74th EAAP ANNUAL MEETING
LYON France 2023
August 26th / September 1st, 2023

EAAP European Federation of Animal Science

UNIVERSITY of IOANNINA

ARISTOTLE UNIVERSITY OF THESSALONIKI

Effects of dietary *Tenebrio molitor* meal and chitosan on health and meat quality of weaned piglets

C. Zacharis[1], E. Bonos[1], A. Tzora[1], I. Skoufos[1], G. Magklaras[1], I. Giavasis[2], I. Giannenas[3], E. Antonopoulou[4], C. Athanasiou[5], A. Tsinas[1]

[1] Department of Agriculture, University of Ioannina, Arta, Greece.

[2] Department of Food Science and Nutrition, University of Thessaly, Karditsa, Greece.

[3] School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece.

[4] Department of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece.

[5] Department of Agriculture, Plant Production and Rural Development, University of Thessaly, Volos, Greece.

Δράση ΕΡΕΥΝΩ-ΔΗΜΙΟΥΡΓΩ-ΚΑΙΝΟΤΟΜΩ
Κωδικός έργου:Τ2ΕΔΚ-02356
InsectFeedAroma

Ευρωπαϊκή Ένωση
Ευρωπαϊκό Ταμείο Περιφερειακής Ανάπτυξης
ΕΠΑνΕΚ 2014-2020
ΕΠΙΧΕΙΡΗΣΙΑΚΟ ΠΡΟΓΡΑΜΜΑ
ΑΝΤΑΓΩΝΙΣΤΙΚΟΤΗΤΑ ΕΠΙΧΕΙΡΗΜΑΤΙΚΟΤΗΤΑ ΚΑΙΝΟΤΟΜΙΑ
ΓΓΕΚ
ΓΕΝΙΚΗ ΓΡΑΜΜΑΤΕΙΑ ΕΡΕΥΝΑΣ ΚΑΙ ΚΑΙΝΟΤΟΜΙΑΣ
ΕΣΠΑ 2014-2020
ανάπτυξη - εργασία - αλληλεγγύη

Introduction (1)

- **Soybean meal** is the most frequently used protein source for pig compound feeds
  - Globally, the land availability for soya cultivation is limited
  - In recent years, the high price of soybean meal has become a serious issue for the economic sustainability of the production
- **Fishmeal** and other **processed animal proteins** have been used extensively in compound feeds
  - However, because of the European regulations, the use of processed animal proteins in pig diets is prohibited due to the transmissible spongiform encephalopathy (TSE) legislation
  - Moreover, marine overexploitation has reduced the abundance of small pelagic forage fish from which fish meal and fish oil are derived
- For these reasons, the **search for new feed material** with high nutritional value for animal consumption is of fundamental importance

Introduction (2)

- Another major problem of the **pork industry** today is the **post weaning stress**
- The period of weaning exposes growing pigs to sudden shifts in their **environment**, **behavior**, and **diet**
- This transition can lead to various negative outcomes, including **gastrointestinal issues**, dysfunctions in **the immune system**, and imbalances in the **endocrine system**
- These effects can be observed as **diarrhea**, increased vulnerability to **illnesses**, increased **morbidity**, and **mortality**, as well as **inhibited growth** and **overall health**
- Use of **natural bioactive compounds** such as **prebiotics** can address problems arising from **post weaning stress**

The role of insects as feed material

- Currently, **insects** are being considered as a **new protein source** for animal feed
- Use of insects for feeding farmed animals represents a **promising alternative** because of the nutritional properties of insects and the possible environmental benefits
- Insects are a part of the **natural diet** of several animal species and especially of monogastric farm animals
  - They can meet the nutritional requirements of monogastric animal due to the amino acid profile that they have
- 22 insect species have **similar crude protein** to that of fishmeal
- The body and especially the exoskeleton of some insects is rich in **substances with prebiotic** activity like chitin and chitosan

Rearing of insects

The **mass production** of insect could potentially offer a lot of benefits:

- Ability to feed on waste biomass and transform this into high value food and feed resource
- Grow and reproduce easily
- High feed conversion ratio
- Small land area is needed to produce 1 kg of protein
- Ability to convert side streams into high–value protein products
- Low emission levels of greenhouse gases

Insect larvae

Mealworm beetle - *Tenebrio molitor*

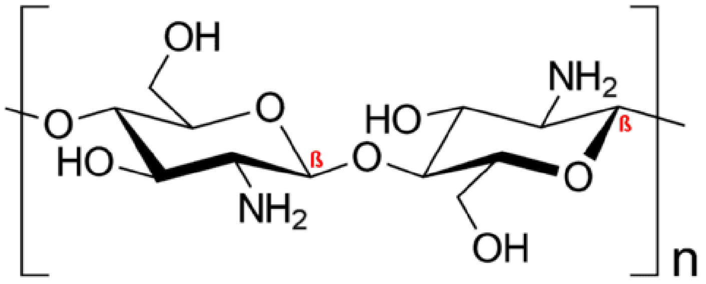
- Acceptable protein source for diets of monogastric animals
- Can be fed and reared easily
- Are omnivorous and can eat all kinds of plant materials as well as animal products such as meat and feathers
- The nutritional value of the larvae is comparable to that of fishmeal
  - Crude protein: 44% - 69% (DM basis)
  - Crude fat: 23% - 47% (DM basis)

The role of chitosan

- Chitosan, derived from chitin, is a biopolymer with intriguing potential in various fields, including agriculture

- Incorporating chitosan into pig diets has been explored for several reasons:

    - Growth promotion
    - Immune stimulation
    - Mycotoxin binding
    - Antimicrobial effects
    - Environmental impact



Structure of completely deacetylated chitosan
(https://en.wikipedia.org/wiki/Chitosan#/media/File:Chitosan_chair.png)

Our objectives

The objectives of the experimental study were to examine the dietary use of *Tenebrio molitor* larvae and chitosan on growth performance, health status and meat quality parameters of growing pigs:

1. Dried *T. molitor larvae*

2. Chitosan (low molecular weight: 50,000; ≥ 90% purity)

Dried *T. molitor* larvae

Low molecular weight chitosan

Material and methods (1)

- Forty-eight 34-days-old healthy crossbreed weaned pigs (¼ Large White, ¼ Landrace, and ½ Duroc) were selected from a commercial pig farm located in the region of Epirus, Greece

- Each pig was uniquely identified using ear tags

- The feeding trial lasted 42 days

- The pigs were randomly allocated into four distinct groups and placed in separate pens with slatted plastic floors:

  - Group A: Control group (commercial maize-barley-based diet)

  - Group B: *T. molitor* meal (10%) addition in the diet

  - Group C: Chitosan (0.5%) addition in the diet

  - Group D: Combined *T. molitor* meal (10%) and chitosan (0.5%) addition in the diet

Diets

Ingredients, g/kg as fed	Group A	Group B	Group C	Group D
Maize	336.0	205.5	335.5	205.0
Barley	347.0	347.0	347.0	347.0
Wheat middlings	30.0	30.0	30.0	30.0
Soybean meal (47% Crude protein)	168.0	188.8	168.0	188.8
Soybean oil	19.0	54.8	19.0	54.8
Vitamin and mineral premix	60.0	60.0	60.0	60.0
Fishmeal (72% Crude protein)	30.0	0.0	30.0	0.0
T. molitor meal	0.0	100.0	0.0	100.0
Benzoic acid	3.0	3.0	3.0	3.0
Zn oxide	3.0	3.0	3.0	3.0
Salt	2.0	2.0	2.0	2.0
Monocalcium phosphate (22% P)	2.0	6.0	2.0	6.0
Chitosan	0.0	0.0	0.5	0.5
Calculated analysis, g/kg as fed				
Digestible energy (DE, MJ/kg)	13.6	13.6	13.6	13.6
Crude protein	186.6	186.5	186.6	186.5
Crude fiber	34.5	34.9	34.5	34.9
Ether extract	39.4	79.0	39.4	79.0
Lysine	12.3	12.2	12.3	12.2
Methionine + Cystine	7.7	7.4	7.7	7.4
Methionine	4.9	4.6	4.9	4.6
Cystine	2.8	2.8	2.8	2.8
Threonine	6.2	6.5	6.2	6.5
Calcium	5.6	5.5	5.6	5.5
Total phosphorus	5.0	5.3	5.0	5.3

Preparation of diets

Pens with slated floors

Material and methods (2)

- Throughout the experimental period, all pigs were weighed individually on the 1st, 21st, and 42nd days
- Daily records were maintained for feed intake
- Evaluated parameters:
  - Weight gain (per pig)
  - Average feed intake (per group)
  - Average feed conversion ratio (per group)
- On the final day of the dietary trial, six pigs were randomly selected from each group and transferred to a nearby commercial abattoir for processing

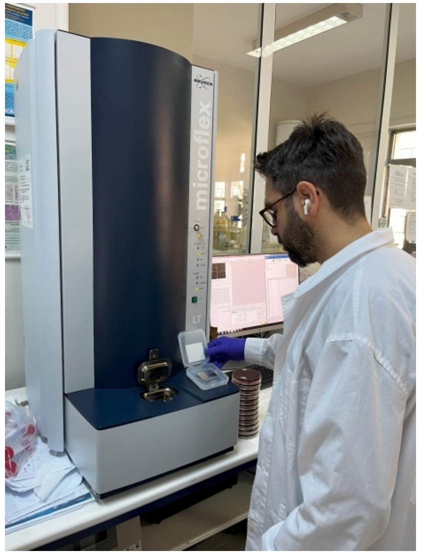
Pig pens

Weighting cage

Analysis of fecal microbiota

On the last day of the 42-day trial, fresh fecal (stool) samples were collected from each pig for microbial analysis:

- Use of culture selective media (Agar)
  - *Escherichia coli*
  - Enterococci
  - Lactobacilli
  - Total aerobes
  - Total anaerobes
- Identification with a **MALDI-TOF MS Biotyper mass spectrometer** (Bruker Daltonics)

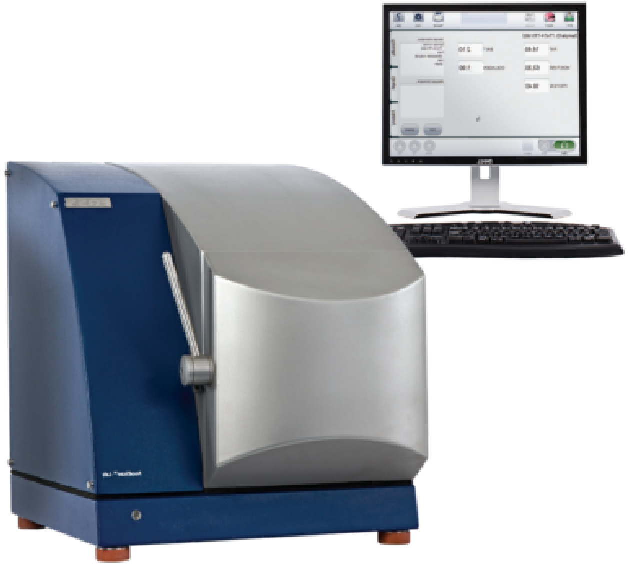


Sampling of meat cuts

- From each pig carcass the following meat samples were selected:
    - Ham meat sample (*Biceps femoris* and *semimebranosus*)
    - Pancetta meat sample (*External abdominal* και *oblique*)
    - Shoulder meat sample (*Trapezius* and *Triceps branchi)*
    - Boneless steak meat sample (*M. longissimus thoracis et lumborum*)

Meat chemical analysis

- Meat samples of 200 g
- NIR FoodScan Pro/Lab (Type 78800, FOSS, Denmark)



Meat microbial populations

- Use of culture selective media (Agar)
  - Total microbes
  - *Escherichia coli*
  - *Clostridium* spp.
  - *Campylobacter jejuni*
  - *Staphylococcus* spp.
  - *Staphylococcus aureus*

Petri dish (agar)

Meat total phenols

- Use of *Folin – Ciocalteu* method (Vasilopoulos et al. 2023)
- Spectrophotometer UV – Vis (DR 5000, Hach Lange)

Meat oxidative stability (TBARS method)

- 2-Thiobarbituric acid method (**TBARS,** thiobarbituric acid reactive substances) (Dias et al. 2020)

- Spectrophotometer Shimadzu UV-1700

Statistical analysis

- The experimental units were the replications (pens)
- Statistical tests:
  - Two-way ANOVA (Analysis of Variance)
    - ✓Supplementation of insect meal
    - ✓Supplementation of chitosan
    - ✓Interaction
  - Tukey's test (post-hoc)
- Significance level of 5% ($P \leq 0.05$)
- Software: IBM SPSS Statistics (Ver. 20)

Results

Effect of dietary *Tenebrio molitor* meal and chitosan supplementation on performance and carcass parameters of pigs

	Treatments					Effect of *T. molitor* meal	Effect of chitosan	Interaction
Body weight on day (kg)	**Group A**	**Group B**	**Group C**	**Group D**	**SEM**	**P**	**P**	**P**
1	8.41	8.51	8.31	8.31	0.114	0.831	0.525	0.833
21	14.77	16.86	15.15	17.46	0.277	<0.001 ⬆	0.298	0.814
42	24.86^{ab}	24.98^{ab}	23.12^{a}	26.96^{b}	0.412	0.011 ⬆	0.875	0.017
Weight gain for period (kg)								
1 to 21 days	6.36	8.35	6.84	9.15	0.231	<0.001 ⬆	0.066	0.640
21 to 42 days	10.09 ^{b}	8.13 ^{ab}	7.97 ^{a}	9.61 ^{ab}	0.368	0.819	0.654	0.014
1 to 42 days	16.45 ^{a}	16.48 ^{ab}	14.81 ^{a}	18.63 ^{b}	0.417	0.015 ⬆	0.739	0.017
Feed intake per pig for period (kg)								
1 to 21 days	14.56	14.02	13.53	13.87	-	-	-	-
21 to 42 days	21.19	20.46	19.65	20.25	-	-	-	-
1 to 42 days	35.75	34.48	33.18	34.12	-	-	-	-
Feed conversion ratio (FCR) for period (kg feed / kg weight gain)								
1 to 21 days	2.29	1.68	1.98	1.52	-	-	-	-
21 to 42 days	2.10	2.52	2.47	2.30	-	-	-	-
1 to 42 days	2.17	2.09	2.24	1.82	-	-	-	-
Carcass parameters								
Carcass weight (kg)	14.94	15.66	16.72	18.26	0.538	0.272	0.041 ⬆	0.685
Carcass dressing percentage (%)	0.63	0.63	0.74	0.67	0.022	0.420	0.103	0.394

Group A, commercial diet; Group B, diet containing 10% *T. molitor* meal; Group C, diet containing 0.5% chitosan; Group D, diet containing 10% *T. molitor* meal and 0.5% chitosan.

^{a,b} Means (n = 6 per treatment) with no common superscript differ significantly (Tukey's test, p ≤ 0.05).

Effect of dietary *Tenebrio molitor* meal and chitosan supplementation on fecal microflora populations of pigs

	Treatments					Effect of *T. molitor* meal	Effect of chitosan	Interaction
Day 42 (Log_{10} CFU/g)	**Group A**	**Group B**	**Group C**	**Group D**	**SEM**	**P**	**P**	**P**
Escherichia coli	6.46	6.90	6.08	5.89	0.141	0.642	0.014 ↓	0.251
Enterococci	4.06	4.06	3.87	3.88	0.098	0.982	0.413	0.997
Lactobacilli	8.12^{ab}	6.96^{a}	7.44^{ab}	8.60^{b}	0.228	0.988	0.275	0.010
Total aerobes	8.34^{c}	8.63^{c}	7.43^{b}	6.64^{a}	0.143	0.128	<0.001 ↓	0.002
Total anaerobes	8.56	8.74	8.93	9.23	0.100	0.226	0.031 ↑	0.744

Group A, commercial diet; Group B, diet containing 10% *T. molitor* meal; Group C, diet containing 0.5% chitosan; Group D, diet containing 10% *T. molitor* meal and 0.5% chitosan.

^{a,b} Means (n = 6 per treatment) with no common superscript differ significantly (Tukey's test, $p \leq 0.05$).

Effect of dietary *Tenebrio molitor* meal and chitosan supplementation on microbial populations of pig meat

	Treatments					Effect of *T. molitor* meal	Effect of chitosan	Interaction
Shoulder meat (Log_{10} CFU/g)	**Group A**	**Group B**	**Group C**	**Group D**	**SEM**	**P**	**P**	**P**
Total microbes	5.82	5.11	5.15	5.51	0.157	0.584	0.674	0.101
Escherichia col	4.27	2.44	3.95	2.60	0.227	<0.001 ↓	0.780	0.413
Clostridium spp.	3.24	2.01	2.32	2.38	0.191	0.112	0.441	0.081
Campylobacter jejuni	3.44	2.31	3.13	2.47	0.162	0.004 ↓	0.738	0.390
Staphylococcus spp.	4.80	4.61	4.27	4.21	0.099	0.496	0.019 ↓	0.700
Staphylococcus aureus	2.60	2.46	1.96	2.63	0.121	0.263	0.309	0.088
Pancetta meat (Log_{10} CFU/g)								
Total microbes	6.04	6.21	5.92	6.03	0.149	0.676	0.649	0.932
Escherichia coli	4.31	3.37	3.62	3.17	0.212	0.110	0.290	0.560
Clostridium spp.	3.24	2.01	2.32	2.38	0.191	0.112	0.441	0.081
Campylobacter jejuni	3.41	2.98	2.84	3.25	0.155	0.982	0.651	0.213
Staphylococcus spp.	4.17	4.09	4.15	3.61	0.753	0.384	0.478	0.514
Staphylococcus aureus	2.40	2.46	2.22	1.93	0.134	0.683	0.211	0.528
Boneless steak meat (Log_{10} CFU/g)								
Total microbes	4.35	4.34	4.12	3.90	0.097	0.555	0.091	0.584
Escherichia coli	2.74	2.08	1.89	1.58	0.186	0.178	0.066	0.625
Clostridium spp.	1.45	1.47	1.53	1.63	0.070	0.692	0.432	0.770
Campylobacter jejuni	3.32	2.98	2.85	2.59	0.158	0.361	0.192	0.897
Staphylococcus spp.	3.09	2.32	3.07	2.47	0.173	0.059	0.848	0.811
Staphylococcus aureus	2.91	2.11	2.06	1.80	0.228	0.254	0.221	0.562

Group A, commercial diet; Group B, diet containing 10% *T. molitor* meal; Group C, diet containing 0.5% chitosan; Group D, diet containing 10% *T. molitor* meal and 0.5% chitosan.

Effect of dietary *Tenebrio molitor* meal and chitosan supplementation on chemical composition of pig meat

	Treatments					Effect of *T. molitor* meal	Effect of chitosan	Interaction
Ham meat (%)	**Group A**	**Group B**	**Group C**	**Group D**	**SEM**	**P**	**P**	**P**
Fat	2.64	3.2	2.94	3.06	0.156	0.310	0.813	0.504
Protein	19.56	20.06	19.82	20.07	0.115	0.118	0.550	0.576
Moisture	76.89	76.09	76.93	76.32	0.137	0.009 ⬇	0.586	0.692
Collagen	1.02	0.89	1.11	1.03	0.038	0.153	0.133	0.715
Ash	0.98	0.97	1.03	1.04	0.026	0.958	0.322	0.791
Boneless steak meat (%)								
Fat	3.18	2.57	2.75	2.98	0.121	0.434	0.967	0.097
Protein	19.80	20.61	20.34	20.47	0.140	0.094	0.463	0.210
Moisture	75.97	76.05	76.22	75.79	0.119	0.491	0.984	0.313
Collagen	1.17	1.08	1.20	1.27	0.039	0.857	0.178	0.301
Ash	1.05	0.98	0.96	0.93	0.019	0.153	0.041 ⬇	0.519
Shoulder meat (%)								
Fat	5.22	5.50	5.41	5.91	0.169	0.275	0.395	0.745
Protein	18.43	18.21	18.42	18.32	0.068	0.261	0.713	0.683
Moisture	75.56	75.55	75.42	75.17	0.149	0.692	0.419	0.715
Collagen	1.31	1.33	1.21	1.10	0.046	0.594	0.078	0.452
Ash	0.97	0.90	0.93	0.94	0.020	0.437	0.963	0.411
Pancetta meat (%)								
Fat	9.87	8.61	8.61	8.99	0.274	0.424	0.430	0.149
Protein	16.93	17.55	17.33	17.49	0.175	0.300	0.646	0.542
Moisture	72.27	72.89	73.09	72.84	0.208	0.670	0.382	0.322
Collagen	1.66	1.67	1.62	1.50	0.062	0.706	0.427	0.608
Ash	1.00	0.90	0.93	0.81	0.024	0.015 ⬇	0.064	0.922

Group A, commercial diet; Group B, diet containing 10% *T. molitor* meal; Group C, diet containing 0.5% chitosan; Group D, diet containing 10% *T. molitor* meal and 0.5% chitosan.

Effect of dietary *Tenebrio molitor* meal and chitosan supplementation on oxidative stability of pig meat

	Treatments					Effect of *T. molitor* meal	Effect of chitosan	Interaction
Total phenols (g/L)	**Group A**	**Group B**	**Group C**	**Group D**	**SEM**	**P**	**P**	**P**
Shoulder meat	1.96	5.31	4.54	5.27	0.448	0.010 ↑	0.088	0.079
Pancetta meat	1.83	2.04	2.36	2.38	0.088	0.463	0.013 ↑	0.535
Boneless steak meat	3.54	5.25	4.34	4.18	0.283	0.169	0.809	0.100
TBARS (mg MDA/kg)								
Shoulder meat	0.06	0.03	0.03	0.03	0.004	0.015 ↓	0.018 ↓	0.071
Pancetta meat	0.05	0.05	0.05	0.05	0.001	0.498	0.506	0.733
Boneless steak meat	0.11	0.13	0.11	0.13	0.003	0.002 ↑	0.379	0.499

Group A, commercial diet; Group B, diet containing 10% *T. molitor* meal; Group C, diet containing 0.5% chitosan; Group D, diet containing 10% *T. molitor* meal and 0.5% chitosan.

CONCLUSION

- According to our knowledge it was the first time that a combined use of *T. molitor* meal and chitosan was carried out on weaned pig diets

- Based on the results of this feeding trial, the combined use of *T. molitor* meal and chitosan improved growing pigs' performance, gut microbiota and meat quality parameters

- Further research is necessary to assess the optimal use of *T. molitor* meal and chitosan in feed formulations

Acknowledgments

This research has been co-financed by Greece and the European Union (European Regional Development Fund) in context “Research – Create – Innovate” within the Operational Program (Competitiveness, Entrepreneurship and Innovation (ΕΠΑΝΕΚ) of the NSRF 2014-2020.

Project Code: **Τ2ΕΔΚ-02356**

Acronym: **InsectFeedAroma**

Thank you for your attention

The Historical Bridge of Arta, Greece