos, I.; Nelli, A.; Venardou, B.; Lagkouvardos, I.; Giannenas, I.; Magklaras, G.; Zacharis, C.; Jin, L.; Wang, J.; Gouva, E.; et al. Use of an Innovative Silage of Agro-Industrial Waste By-Products in Pig Nutrition: A Pilot Study of Its Effects on the Pig Gastrointestinal Microbiota. Microorganisms 2023, 11, 1723.

61. Anderson, S. Determination of fat, moisture, and protein in meat and meat products by using the FOSS FoodScan near-infrared spectrophotometer with FOSS artificial neural network calibration model and associated database: Collaborative study. J. AOAC Int. 2007, 90, 1073–1083.

62. Magklaras, G.; Skoufos, I.; Bonos, E.; Tsinas, A.; Zacharis, C.; Giavasis, I.; Petrotos, K.; Fotou, K.; Nikolaou, K.; Vasilopoulou, K.; et al. Innovative Use of Olive, Winery and Cheese Waste By-Products as Novel Ingredients in Weaned Pigs Nutrition. Vet. Sci. 2023, 10, 397.

63. Florou-Paneri, P.; Palatos, G.; Govaris, A.; Botsoglou, D.; Giannenas, I.; Ambrosiadis, I. Oregano Herb versus Oregano Essential Oil as Feed Supplements to Increase the Oxidative Stability of Turkey Meat. Int. J. Poult. Sci. 2005, 4, 866–871.

64. Giannenas, I.; Bonos, E.; Skoufos, I.; Tzora, A.; Stylianaki, I.; Lazari, D.; Tsinas, A.; Christaki, E.; Florou-Paneri, P. Effect of Herbal Feed Additives on Performance Parameters, Intestinal Microbiota, Intestinal Morphology and Meat Lipid Oxidation of Broiler Chickens. Br. Poult. Sci. 2018, 59, 545–553.

65. Minelli, G.; D'Ambra, K.; Macchioni, P.; Lo Fiego, D. Pietro Effects of Pig Dietary n-6/n-3 Polyunsaturated Fatty Acids Ratio and Gender on Carcass Traits, Fatty Acid Profiles, Nutritional Indices of Lipid Depots and Oxidative Stability of Meat in Medium–Heavy Pigs. Foods 2023, 12, 4106.

66.SPSS. SPSS Statistics for Windows; IBM: Armonk, NY, USA, 2018.

67. Grunert, K.G.; Sonntag, W.I.; Glanz-Chanos, V.; Forum, S. Consumer Interest in Environmental Impact, Safety, Health and Animal Welfare Aspects of Modern Pig Production: Results of a Cross-National Choice Experiment. Meat Sci. 2018, 137, 123–129.

68. Apostolidis, C.; McLeay, F. To Meat or Not to Meat? Comparing Empowered Meat Consumers' and Anti-Consumers' Preferences for Sustainability Labels. Food Qual. Prefer. 2019, 77, 109–122.

69. Carlsson, F.; Kataria, M.; Lampi, E. Sustainable Food: Can Information from Food Labels Make Consumers Switch to Meat Substitutes? Ecol. Econ. 2022, 201, 107567.

70. Zacharis, C.; Bonos, E.; Giannenas, I.; Skoufos, I.; Tzora, A.; Voidarou, C.; Tsinas, A.; Fotou, K.; Papadopoulos, G.; Mitsagga, C.; et al. Utilization of Tenebrio Molitor Larvae Reared with Different Substrates as Feed Ingredients in Growing Pigs. Vet. Sci. 2023, 10, 393.

71. Dugan, M.E.R.; Vahmani, P.; Turner, T.D.; Mapiye, C.; Juárez, M.; Prieto, N.; Beaulieu, A.D.; Zijlstra, R.T.; Patience, J.F.; Aalhus, J.L. Pork as a Source of Omega-3 (n-3) Fatty Acids. J. Clin. Med. 2015, 4, 1999–2011.

72. Henchion, M.; Moloney, A.P.; Hyland, J.; Zimmermann, J.; McCarthy, S. Review: Trends for Meat, Milk and Egg Consumption for the next Decades and the Role Played by Livestock Systems in the Global Production of Proteins. Animal 2021, 15, 100287.

73. Yang, C.; Zhang, L.; Cao, G.; Feng, J.; Yue, M.; Xu, Y.; Dai, B.; Han, Q.; Guo, X. Effects of Dietary Supplementation with Essential Oils and Organic Acids on the Growth Performance, Immune System, Fecal Volatile Fatty Acids, and Microflora Community in Weaned Piglets. J. Anim. Sci. 2019, 97, 133–143.

74. Froehlich, K.A.; Abdelsalam, K.W.; Chase, C.; Koppien-Fox, J.; Casper, D.P. Evaluation of Essential Oils and Prebiotics for Newborn Dairy Calves. J. Anim. Sci. 2017, 95, 3772.

75. Zhao, B.C.; Wang, T.H.; Chen, J.; Qiu, B.H.; Xu, Y.R.; Li, J.L. Essential Oils Improve Nursery Pigs' Performance and Appetite via Modulation of Intestinal Health and Microbiota. Anim. Nutr. 2024, 16, 174–188.

76. Sánchez, C.J.; Martínez-Miró, S.; Ariza, J.J.; Madrid, J.; Orengo, J.; Aguinaga, M.A.; Baños, A.; Hernández, F. Effect of Alliaceae Extract Supplementation on Performance and Intestinal Microbiota of Growing-Finishing Pig. Animals 2020, 10, 1557.

77. Huang, C.; Chen, D.; Tian, G.; He, J.; Zheng, P.; Yu, J.; Mao, X.; Huang, Z.; Yan, H.; Wang, Q.; et al. Effects of Dietary Plant Essential Oil Supplementation on Growth Performance, Nutrient Digestibility and Meat Quality in Finishing Pigs. J. Anim. Physiol. Anim. Nutr. 2022, 106, 1246–1257.

78. Li, Y.; Cao, H.; Zhang, S.; Guo, P.; Zhao, J.; Zhang, D.; Zhang, S. Effects of the Supplementation of Essential Oil Mixtures on Growth Performance, Nutrient Digestibility, Immune Status and Microbial Community in Weaned Piglets. Animals 2023, 13, 3697.

79. Dokou, S.; Vasilopoulou, K.; Bonos, E.; Grigoriadou, K.; Savvidou, S.; Stefanakis, M.K.; Christaki, S.; Kyriakoudi, A.; Mourtzinos, I.; Tzora, A.; et al. Effects of Dietary Supplementation with Phytobiotic Encapsulated Plant Extracts on Broilers' Performance Parameters, Welfare Traits and Meat Characteristics. Ann. Anim. Sci. 2023, 23, 1105–1118.

80. Chen, K.; Dai, Z.; Zhang, Y.; Wu, S.; Liu, L.; Wang, K.; Shen, D.; Li, C. Effects of Microencapsulated Essential Oils on Growth and Intestinal Health in Weaned Piglets. Animals 2024, 14, 2705.

81. Heckert Bastos, L.P.; Vicente, J.; Corrêa dos Santos, C.H.; Geraldo de Carvalho, M.; Garcia-Rojas, E.E. Encapsulation of Black Pepper (Piper nigrum L.) Essential Oil with Gelatin and Sodium Alginate by Complex Coacervation. Food Hydrocoll. 2020, 102, 105605.

82. Sousa, V.I.; Parente, J.F.; Marques, J.F.; Forte, M.A.; Tavares, C.J. Microencapsulation of Essential Oils: A Review. Polymers 2022, 14, 1730.

83. Stevanovic´, Z.D.; Bošnjak-Neumüller, J.; Pajic´-Lijakovic´, I.; Raj, J.; Vasiljevic´, M. Essential Oils as Feed Additives—Future Perspectives. Molecules 2018, 23, 1717.

84. Costa, L.B.; Luciano, F.B.; Miyada, V.S.; Gois, F.D. Herbal Extracts and Organic Acids as Natural Feed Additives in Pig Diets. S. Afr. J. Anim. Sci. 2013, 43, 181–193.

85. Ali, A.; Ponnampalam, E.N.; Pushpakumara, G.; Cottrell, J.J.; Suleria, H.A.R.; Dunshea, F.R. Cinnamon: A Natural Feed Additive for Poultry Health and Production—A Review. Animals 2021, 11, 2026.

86. Kumar, A.; Dev, K.; Sourirajan, A. Essential Oils of Rosmarinus officinalis L., Cymbopogon Citratus (DC.) Stapf., and the Phyto-Compounds, Delta-Carene and Alpha-Pinene Mediate Cell Cycle Arrest at G2/M Transition in Budding Yeast Saccharomyces Cerevisiae. S. Afr. J. Bot. 2021, 141, 296–305.

87. Long, S.; Liu, S.; Wang, J.; Mahfuz, S.; Piao, X. Natural Capsicum Extract Replacing Chlortetracycline Enhances Performance via Improving Digestive Enzyme Activities, Antioxidant Capacity, Anti-Inflammatory Function, and Gut Health in Weaned Pigs. Anim. Nutr. 2021, 7, 305–314.

88. Hossain, M.M.; Bo Cho, S.; Kim, I.H. Silybum Marianum Seed Extract as a Potential Phytogenic Feed Additive for Improving Growth Performance and Nutrient Digestibility in Growing Pigs. Can. J. Anim. Sci. 2024, 104, 80–85.

89. Liu, Y.; Song, M.; Che, T.M.; Lee, J.J.; Bravo, D.; Maddox, C.W.; Pettigrew, J.E. Dietary Plant Extracts Modulate Gene Expression Profiles in Ileal Mucosa of Weaned Pigs after an Escherichia coli Infection. J. Anim. Sci. 2014, 92, 2050–2062.

90. Long, S.F.; Wu, D.; He, T.F.; Piao, X.S. Dietary Supplementation with Forsythia Suspensa Extract during Late Gestation Improves Reproductive Performance, Colostrum Composition,

Antioxidant Status, Immunoglobulin, and Inflammatory Cytokines in Sows and Newborn Piglets. Anim. Feed. Sci. Technol. 2021, 271, 114700.

91. Rabelo-Ruiz, M.; Teso-Pérez, C.; Peralta-Sánchez, J.M.; Ariza, J.J.; Martín-Platero, A.M.; Casabuena-Rincón, Ó.; Vázquez-Chas, P.; Guillamón, E.; Aguinaga-Casañas, M.A.; Maqueda, M.; et al. Allium Extract Implements Weaned Piglet's Productive Parameters by Modulating Distal Gut Microbiota. Antibiotics 2021, 10, 269.

92. Zhang, Q.; Kim, H.I. Micelle Silymarin Supplementation to Fattening Diet Augments Daily Gain, Nutrient Digestibility, Decreases Toxic Gas Emissions, and Ameliorates Meat Quality of Fattening Pigs. Czech J. Anim. Sci. 2022, 67, 125–136.

93. Yang, Y.; Li, F.; Guo, Q.; Wang, W.; Zhang, L.; Yin, Y.; Gong, S.; Han, M.; Yin, Y. Effects of Different Supplemental Levels of Eucommia Ulmoides Leaf Extract in the Diet on Carcass Traits and Lipid Metabolism in Growing–Finishing Pigs. Front. Vet. Sci. 2022, 8, 828165.

94. Ahmed, S.T.; Mun, H.S.; Islam, M.M.; Ko, S.Y.; Yang, C.J. Effects of Dietary Natural and Fermented Herb Combination on Growth Performance, Carcass Traits and Meat Quality in Grower-Finisher Pigs. Meat Sci. 2016, 122, 7–15.

95. Peng, X.; Zhou, Q.; Wu, C.; Zhao, J.; Tan, Q.; He, Y.; Hu, L.; Fang, Z.; Lin, Y.; Xu, S.; et al. Effects of Dietary Supplementation with Essential Oils and Protease on Growth Performance, Antioxidation, Inflammation and Intestinal Function of Weaned Pigs. Anim. Nutr. 2022, 9, 39–48.

96. Muhl, A.; Liebert, F. No Impact of a Phytogenic Feed Additive on Digestion and Unspecific Immune Reaction in Piglets. J. Anim. Physiol. Anim. Nutr. 2007, 91, 426–431.

97. Patil, Y.; Gooneratne, R.; Ju, X.H. Interactions between Host and Gut Microbiota in Domestic Pigs: A Review. Gut Microbes 2020,
11, 310–334.

98. Rueda, A.F.; Samuel, R.; St-Pierre, B. Investigating the Effects of a Phytobiotics-Based Product on the Fecal Bacterial Microbiome of Weaned Pigs. Animals 2021, 11, 1950.

99. Chen, G.; Li, Z.; Liu, S.; Tang, T.; Chen, Q.; Yan, Z.; Peng, J.; Yang, Z.; Zhang, G.; Liu, Y.; et al. Fermented Chinese Herbal Medicine Promoted Growth Performance, Intestinal Health, and Regulated Bacterial Microbiota of Weaned Piglets. Animals 2023, 13, 476.

100. Cheng, C.; Xia, M.; Zhang, X.; Wang, C.; Jiang, S.; Peng, J. Supplementing Oregano Essential Oil in a Reduced-Protein Diet Improves Growth Performance and Nutrient

Digestibility by Modulating Intestinal Bacteria, Intestinal Morphology, and Antiox- idative Capacity of Growing-Finishing Pigs. Animals 2018, 8, 159.

101. Li, N.; Huang, S.; Jiang, L.; Wang, W.; Li, T.; Zuo, B.; Li, Z.; Wang, J. Differences in the Gut Microbiota Establishment and Metabolome Characteristics between Low- and Normal-Birth-Weight Piglets during Early-Life. Front. Microbiol. 2018, 9, 1798.

102. Wang, M.; Huang, H.; Hu, Y.; Liu, Y.; Zeng, X.; Zhuang, Y.; Yang, H.; Wang, L.; Chen, S.; Yin, L.; et al. Effects of Dietary Supplementation with Herbal Extract Mixture on Growth Performance, Organ Weight and Intestinal Morphology in Weaning Piglets. J. Anim. Physiol. Anim. Nutr. 2020, 104, 1462–1470.

103. Mosolov, A.A.; Starodubova, Y.V.; Miroshnik, A.S.; Slozhenkina, M.I.; Komlatsky, V.I.; Struk, A.N. Phytobiotics in Pig Feeding Architecture for the Organic Animal Husbandry Development. In IOP Conference Series: Earth and Environmental Science; IOP Publishing: Bristol, UK, 2022.

104. Szabó, C.; Kachungwa Lugata, J.; Ortega, A.D.S.V. Gut Health and Influencing Factors in Pigs. Animals 2023, 13, 1350.

105. Saad, N.; Delattre, C.; Urdaci, M.; Schmitter, J.M.; Bressollier, P. An Overview of the Last Advances in Probiotic and Prebiotic Field. LWT 2013, 50, 1–16.

106. Lan, R.X.; Park, J.W.; Lee, D.W.; Kim, I.H. Effects of Astragalus Membranaceus, Codonopsis Pilosula and Allicin Mixture on Growth Performance, Nutrient Digestibility, Faecal Microbial Shedding, Immune Response and Meat Quality in Finishing Pigs. J. Anim. Physiol. Anim. Nutr. 2017, 101, 1122–1129.

107. Su, G.; Zhou, X.; Wang, Y.; Chen, D.; Chen, G.; Li, Y.; He, J. Effects of Plant Essential oil Supplementation on Growth Performance, Immune Function and Antioxidant Activities in Weaned Pigs. Lipids Health Dis. 2018, 17, 139.

108. Ma, J.; Ma, H.; Liu, S.; Wang, J.; Wang, H.; Zang, J.; Long, S.; Piao, X. Effect of Mulberry Leaf Powder of Varying Levels on Growth Performance, Immuno-Antioxidant Status, Meat Quality and Intestinal Health in Finishing Pigs. Antioxidants 2022, 11, 2243.

109. Wang, M.; Wu, H.; Lu, L.; Jiang, L.; Yu, Q. Lactobacillus reuteri Promotes Intestinal Development and Regulates Mucosal Immune Function in Newborn Piglets. Front. Vet. Sci. 2020, 7, 42.

110. Yin, Z.; Wang, K.; Liu, Y.; Li, Y.; He, F.; Yin, J.; Tang, W. Lactobacillus johnsonii Improves Intestinal Barrier Function and Reduces Post-Weaning Diarrhea in Piglets: Involvement of the Endocannabinoid System. Animals 2024, 14, 493.

111. Luo, W.; Yin, Z.; Zhang, M.; Huang, X.; Yin, J. Dietary Lactobacillus delbrueckii Affects Ileal Bacterial Composition and Circadian Rhythms in Pigs. Animals 2024, 14, 412.

112. Nse Abasi, N.; Etim, E.E.; Offiong, A.; Mary, E.; Williams, L.E.A. Influence of Nutrition on Blood Parameters of Pigs. Am. J. Biol. Life Sci. 2014, 2, 46–52.

113. Zhang, S.; Yu, B.; Liu, Q.; Zhang, Y.; Zhu, M.; Shi, L.; Chen, H. Assessment of Hematologic and Biochemical Parameters for Healthy Commercial Pigs in China. Animals 2022, 12, 2464.

114. Lan, R.; Tran, H.; Kim, I. Effects of Probiotic Supplementation in Different Nutrient Density Diets on Growth Performance, Nutrient Digestibility, Blood Profiles, Fecal Microflora and Noxious Gas Emission in Weaning Pig. J. Sci. Food Agric. 2017, 97, 1335–1341.

115. Long, S.; Piao, X. Effects of Dietary Forsythia Suspensa Extract Supplementation to Lactating Sows and Nursery Pigs on Post- Weaning Performance, Antioxidant Capacity, Nutrient Digestibility, Immunoglobulins, and Intestinal Health. J. Anim. Sci. 2021, 99, skab142.

116. Li, P.; Piao, X.; Ru, Y.; Han, X.; Xue, L.; Zhang, H. Effects of Adding Essential Oil to the Diet of Weaned Pigs on Performance, Nutrient Utilization, Immune Response and Intestinal Health. Asian-Australas. J. Anim. Sci. 2012, 25, 1617–1626.

117. Janacua-Vidales, H.; Peña-González, E.; Alarcon-Rojo, A.D.; Ortega-Gutiérrez, J.; Aguilar-Palma, N. Determination of Carcase Yield, Sensory and Acceptance of Meat from Male and Female Pigs with Dietary Supplementation of Oregano Essential Oils. Ital. J. Anim. Sci. 2019, 18, 668–678.

118. Han, M.; Yin, Y.; Gong, S.; Shi, H.; Li, Q.; Lian, X.; Duan, Y.; Li, F.; Guo, Q. Effects of Dietary Eucommia Ulmoides Leaf Extract Supplementation on Growth Performance, Meat Quality, Antioxidant Capacity, and Lipid Metabolism of Finishing Pigs. Antioxidants 2024, 13, 320.

119. Zhang, T.; Si, B.; Tu, Y.; Cui, K.; Zhou, C.; Diao, Q. Effect of Including Different Levels of Moringa (Moringa oleifera) Leaf Meal in the Diet of Finishing Pigs: Performance, Pork Quality, Fatty Acid Composition, and Amino Acid Profile. Czech J. Anim. Sci. 2019,
64, 141–149.

120. Chen, Z.; Xie, Y.; Luo, J.; Chen, T.; Xi, Q.; Zhang, Y.; Sun, J. Dietary Supplementation with Moringa oleifera and Mulberry Leaf Affects Pork Quality from Finishing Pigs. J. Anim. Physiol. Anim. Nutr. 2021, 105, 72–79.

121. Karamucki, T.; Jakubowska, M.; Rybarczyk, A.; Gardzielewska, J. The Influence of Myoglobin on the Colour of Minced Pork Loin. Meat Sci. 2013, 94, 234–238.

122. Ngapo, T.M.; Fortin, J.; Martin, J.F. Do Pig Farmers Preferences Bias Consumer Choice for Pork? Response to Critique of the Pork Preference Studies. Meat Sci. 2010, 85, 788–791.

123. Simitzis, P.E.; Deligeorgis, S.G.; Bizelis, J.A.; Dardamani, A.; Theodosiou, I.; Fegeros, K. Effect of Dietary Oregano Oil Supplemen- tation on Lamb Meat Characteristics. Meat Sci. 2008, 79, 217–223.

124. Li, T.S.; Liu, W.C.; Zhao, P.Y.; Kim, I.H. Evaluation of Essential Oil or/and Emulsifier in Low Energy Density Diets on Growth Performance, Nutrient Digestibility, Blood Cholesterol and Meat Quality in Finishing Pigs. Ital. J. Anim. Sci. 2017, 16, 624–630.

125. Campbell, J.M.; Crenshaw, J.D.; Polo, J. The Biological Stress of Early Weaned Piglets. J. Anim. Sci. Biotechnol. 2013, 4, 19.

126. Tang, X.; Xiong, K.; Fang, R.; Li, M. Weaning Stress and Intestinal Health of Piglets: A Review. Front. Immunol. 2022, 13, 1042778.

127. Guo, Z.; Chen, X.; Huang, Z.; Chen, D.; Yu, B.; Chen, H.; Yu, J.; Yan, H.; Zheng, P.; Luo, Y. Dietary Dihydromyricetin Supplementation Enhances Antioxidant Capacity and Improves Lipid Metabolism in Finishing Pigs. Food Funct. 2021, 12, 6925–6935.

128. Giannenas, I.; Bonos, E.; Christaki, E.; Florou-Paneri, P. Chapter 6—Oregano: A Feed Additive with Functional Properties. In Handbook of Food Bioengineering; Holban, A.M., Grumezescu, A.M.B.T.-T.F., Eds.; Academic Press: Cambridge, MA, USA, 2018; pp. 179–208, ISBN 978-0-12-811517-6.

129. Wang, S.; Tang, C.; Li, J.; Wang, Z.; Meng, F.; Luo, G.; Xin, H.; Zhong, J.; Wang, Y.; Li, B.; et al. The Effects of Dietary Inclusion of Mulberry Leaf Powder on Growth Performance, Carcass Traits and Meat Quality of Tibetan Pigs. Animals 2022, 12, 2743.

130. Feng, Y.; Chen, X.; Chen, D.; He, J.; Zheng, P.; Luo, Y.; Yu, B.; Huang, Z. Dietary Grape Seed Proanthocyanidin Extract Supplementation Improves Antioxidant Capacity and Lipid Metabolism in Finishing Pigs. Anim. Biotechnol. 2023, 34, 4021–4031.

131. Madeira, M.S.; Alfaia, C.M.; Lopes, P.A.; Pestana, J.; Coelho, D.; Fontes, C.M.G.A.; Prates, J.A.M. Ameliorating Pork Marbling and Quality with Novel Feeding Approaches. In Advances in Animal Health, Medicine and Production: A Research Portrait of the Centre for Interdisciplinary Research in Animal Health (CIISA); Freitas Duarte, A., Lopes da Costa, L., Eds.; Springer International Publishing: Cham, Switzerland, 2020; pp. 161–177.

132. Fanalli, S.L.; da Silva, B.P.M.; Petry, B.; Santana, M.H.A.; Polizel, G.H.G.; Antunes, R.C.; de Almeida, V.V.; Moreira, G.C.M.; Luchiari Filho, A.L.; Coutinho, L.; et al. Dietary Fatty Acids Applied to Pig Production and Their Relation to the Biological Processes: A Review. Livest. Sci. 2022, 265, 105092.

133. Bengmark, S. Ecoimmunonutrition: A Challenge for the Third Millennium. Nutrition 1998, 14, 563–572.

134. Simopoulos, A.P. The Importance of the Omega-6/Omega-3 Fatty Acid Ratio in Cardiovascular Disease and Other Chronic Diseases. Exp. Biol. Med. 2008, 233, 674–688.

135. Guasch-Ferré, M.; Babio, N.; Martínez-González, M.A.; Corella, D.; Ros, E.; Martín-Peláez, S.; Estruch, R.; Arós, F.; Gómez-Gracia, E.; Fiol, M.; et al. Dietary Fat Intake and Risk of Cardiovascular Disease and All-Cause Mortality in a Population at High Risk of Cardiovascular Disease. Am. J. Clin. Nutr. 2015, 102, 1563–1573.

136. Maki, K.C.; Dicklin, M.R.; Kirkpatrick, C.F. Saturated Fats and Cardiovascular Health: Current Evidence and Controversies. J. Clin. Lipidol. 2021, 15, 765–772.

137. Cao, M.; Yang, F.; McClements, D.J.; Guo, Y.; Liu, R.; Chang, M.; Wei, W.; Jin, J.; Wang, X. Impact of Dietary N-6/n-3 Fatty Acid Ratio of Atherosclerosis Risk: A Review. Prog. Lipid Res. 2024, 95, 101289.

138. Simopoulos, A.P. Omega-6/Omega-3 Essential Fatty Acid Ratio and Chronic Diseases. Food Rev. Int. 2004, 20, 77–90.

139. Ma, X.; Jiang, Z.; Lai, C. Significance of Increasing N-3 PUFA Content in Pork on Human Health. Crit. Rev. Food Sci. Nutr. 2016,
56, 858–870.

140. Gonzalez-Becerra, K.; Barron-Cabrera, E.; Muñoz-Valle, J.F.; Torres-Castillo, N.; Rivera-Valdes, J.J.; Rodriguez-Echevarria, R.; Martinez-Lopez, E. A Balanced Dietary Ratio of N-6:N-3 Polyunsaturated Fatty Acids Exerts an Effect on Total Fatty Acid Profile in RBCs and Inflammatory Markers in Subjects with Obesity. Healthcare 2023, 11, 2333.

141. Department of Health. Nutritional Aspects of Cardiovascular Disease; Report on Health and Social Subjects No. 46; Her Majesty's Stationery Office: London, UK, 1994; ISBN 0113218753.
142. Ratusz, K.; Symoniuk, E.; Wroniak, M.; Rudzin´ska, M. Bioactive Compounds, Nutritional Quality and Oxidative Stability of Cold-Pressed Camelina (Camelina sativa L.) Oils. Appl. Sci. 2018, 8, 2606.
143. Ahmed, S.T.; Islam, M.M.; Bostami, A.B.M.R.; Mun, H.S.; Kim, Y.J.; Yang, C.J. Meat Composition, Fatty Acid Profile and Oxidative Stability of Meat from Broilers Supplemented with Pomegranate (Punica granatum L.) by-Products. Food Chem. 2015, 188, 481–488.
144. Chen, J.; Liu, H. Nutritional Indices for Assessing Fatty Acids: A Mini-Review. Int. J. Mol. Sci. 2020, 21, 5695.
145. Cheng, C.; Liu, Z.; Zhou, Y.; Wei, H.; Zhang, X.; Xia, M.; Deng, Z.; Zou, Y.; Jiang, S.; Peng, J. Effect of Oregano Essential Oil Supplementation to a Reduced-Protein, Amino Acid-Supplemented Diet on Meat Quality, Fatty Acid Composition, and Oxidative Stability of Longissimus Thoracis Muscle in Growing-Finishing Pigs. Meat Sci. 2017, 133, 103–109.

Chapter 5 : Summary, limitations and future perspectives

5.1. Summary of the dissertation and general conclusions

The integration of agro-industrial by-products and phytobiotics into pig diets represents a strategic pathway toward more sustainable, antibiotic-free, and high-quality pork production. By-products derived from agro-industrial processes, such as grape pomace, olive mill residues, and cheese whey, supply valuable nutrients and bioactive compounds, while simultaneously reducing waste streams and supporting the principles of circular economy and resource efficiency [1]. At the same time, phytobiotics—including oregano essential oil, rock samphire essential oil, garlic flour, and camelina flour—offer antimicrobial, antioxidant, and immunomodulatory properties that contribute to improved gut health, enhanced anti-inflammatory responses, and better meat quality characteristics [2].

In this context, firstly, a novel silage created from Greek olive, winery, and feta cheese waste by-products was evaluated as a feed ingredient at different inclusion rates (0%, 5% or 10%) by feeding it to 34-day-old weaned piglets in a commercial farm. The potential beneficial effects on weaners' performance and health, as well as meat quality, were evaluated.

The main conclusions obtained from this experimental study (Chapter 2) were:

1. No detrimental effects (P>0,050) on the piglets' performance and no significant changes (P>0,050) in meat pH, color and chemical analysis.
2. Intestinal microflora populations were positively affected by the dietary usage of the silage (P≤0.05).
3. The microbial populations of different meat cuts were positively modified (P≤0.01),
4. The concentration of total phenols in the meat cuts was increased (P≤0.05),
5. The resistance to oxidation of the meat was improved (P≤0.05),
6. The fatty acid profile of the meat lipids was positively modified (P≤0.001).

Secondly, we investigated the effects of the nutritional addition of the previously used on piglets' novel silage (containing a mixture of olive, winery, and feta cheese waste by-products) on 120-day-old finishing pig diets at three inclusion levels (Control Group Silage-0%, Group Silage-5% and Group Silage-10%). Performance, health parameters, meat and lipid quality characteristics and intestinal microflora populations were documented.

The main conclusions obtained from this experimental study (Chapter 3) were:

1. Pig end weight did not differ (P>0.05) for all groups.
2. Fat, protein, collagen and ash content did not differ (P>0.05) between the three groups for the triceps brachii and external abdominal samples.
3. Biochemical analysis revealed that ALT (Alanine Transaminase) was significantly (P≤0.05) different between group C and control A.
4. The pH of triceps brachii and external abdominal oblique samples did not differ significantly (P>0.05) between all groups.
5. In the ileum, total numbers of *Enterobacteriaceae* and Enterococci were significantly reduced (P≤0.05) in both B and C groups compared to control group A.
6. Total numbers of *Lactobacilli* significantly increased (P≤0.05) in both treatment groups B and C.
7. In the cecum, total aerobes in group B were significantly reduced (P≤0,05) compared to group C but not to the control group A.
8. Total numbers of Enterococci were significantly reduced (P≤0.01) in both groups B and C. *Lactobacilli* in group B tended to increase (0.05<P≤0.10) compared to the other two groups.
9. Total phenolic content in the triceps brachii samples was significantly higher (P≤0.05) for group C comped to control group A.
10. Thiobarbituric acid reactive substances (TBARS) did not differ (P>0.05) between the three groups.
11. The n-6/n-3 fatty acid ratio was significantly improved (P≤0.05) in group C of external abdominal samples.
12. In all treatments, there was absence of *Salmonella sp*. and *Listeria monocytogenes* per 25gr of meat tissue sample.
13. In the triceps brachii samples *Campylobacter jejuni* was significantly lower (P≤0.05) in treatment C compared to A.
14. Escherichia coli was not detected in any treatment concerning the triceps brachii, however a low population (1.04-1.46 log cfu/g) was found in external abdominal samples.

15. The color of the triceps brachii samples was significantly (P≤0.001) redder (increased a* value) in both groups B and C, while it was whiter (increased L* value) in control group A.

Furthermore, to extend our understanding of novel feeding systems using alternative additives (in this case, phytobiotics), we studied the effects of medicinal plant extracts and essential oils on pig performance parameters, health indices and meat quality. A phytobiotic mixture (PM) consisting of oregano (*Origanum vulgare* subsp. *hirtum*) essential oil, rock samphire (*Crithmum maritimum* L.) essential oil, garlic flour (*Allium sativum* L.) and false flax flour (*Camelina sativa* L. Crantz) was used, for the first time in pig diets, containing in the experimental trial two different proportions of the oregano essential oil (200 mL/t of feed vs. 400 mL/t of feed) in a micro-encapsulated form. Three groups of weaned pigs were fed either the control diet (CONT) or one of the enriched diets (PM-A or PM-B, 2 g/kg).

The main conclusions obtained from this experimental study (Chapter 4) were:

1. The statistical analysis revealed no differences ($p > 0.05$) in the body weights and growth rates among the groups.
2. The meat proximate analysis, as well as hematological and biochemical parameters, did not identify any differences ($p > 0.05$) between the groups.
3. An increase ($p < 0.05$) in total aerobic bacteria was detected in the ileum of group PM-A.
4. *Escherichia coli* (*E. coli)* counts were reduced ($p < 0.05$) in group PM-B.
5. In the caecum, reductions in *Enterobacteriaceae* and *Lactobacillaceae* counts were observed in groups PM-A and PM-B.
6. Concentrations of malondialdehyde (MDA) as an indicator of lipid peroxidation were significantly reduced ($p < 0.05$) in triceps brachii and biceps femoris for both groups PM-A and PM-B (day 0).
7. A reduction ($p < 0.05$) in MDA was noticed in triceps brachii and external abdominal meat samples (day 7) for groups PM-A and PM-B.
8. The fatty acid profile of the meat lipids (ΣPUFA, h/H and PUFA/SFA ratios) was positively modified ($p < 0.05$) in the ham and belly cuts.

9. The addition of the PM significantly ($p < 0.05$) affected the redness of the ham and shoulder meat (a* value increased), the yellowness of only the ham (b* value decreased) and the lightness of both belly (L* value increased) and ham samples (L* value decreased).

Overall, in this study, we demonstrated that the addition of the novel silage in the feed of weaned pigs exhibited a positive correlation between changes in the gastrointestinal tract and the level of their health and welfare, while it notably improved important quality characteristics of the meat, providing an added value to the end product (Experiment 1). Extending the research to finishing pigs, we indicated that the use of the novel silage based on a mixture of olive, winery, and cheese waste by-products retrieved from the agro-feed industry configured a favorable ratio of omega-6 to omega-3 fatty acids in the muscle tissue, increased the concentration of total phenols and microbiologically formed a beneficial profile of eubiotic microbial organisms that strongly correlates with the zootechnical results, health and welfare of the pigs (Experiment 2). Furthermore, the phytobiotic mixture under investigation may be used in the diet of weaned piglets, potentially exerting beneficial effects on their gut microbiota, oxidative stability, fatty acid profile, and the color characteristics of the produced meat (Experiment 3).

In conclusion, the use of agro-industrial by-products and phytobiotics in pig production holds substantial potential to transform conventional feeding systems into more resilient, sustainable, and consumer-oriented models. By aligning animal nutrition with circular economy principles, environmental sustainability goals, and public health considerations, these strategies may contribute significantly to shaping the future of the swine industry in an era increasingly defined by resource efficiency, regulatory pressures, and consumer demand for healthier and more sustainable food products.

5.2. Challenges, Limitations, and Future Perspectives

Although the incorporation of agro-industrial by-products and phytobiotics into pig diets has shown promising results, their widespread adoption in commercial production systems is still constrained by multiple barriers. These limitations can be grouped into technical, economic, regulatory, and knowledge-related categories, each of which requires targeted solutions to enable successful implementation.

5.2.1 Variability in Composition and Quality

One of the major challenges associated with agro-industrial by-products is the variability in their nutritional and functional composition. This heterogeneity arises from factors such as crop genotype, environmental and agronomic conditions, harvesting practices, processing methods, and storage duration. Such influences directly affect the concentrations of proteins, fibers, lipids, minerals, and bioactive compounds [3,4]. For instance, the polyphenol content of grape pomace or the phenolic composition of olive mill residues can fluctuate markedly across seasons and geographic locations, complicating the establishment of consistent and standardized feeding strategies [5]. Similarly, the nutritional profile and functional value of cheese whey depend on the milk source, type of cheese produced, and processing intensity applied during manufacture [6]. This variability poses difficulties not only in formulating balanced diets but also in ensuring reproducible animal responses, thereby limiting the scalability of the research.

5.2.2. Presence of Anti-Nutritional Factors and Contaminants

Another important limitation is the occurrence of anti-nutritional factors (ANFs) in certain by-products. These compounds can impair nutrient digestibility, reduce palatability, and negatively impact animal performance if not properly managed. Notable examples include tannins in grape pomace, glucosinolates in camelina, and excessive fiber fractions in olive cake [7,8]. Additionally, there is a risk of contamination by undesirable agents, including pathogenic microorganisms, mycotoxins, heavy metals, or chemical residues. Such hazards necessitate stringent quality control measures, including advanced processing techniques, detoxification methods, and continuous monitoring systems, to guarantee feed safety and protect animal and human health [9].

5.2.3. Economic and Logistical Constraints

From an economic perspective, while the use of agro-industrial by-products may lower feed costs at the farm level, their handling and stabilization often require significant investment. Processing techniques such as drying, ensiling, pelletizing, or fermentation enhance stability and nutritional availability but simultaneously increase production costs, which may erode their competitiveness compared with conventional feed ingredients [10]. Logistical challenges further complicate adoption. Seasonal fluctuations in availability, lack of reliable supply chains, and limited access to local processing or storage facilities can all hinder the consistent and long-term

inclusion of these by-products in swine diets [11]. These issues highlight the need for innovative supply chain solutions and cost-effective stabilization technologies to ensure sustainable utilization.

5.2.4. Regulatory and Consumer Acceptance

The use of novel feed ingredients and phytobiotics is also subject to complex regulatory frameworks, which differ across jurisdictions. For example, in the European Union, the European Food Safety Authority (EFSA) requires comprehensive assessments of safety, efficacy, and quality before approving any new feed additive [12]. Such requirements, while critical for maintaining food chain safety, may delay commercialization and discourage adoption by farmers due to lengthy approval processes and high compliance costs. Beyond regulation, consumer perception and market acceptance are equally decisive. The success of meat products derived from animals fed agroindustrial by-products largely depends on public trust, transparency in production practices, safety assurances, and effective communication or marketing strategies that emphasize sustainability and product quality [13].

5.2.5. Research Gaps and Opportunities

Despite the growing body of literature supporting the inclusion of by-products and phytobiotics in pig nutrition, significant research gaps remain. Much of the existing evidence is derived from in vitro studies or small-scale in vivo experiments, whereas large-scale, long-term trials under commercial farming conditions are still limited [14,15]. To address these gaps, several opportunities for future research can be identified:

- Precision feeding strategies: The integration of omics technologies (e.g., genomics, transcriptomics, metabolomics) offers the potential to match specific by-products and phytobiotics with particular pig genetic lines, physiological stages, and production objectives [16].
- Encapsulation and delivery systems: Advanced formulations, including microencapsulation and nanoencapsulation, can enhance the stability, bioavailability, and targeted release of phytobiotics within the gastrointestinal tract, thereby improving efficacy [17].

- Life cycle assessment (LCA): Comprehensive LCA studies are needed to quantify the environmental benefits of valorizing agro-industrial by-products in pig diets, including reductions in greenhouse gas emissions, land use, and waste generation [18].
- Integration with digital farming technologies: The deployment of precision livestock farming tools, such as sensors, artificial intelligence, and big data analytics, can facilitate real-time monitoring of animal responses and enable dynamic optimization of feeding strategies [19].

5.2.6. Future Directions

Our future research will focus on evaluating dietary strategies that combine agro-industrial by-products with phytobiotics. Such an approach may yield synergistic effects, not only enhancing growth performance and animal welfare but also reducing dependence on antibiotic growth promoters, thereby addressing a key challenge in contemporary livestock production. In addition, the incorporation of these feed resources may contribute to lowering the environmental footprint of pig farming through the valorization of agro-industrial residues, reduced reliance on conventional feed ingredients, and the enrichment of pork with bioactive compounds that could provide health-promoting benefits for consumers. Collectively, these outcomes highlight the potential of such strategies to align animal health, environmental sustainability, and human well-being.

Looking ahead, the combined use of agro-industrial by-products and phytobiotics has strong potential to become a cornerstone of sustainable pig production systems. However, realizing this potential will require coordinated efforts to overcome technical and logistical barriers, develop standardized processing and quality assurance protocols, and establish economically viable supply chains. Moreover, supportive regulatory frameworks, aligned with scientific evidence and market demands, are essential to accelerate safe adoption. Finally, close collaboration between academia, the feed industry, policymakers, and farmers will be critical to translating experimental findings into practical and scalable applications. Such multi-stakeholder engagement will ensure that these innovative feeding strategies not only enhance animal performance and health but also contribute to economic resilience and environmental sustainability in the pig industry.

5.3 References

1. Prates, J.A.M. Enhancing Meat Quality and Nutritional Value in Monogastric Livestock Using Sustainable Novel Feed Ingredients. Foods 2025, 14, 146. https://doi.org/10.3390/foods14020146
2. Madesh, M.; Yan, J.; Jinan, G.; Hu, P.; Kim, I.H.; Liu, H.-Y.; Ennab, W.; Jha, R.; Cai, D. Phytogenics in swine nutrition and their effects on growth performance, nutrient utilization, gut health, and meat quality: a review. Stress Biol. 2025, 5, 1–20, https://doi.org/10.1007/s44154-024-00209-2.
3. Foti, P.; Romeo, F.V.; Russo, N.; Pino, A.; Vaccalluzzo, A.; Caggia, C.; Randazzo, C.L. Olive Mill Wastewater as Renewable Raw Materials to Generate High Added-Value Ingredients for Agro-Food Industries. Appl. Sci. 2021, 11, 7511. https://doi.org/10.3390/app11167511
4. Lopes JDC, Madureira J, Margaça FMA, Cabo Verde S. Grape Pomace: A Review of Its Bioactive Phenolic Compounds, Health Benefits, and Applications. Molecules. 2025;30(2):362. Published 2025 Jan 17. doi:10.3390/molecules30020362
5. Blasi, F.; Trovarelli, V.; Mangiapelo, L.; Ianni, F.; Cossignani, L. Grape Pomace for Feed Enrichment to Improve the Quality of Animal-Based Foods. Foods 2024, 13, 3541. https://doi.org/10.3390/foods13223541
6. Pires, A.F.; Marnotes, N.G.; Rubio, O.D.; Garcia, A.C.; Pereira, C.D. Dairy By-Products: A Review on the Valorization of Whey and Second Cheese Whey. Foods 2021, 10, 1067. https://doi.org/10.3390/foods10051067
7. Iyakutye, J.N.; Owen, O.J.; Friday, O. Anti-Nutritional Factors in Animal Feedstuffs: A Review. Int. J. Res. Rev. 2023, 10(2), 226–244. doi:10.52403/ijrr.20230229.
8. Hilbrands AM, Johnston LJ, Cox RB, Forcella F, Gesch R, Li YZ. Effects of increasing dietary inclusion of camelina cake on growth performance of growing-finishing pigs. Transl Anim Sci. 2021;5(3):txab140. Published 2021 Aug 26. doi:10.1093/tas/txab140
9. Akinmoladun OF, Fon FN, Nji Q, Adeniji OO, Tangni EK, Njobeh PB. Multiple Mycotoxin Contamination in Livestock Feed: Implications for Animal Health,

Productivity, and Food Safety. Toxins (Basel). 2025 Jul 25;17(8):365. doi: 10.3390/toxins17080365. PMID: 40864041; PMCID: PMC12389956.

10. Yang, K.; Qing, Y.; Yu, Q.; Tang, X.; Chen, G.; Fang, R.; Liu, H. By-Product Feeds: Current Understanding and Future Perspectives. Agriculture 2021, 11, 207. https://doi.org/10.3390/agriculture11030207
11. Y. Mohammady E, Elshobary M, Ashour M. From Wastes to Resources: Sustainable Applications of Agro-Industrial Byproducts for a Greener Future [Internet]. Circular Bioeconomy - Integrating Biotechnology and Sustainability for a Greener Planet. IntechOpen; 2025. Available from: http://dx.doi.org/10.5772/intechopen.1010715
12. EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP). Guidance on the Assessment of the Safety and Efficacy of Feed Additives. EFSA J. 2017, 15, e5021. https://doi.org/10.2903/j.efsa.2017.5021
13. Ornelas Herrera, S.I., Baba, Y., Kallas, Z. et al. The role of environmental attitudes and consumption patterns in consumers' preferences for sustainable food from circular farming system: a six EU case studies. Agric Econ 13, 7 (2025). https://doi.org/10.1186/s40100-025-00350-0
14. Garavito-Duarte Y, Deng Z, Kim SW. Literature Review: Opportunities with Phytobiotics for Health and Growth of Pigs. Annals of Animal Science. National Research Institute of Animal Production, 2025;25(4): 1237-1247. https://doi.org/10.2478/aoas-2024-0119
15. Papatsiros, V.G.; Tsekouras, N.; Papakonstantinou, G.I.; Kamvysi, K.; Eliopoulos, C.; Fotos, L.; Arapoglou, D.; Meletis, E.; Michailidis, G.; Gougoulis, D. The Use of Food Industry By-Products in Pig Diets as a Challenge Option to Reduce the Environmental Footprint. Agriculture 2025, 15, 2390. https://doi.org/10.3390/agriculture15222390
16. Chen LH, Canibe N, Curtasu MV, Hedemann MS. Untargeted metabolomics as a tool to assess the impact of dietary approaches on pig gut health: a review. J Anim Sci Biotechnol. 2025;16(1):106. Published 2025 Jul 22. doi:10.1186/s40104-025-01238-1
17. Nantapo, C.W.T.; Marume, U. Strategic technologies to improve phytogenic feed additive efficacy in pigs and poultry. Animal Nutrition 2025, xx(xx), xx–xx. doi:10.1016/j.aninu.2024.06.010.

18. Valenti, F.; Liao, W.; Porto, S.M.C. Life cycle assessment of agro-industrial by-product reuse: a comparison between anaerobic digestion and conventional disposal treatments. Green Chem. 2020, 22, 7119–7139. doi:10.1039/D0GC01918F.
19. Papakonstantinou, G.I.; Voulgarakis, N.; Terzidou, G.; Fotos, L.; Giamouri, E.; Papatsiros, V.G. Precision Livestock Farming Technology: Applications and Challenges of Animal Welfare and Climate Change. Agriculture 2024, 14, 620. https://doi.org/10.3390/agriculture14040620

A. Appendices

A. General workflow

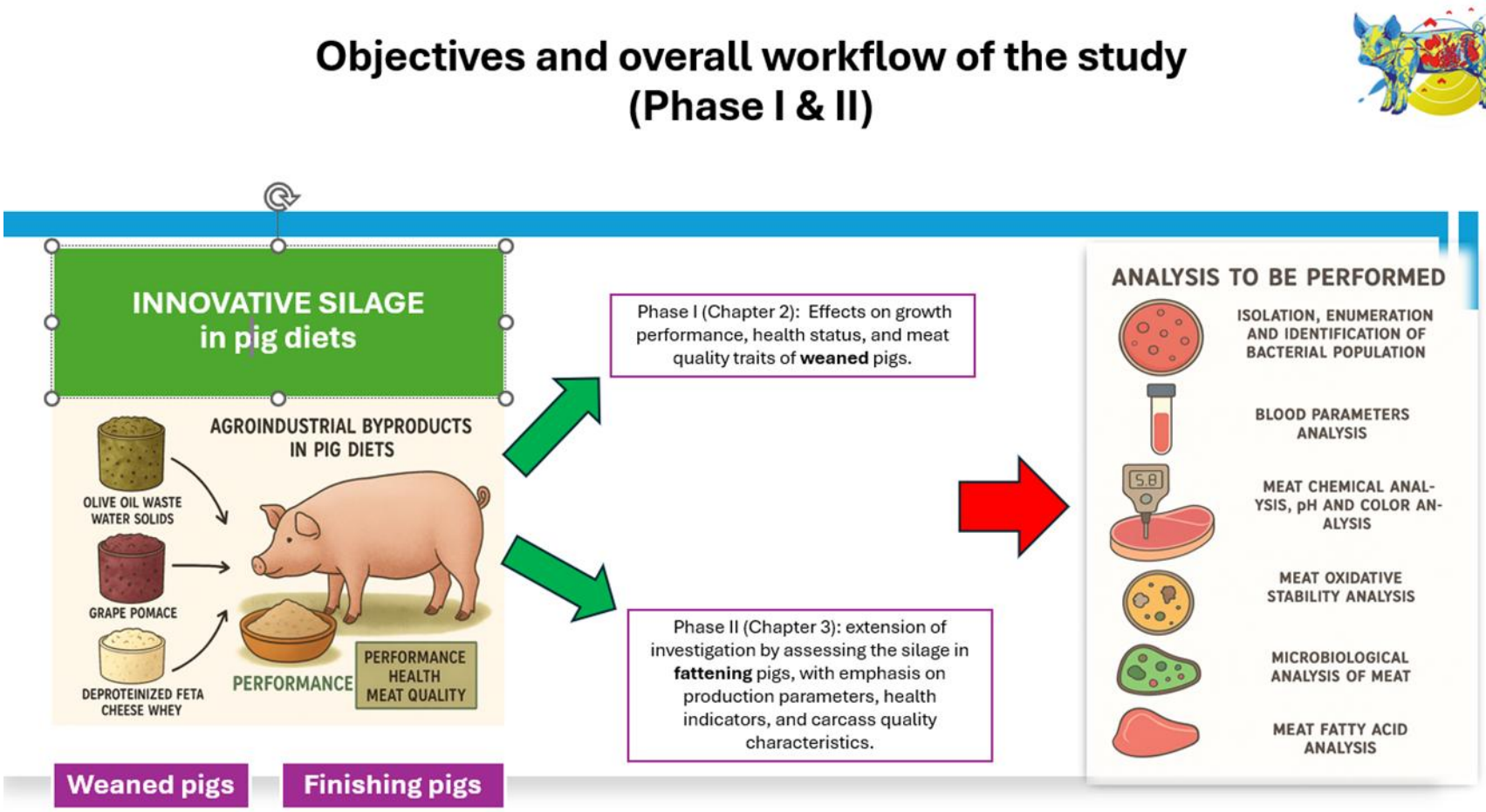


Figure A.1. Objectives and overall workflow of the study (Phase I & Phase II).

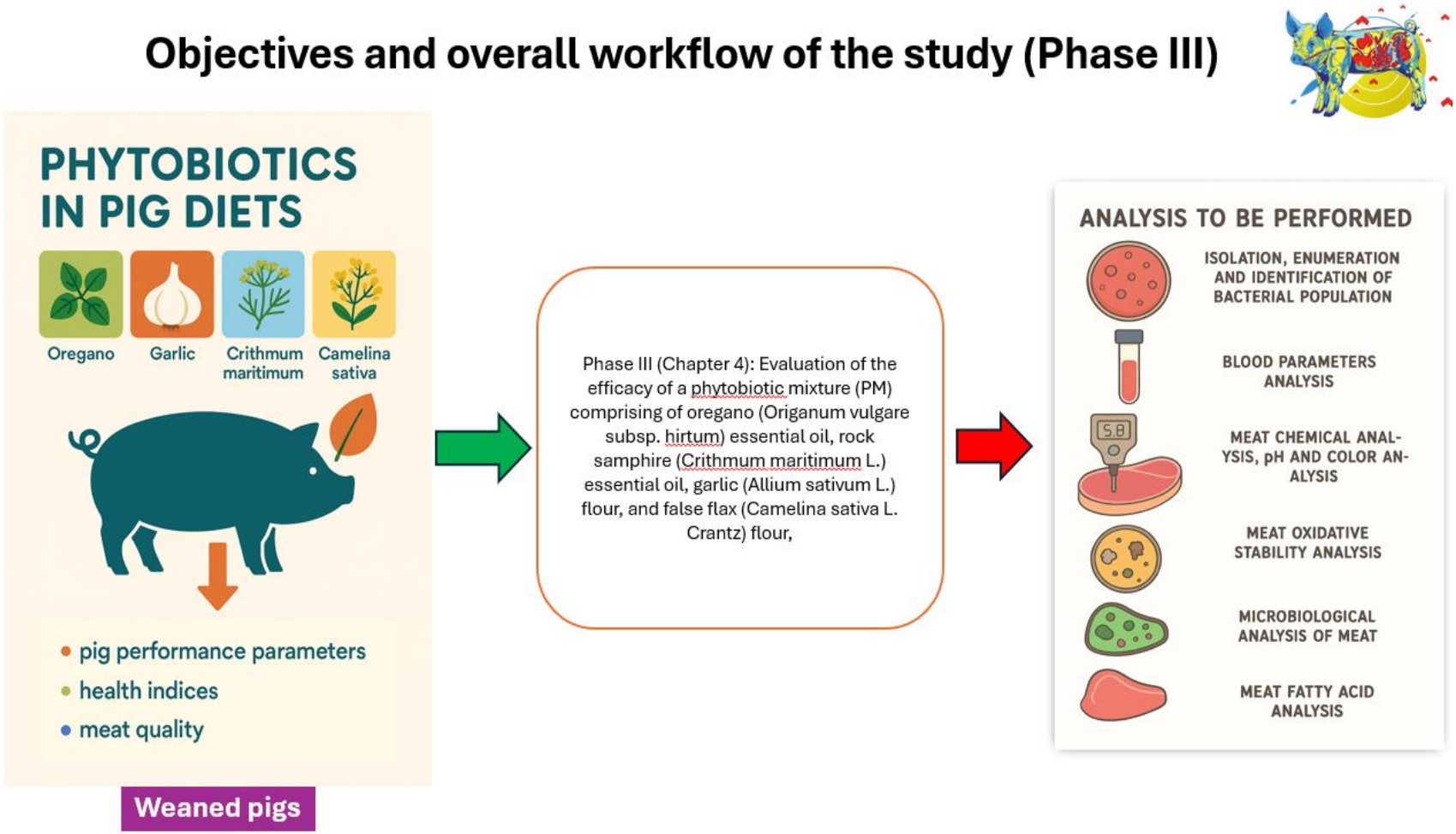


Figure A.2. Objectives and overall workflow of the study (Phase III).

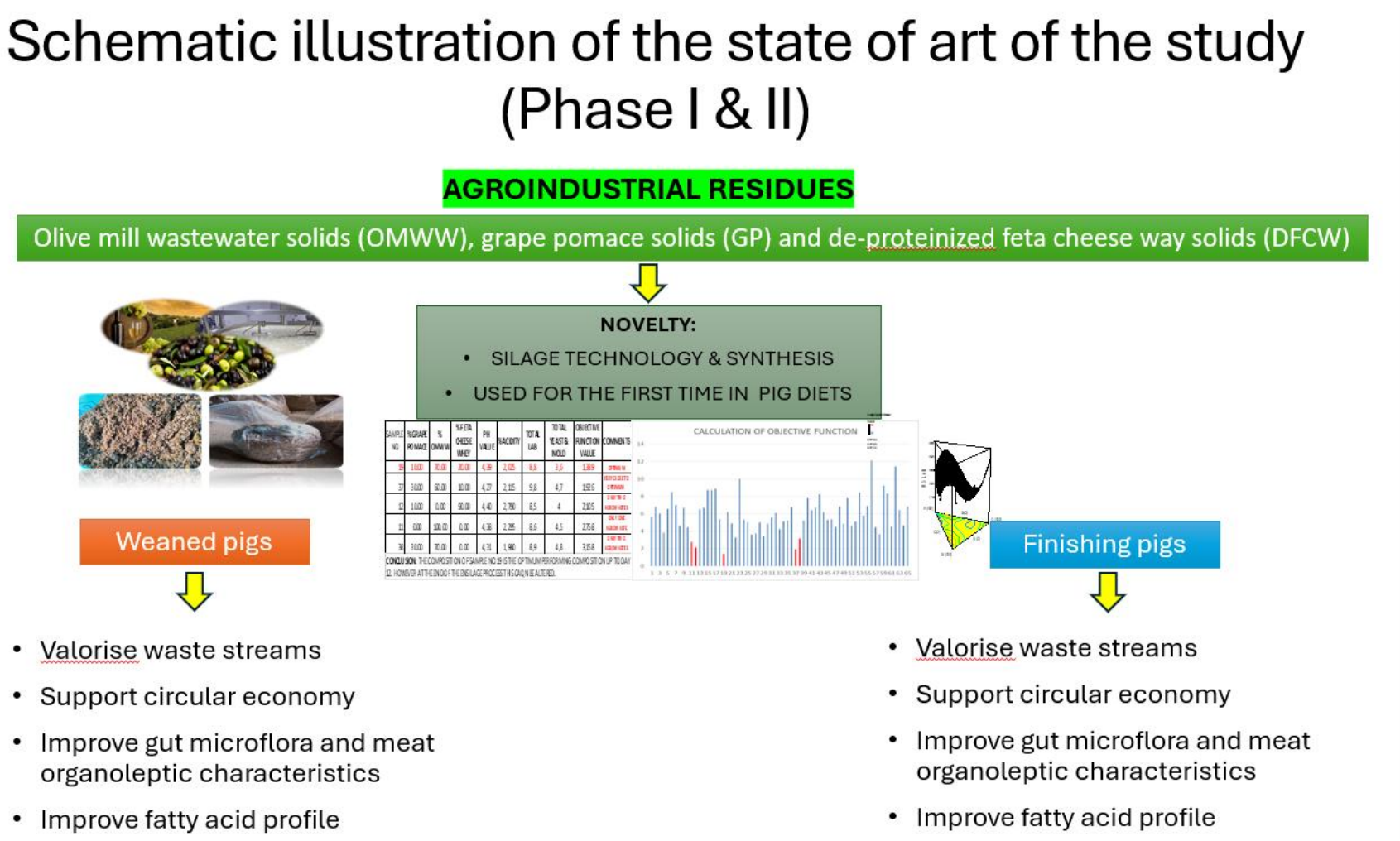


Figure A.3. Schematic illustration of the state of art of the study (Phase I & Phase II).

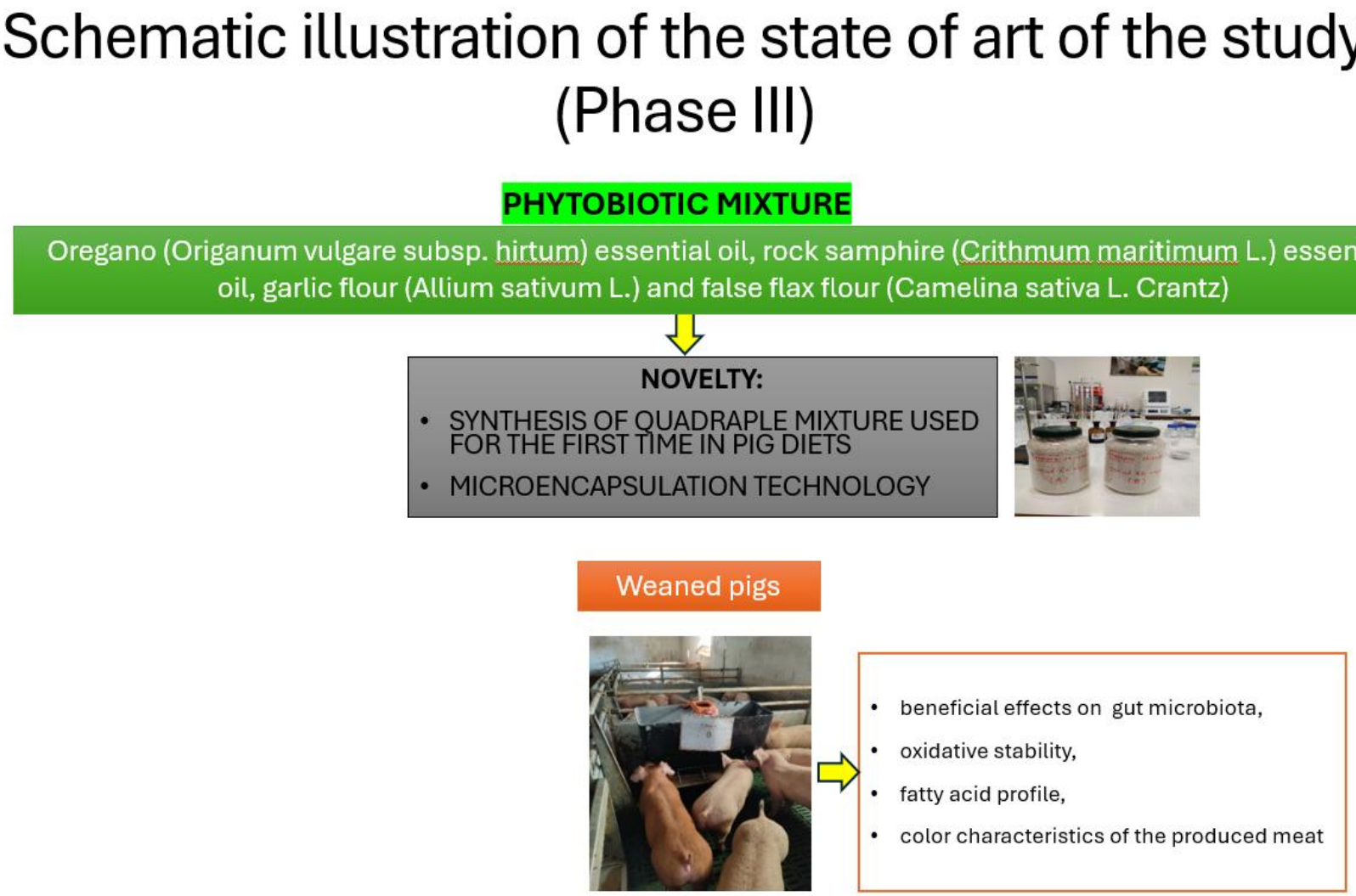


Figure A.4. Schematic illustration of the state of art of the study (Phase III).

B. List of Protocols

B.1. Microbial Analysis of Pig Ileal & Caecal Digesta

Sample collection

1. Euthanasia & aseptic sampling: Immediately after slaughter, open the gastrointestinal tract aseptically and collect digesta from the ileum and caecum.
2. Aliquoting: Place ~5–10 g digesta per site into pre-labelled, sterile containers.
3. Hold & transport: Keep on ice/4 °C and transfer to the lab for processing as soon as possible (ideally ≤2–4 h).

Homogenization & serial dilutions

4. Primary homogenate: Weigh 1.00 g digesta into a sterile tube/homogenization bag; add 9 mL of 0.1% sterile peptone water. Homogenize thoroughly (vortex/stomacher) → 10^{-1}.
5. Serial ten-fold dilutions (10^{-2} to 10^{-12}): Using a 96-well microdilution plate (or tubes), transfer 100 µL into 900 µL 0.1% peptone water (or equivalent scheme) per step, changing tips at each dilution. Mix well at every step.

Miles & Misra surface-drop plating

6. Plate set-up (per sample/dilution):
    - *Enterobacteriaceae*: MacConkey agar (aerobic, 37 °C, 24–48 h).
    - *Enterococci*: KAA agar (Kanamycin Aesculin Azide) (aerobic, 37 °C, 24–48 h).
    - *Lactobacillaceae*: MRS agar and M17 agar (anaerobic, 37 °C, 48 h).
    - Total counts: Plate Count Agar (PCA)
        - Aerobes: 30 °C, 48 h (aerobic)
        - Anaerobes: 37 °C, 48–72 h (anaerobic).
7. Spot inoculation: For each selected dilution, dispense 10 µL drops (≥3 technical replicates/dilution) onto the agar surface (Miles & Misra). Allow drops to absorb with plates level and lids ajar (~10–15 min in biosafety cabinet).
8. Incubation: Invert plates. For anaerobic plates, place in anaerobic jars with gas packs and indicators.

Enumeration & data handling

9. Counting: Select drops yielding countable colonies (typically 3–30 colonies per 10 µL drop is practical for this method). Record counts for each medium and dilution.
10. Calculations:
    - CFU/mL of dilution = (mean colonies per drop) ÷ 0.01 mL.
    - CFU/g (wet weight) = CFU/mL × dilution factor × 10 (for the initial 1:10 homogenate).
    - Report as $\log_{10}$ CFU per g (mean ± SD).
11. Representative colony selection: From each medium, pick typical colonies, streak to purity on the same medium, and re-incubate under the appropriate atmosphere.

Identification

12. Prep for Vitek 2: Prepare pure isolates as per manufacturer's McFarland standard.
13. Automated ID: Load onto Vitek® 2 Compact using:
    - ID-GN (Gram-negative), ID-GP (Gram-positive) cards, and ANC/CBC cards as applicable.
14. Target taxa: Confirm *Enterobacteriaceae, Enterococcaceae, Lactobacillaceae, Bifidobacteriaceae*, and others as recovered.

15. QC & documentation: Include appropriate positive/negative controls, record incubation conditions, plate IDs, dilution series, and instrument run data.

B.2. Hematological and Biochemical Analysis of the Blood

Sample collection

1. Animal preparation: Withhold feed for 4 h prior to sampling on the final day of the trial.
2. Blood sampling: Collect ~4 mL of blood aseptically from the jugular vein of each pig.
3. Collection tubes: Transfer blood immediately into sterile EDTA-coated vacutainer tubes to prevent clotting.
4. Labeling: Properly label each tube with animal ID, treatment, and sampling time.
5. Mixing: Gently invert tubes several times to ensure anticoagulant mixing.

Hematological analysis

6. Analyzer preparation: Calibrate and prepare MS4 automated hematology analyzer (Melet Schloesing Lab, Osny, France).
7. Sample loading: Introduce whole blood (EDTA-preserved) into the analyzer.
8. Parameters measured: Hemoglobin (Hb), erythrocytes (RBC), hematocrit (Hct), leucocytes (WBC), lymphocytes.
9. Data recording: Export/record hematological values per animal.

Biochemical analysis

10. Serum preparation: Centrifuge a portion of blood sample (without anticoagulant, in serum separator tubes if available) at ~3000 rpm for 10–15 min.
11. Serum collection: Carefully collect the serum supernatant into clean tubes.
12. Analyzer preparation: Prepare IDEXX VETTEST 8008 biochemical analyzer (IDEXX Labs, Westbrook, ME, USA) with required reagent slides.
13. Sample loading: Introduce serum samples into the analyzer.
14. Parameters measured: Albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, creatine kinase (CK), glucose, total bilirubin, triglycerides.
15. Data recording: Export/record biochemical results per animal.

Quality control & reporting

16. Controls: Run analyzer QC checks with commercial control materials.
17. Data management: Compile hematological and biochemical data into structured dataset per treatment.
18. Sample disposal: Dispose of all biological samples, sharps, and vacutainers according to biosafety protocols.

B.3. Chemical Analysis, pH Measurement and Color Analysis of the Meat

Sample collection & preparation

1. Tissue sampling: Collect meat samples (~200 g each) from:
    - Ham (*Biceps femoris*)
    - Shoulder (*Triceps brachii*)
    - Belly (*External abdominal*)
2. Storage: Store samples at –20 °C until analysis.
3. Grinding: Thaw and homogenize samples using an industrial meat grinder (Bosch, Gerlingen, Germany).

Chemical composition analysis (FoodScan™ NIR)

4. Sample preparation: Ensure ~200 g homogenized meat per sample.

5. Instrument setup: Use FoodScan™ Lab (FOSS, Hillerød, Denmark) in transmittance mode.
6. Analysis: Determine moisture, crude protein, fat, collagen, and ash content.
7. Reference method: Follow AOAC Official Method 2007.04 for meat and meat products.
8. Data recording: Record results for each sample and calculate group averages.

pH measurement

9. Instrument preparation: Calibrate the Hanna HI981036 portable pH meter using standard buffers (pH 4.0, 7.0, 10.0).
10. Measurement: For each sample type (ham, shoulder, belly), measure 6 replicates/group by inserting the stainless-steel probe deep into the tissue.
11. Data handling: Record values and compute mean pH for each group.

Meat color analysis (Hunter scale)

12. Instrument preparation: Calibrate the CAM-System 500 Chromatometer (Lovibond, UK) with standard white/black calibration tiles.
13. Measurement: Place the probe on the surface of each meat sample and record *L, a*, b*** values according to the Hunter scale.
14. Replicates: Take at least three readings per sample and compute the mean values.
15. Data handling: Report average L*, a*, b* values for each group and muscle type.

Quality control & reporting

16. Calibration checks: Perform routine instrument calibration before each set of measurements.
17. Replicates: Ensure multiple measurements per group for statistical validity.
18. Data compilation: Combine chemical, pH, and color results into a single dataset for further analysis.

B.4. Oxidative Stability Analysis of the Meat

Part 1 – Total Polyphenols (Folin–Ciocalteu Method)

1. Preparation of gallic acid stock solution: Dissolve 0.2 g gallic acid (Merck, Germany) in 100 mL distilled water (stock: 2 g/L).
2. Preparation of standards: Dilute stock to prepare standard solutions of 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, and 1 g/L gallic acid.
3. Reaction setup (standards): In 50 mL Falcon tubes, add:
    - 0.2 mL standard solution
    - 10.8 mL distilled water
    - 8 mL Na_2CO_3 (75 g/L in water, Penta Chemicals)
    - 1 mL Folin–Ciocalteu reagent (PanReac Applichem, Germany)
    - Mix by vortex.
4. Control (blank): Replace standard with 0.2 mL distilled water.
5. Incubation: Place all tubes in the dark at room temperature for 1 h.
6. Spectrophotometry (standards): Use the blank to calibrate the UV–Vis spectrophotometer (DR 5000, Hach Lange) at 750 nm. Measure absorbance of all standards.
7. Standard curve: Construct calibration curve (concentration vs absorbance, $R^2 > 0.998$).
8. Sample preparation: Homogenize 5 g meat (shoulder, belly, ham) with 10 mL distilled water using a blender. Filter through filter paper.
9. Reaction setup (samples): In 50 mL Falcon tubes, add:
    - 0.2 mL filtrate
    - 10.8 mL distilled water
    - 8 mL Na_2CO_3 solution (75 g/L)

- 1 mL Folin–Ciocalteu reagent
- Mix by vortex.

10. Sample blank: Replace sample with 0.2 mL distilled water.
11. Incubation: Place in dark at room temperature for 1 h.
12. Spectrophotometry (samples): Calibrate with blank at 750 nm. Measure absorbance of samples.
13. Calculation: Express results as mg gallic acid equivalents (GAE) per g meat, using the calibration curve.

Part 2 – Lipid Oxidation (TBARS Method)

1. Sample homogenization: Homogenize 5 g meat (shoulder, ham, belly) with 25 mL trichloroacetic acid (TCA) solution in a blender.
2. Incubation: Transfer homogenate into glass bottles, leave for 20 min.
3. Filtration: Filter through filter paper to obtain clear filtrate.
4. Reaction setup: Transfer 5 mL filtrate into glass test tubes, add 5 mL 2-thiobarbituric acid (TBA) solution.
5. Blank: Replace sample with 5 mL TCA solution.
6. Mixing: Vortex all tubes thoroughly.
7. Incubation: Place tubes in water bath at 60 °C for 15 min.
8. Spectrophotometry: Calibrate UV–Vis spectrophotometer with blank at 532 nm, then measure absorbance of all samples.

B.5. Fatty Acid Analysis of the Meat

Sample preparation (GC-FAME Method)

1. Sample collection: Use homogenized shoulder, ham, and belly meat cuts.
2. Lipid extraction & methylation:
   - Weigh ~1 g of homogenized meat.
   - Perform direct transesterification according to O'Fallon et al.: add methanolic base/acid reagents to simultaneously extract and methylate fatty acids into fatty acid methyl esters (FAMEs).
   - Heat the mixture as per protocol (e.g., ~80 °C for 1 h) and cool.
   - Extract FAMEs with hexane or similar solvent.
   - Collect the supernatant containing FAMEs.

Gas Chromatography

3. FFAP Column conditions: Set appropriate capillary column parameters (length, stationary phase, and temperature program as per validated method).
4. Injection: Inject FAME extracts into GC system.
5. Detection & quantification: Acquire chromatograms; peaks correspond to individual fatty acids.
6. Reference standard: Identify retention times and elution order using Supelco 37 Component FAME Mix (Sigma-Aldrich, Darmstadt, Germany).
7. Calculation of fatty acid composition:
   - Express each fatty acid as % of total:
     % FA = (peak area of individual FA ÷ total peak area of all identified FAs) × 100.

Nutritional indices

9. PUFA/SFA ratio: Calculate from total identified fatty acids.
10. n-6/n-3 PUFA ratio: Calculate from identified ω-6 and ω-3 fatty acids.

11. h/H index: Calculate as:
h/H=(C18:1n−9+PUFA)(C12:0+C14:0+C16:0)h/H = \frac{(C18:1n-9 + \text{PUFA})}{(C12:0 + C14:0 + C16:0)}h/H=(C12:0+C14:0+C16:0)(C18:1n−9+PUFA)
This evaluates the cholesterolemic potential of lipids.

B.6. Microbiological Analysis of Pig Meat

Sample preparation

1. Collect 10 g meat (shoulder, belly, ham) aseptically.
2. Place sample into sterile stomacher bag with 90 mL Maximum Recovery Diluent (MRD).
3. Homogenize using BagMixer 400 (Interscience, France).

Serial dilution

4. Prepare 10-fold dilutions by transferring homogenate into sterile glass tubes with 9 mL MRD.
5. From the selected dilution, inoculate either 1 mL or 0.1 mL into Petri dishes (pour or spread plating).

Microorganism-specific enumeration

6. *Escherichia coli*: Plate on TBX agar (Tryptone Bile X-Glucuronide, Oxoid). Incubate aerobically, 37 °C, 24 h.
7. Sulfite-reducing Clostridia: Plate on Perfringens Agar Base (Oxoid). Incubate anaerobically at 37 °C, 48 h (Anaerobic jars + Anaerocult A, Oxoid).
8. *Staphylococcus aureus* / spp.: Plate on Baird Parker agar (Oxoid) supplemented with egg yolk tellurite emulsion (Oxoid). Incubate aerobically at 37 °C, 48 h.
9. Total Mesophilic Count: Plate on Plate Count Agar (PCA, Oxoid). Incubate aerobically at 30 °C, 48 h.
10. *Campylobacter jejuni*: Plate on Campy Blood-Free Selective Agar (CCDA, Acumedia – Lab M, USA/UK) supplemented with Campylobacter Selective Supplement. Incubate microaerophilically at 37 °C, 72 h, 10% CO_2 atmosphere.

Pathogen detection (ISO methods)

11. *Salmonella spp*.: Analyze 25 g meat following ISO 6579:2002 method.
12. *Listeria monocytogenes*: Analyze 25 g meat following ISO 4833:2001 method.

Incubation

13. All plates incubated in Binder BD 115 incubators (Binder GmbH, Germany), using appropriate aerobic, anaerobic, or microaerophilic conditions.

Data collection

14. Enumerate colonies from plates with suitable dilutions.

Express results as log CFU/g meat.

B.7. Phenolic content and antioxidant capacity determination of the feed

A. Total Phenolic Content – Folin–Ciocalteu Method (after Vasilopoulos et al.)

1. Weigh feed sample (as per standardized method).
2. Extract phenolic compounds (aqueous or methanolic extraction depending on lab SOP).
3. Mix aliquot with Folin–Ciocalteu reagent.
4. After 3–5 min, add sodium carbonate solution.
5. Incubate in the dark (room temp) for ~30–60 min.
6. Measure absorbance at 750 nm using UV–Vis spectrophotometer.
7. Quantify total phenols using gallic acid calibration curve.

B. Lipid Oxidation – TBARS Method (adapted from Ahn et al.)

1. Weigh 1.0 g feed sample into homogenizer tube.
2. Add 8 mL of 5% (w/v) TCA and 5 mL of 0.8% (w/v) BHT in hexane.
3. Homogenize thoroughly.
4. Centrifuge homogenate at 3,000 × g for 5 min.
5. Collect 1.5 mL of the lower aqueous phase.
6. Add 2.5 mL of 0.8% (w/v) TBA.
7. Incubate mixture in water bath at 70 °C for 30 min.
8. Cool samples to room temperature.
9. Measure absorbance at 532 nm.
10. Express oxidative status as TBARS (ng MDA/g feed) using an MDA calibration curve.

C. List of Consumables

Category	Consumable / Instrument	Supplier / Distributor
Media, Reagents & Standards	Maximum Recovery Diluent (MRD)	Oxoid (UK) → Bioline Scientific (GR)
	Malondialdehyde (MDA) standard solution	Sigma-Aldrich (Analysi, GR)
	0.1% Peptone Water	Oxoid (UK) → Bioline Scientific (GR)
	MacConkey Agar	Merck / Sigma-Aldrich (DE) → Analysi Scientific Instruments (GR)
	KAA Agar (Kanamycin Aesculin Azide)	Merck (DE) → Analysi (GR)
	MRS Agar	Oxoid (UK) → Bioline Scientific (GR)
	M17 Agar	Lab M (Acumedia/Neogen, UK/US) → Elvetiki (GR)
	Plate Count Agar (PCA)	Oxoid (UK) → Lab Supplies Hellas (GR)
	Baird-Parker Agar	Oxoid (UK) → Lab Supplies Hellas / Bioline Scientific (GR)
	Egg Yolk Tellurite Emulsion	Oxoid (UK) → Bioline Scientific (GR)
	TBX Agar (Tryptone Bile X-Glucuronide)	Oxoid (UK) → Bioline Scientific (GR)
	Perfringens Agar Base	Oxoid (UK) → Bioline Scientific (GR)
	CCDA + Campylobacter Selective Supplement	Lab M / Acumedia (Neogen) → Elvetiki (GR)
	Anaerocult A (Gas Packs)	Merck (DE) → Analysi (GR)
	CampyGen (Microaerophilic system)	Oxoid (UK) → Bioline Scientific (GR)
	Folin–Ciocalteu Reagent	PanReac AppliChem → Analysi (GR)
	Gallic Acid standard (≥99%)	Merck / Sigma-Aldrich (DE) → Analysi (GR)
	Sodium Carbonate (Na_2CO_3)	Penta Chemicals (CZ) → Lab Supplies Hellas / Chemland (GR)
	Trichloroacetic Acid (TCA) ≥99%	Merck / Sigma-Aldrich (DE) → Analysi (GR)
	Butylated hydroxytoluene (BHT)	Merck / Sigma-Aldrich (Analysi, GR)

	Thiobarbituric Acid (TBA) ≥98%	Merck / Sigma-Aldrich (DE) → Analysi (GR)
	Hexane (HPLC grade)	Merck / Sigma-Aldrich (DE) → Analysi (GR)
	Methanol (HPLC grade)	Merck / Sigma-Aldrich (DE) → Analysi (GR)
	Methanolic reagents for transesterification	Merck / Sigma-Aldrich (DE) → Analysi (GR)
	Supelco 37-Component FAME Mix	Supelco / Merck (DE) → Analysi (GR)
	QC Control Strains (e.g., E. coli)	ATCC / Microbiologics → LGC Standards Hellas (GR)
	pH Calibration Buffers (4, 7, 10)	Hanna Instruments → Hanna Instruments Hellas (GR)
	Distilled/Deionized Water	Local suppliers (GR)
Labware & Disposables	Sterile Stomacher Bags	Interscience (FR) → Bioline Scientific (GR)
	Petri Dishes (90 mm)	Sarstedt (DE) → Sarstedt Greece (GR)
	Glass Dilution Tubes (9 mL)	DURAN (DE) → Sarstedt Greece (GR)
	50 mL Conical Tubes	Corning / Sarstedt → Sarstedt Greece (GR)
	Glass Test Tubes (screw-cap)	DURAN / Kimble → Sarstedt Greece (GR)
	Filter Papers	Whatman / Cytiva → Analysi (GR)
	Pipettes & Filter Tips	Eppendorf / Sarstedt → Elvetiki / Sarstedt Greece (GR)
	Inoculating Loops/Spreaders	Copan / VWR → Lab Supplies Hellas (GR)
	Cryotubes / Microtubes	Sarstedt → Sarstedt Greece (GR)
	Weigh Boats / Dishes	VWR / Fisherbrand → Lab Supplies Hellas (GR)
	Labels & Markers	Brady / VWR → Lab Supplies Hellas (GR)
	PPE (Gloves, Coats, Goggles)	Ansell / 3M / Honeywell → Sarstedt Greece (GR)

	Biohazard Bags, Sharps Containers	Sarstedt / Thermo → Sarstedt Greece (GR)
	Disinfectants (70% EtOH, Bleach)	Lab grade → Local suppliers (GR)
Instruments & Accessories	BagMixer 400 Stomacher	Interscience (FR) → Bioline Scientific (GR)
	Anaerobic Jars/Canisters	Oxoid (UK) → Lab Supplies Hellas / Bioline (GR)
	Binder BD 115 Incubators	Binder (DE) → Analysi (GR)
	CO_2/Microaerophilic Incubators	Binder / Oxoid → Analysi / Bioline (GR)
	Vitek 2 Compact + ID Cards	bioMérieux (FR) → bioMérieux Hellas (GR)
	Hematology Analyzer (MS4)	Melet Schloesing (FR) → Authorized Greek distributor
	Biochemical Analyzer (VETTEST 8008)	IDEXX (US) → IDEXX regional distributor (GR)
	FoodScan Lab (NIR)	FOSS (DK) → FOSS Hellas (GR)
	Portable pH Meter HI981036	Hanna Instruments → Hanna Instruments Hellas (GR)
	CAM-System 500 Chromatometer	Lovibond / Tintometer → Analysi / partner (GR)
	Centrifuge (capable of 3,000 × g), model	Eppendorf centrifuge, 5702 series→ Elvetiki S.A.
	UV–Vis Spectrophotometer DR 5000	Hach → Antisel SA (GR)
	Water Bath (up to 70 °C)	Julabo / Memmert → Analysi / Antisel (GR)
	Blender/Homogenizer	Bosch / Waring → Local suppliers (GR)
	GC TraceGC + FID	Thermo Fisher → Antisel SA (GR)
	GC FAME Column SP-2560	Supelco / Merck → Analysi (GR)
	Vortex Mixer, Balance, Micropipettes	Eppendorf / Sartorius → Elvetiki (GR)

Method-Specific Kits	ISO 6579:2002 (Salmonella)	Oxoid / Merck → Bioline / Analysi (GR)
	ISO 4833:2001 (Listeria)	Oxoid / Merck → Bioline / Analysi (GR)
	Supelco FAME Mix (37)	Merck → Analysi (GR)

D. Representative images of the experimental procedures

D.1. Phase I (Chapter 2): EXPERIMENT 1_SILAGE_PIGLETS

Silage in 25kg vaccumed bags

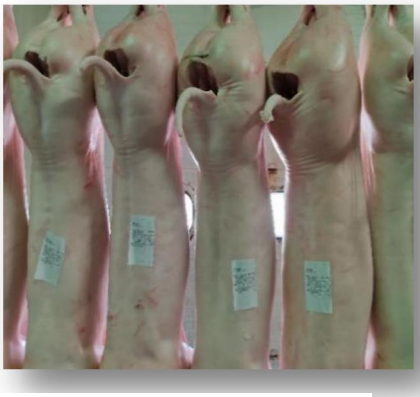
Silage in 0%, 5% and 10% ratios

Group of weaned pigs (Control)

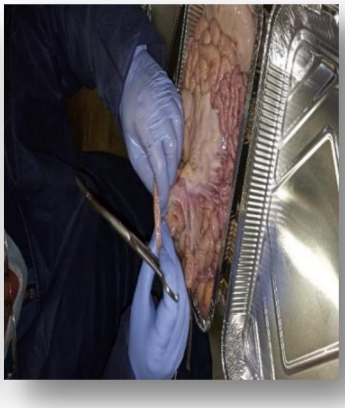
Weighing piglets (Mini–L 3510 animal scale)

Biochemical and Hematological analysis

After a busy day at the abattoir



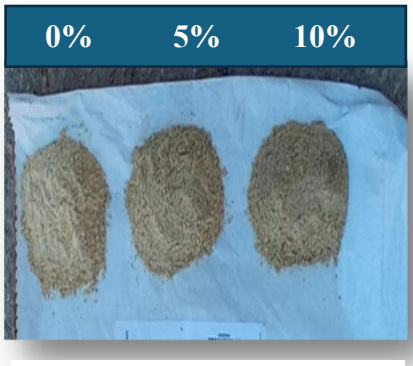
Pig carcasses after slaughter and grading procedures



Collection of intestinal tissues

Collection of fresh ileal and caecal digesta

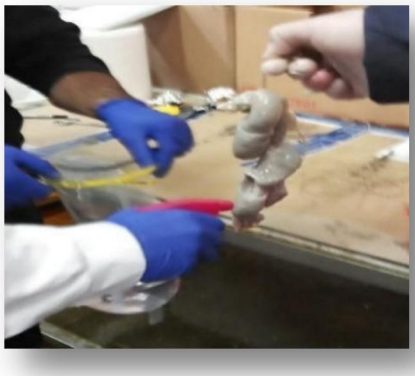
D.2. Phase II (Chapter 3): EXPERIMENT 2_SILAGE_FINISHING PIGS



Innovative silage after mixing (Control-0%, 5%, 10%)

Weighing fatteners (Mini–L 3510 animal scale)

Finishing pig carcasses prior to tissue collection



Collection of intestinal tissues at abattoir

Blood biochemical analysis

Preparation of meat cuts

Meat chemical analysis (FoodScan™ Lab, FOSS

Culture media for the isolation and enumeration of microorganisms from intestinal content

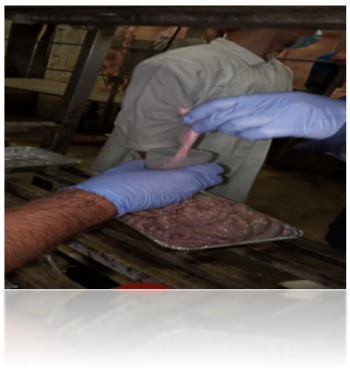
Isolation & enumeration of *E.coli* (MacConkey agar) and Lactic acid bacteria (MRS agar)

D.3. Phase III (Chapter 4): EXPERIMENT 3_PHYTOBIOTICS_PIGLETS

Phytobiotic mixture (PM-A & PM-B)

Piglet group on farm

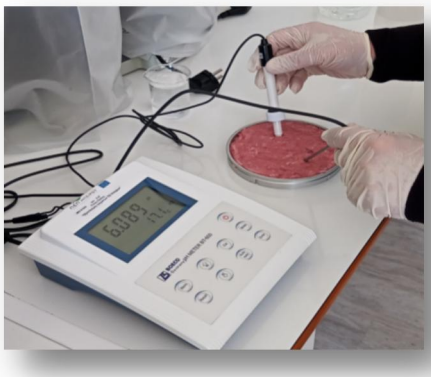
Piglet transport to abattoir (d77)



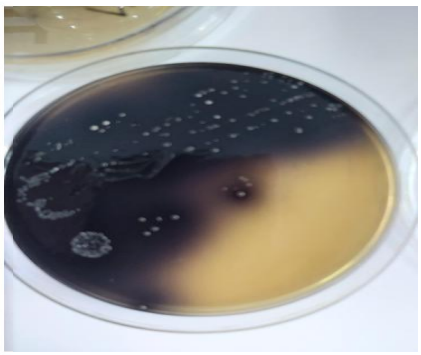
Gut tiisue collection (Ileum & Caecum)

Piglet carcasses at local abattoir

At the abattoir after meat cut collection



pH measurement



Isolation & enumeration of *Enterococci* [Kanamycin Aesculin Azide (KAA) agar]

In the Laboratory ofAnimal Health, Hygiene and Food Quality, Department of Agriculture, University of Ioannina

Chapter 6 : Scientific outputs

This work has been published in three (3) peer-reviewed scientific journals, presented orally in four (4) national congresses, as well as in six (6) world, international and national congresses as poster presentations.

6.1 Published manuscripts

Magklaras, G., Skoufos, I., Bonos, E., Tsinas, A., Zacharis, C., Giavasis, I., Petrotos, K., Fotou, K., Nikolaou, K., Vasilopoulou, K., Giannenas, I., & Tzora, A. (2023). Innovative Use of Olive, Winery and Cheese Waste By-Products as Novel Ingredients in Weaned Pigs Nutrition. *Veterinary Sciences*, *10*(6), 397. https://doi.org/10.3390/vetsci10060397

Magklaras, G., Tzora, A., Bonos, E., Zacharis, C., Fotou, K., Wang, J., Grigoriadou, K., Giannenas, I., Jin, L., & Skoufos, I. (2025). Nutritional Use of Greek Medicinal Plants as Diet Mixtures for Weaned Pigs and Their Effects on Production, Health and Meat Quality. *Applied Sciences*, *15*(17), 9696. https://doi.org/10.3390/app15179696

Magklaras, G., Skoufos, I., Bonos, E., Zacharis, C., Nikolaou, K., Gouva, E., Giannenas, I., Giavasis, I., & Tzora, A. (2026). Sustainable Use of Agro-Industrial By-Products as Feed in Finishing Pigs. *Veterinary Sciences*, *13*(1), 39. https://doi.org/10.3390/vetsci13010039

6.2 Oral presentations

Georgios Magklaras, Ioannis Skoufos, Christos Zacharis, Ioannis Giavasis, Anastasios Tsinas, Athina Tzora, Eleftherios Bonos, Ilias Giannenas. Effect of dietary use of innovative silage on health parameters and meat quality characteristics of weaned piglets. 7th Panhellenic Conference on Meat and Meat Products, Thessaloniki, February 3–5, 2023.

Georgios Magklaras, Ioannis Skoufos, Christos Zacharis, Ioannis Giavasis, Eleftherios Bonos, Chrysoula (Chrysa) Voidarou, Konstantina Nikolaou, Ilias Giannenas, Athina Tzora. Effect of a

dietary novel silage on health parameters and meat quality characteristics of finishing pigs. 8th Panhellenic Conference on Meat and Meat Products, Thessaloniki, February 1–2, 2025.

Georgios Magklaras, Athina Tzora, Eleftherios Bonos, Christos Zacharis, Konstantina Fotou, Jing Wang, Katerina Grigoriadou, Ilias Giannenas, Lizhi Jin, Ioannis Skoufos. Dietary Use of a Mixture of Greek Aromatic/Medicinal Plants in Diets of Weaned Piglets. 39th Annual Scientific Conference of Hellenic Society of Animal Production (H.A.S.P.), Ioannina, October 15–17, 2025.

Georgios Magklaras, Ioannis Skoufos, Eleftherios Bonos, Christos Zacharis, Evagelia Gouva, Ilias Giannenas, Ioannis Giavasis, Athina Tzora. Alternative Feeds and Circular Economy: Effects of Using Agri-Food By-Products in Fattening Pigs. 16th Panhellenic Veterinary Conference, Thessaloniki, October 31–November 2, 2025.

6.3 Poster presentations

Georgios Magklaras, Ioannis Skoufos; Christos Zacharis, Kostas Petrotos, Ioannis Giavasis, Chrysanthi Mitsagga, Athina Tzora, Anastasios Tsinas, Ilias Giannenas, Angela Gabriella D'Alessandro, Eleftherios Bonos Effects of using olive, winery, and cheese waste by-products in the diet of weaned piglets on their performance parameters IPVS 26th international pig veterinary society congress, 21-24 June 2022, Rio de Janeiro, Brazil, proceedings p.316.

Georgios Magklaras, Chrysanthi Mitsagga, Ioannis Giavasis, Kostas Petrotos, Christos Zacharis, Athina Tzora, Evaggelia Gouva, Nikolaos Kasalias, Eleftherios Bonos, Ioannis Skoufos. Effects on meat quality characteristics of 74-day-old piglets fed by products rich in polyphenols, beneficial bacteria, and antimicrobial peptides IPVS 26th international pig veterinary society congress, 21-24 June 2022, Rio de Janeiro, Brazil, proceedings p.317

Georgios Magklaras, Eleftherios Bonos, Christos Zacharis, Kostas Petrotos, Ioannis Giavasis, Athina Tzora, Anastasios Tsinas, Ilias Giannenas, Angela Gardinali, Ioannis Skoufos . Sustainable use of agro-industrial by-products as feed in finishing pigs' diets IPVS 26th

international pig veterinary society congress, 21-24 June 2022, Rio de Janeiro, Brazil, proceedings p.334

Georgios Magklaras, Athina Tzora, Ioannis Skoufos, Chrysanthi Mitsagga, Kostas Petrotos, Christos Zacharis, Evaggelia Gouva, Nikolaos Kasalias, Eleftherios Bonos, Ioannis Giavasis Microbiological data of meat of weaned piglets fed a silage containing cheese whey, grape pomace, and olive oil wastewater, IPVS 26th international pig veterinary society congress, 21-24 June 2022, Rio de Janeiro, Brazil, proceedings p.127

Georgios Magklaras, Konstantina Fotou, Eleftherios Bonos, Christos Zacharis, Ilias Giannenas, Jing Wang, Katerina Grigoriadou, L.Z. Jin, Anastasios Tsinas, Athina Tzora, Ioannis Skoufos. Effect of dietary use of specific mixtures of Greek aromatic medicinal plants on zootechnical parameters, meat quality, and health indicators of weaned piglets, 15th Panhellenic Veterinary Congress, Athens, November 4–6, 2022.

Georgios Magklaras, Christos Zacharis, Konstantina Fotou, Eleftherios Bonos, Ilias Giannenas, Jing Wang, Lizhi Jin, Athina Tzora and Ioannis Skoufos. Effects of Greek aromatic/medicinal plants on health and meat quality characteristics of piglets, 74th EAAP Annual Meeting, Lyon, France, 26th August – 1st September 2023.