FACULTAD DE CIENCIAS AGRARIAS Y AMBIENTALES
DEPARTAMENTO DE CONSTRUCCIÓN Y AGRONOMÍA

Efecto de la harina de *Tenebrio molitor* y *Acheta domesticus* sobre los rendimientos productivos y la calidad de carne de pollos de engorde de crecimiento lento

.....

Effect of *Tenebrio molitor* and *Acheta domesticus* meal on the growth performance and meat quality of slow-growing chickens

TESIS DOCTORAL

Programa de Doctorado en Ciencia y Tecnología Químicas

Jaime Nieto de la Losa

Salamanca, España 2024

D/Dª. Jaime Nieto de la Losa, con DNI 70827216 G, matriculado en el Programa de Doctorado en Ciencia y Tecnología Químicas de la Universidad de Salamanca,

EXPONE

Según el Reglamento de Doctorado de la Universidad de Salamanca para la presentación de Tesis Doctoral en la modalidad como **compendio de artículos/publicaciones**,

SOLICITA

La autorización de la Comisión Académica para la presentación de la Tesis Doctoral titulada **"Efecto de la harina de *Tenebrio molitor* y *Acheta domesticus* sobre los rendimientos productivos y la calidad de carne de pollos de engorde de crecimiento lento"** en el formato como compendio de artículos/publicaciones (Comisión de Doctorado y Posgrado, 15 de febrero de 2013).

Los siguientes artículos han sido publicados en revistas internacionales de alto impacto revisadas por pares e indexadas en JCR (*Journal Citation Reports*) de la *Web of Science*. En ellos el doctorando Jaime Nieto de la Losa aparece como el primer autor en todos ellos. Las publicaciones aportadas a la Tesis son:

1. **Performance of Slow-Growing Chickens Fed with *Tenebrio molitor* Larval Meal as a Full Replacement for Soybean Meal.**

   Jaime Nieto[1], Javier Plaza[1], Javier Lara[2], José-Alfonso Abecia[3], Isabel Revilla[4], Carlos Palacios[1]

   [1] Area of Animal Production, Faculty of Environmental and Agrarian Sciences, University of Salamanca, Avenida Filiberto Villalobos 119-129, 37007 Salamanca, Spain.

   [2] Animal Husbandry and Animal Health Assistance, High-School Torres Villarroel, Avenida Hilario Goyenechea, 44, 37008 Salamanca, Spain.

[3] Institute of Research in Environmental Sciences of Aragón (IUCA), University of Zaragoza, Miguel Servet, 177, 50013 Zaragoza, Spain.

[4] Area of Food Technology, E.P.S. of Zamora, University of Salamanca, Avenida Requejo 33, 49022 Zamora, Spain.

Revista: Veterinary Sciences. 2022, 9(3), 131; https://doi.org/10.3390/vetsci9030131

Referencia: Nieto, J., Plaza, J., Lara, J., Abecia, J.-A., Revilla, I., & Palacios, C. (2022). Performance of Slow-Growing Chickens Fed with *Tenebrio molitor* Larval Meal as a Full Replacement for Soybean Meal. Veterinary Sciences, 9(3), 131. https://doi.org/10.3390/vetsci9030131

Índices de calidad de la revista:

- ISSN: N/A
- EISSN: 2306-7381
- Journal Impact Factor (2022): 2.4
- Journal Citation Indicator (2022): 0.97
- Category: VETERINARY SCIENCES
- JCR 2022. JIF RANK 29/144. JIF QUARTILE: Q1. JIF PERCENTILE: 80.2

2. Use of *Acheta domesticus* meal as a full soybean substitute in the feeding of slow-growing chicks.

Jaime Nieto[1], Javier Plaza[1], Javier Lara[2], José-Alfonso Abecia[3], Isabel Revilla[4], Carlos Palacios[1]

[1] Area of Animal Production, Faculty of Environmental and Agricultural Sciences, University of Salamanca, 37007 Salamanca, Spain.

[2] Animal Husbandry and Animal Health Assistance Educational Program, High-School Torres Villarroel, 37008 Salamanca, Spain.

[3] Institute of Research in Environmental Sciences of Aragón (IUCA), University of Zaragoza, 50013 Zaragoza, Spain.

[4] Area of Food Technology, E.P.S. of Zamora, University of Salamanca, 49022 Zamora, Spain.

Revista: Poultry Science. 2023, 102(4), 102503; https://doi.org/10.1016/j.psj.2023.102503

Referencia: Nieto, J., Plaza, J., Lara, J., Abecia, J.-A., Revilla, I., & Palacios, C. (2023). Use of *Acheta domesticus* meal as a full soybean substitute in the feeding of slow-growing chicks. Poultry Science, 102(4), 102503. https://doi.org/10.1016/j.psj.2023.102503

Índices de calidad de la revista:

- ISSN: 0032-5791
- EISSN: 1525-3171
- Journal Impact Factor (2022): 4.4
- Journal Citation Indicator (2022): 1.84
- Category: AGRICULTURE, DAIRY & ANIMAL SCIENCE
- JCR 2022. JIF RANK 4/62. JIF QUARTILE: Q1. JIF PERCENTILE: 94.4

3. **Carcass traits and meat quality assessment of two slow-growing chicks strains fed *Acheta domesticus* larval meal.**

Jaime Nieto[1], Javier Plaza[1], Miriam Hernández-Jiménez[2], Isabel Revilla[2], Carlos Palacios[1]

[1] Area of Animal Production, Faculty of Environmental and Agricultural Sciences, University of Salamanca, Salamanca 37007, Spain.

[2] Area of Food Technology, E.P.S. of Zamora, University of Salamanca, Zamora 49022, Spain.

Revista: Poultry Science. 2024, 103(6), 103684 https://doi.org/10.1016/j.psj.2024.103684

Referencia: Nieto, J., Plaza, J., Hernández-Jiménez, M., Revilla, I., & Palacios, C. (2024). Carcass traits and meat quality assessment of two slow-growing chicks strains fed *Acheta domesticus* larval meal. Poultry Science, 103(6), 103684. https://doi.org/10.1016/j.psj.2024.103684

Índices de calidad de la revista:

- ISSN: 0032-5791
- EISSN: 1525-3171
- Journal Impact Factor (2022): 4.4
- Journal Citation Indicator (2022): 1.84
- Category: AGRICULTURE, DAIRY & ANIMAL SCIENCE
- JCR 2022. JIF RANK 4/62. JIF QUARTILE: Q1. JIF PERCENTILE: 94.4

4. **Substitution of soybean meal for yellow mealworm meal in the diet of slow-growing chickens provides comparable carcass traits and meat quality**

Jaime Nieto[1], Javier Plaza[1], Miriam Hernández-Jiménez[2], Isabel Revilla[2], Carlos Palacios[1]

[1] Area of Animal Production, University of Salamanca, Avenida Filiberto Villalobos 119, Faculty of Agricultural and Environmental Sciences, 37007, Salamanca, Spain.

[2] Area of Food Technology, University of Salamanca, Avenida Requejo 33, Superior Polytechnic School of Zamora, 49022, Zamora, Spain.

Revista: British Poultry Science. 2024, Accepted: 26 May 2024.

Índices de calidad de la revista:

- ISSN: 0007-1668
- EISSN: 1466-1799
- Journal Impact Factor (2022): 2.0
- Journal Citation Indicator (2022): 0.79
- Category: AGRICULTURE, DAIRY & ANIMAL SCIENCE
- JCR 2022. JIF RANK 24/62. JIF QUARTILE: Q2. JIF PERCENTILE: 62.1

Salamanca, a fecha de firma electrónica.

[redacted]

NIETO DE LA LOSA JAIME - [redacted]

Firmado digitalmente por NIETO DE LA LOSA JAIME - [redacted]
Fecha: 2024.06.14 12:54:42 +02'00'

Copyright © 2024 por Jaime Nieto de la Losa

Reservados todos los derechos. No se permite la reproducción total o parcial de esta obra, ni su incorporación a un sistema informático, ni su transmisión en cualquier forma o por cualquier medio (electrónico, mecánico, fotocopia, grabación u otros) sin autorización previa y por escrito del autor (jaimenl@usal.es; jaimenietodelalosa93@gmail.com).

Dña. Isabel Revilla Martín, Catedrática de Universidad del Área de Tecnología de Alimentos perteneciente al Departamento de Construcción y Agronomía de la Universidad de Salamanca.

D. Carlos Palacios Riocerezo, Profesor Titular del Área de Producción Animal del Área perteneciente al Departamento de Construcción y Agronomía de la Universidad de Salamanca.

HACEN CONSTAR QUE:

La Tesis Doctoral titulada "**Efecto de la harina de *Tenebrio molitor* y *Acheta domesticus* sobre los rendimientos productivos y la calidad de carne de pollos de engorde de crecimiento lento"**, ha sido realizada por D. Jaime Nieto de la Losa bajo nuestra dirección para optar al título de doctor por la Universidad de Salamanca en el programa de doctorado de Ciencia y Tecnología Químicas.

Cumpliéndose todos los requisitos recogidos en la normativa correspondiente, damos nuestra conformidad para que sea presentada y defendida bajo el formato de Compendio de Artículos.

Y para que conste, firmamos el presente certificado en Salamanca, a fecha de firma electrónica.

REVILLA MARTIN ISABEL - [redacted]
Firmado digitalmente por REVILLA MARTIN ISABEL - [redacted]
Fecha: 2024.06.14 16:03:37 +02'00'

Fdo: Dña. Isabel Revilla Martín

PALACIOS RIOCEREZO CARLOS - [redacted]
Firmado digitalmente por PALACIOS RIOCEREZO CARLOS [redacted]
Fecha: 2024.06.14 17:24:51 +02'00'

Fdo: D. Carlos Palacios Riocerezo

Dña. Encarnación Rodríguez Gonzalo, Coordinadora del programa de Doctorado en Ciencia y Tecnología Químicas.

HACE CONSTAR QUE:

La Tesis Doctoral titulada "**Efecto de la harina de *Tenebrio molitor* y *Acheta domesticus* sobre los rendimientos productivos y la calidad de carne de pollos de engorde de crecimiento lento"**, ha sido realizada por D. Jaime Nieto de la Losa bajo en el programa de doctorado de Ciencia y Tecnología Químicas.

A sí mismo, autoriza la presentación de dicha Tesis Doctoral por compendio de artículos solicitada por D. Jaime Nieto de la Losa cumpliendo los requisitos exigidos por la Universidad de Salamanca *"Procedimiento para la presentación de la Tesis Doctoral en la Universidad de Salamanca en el formato de compendio de artículos/publicaciones, aprobado por la Comisión de Doctorado y Postgrado, 15 de febrero de 2013"* para optar al grado de Doctor por dicha Universidad.

Y para que conste, firmo el presente documento en Salamanca, a fecha de firma electrónica.

RODRIGUEZ GONZALO ENCARNACION - [redacted]

Firmado digitalmente por RODRIGUEZ GONZALO ENCARNACION - [redacted]
Fecha: 2024.06.18 13:46:23 +02'00'

Fdo: Dña. Encarnación Rodríguez Gonzalo

COORDINADORA DEL PROGRAMA DE DOCTORADO

A mi familia,

por su apoyo incondicional durante todo mi camino

Agradecimientos

Llega el momento de expresar mi gratitud a todas aquellas que han formado parte de esta gran etapa de mi vida: la realización de mi Tesis Doctoral. En primer lugar, quiero dar las gracias a mis directores de tesis Isabel y Carlos, no solo por la dirección de esta memoria, sino por muchos otros logros académicos que sin ellos habría sido imposible conseguir. Siento una gran admiración por ellos; Carlos con su toque de locura complementado con la cautela de Isabel; sin duda el tándem perfecto.

Carlos, fuiste quien me dio la oportunidad de comenzar en este mundo y me permitiste ver y disfrutar la Producción Animal desde otro punto de vista. Muchas horas compartidas (también de risas), han creado un vínculo que va más allá de la relación entre un director y compañero de trabajo.

Dar las gracias también a mi "socio" Javier Plaza, con quien he compartido casi todas las etapas de este proceso, ya no solo es un compañero sino también un amigo. Espero que podamos seguir aunando esfuerzos por mucho tiempo.

No me olvido del resto de compañeros y profesores, como son los docentes del Grado Superior de Ganadería y Asistencia en Sanidad Animal de Salamanca Toñi, Elena y Javier, que han colaborado conmigo en la cría de los pollitos o las compañeras de Agroalimentarias de Zamora, haciendo que los trabajos en el laboratorio estuvieran bajo control.

La mención especial de estas palabras va dedicada a mi familia: Papá, Mamá y Diego. Ellos son los que siempre me han apoyado en esta batalla, sin oponerse a todas las "locuras" que hacía en mis ensayos. Mi madre, la más pendiente, con su… Hijo, ¿Cómo vas con lo tuyo?, me trasmitía su preocupación y su interés, dándome fuerzas para seguir adelante.

No puedo olvidarme de mis abuelos. Tengo la gran suerte de contar con mi abuela, quien me transmitió el cariño por la avicultura. Y a mis otros abuelos, que me ven desde allí arriba, especialmente a ella, que se marchó cuando todo esto empezaba a despegar. Estoy seguro de que me estás viendo; esta Tesis también es para ti.

Agradezco también al resto de mi familia, incluida mi familia política, que ha estado tan pendiente de mi como el resto. También agradezco a Jacin y Sonso, a las que incluyo en la familia.

Por último, y no por ello menos importante, quiero expresar mi inmensa gratitud a mi compañera de vida, Susi. Ella ha sido quien más ha soportado los altibajos de este largo camino, sin dejar de apoyarme en ningún momento. Su gran sustento ha sido primordial para no desistir en este arduo recorrido, ofreciéndome apoyo tanto emocional como físico, estando al pie del cañón siempre que la he necesitado. Espero que la vida nos permita disfrutar de grandes logros en común.

ÍNDICE GENERAL / GENERAL INDEX

Siglas y Acrónimos / Acronyms and Abbreviations 25

Resumen 29

Abstract 33

Capítulo 1: Introducción 39

1.1. Hipótesis de partida 39

1.2 Objetivos 41

Capítulo 2: Revisión bibliográfica 45

2.1 Sistemas productivos en la avicultura de carne. Sistemas alternativos 45

2.2 Pollos de crecimiento lento 50

2.3 Problemática de las fuentes proteicas. 52

2.4 Uso de insectos como alternativas proteicas 54

2.5 Especies autorizadas 57

2.6 Factores productivos que afectan a la calidad de carne 63

Capítulo 3: Performance of Slow-Growing Chickens Fed with *Tenebrio molitor* Larval Meal as a Full Replacement for Soybean Meal 71

Resumen 71

Abstract 73

3.1 Introduction 74

3.2 Materials and Methods 75

3.3 Results 79

3.4 Discussion 84

3.5 Conclusions 87

Capítulo 4: Use of *Acheta domesticus* meal as a full soybean substitute in the feeding of slow-growing chicks 91

Resumen 91

Abstract 94
4.1 Introduction 95
4.2 Material and Methods 96
4.3 Results 101
4.4 Discussion 107
Capítulo 5: Carcass traits and meat quality assessment of two slow-growing chicks strains fed *Acheta domesticus* larval meal 113
Resumen 113
Abstract 116
5.1 Introduction 117
5.2 Material and Methods 118
5.3 Results and Discussion 128
Capítulo 6: Substitution of soybean meal for yellow mealworm meal in the diet of slow-growing chickens provides comparable carcass traits and meat quality 141
Resumen 141
Abstract 144
6.1 Introduction 145
6.2 Material and Methods 146
6.3 Results 154
6.4 Discussion 159
Capítulo 7: Conclusiones 167
Conclusions 169
Capítulo 8: Líneas futuras de investigación 173
Capítulo 9: Otras contribuciones científicas 177
Referencias bibliográficas 183

ÍNDICE DE FIGURAS / INDEX OF FIGURES

Figura 2.1. Pollos criados al aire libre. 45
Figura 2.2. Producción de carne de pollo de forma intensiva. 47
Figura 2.3. Pollos camperos. 48
Figura 2.4. Estirpes de crecimiento lento. 51
Figura 2.5. Larva de *Tenebrio molitor*..... 61
Figura 2.6. Grillo doméstico (*Acheta domesticus*) 62
Figure 3. 1. Graphical Abstract..... 73
Figure 3. 2. LW curves for the two studied groups (C, Control group and TM, Tenebrio group) throughout the growing period (95 days). (a) Group C and (b) group TM. LW: live weight, Max Dev.: maximum live weight deviation and Min. Dev.: minimum live weight deviation. 82
Figure 3. 3. BWG (Body Weight Gain) percentage increase in each studied group (C and TM) between two consecutive weeks throughout the whole study period. 83
Figure 3. 4. BWG percentage increase in the C group over the TM group throughout the whole study period. NS or **: non-significant or significant at $p < 0.01$, respectively. 83
Figure 3.5. FI percentage increase in the C group over the TM group throughout the whole study period. NS, * or **: non-significant or significant at $p < 0.05$ or 0.01, respectively……..…84

Figure 4.1. LBW curves for two groups of chicks fed diets that included soybean meal (group C) or *Acheta domesticus* insect meal (group AD) as the main protein source throughout the growing period (14 wk). Discontinuous lines reflect degree 2 polynomial trend lines……...105

Figure 4.2. LBW increase (%) for two groups of chicks fed diets that included soybean meal (group C) or *Acheta domesticus* insect meal (group AD) as the main protein source between two consecutive weeks over the entire studied period. 106
Figure 4. 3. BWG increase (%) for two groups of chicks fed diets that included soybean meal (group C) relative to the group fed *Acheta domesticus* insect meal (group AD) as the main protein source over the entire period studied. NS, * or **: non significant or significant at $P < 0.05$ or 0.01, respectively..... 106

Figure 5.1. NIRS spectra of poultry meat from the different experimental treatments of slow-growing chicks fed diets including either soybean meal (treatments R-C and NN-C) or *Acheta domesticus* insect meal (treatments R-AD and NN-AD) as the main protein source, and slaughtered at 95 d of age. R-C: Red Control group, NN-C: Naked Neck Control group, R-AD: Red *Acheta domesticus* group, NN-AD: Naked Neck *Acheta domesticus* group……...129

Figure 6.1. NIRS spectra of poultry meat from slow-growing chickens fed with different percentages of *Tenebrio molitor* meal inclusión…….159

ÍNDICE DE TABLAS / INDEX OF TABLES

Tabla 2. 1. Principales características nutricionales de las especies de insectos autorizadas en alimentación animal. 58

Table 3.1. Compositions of the experimental diets for the two studied groups of chickens expressed as percentages of raw matter……77

Table 3.2. Chemical/nutritional characteristics of the experimental diets for the two studied groups of chickens. 78

Table 3.3. Total production performance parameters of the two studied chicken groups (C and TM) for the three different diets (mean±SEM)..... 81

Table 3.4. Weekly production performance parameters of the two studied chicken groups (C and TM) for the three different diets (mean ± SEM)..... 81

Table 4.1. Composition of the experimental diets for two groups of chicks (% ingredients), fed diets including either soybean meal (group C) or A*cheta domesticus* insect meal (group AD) as the main protein source……...99

Table 4.2. Chemical/nutritional characteristics of the experimental diets for two groups of chicks fed diets including either soybean meal (group C) or A*cheta domesticus* insect meal (group AD) as the main protein source. 100

Table 4.3. Total production performance parameters of two chicken groups fed diets including either soybean meal (group C) or *Acheta domesticus* insect meal (group AD) as the main protein source for the three different diets offered (mean ± SEM[1]). 102

Table 4.4. Weekly production performance parameters of the chicken groups fed diets including either soybean meal (group C) or *Acheta domesticus* insect meal (group AD) as the main protein source. for the three different diets offered (mean ± SEM[1]). 104

Table 5.1. Composition of the diets for the four experimental treatments (g/Kg ingredients), fed diets including either soybean meal (treatments R-C and NN-C) or *Acheta domesticus* insect meal (treatments R-AD and NN-AD) as the main protein source. These diets align with those utilized in the study by Nieto, et al. (2023a)……...120

Table 5.2. Chemical/nutritional characteristics of the diets for the 4 experimental treatments, fed diets including either soybean meal (treatments R-C and NN-C) or A*cheta domesticus* insect meal (treatments R-AD and NN-AD) as the main protein source (expressed in grams/100 grams). These diets align with those utilized in the study by Nieto, et al. (2023a). 121
Table 5.3. Fatty acids composition (as g/100 g of fatty acid methyl esters) of the F3 (60–95 d) experimental feed for the 4 studied treatments of chicks, fed diets including either soybean meal (treatments R-C and NN-C) or *Acheta domesticus* insect meal (treatmentR-AD and NN-AD) as the main protein source. 122
Table 5.4. Slaughter weight (g) and weights of several carcass quality parameters (g) and cold carcass yield (g/kg) of the different experimental treatments of slow-growing chickens fed diets including either soybean meal (treatments R-C and NN-C) or *Acheta domesticus* insect meal (treatments R-AD and NN-AD) as the main protein source, and slaughtered at 95 d of age.[1] 131
Table 5.5. Technological meat characteristics of the different experimental treatments of slow-growing chicks fed diets including either soybean meal (treatments R-C and NN-C) or *Acheta domesticus* insect meal (treatments R-AD and NN-AD) as the main protein source and slaughtered at 95 d of age[1] 132
Table 5.6. Chemical proximal meat characteristics (%) of the different experimental treatments of slow-growing chicks fed diets including either soybean meal (treatments R-C and NN-C) or *Acheta domesticus* insect meal (treatments R-AD and NN-AD) as the main protein source, and slaughtered at 95 d of age.[1] 133
Table 5.7. Fatty acids composition (as g/100 g of fatty acid methyl esters) of the meat of the different experimental treatments of slow-growing chicks fed diets including either soybean meal (treatments R-C and NN-C) or *Acheta domesticus* insect meal (treatments R-AD and NN-AD) as the main protein source, and slaughtered at 95 d of age.[1] 135
Table 6. 1. Composition of the experimental feeds (F1, F2 and F3) for the four studied treatments of slow-growing chickens (C, T1, T2 and T3), which were fed feeds including either soybean meal and/or yellow mealworm insect meal as the main protein source. Composition of C and T3 feeds coincide with the ones used in Nieto et al. (2022)…….148

Table 6. 2. Chemical-nutritional composition of the experimental feeds (F1, F2 and F3) for the four studied treatments of chickens (C, T1, T2 and T3) and of the soybean and yellow mealworm meals separately, expressed as g/kg of dry matter, except for metabolizable energy (ME), which is expressed as MJ/kg of dry matter. Composition of C and T3 feeds coincide with the ones used in Nieto et al. (2022). 149
Table 6. 3. Fatty acids composition (as g/kg of fatty acids) of the F3 experimental feed for the four studied treatments of chickens (C, T1, T2 and T3). 150
Table 6. 4. Growth performances of slow-growing chickens fed different proportions of soybean meal and/or yellow mealworm meal and slaughtered at 92 days of age[1]..... 154
Table 6. 5. Weights of several carcass quality parameters (g) and cold carcass yield (g/kg) of slow-growing chickens fed different proportions of soybean meal and/or yellow mealworm meal and slaughtered at 92 days of age[1]..... 155
Table 6.6. Physical meat quality characteristics of slow-growing chickens fed different proportions of soybean meal and/or yellow mealworm meal and slaughtered at 92 days of age[1]. 156
Table 6. 7. Chemical meat quality characteristics of slow-growing chickens, expressed as g/kg of dry matter, fed different proportions of soybean meal and/or yellow mealworm meal and slaughtered at 92 days of age[1]. 156
Table 6. 8. Fatty acids (as g/kg of fatty acids) of slow-growing chickens fed different proportions of soybean meal and/or yellow mealworm meal and slaughtered at 92 days of age[1]. 158

Siglas y Acrónimos / Acronyms and Abbreviations

a*→ Enrojecimiento / Redness

AD→ *Acheta domesticus*

AOAC→ Asociación Oficial de Químicos Analíticos / Association of Official Analytical Chemists

b*→ Amarillez / Yellowness

BWG→ Ganancia de peso corporal / Body weight gain

C→ Control

Ca→ Calcio / Calcium

CE→ Comunidad Europea / European Community

CEE→ Comunidad Económica Europea / European Economic Community

cm→ Centímetros /Centrimetres

d→ Día / Day

DM→ Materia Seca / Dry matter.

DT→ Corrección de la tendencia / Detrend

FCR→ Índice de conversión / Feed conversion ratio

FI→ Consumo de pienso / Feed intake

F1, F2, F3→ Diferentes raciones alimenticias mensuales / Different monthly feeds

g→ Gramo / Gram

GC→ Cromatografía de Gases / Gas Chromatography

GLM→ Modelo Lineal General / General linear model

h→ Hora / Hour

Kcal→ Kilocaloría / Kilocalorie

Kg→ Kilogramo / Kilogram

L*→ Luminosidad / Lightness

LBW-LW→ Peso vivo /: Live body weight

m→ Metro / Metre

MANOVA→ Análisis multivariante de la varianza / Multivariate analysis of variance

Max Dev→ Desviación máxima / Maximum deviation

ME→ Energía metabolizable / Metabolizable energy

Min Dev→ Desviación mínima / Minimum deviation

mm→ Milímetros / Millimetres

MSC→ Efecto Multiplicativo de la Dispersión / Multiplicative Scatter Correction

MUFA→ Ácidos grasos monoinsaturados / Monounsaturated fatty acids

NIRS→ Espectroscopía de infrarrojo cercano / Near-infrared spectroscopy

NN→ Cuello desnudo / Naked Neck

NRC→ Consejo Nacional de Investigación / National Research Council

n-3→ Ácidos grasos n 3 / n-3 polyunsaturated fatty acids

n-6→ Ácidos grasos n 6 / n-6 polyunsaturated fatty acids

OGM→ Organismos genéticamente modificados / Genetically modified organisms

P→ Fósforo / Phosphorous

***P*→** P-valor / P-value

PSEM→ Error estándar de la media agrupada / Pooled standard error of the mean

PUFA→ Ácidos grasos poliinsaturados / Polyunsaturated fatty acids

R→ Rojo / Red

RMS-X Residuals→ Raíz Cuadrada de la Media Cuadrática Residual / Square Root of the Residual Root Mean Square

S→ Estirpe / Strain

SB→ Harina de soja / Soymeal

SEM→ Error estándar de la media / Standard error of the mean

SFA→ Ácidos grasos saturados / Saturated fatty acids

Sig→ Significación / Significance

SNV→ Variación normal estándar / Standard Normal Variate

T→ Tratamiento / Treatment

TM→ *Tenebrio molitor*

Trans FA→ Ácidos grasos trans / Trans fatty acids

T1, T2, T3→ Inclusión del 50, 75 y 100 % de TM respectivamente / Inclusion of 50, 75 and 100 % of TM respectively

UE→ Unión Europea / European Union

Vs→ *Versus*

W→ Semana / Week

WBSF→ Fuerza de cizallamiento Warner-Braztler / Warner-Braztler shear force.

WI→ Consumo de agua / Water intake

ºC→ Grados Centígrados / Celsius degrees

Resumen

La producción avícola es una gran fuente de proteína a la hora de satisfacer el aumento del consumo mundial de carne debido al aumento poblacional y los cambios alimentarios. El aumento de la demanda de carne conlleva una intensificación de la ganadería, en detrimento de la producción extensiva en la que predomina la crianza al aire libre en amplios espacios y con una menor dependencia de recursos externos.

Actualmente, la producción de carne de ave se realiza mayoritariamente en sistemas intensivos estandarizados. Por suerte, cada vez existe un mayor interés por la crianza en sistemas alternativos, en los que prima la sostenibilidad ambiental y el bienestar animal. Para adaptarse a estos sistemas se requiere el uso de razas o estirpes de crecimiento lento, en contraposición a las estirpes de rápido crecimiento (broilers) de los sistemas intensivos. Estas estirpes presentan un ciclo de producción más largo y una mayor rusticidad y poder de adaptación a la crianza al aire libre.

La alimentación es un aspecto fundamental en cualquier ganadería, tanto en el bienestar animal como en los resultados productivos. Las fuentes proteicas en la alimentación avícola desempeñan un papel crucial en los rendimientos de los animales, siendo los componentes más limitantes y costosos de las granjas. La harina de soja es la fuente proteica más empleada, pero sus altos precios unidos a los graves problemas ambientales ligados a su producción están provocando la necesidad de buscar nuevas fuentes proteicas para incluir en las raciones avícolas.

Los insectos se proponen como una fuente alternativa y sostenible para aportar a las dietas debido a su alto nivel de proteína, con bajos requisitos de tierra y agua para su producción, ciclos cortos de producción y baja huella ecológica. Su uso en aves de corral está ganando interés comercial desde la reciente regulación de la UE que ha permitido la alimentación con insectos en las dietas de monogástricos.

La aceptabilidad de la carne de ave está influenciada por su calidad. La nutrición de dichas aves tiene un gran impacto en el desarrollo de los animales, así como en las cualidades de en su carne. Además, las estirpes de crecimiento lento presentan características diferentes frente a las estirpes convencionales, tanto en sus rendimientos como en la calidad de carne.

En este escenario, esta Tesis Doctoral propone evaluar el efecto de la sustitución de la harina de soja por harina de *Tenebrio molitor* y de *Acheta domesticus* sobre los índices productivos y la calidad de carne de pollos de engorde de crecimiento lento, además de aplicar la metodología NIRS para intentar discriminar si se incluyó la harina de insectos en la dieta.

En los dos primeros artículos se realizó una sustitución completa de la harina de soja por harina de *Tenebrio molitor* en el primer caso y por harina de *Acheta domesticus* en el segundo, para evaluar su efecto en el desarrollo de pollos de engorde de crecimiento lento (*Coloryeld*). Los animales se criaron desde el primer día de vida hasta los 95 días, de modo que la primera mitad del ensayo estuvieron en un alojamiento cerrado y posteriormente contaron con acceso al aire libre. En cada ensayo, un grupo estaba formado por 64 pollos machos divididos en 8 corrales de 8 pollos cada uno que recibió harina de soja como fuente proteica y el otro grupo recibió la harina de cada insecto respectivamente. Los grupos fueron alimentados con dietas isoproteicas e isoenergéticas empleando 3 raciones diferentes cada mes.

En ambos casos, durante el primer mes la harina de insectos se tradujo en unos rendimientos productivos inferiores frente a la soja, con resultados similares para el segundo y tercer mes. Por tanto, puede recomendarse una sustitución parcial durante el primer periodo y usarse como sustituto completo a partir del segundo mes hasta el final del ciclo productivo.

En el tercer artículo se evaluó el impacto de la incorporación de harina de *Acheta domesticus* frente la harina de soja en la dieta de dos estirpes de pollo de crecimiento lento sobre las características de la canal y la calidad de su carne. Para ello se utilizaron 256 pollitos machos de crecimiento lento de un día de vida, la mitad pertenecientes a la estirpe Roja y la otra mitad Cuello desnudo. Los pollos de cada estirpe se asignaron de forma aleatoria en 16 réplicas de 8 polluelos cada una. A su vez se asignaron 8 réplicas a 2 tratamientos distintos (n=64 pollitos/tratamiento) en función de la dieta empleada: los tratamientos de control tanto para la estirpe roja como para la cuello desnudo fueron alimentados con harina de soja, mientras que los otros 2 tratamientos fueron alimentados con harina de *Acheta domesticus* en ambas estirpes.

La crianza se realizó al inicio en un alojamiento cerrado y al final de su ciclo tuvieron acceso al exterior. Los pollos fueron sacrificados a los 95 días de edad y se eligieron al azar ocho pollos por tratamiento para evaluar las características de la canal y la calidad de la carne.

La inclusión de la harina de este insecto generó características de la canal menos favorables en ambos genotipos. Sin embargo, a pesar de las variaciones en el pH, las pérdidas por cocción, la humedad y el contenido de proteína cruda, la calidad general de la carne se mantuvo en niveles satisfactorios. El contenido de grasa mostró valores más bajos para los pollos cuello desnudo.

En el cuarto trabajo se evaluaron los efectos de la harina de larva de *Tenebrio molitor* como sustitutivo parcial y/o completo de la harina de soja sobre la calidad de la canal y la carne en pollos de crecimiento lento. Un total de 256 pollos machos de un día *(Coloryeld*) se distribuyeron aleatoriamente en 32 unidades experimentales (8 pollos cada una). Estas unidades se distribuyeron uniformemente entre cuatro tratamientos (n=8): el tratamiento control (C) en el que la harina de soja fue la única fuente de proteína y tres tratamientos experimentales en los que la harina de soja se sustituyó por harina de larvas de *Tenebrio molitor* a niveles del 50%, 75% y 100% (T1, T2 y T3 respectivamente). Para cada tratamiento se utilizaron tres piensos diferentes según el periodo de crecimiento. Todos los pollos fueron sacrificados a los 92 días de edad, con 8 animales por tratamiento seleccionados aleatoriamente para evaluar la calidad de la canal y la carne.

Los rasgos de la canal apenas mostraron diferencias entre tratamientos. El tratamiento T2 mostró una menor intensidad del color amarillo de la carne, mientras que el jugo exprimible y las pérdidas por cocción fueron menores en los tratamientos T1 y T2, ambos con menor fuerza de cizallamiento, mayor contenido de humedad y menores valores de proteína total en comparación con los tratamientos C y T3, siendo este último el de mayor contenido de cenizas. Los pollos que consumieron insectos presentaron niveles más altos de ácidos grasos monoinsaturados (MUFA) y más bajos de ácidos grasos poliinsaturados (PUFA) y (n-6) PUFA.

En estos dos últimos trabajos se empleó la metodología NIRS para clasificar la carne en función del alimento y la cepa del pollito, logrando una correcta discriminación del 100% de las muestras.

Las conclusiones que derivan de los estudios realizados determinan que las harinas de *Tenebrio molitor* y *Acheta domesticus* pueden ser una fuente proteica interesante para incluir en la dieta de pollitos de lento crecimiento como sustitución parcial de la harina de soja durante el primer mes de vida de las aves y como sustitución total a partir de ese momento hasta el final de su ciclo productivo. Además, proporciona características de calidad de la carne comparables a las obtenidas en sistemas convencionales.

Abstract

Poultry production is an important source of protein to meet the increasing global meat consumption driven by population growth and dietary changes. The rise in meat demand is leading to the intensification of livestock production, often at the expense of extensive production systems, which are characterized by free-range rearing over large areas with less dependence on external resources.

Currently, most poultry meat is produced in standardized intensive systems. Fortunately, there is growing interest in alternative systems that prioritize environmental sustainability and animal welfare. Adapting to these systems requires the use of slow-growing breeds or strains, as opposed to the fast-growing strains (broilers) used in intensive systems. These breeds have longer production cycles and are more robust and adaptable to outdoor rearing.

Feeding is a fundamental aspect of any livestock production system, both in terms of animal welfare and production outcomes. Protein sources in poultry diets are critical for animal performance and are among the most limiting and costly components on farms. Soybean meal is the most widely used protein source, but its high price, coupled with the serious environmental problems associated with its production, necessitates the search for new protein sources for poultry rations.

Insects have been proposed as an alternative and sustainable protein source for diets due to their high protein content, low land and water requirements for production, short production cycles, and low environmental footprint. Their use in poultry is gaining commercial interest since the recent EU regulation allowing the feeding of insects in monogastric diets.

The acceptance of poultry meat is influenced by its quality, which is heavily impacted by poultry nutrition. Slow-growing strains have different characteristics compared to conventional strains, both in terms of performance and meat quality. In this context, this thesis aims to evaluate the effect of replacing soybean meal with *Tenebrio molitor* and *Acheta domesticus* meal on the production indices and meat quality of slow-growing broilers, as well as using NIRS methodology to determine whether insect meal was included in the diet.

In the first two studies, a complete substitution of soybean meal with *Tenebrio molitor* meal in one case and with *Acheta domesticus* meal in the other was carried out to evaluate their effects on the development of slow-growing broilers (*Coloryeld*). The broilers were reared from the first day of life until 95 days of age, kept indoors for the first half of the experiment, and then given access to the outdoors. Each experiment involved two groups: one consisting of 64 male chicks divided into 8 pens of 8 chicks each, receiving soybean meal as a protein source, and the other group receiving the insect meal. The groups were fed isoprotein and isoenergetic diets with three different rations each month.

In both cases, the insect meal resulted in lower production yields than soybean meal in the first month, with similar results in the second and third months. Therefore, a partial substitution during the first period and a complete substitution from the second month until the end of the production cycle is recommended.

In the third study, the effect of including *Acheta domesticus* meal versus soybean meal in the diet of two slow-growing chicken strains on carcass characteristics and meat quality was evaluated. For this purpose, 256 day-old slow-growing male chicks were used, half of which belonged to the Red strain and half to the Naked Neck strain. The chicks of each strain were randomly divided into 16 replicates of 8 chicks each. In turn, 8 replicates were assigned to 2 different treatments (n=64 chicks/treatment) depending on the diet used: the control treatments for both Red and Naked Neck were fed soybean meal, while the other 2 treatments were fed *Acheta domesticus* meal. The chickens were reared in an enclosed barn at the beginning of the rearing period and given access to the outdoors at the end. Chickens were slaughtered at 95 days of age and eight chickens per treatment were randomly selected to evaluate carcass characteristics and meat quality.

The inclusion of insect meal resulted in less favorable carcass characteristics in both genotypes. However, despite variations in pH, cooking losses, moisture, and crude protein content, overall meat quality remained satisfactory. Fat content was lower in the Naked Neck chickens.

In the fourth study, the effects of *Tenebrio molitor* larvae meal as a partial and/or complete substitute for soybean meal on carcass and meat quality were evaluated in slow-growing broilers. A total of 256 male day-old chicks (*Coloryeld*) were randomly distributed into 32 experimental units (8 chicks each). These units were evenly distributed among four treatments (n=8): the control treatment (C) where soybean meal was the sole protein source and three experimental treatments where soybean meal was replaced by *Tenebrio molitor* larval meal at levels of 50%, 75%, and 100% (T1, T2, and T3 respectively). For each treatment, three different feeds were used depending on the growth period. All chickens were slaughtered at 92 days of age, with 8 animals per treatment randomly selected to assess carcass and meat quality.

Carcass characteristics showed little difference between treatments. Treatment T2 showed a lower intensity of yellow meat color, while squeezable juice and cooking losses were lower in treatments T1 and T2, both with lower shear strength, higher moisture content, and lower total protein values compared to treatments C and T3, the latter having the highest ash content. Insect-fed chickens had higher levels of monounsaturated fatty acids (MUFA) and lower levels of polyunsaturated fatty acids (PUFA) and (n-6) PUFA.

In the last two studies, the NIRS method was used to classify meat according to feed and chick strain, with 100% of the samples being correctly classified.

The conclusions drawn from these studies indicate that *Tenebrio molitor* and *Acheta domesticus* meal can be interesting protein sources to include in the diet of slow-growing chicks. A partial replacement of soybean meal during the first month of the birds' life and a total replacement from that time until the end of their productive cycle is recommended. Additionally, meat quality characteristics are comparable to those obtained in conventional systems.

CAPÍTULO 1:

Introducción

Capítulo 1: Introducción

1.1. Hipótesis de partida

El consumo mundial de carne está aumentando, potenciado principalmente por el crecimiento económico y los cambios en los hábitos alimenticios (especialmente en los países en desarrollo). Este crecimiento económico aumenta los ingresos, favoreciendo que más personas tengan acceso a una dieta rica y variada pudiendo permitirse el consumo de carne. También el aumento de la clase media y la urbanización tienden al consumo de dietas más occidentales, en las que la inclusión de carne es más elevada que en las dietas más tradicionales. La búsqueda de dietas más equilibradas y saludables incluyen a la carne como una fuente de proteínas de alta calidad. Este aumento del consumo de carne viene influenciado también por una mejora de la tecnología agrícola-ganadera y de la cadena de suministro, provocando un mayor disponibilidad y acceso a esta (Allegretti et al., 2018).

Sin embargo, este aumento en el consumo de carne plantea preocupaciones sobre su impacto en la salud humana y el medio ambiente, como la deforestación, la emisión de gases de efecto invernadero y la pérdida de biodiversidad. A nivel ético preocupa también el bienestar de los animales, principalmente de aquellos criados de forma intensiva (Thornton, 2010).

El aumento de la producción de carne debido al aumento en el consumo conlleva una intensificación de la ganadería, con menos dependencia del entorno natural y un mayor uso de energía y materias primas importadas. Esto conduce a su vez a la reducción de la ganadería extensiva, caracterizada por la cría de los animales en áreas más amplias y la mayor dependencia de los recursos locales (Ruiz-Mirazo, 2011). Esto se une a la disminución tanto del potencial de recursos naturales como de la población rural, además de unas mayores exigencias hacia unas prácticas agrícolas correctas, requiriendo nuevas soluciones para mitigar los desafíos externos y los factores que afectan la eficiencia de la producción (Hovhannisyan & Grigoryan, 2016).

Para abordar estos desafíos es necesario promover prácticas agroganaderas responsables y éticas en la producción de carne. Las ganaderías extensivas y/o alternativas promueven estos intereses, ya que su producción está ligada al medio y dan prioridad al bienestar animal tratando de alcanzar la mayor calidad de sus productos. Estas ganaderías satisfacen las demandas de un número cada vez mayor de consumidores que critican los métodos de producción convencionales (Siegford et al., 2008; Sundrum, 2001)

La carne de ave se considera una fuente indispensable de proteínas asequibles y económicas para los seres humanos, (Elahi et al., 2020), por lo que tiene una gran demanda a nivel mundial (Wahyono & Utami, 2018). El sector avícola de carne se encuentra liderado por la producción intensificada a través de grandes sistemas de integración, donde la base genética y sistema de crianza está muy estandarizada. Se ha convertido en el producto cárnico más popular por su valor nutricional y precio. La mayor concienciación medioambiental también ha empujado su consumo, posicionándola como la segunda carne más consumida en la UE presagiando un mayor crecimiento en el futuro en términos de producción, comercio y consumo (Vida & Szakály, 2024).

Además, el aumento en la escasez de recursos para la producción de piensos y las preocupaciones ambientales han puesto de manifiesto la insostenibilidad de las fuentes de alimentación convencionales, la competencia por las materias primas entre los alimentos y los piensos y la disponibilidad de insumos, ya que la producción de piensos requiere de tierras de cultivo, además de fertilizantes, agua y energía (Abro et al., 2020; Flachowsky et al., 2017; te Pas et al., 2021), generando un aumento de los costes. Por lo tanto, es necesario buscar alternativas más sostenibles que incluyan prácticas que mejoren la salud del suelo y reduzcan la dependencia de insumos externos. Además, por otro lado, abordar la insostenibilidad de las fuentes de piensos convencionales requiere un enfoque integral que promueva la conservación de recursos naturales, la protección del medio ambiente y el bienestar animal (Abro et al., 2020).

En este contexto y unida a mi tradición familiar y pasión por la ganadería extensiva de diferentes especies incluyendo la avicultura de carne, surge la presente tesis doctoral.

La hipótesis de partida de dicha Tesis Doctoral es que la inclusión en la dieta de pollos de engorde de crecimiento lento de proteínas que pueden ser consideradas más sostenibles o alternativas en sustitución de la harina de soja, no afecta a los parámetros productivos y de calidad.

1.2 Objetivos

El objetivo general de esta Tesis Doctoral es evaluar el efecto de la inclusión de dos tipos de harinas de insectos autorizadas (*Tenebrio molitor* y *Acheta domesticus*) en la alimentación de monogástricos como sustitutivo de la harina de soja como principal fuente proteica en las raciones de pollos de engorde de crecimiento lento.

Los objetivos específicos se centran en:

- Evaluar los parámetros productivos para determinar si estos se ven afectados por la inclusión de estas fuentes proteicas alternativas.
- Evaluar si los rasgos de canal y la calidad de carne se ven afectados por la inclusión de ambas harinas de insectos.
- Establecer si técnicas multivariantes, como la espectroscopía de infrarrojo, es capaz de discriminar la carne de pollo en función del tipo de dieta suministrada.

CAPÍTULO 2:

Revisión bibliográfica

Capítulo 2: Revisión bibliográfica

2.1 Sistemas productivos en la avicultura de carne. Sistemas alternativos

La mayor parte de la producción de carne de ave se realiza mediante sistemas de producción intensivos con métodos de crianza estandarizada, empleando animales denominados broilers. En cambio, los sistemas alternativos, que priorizan la crianza al aire libre (Figura 2.1), constituyen solo aproximadamente el 5% de la producción avícola en la Unión Europea (Baéza et al., 2022; Dal Bosco et al., 2021). Afortunadamente, existe un interés creciente en estos sistemas de cría para mantener la sostenibilidad ambiental, mejorar el bienestar animal y la calidad de la carne. Esto ha motivado una mayor voluntad por parte de los consumidores hacia la compra de aves criadas en sistemas alternativos (Fanatico et al., 2007), en los que el acceso al aire libre es un factor que los consumidores consideran de suma importancia (Wang et al., 2009).

Figura 2.1. *Pollos criados al aire libre.*

Partiendo de los sistemas intensivos que emplean estirpes de crecimiento lento, existen otras denominaciones que difieren en el sistema de producción y los tiempos de crecimiento hasta llegar a los sistemas ecológicos, que son los que más garantías de sostenibilidad ofrecen en este momento en Europa (Palacios, 2021), recogidos en el Reglamento (UE) N ° 1308/2013 del Parlamento Europeo y del Consejo, de 17 de diciembre de 2013 , por el que se crea la organización común de mercados de los productos agrarios y por el que se derogan los Reglamentos (CEE) N ° 922/72, (CEE) N ° 234/79, (CE) N ° 1037/2001 y (CE) N ° 1234/2007; con las especificaciones del Reglamento (CE) Nº 543/2008 de la Comisión, de 16 de junio de 2008, por el que se establecen normas de desarrollo del Reglamento (CE) Nº 1234/2007 del Consejo en lo que atañe a la comercialización de carne de aves de corral. Dentro de los distintos sistemas de producción en la avicultura de carne podemos diferenciar (Palacios, 2021):

a) Sistema de producción convencional

El sistema convencional cerrado es el más extendido, en el que predominan las relaciones de integración vertical. Esto supone que las empresas avícolas controlan las diferentes etapas del proceso (suministro de animales y de pienso, directrices de crianza acorde a sus exigencias sanitarias y de calidad, servicios veterinario y comercialización) y el avicultor aporta sus instalaciones y mano de obra. Los animales permanecen en instalaciones cerradas durante todo su ciclo (42-45 días), las cuales están equipadas con sistemas informáticos que regulan y monitorizan la luz, humedad y temperatura, junto con extractores e inyectores de aire, humidificadores, pulverizadores, calentadores de aire y reguladores de intensidad y cantidad de luz (Figura 2.2).

Estos animales (broilers), se crían es espacios cerrados con densidades altas (16 pollos/$m^{2)}$, si bien la legislación permite hasta un máximo de 33 Kg/m^2 pudiendo llegar hasta 39 o 42 Kg/m^2 siempre que cumpla con requisitos determinados en la legislación de la UE (EC 43, 2007). Sin embargo, este método necesita un alto consumo de energía, tanto eléctrica como de combustibles fósiles, lo que implica costos ambientales significativos. Los animales se crían en libertad sobre el suelo cubierto por material de cama. Distribuidos por toda la instalación se encuentran los comederos y bebederos adaptados a su altura.

Figura 2.2. *Producción de carne de pollo de forma intensiva.*

Fuente: https://avicultura.com/la-provincia-de-castellon-ya-supera-las-500-000-plazas-de-pollos-con-la-autorizacion-ampliacion-granja-en-viver-por-parte-de-la-generalitat-valenciana/

Existen también los sistemas de producción convencional abiertos, propios de lugares con temperaturas medias-altas con bajas variaciones térmicas. Las diferencias con el sistema cerrado se centran únicamente en la existencia de ventanas para regular la temperatura de forma automática y una densidad de 15 pollos/m^2.

b) Sistema de producción extensivo en interior

Es el primer sistema reconocido como cría alternativa por el reglamento europeo. Las condiciones de iluminación y los controles ambientales son comparables al sistema anterior, con la presencia de ventanas y aireación natural y densidades similares (15 pollos/m^2).

Su diferencia radica en que la edad al sacrifico debe ser al menos de 52 días, empleando generalmente estirpes de crecimiento lento. Ante este hecho se alcanzan mayores pesos finales, restringiendo la alimentación para evitar altos niveles de engrasamiento.

c) Sistema de producción campero

Este sistema permite la salida al exterior durante el día y recogerse en la instalación durante la noche. Esto exige que los animales deben tener una mayor capacidad de adaptación al entorno y no existe un control total del ambiente respecto los sistemas anteriores; por lo que los animales son más vulnerables a las inclemencias climatológicas pudiendo presentar diferentes patologías frente los sistemas convencionales (Figura 2.3).

Figura 2.3. *Pollos camperos.*

Fuente:https://carnisima.com/blogs/carne-y-salud/carne-de-pollo-ecologico-de-corral-campero-o-industrial

Este sistema mantiene los 52 días de vida mínimos que deben alcanzar los animales, pero limita el uso de concentrados proteicos en la ración (mínimo 70 % de cereales). La densidad animal en el alojamiento interior no debe superar los 13 pollos/m^2 y al menos la mitad de su vida deben tener acceso continuo durante el día a un espacio libre con vegetación (1 m^2/pollo). Por tanto, este sistema exige la existencia de trampillas que conecten ambas zonas y toda la instalación contará con cercados para salvaguardarlos de las alimañas.

d) Sistema de producción campero tradicional

Las instalaciones son similares al pollo campero anterior, manteniendo la obligatoriedad de aportar al menos un 70 % de cereales. En este caso se aumenta la duración del crecimiento pudiendo llegar hasta los 81 días de crianza. Por este motivo es necesario utilizar razas de crecimiento lento basadas en razas autóctonas con cierto grado de selección, siendo Francia el país con mayor desarrollo de este sistema gracias a sus marcas de garantía.

La densidad animal se limita a 12 pollos/m^2, ampliando los patios de recreo a 2m^2/pollo. Cada 100 m^2 de instalación cerrada se necesitan 4 metros de trampilla de salida. Las condiciones son como las del sistema campero, pero deben poder salir de forma continua a partir de la sexta semana de vida. La principal restricción normativa de este tipo de instalación se refiere a las dimensiones máximas del gallinero, que están limitadas a una superficie interior de 1.600 m^2 y una ocupación máxima de 4.800 pollos.

e) Sistema de producción campero tradicional en total libertad

Este sistema es similar al campero tradicional anterior, salvo que las dimensiones de los patios de ejercicio deben ser mayores a 2 m^2/pollo y que las aves deben tener acceso al exterior de forma libre y continua desde las 4,5 semanas de vida. Debido a que se prolonga el tiempo en el exterior se recomienda proveer sombras y refugios en el área de recreo para animales que permanecen fuera de la instalación.

f) Sistema de producción ecológico (*Organic farming*)

Este sistema no está recogido en el Reglamento (UE) 1308/2013, sino en el Reglamento (UE) 2018/848 del parlamento europeo y del consejo de 30 de mayo de 2018 sobre producción ecológica y etiquetado de los productos ecológicos y por el que se deroga el Reglamento (CE) N.º 834/2007 del Consejo. Destaca por su estricta regulación, abordando aspectos que incluyen las instalaciones, el origen de los animales, la alimentación, el entorno en el que se desarrollan, asegurando el bienestar animal en todas las etapas desde la crianza hasta el transporte y la matanza.

Se establece una edad mínima de sacrificio de 81 días, lo que implica el uso de estirpes de crecimiento lento. Las instalaciones abiertas tienen una ocupación máxima de 10 pollos/m^2 en gallineros de hasta 1.600 m^2, con una capacidad máxima de 4.800 pollos. Las horas de luz dentro de la instalación están limitadas a 16 horas, con prohibición de iluminación exterior adicional. Se requiere la instalación de comederos, bebederos y asideros dentro de la instalación, junto con un suelo mayormente compuesto por cama caliente.

Se requiere una superficie vegetal en el exterior sincronizada con el proceso de cría. La superficie mínima por pollo es de 4 m^2, con presencia de árboles y zonas de sombra, así como bebederos y comederos. La densidad de las áreas de pastoreo no debe exceder los 580 animales/ha. También se precisa de una zona adicional, similar a la utilizada por los pollos para la espera y recuperación vegetal y del suelo (sin presencia de animales). Todas las instalaciones salvaguardarán la entrada de depredadores.

Asimismo, se exige que la alimentación sea completamente certificada como ecológica, con al menos el 30% proveniente de la propia granja o de granjas certificadas cercanas. Además, queda estrictamente prohibido el uso de antibióticos y sustancias sintéticas en la cría de los animales.

2.2 Pollos de crecimiento lento

La creciente demanda de aves criadas en sistemas alternativos ligada a la producción al aire libre, refleja la preocupación cada vez mayor por el bienestar animal y la calidad de los productos avícolas (Wang et al., 2009). Para adaptarse a estos sistemas se requiere el uso de razas o estirpes de crecimiento lento que puedan desarrollarse correctamente en estos sistemas (Figura 2.4). Esto contrasta con las razas de crecimiento rápido (broilers) empleadas en sistemas intensivos, que están seleccionadas para maximizar la eficiencia en la conversión de alimento en el menor tiempo posible, mantenidolas en alojamientos cerrados con condiciones ambientales controladas y densidades de población alta (Vissers et al., 2019).

Figura 2.4. *Estirpes de crecimiento lento.*

El ciclo de producción de las razas o estirpes de crecimiento lento es más largo, debido a que sus tasas de crecimiento y desarrollo son menores comparadas con los pollos intensivos. Sin embargo, estas razas muestran una notable capacidad de adaptación a la crianza al aire libre, respaldada por su resistencia a enfermedades, habilidad para obtener alimento en ambientes más naturales y una tasa de crecimiento que permita un desarrollo adecuado sin comprometer el bienestar animal. Esto les permite ser criadas en entornos más naturales y con mayor libertad de movimientos. A pesar de requerir un tiempo adicional para alcanzar el peso de mercado, estas razas ofrecen características de carne más valoradas por los consumidores, lo que las convierte en una elección preferida para la cría al aire libre (Allais et al., 2019; Filho et al., 2021).

Estas razas alcanzan rangos de peso vivo de 2 a 4 Kg entre los 56 y los 150 días de vida. Las principales estirpes de crecimiento lento utilizadas en estos sistemas alternativos o sus cruces son las razas Colorield, Redbro, Redbro cuello desnudo, Tricolor y Grey Barred, las cuales presentan tonalidades entre rojizas y negras, muy alejadas de los broilers de color blanco (Hubbard, 2024).

2.3 Problemática de las fuentes proteicas.

En todas las ganaderías, la alimentación juega un papel fundamental, tanto en los resultados productivos como en el bienestar animal. La producción de alimentos enfrenta una serie de desafíos que afectan en su eficiencia y sostenibilidad. Por tanto, estos factores plantean retos importantes para la industria alimentaria animal en su búsqueda por mantener una producción eficiente y ambientalmente responsable (Abro et al., 2020; te Pas et al., 2021).

Las fuentes proteicas utilizadas en la alimentación avícola son los componentes alimenticios más costosos y limitantes en comparación con las fuentes de energía (Selaledi et al., 2020). Su presencia es esencial en las dietas avícolas, ya que desempeñan un papel crucial en el desarrollo y crecimiento de las aves, influyendo directamente en su salud, bienestar y, como resultado, en los niveles de producción (Kim et al., 2019). La harina de soja es la fuente proteica predominante en las raciones de los animales, dominando el mercado mundial.

Los principales productores de harina de soja están concentrados en el continente americano, lo que ha generado una gran dependencia en la Unión Europea respecto a las importaciones de este producto. Esta dependencia conlleva diversos problemas, como la intermitencia en el suministro, la limitada disponibilidad, los altos costos, las disputas comerciales y los factores políticos. Estas dificultades resaltan la importancia de diversificar las fuentes de proteínas en la alimentación animal (Khan et al., 2023).

La rápida expansión del cultivo de soja como la principal fuente proteica para la alimentación animal ha suscitado preocupaciones en la sociedad, ya que ha dado lugar a graves problemas medioambientales (Sánchez-Muros et al., 2014; Vauterin et al., 2021). Su cultivo conlleva la deforestación de áreas de alto valor biológico, poniendo en peligro bosques y sabanas y amenazando la biodiversidad de esas regiones. La expansión de la agricultura de soja ha resultado en la pérdida irreversible de hábitats naturales, lo que ha llevado a la fragmentación de ecosistemas y a la disminución de las poblaciones de especies endémicas.

Estos impactos ecológicos son especialmente preocupantes en áreas de gran biodiversidad, donde la riqueza biológica está en riesgo debido a la expansión descontrolada de los cultivos de soja (Fehlenberg et al., 2017; Richards et al., 2012; Soisontes et al., 2023). El cultivo de soja también demanda grandes volúmenes de agua y recursos fósiles, así como el uso extensivo de pesticidas y fertilizantes para garantizar una producción óptima. Esta intensiva utilización de recursos naturales y químicos tiene un impacto significativo en el medio ambiente, acentuando aún más los problemas ambientales asociados con la producción de soja, subrayando la necesidad urgente de adoptar prácticas agrícolas más sostenibles (Pimentel et al., 2002).

Una preocupación adicional en relación con la importación de soja es el creciente uso de variedades transgénicas. Estos cultivos genéticamente modificados plantean amenazas para la conservación de la biodiversidad y la agricultura sostenible, ya que pueden resultar en la contaminación generalizada de la flora natural con rasgos transgénicos y la erosión de los recursos genéticos. Este riesgo se intensifica con las plantas genéticamente modificadas que poseen características farmacéuticas e industriales, ya que su liberación en el medio ambiente podría tener consecuencias impredecibles y potencialmente dañinas para los ecosistemas y la salud humana. Por lo tanto, es crucial considerar estos riesgos al evaluar las prácticas agrícolas y las políticas relacionadas con la producción y el comercio de soja transgénica (Bonny, 2008; Garcia & Altieri, 2005). Este aspecto limita aún más el uso de la soja en la agricultura ecológica, ya que la normativa orgánica prohíbe específicamente el uso de organismos genéticamente modificados (OGM) (EU 848, 2018; Lombardo & Zelasco, 2016).

Estos problemas también plantean preocupaciones éticas, como la fuerte competencia por la tierra cultivable y los recursos no renovables, como el agua y los combustibles fósiles, que podrían resultar en el acaparamiento de tierras por parte de grandes productores en perjuicio de pequeños campesinos (Flachowsky et al., 2017). Por lo tanto, es necesario optimizar la producción ganadera para reducir la dependencia de los piensos de soja importados, lo que ayudaría a disminuir la demanda de tierras de cultivo en regiones propensas a la deforestación. Al mismo tiempo, esta medida permitiría satisfacer las necesidades nutricionales de las dietas de la Unión Europea de manera más sostenible y ética (Karlsson et al., 2020).

Existen otras leguminosas disponibles como fuentes proteicas distintas a la soja, tales como guisantes, garbanzos, lentejas o judías. Estas legumbres tienen una amplia distribución geográfica y se cultivan en diversas regiones, lo que fomenta los circuitos cortos de distribución y las producciones locales. Sin embargo, es importante tener en cuenta que el contenido proteico de estas legumbres suele ser inferior al de la harina de soja (Boye et al., 2010).

Además, estas leguminosas contienen factores antinutricionales que pueden interferir con la disponibilidad de ciertos nutrientes. Estos factores pueden reducirse o eliminarse mediante diversos métodos de procesamiento, como el calentamiento, la aplicación de enzimas, el remojo, la germinación, la irradiación, la fermentación o métodos mecánicos como el descascarado y la molienda. También existen técnicas más avanzadas como el procesamiento a alta presión, el calentamiento por microondas y la extrusión, que pueden ser efectivas para mitigar estos factores antinutricionales (Abbas & Ahmad, 2018; Nikmaram et al., 2017). Sin embargo, es importante tener en cuenta que estos procesos de procesamiento adicional aumentan tanto los costos como la mano de obra asociada con el uso de leguminosas en la alimentación animal. Por lo tanto, si bien las leguminosas ofrecen una alternativa valiosa a la soja en términos de sostenibilidad y diversificación de fuentes proteicas, es crucial considerar los costos y los recursos necesarios para su procesamiento adecuado antes de su utilización en piensos

Los desafíos mencionados anteriormente plantean serias limitaciones para el uso de la soja y otras fuentes proteicas tradicionales en la alimentación del ganado, lo que resalta la necesidad de buscar alternativas que aborden estos problemas.

2.4 Uso de insectos como alternativas proteicas

En la actualidad, los insectos están emergiendo como una nueva fuente proteica para la alimentación animal debido a su alto contenido en proteínas, posicionándose como una clara alternativa al presente monopolio de la harina de soja (Biasato et al., 2016).

La clase insecta alberga la mayor variedad de especies en el mundo, incluyendo especies que proporcionan un alto contenido de proteínas y aminoácidos que pueden explotarse con éxito (Zou et al., 2024).

Entre ellos, la mosca soldado negra, la mosca doméstica, el escarabajo, los gusanos de la harina, los gusanos de seda, las lombrices de tierra, los grillos y los saltamontes están en el punto de mira porque han sido identificados como una importante fuente futura para la alimentación, satisfaciendo los requisitos nutricionales, composición de aminoácidos esenciales, digestibilidad de los nutrientes y aceptación del alimento (Belhadj Slimen et al., 2023).

Estas nuevas fuentes proteicas utilizadas en las dietas animales ofrecen una solución eficaz para mitigar los impactos negativos sociales y ecológicos derivados de las importaciones masivas de soja desde el extranjero y al mismo tiempo son capaces de lograr una eficiencia alimenticia y una calidad del producto equivalentes (Leiber et al., 2015). Los insectos satisfacen las preocupaciones de las fuentes tradicionales de proteína ofreciendo perspectivas prometedoras y sostenibles en su utilización (Sajid et al., 2023). Esta estrategia viable como fuente no convencional de proteínas contribuye a la seguridad alimentaria y la conservación del medio ambiente (de Castro et al., 2018).

Entre las ventajas de la producción de harinas de insectos resalta su ciclo de vida corto, el control de su producción y un requerimiento menor de recursos naturales, como agua y tierra, en comparación con la cría de ganado convencional (Perera & Bhujel, 2021; Sánchez-Muros et al., 2014). Además, los insectos pueden ser alimentados con desechos orgánicos de baja calidad, lo que reduce la presión sobre los cultivos destinados a la alimentación animal (Veldkamp & Bosch, 2015). Estos aspectos provocan que los insectos puedan contribuir a reducir la huella de carbono debido a su eficiencia en la conversión de alimentos y a la reducción de los gases de efecto invernadero asociados con su producción (Vauterin et al., 2021). Por tanto, su uso no solo promueve la eficiencia en el uso de recursos, sino que también puede ayudar a mitigar los impactos ambientales asociados con la agricultura intensiva.

Las harinas de insectos tienen propiedades nutritivas aceptables con un perfil adecuado de aminoácidos (Sánchez-Muros et al., 2014). Su utilización en las mezclas de pienso se ha probado en diferentes estudios con distintas especies con resultados satisfactorios, ya que no ha tenido efectos negativos sobre el crecimiento y estado de salud (Khan, 2018; Perera & Bhujel, 2021), sin perjudicar tampoco las características de calidad

de carne (Dabbou et al., 2020), mejorando incluso los resultados obtenidos (Khan, 2018; Perera & Bhujel, 2021).

En estos momentos la producción de insectos es limitada y su coste es elevado. Sin embargo, los avances en la tecnología y la eficiencia de la producción de insectos tienen el potencial de aumentar la escala de producción y al mismo tiempo reducir los precios, haciendo que las harinas de insectos sean más asequibles en comparación con las fuentes de proteínas convencionales (Sajid et al., 2023). Su producción lleva asociados riesgos y peligros biológicos, ya que bacterias, virus, hongos, parásitos, priones y también contaminantes químicos podrían estar presentes en insectos no procesados dependiendo del sustrato utilizado para alimentarlos y de las condiciones de cría y de recogida; aunque la prevalencia de algunos de estos patógenos será menor que en otras fuentes de proteínas animales no procesadas dado que la replicación de algunos de estos patógenos en el insecto no parece tener lugar (EFSA, 2015).

Los sistemas de producción alternativa ligados a la tierra, en las que los animales tienen acceso al aire libre, los consumidores asocian este sistema con el bienestar animal. El comportamiento natural de las aves de corral o los cerdos cuando se encuentran en libertad es alimentarse de diferentes insectos, por lo que la inclusión de su harina podría considerarse un sustituto aceptable de la harina de soja y de pescado como fuente de proteínas en las dietas de estos animales, sin que exista rechazo. Por tanto, el uso de insectos en la alimentación animal es una opción alentadora debido a sus cualidades nutricionales, con resultados positivos tanto en rendimientos como en calidad de carne. Otro aspecto fundamental hace referencia a la aceptación por parte de los consumidores de carne obtenida de animales que reciban harina de insectos en su dieta, ya que los insectos generan sentimientos de aversión y antipatías. Para aumentar su aceptación es necesario concienciar a los consumidores de los diversos beneficios de la harina de insectos en la alimentación animal (Szendrő et al., 2020).

2.5 Especies autorizadas

Una práctica extendida en la producción vegetal es el empleo de insectos como arma para luchar contra distintas plagas como método de control biológico, empleo de feromonas para monitorizar poblaciones, liberar insectos estériles o biopesticidas (Baker et al., 2020).

Su empleo en las ganaderías como alimento está menos extendido debido a su moderna regulación. A pesar de ello, se han realizado estudios para evaluar el efecto del empleo de insectos en la dieta de distintas especies con diferentes sistemas de producción, tanto en rendimientos, calidad como su impacto ambiental. Un aspecto fundamental para poder utilizar dichos insectos se basa en la legislación, reglamentos y normas alimentarias que rigen su uso y poder brindar más oportunidades para el desarrollo de los insectos (Zou et al., 2024).

En sus inicios, la UE comenzó a autorizar el uso de insectos de granja para la alimentación animal como proteína animal transformada (PAT) en la producción acuícola, recogiendo un listado de los insectos permitidos (EU 893, 2017). Los insectos están ganando mayor interés comercial a raíz del reciente Reglamento (UE) 2021/1372 de la Comisión, de 17 de agosto de 2021, por el que se modifica el anexo IV del Reglamento (CE) nº. 999/2001 del Parlamento Europeo y del Consejo en lo que respecta a la prohibición de alimentar a animales de granja no rumiantes, distintos de los animales de peletería con proteínas derivadas de animales, en el que se permite el uso de proteína animal transformada en porcinos y aves de corral, además de animales de acuicultura y animales de peletería (a excepción de los rumiantes). Además, los animales de granja de los que derivan dichas proteínas podrán alimentar a cualquiera de las especies mencionadas excepto la suya propia (EU 1372, 2021). Estas normativas recogen las condiciones de la producción y el uso de insectos destinada a la producción de piensos para animales de granja distintos de los animales de peletería.

Los insectos, por lo tanto, están considerados animales de granja, catalogando su cría como cualquier explotación ganadera y pueden usarse como materia prima para el pienso tanto muertos como vivos.

Las especies de insectos autorizadas para la producción de proteína hidrolizada son la mosca soldado negra *(Hermetia illucens*) y mosca común (*Musca domestica*), escarabajo de la cama (*Alphitobius diaperinus*), gusano de la harina (*Tenebrio molitor*), grillo doméstico (*Acheta domesticus*), grillo bicolor (*Gryllus assimilis*) y grillo rayado (*Gryllodes sigillatus*). Estos insectos pueden importarse fuera de la Unión siempre y cuando corresponda a estas especies mencionadas, el sustrato para su alimentación solo contenga productos de origen no animal u otros productos recogidos en el reglamento y si el alimento y los propios insectos no han tenido contacto con materiales de origen animal no permitidos (EU 893, 2017). Posteriormente se ha unido a esta lita de especies autorizadas el gusano de la seda (*Bombyx mori*), recogiendo también el término de "frass" como la mezcla de excrementos de insectos con sus partes muertas y restos del sustrato alimentario en el que se regulan las condiciones para su comercialización (EU 1925, 2021).

Las características nutricionales de los insectos están condicionadas por diversos factores, como son sus condiciones ambientales, alimentación empleada o las características propias de cada especie (Bbosa et al., 2019). La Tabla 2.1 recoge las principales características nutricionales de los insectos autorizados para su uso en la alimentación animal.

Tabla 2. 1. *Principales características nutricionales de las especies de insectos autorizadas en alimentación animal.*

Insecto	**Proteína bruta (%)**	**Grasa bruta (%)**	**Referencia**
Hermetia illucens (larva)	42,0 – 42,4	28,0 – 29,0	(Kozłowski et al., 2021; Wang & Shelomi, 2017)
Musca domestica (larva)	53,3	20,3	(Hall et al., 2018)
Tenebrio molitor (larva)	47,0 - 50,0	29,6 – 29,7	(Kozłowski et al., 2021; Nascimento Filho et al., 2021)
Alphitobius diaperinus (pupa)	70,7	19,3	(Kurečka et al., 2021)
Acheta domesticus, Gryllus assimilis and *Gryllodes sigillatus*	61,2 – 69,6	12,6 – 23,5	(Kulma et al., 2019; Ribeiro et al., 2019; Soares Araújo et al., 2019)
Bombyx mori (pupa)	55,6	32,2	(Tomotake et al., 2010)

Incluso en la producción ecológica, el reglamento (UE) 2018/848 del Parlamento Europeo y del Consejo, de 30 de mayo de 2018, también recoge la producción animal en su definición nº 27 como la producción de animales terrestres domésticos o domesticados, incluidos los insectos (EU 848, 2018). Todo esto ha generado que el uso de insectos en las ganaderías comienza a implementarse, generando un futuro prometedor para ser utilizado en las raciones de animales criados bajo el régimen de producción ecológica.

A continuación, se muestran algunos resultados obtenidos tras su uso en monogástricos y acuicultura.

A) Mosca soldado negra (*Hermetia illucens*)

Las larvas de este insecto son unas de las más utilizadas en la dieta de monogástricos y especies acuícolas (junto con las de *Tenebrio molitor*). Estas larvas son capaces de convertir los desechos en alimento, pueden criarse en instalaciones sencillas y no concentran pesticidas ni micotoxinas (Wang & Shelomi, 2017).

La harina de la mosca soldado se ha utilizado en pollos industriales frecuentemente como sustitutivo parcial y total a la harina de soja, sin efectos negativos sobre el crecimiento y salud de los animales. El uso de la grasa derivada de estas larvas ha garantizado desempeños productivos satisfactorios, características de la canal y calidad general de la carne de aves adecuadas (Bellezza Oddon et al., 2021; Schiavone et al., 2017), ofreciendo incluso menores riesgos para la salud cardiovascular (Mlaga et al., 2022).

La administración de larvas en cerdos de engorde ha tenido impactos beneficiosos sobre el rendimiento del crecimiento y la calidad de la carne (Yu et al., 2019), mejorando también el desarrollo de lechones y su comportamiento al reducir el estrés tras el destete cuando se suministran vivas (Ipema et al., 2021). Su uso en especies acuícolas también afecta a sus rendimientos, aumentando además su inmunidad y su resistencia a enfermedades (Mohan et al., 2022).

B) Mosca común (*Musca domestica*)

El análisis nutricional de las larvas de mosca doméstica revela un contenido alto de proteínas y un perfil de aminoácidos favorable rico en aminoácidos esenciales como lisina y metionina, con resultados exitosos en dietas para pollos de engorde industriales (Hall et al., 2018).

Además, su inclusión en el pienso genera un mayor aumento de peso corporal sin efectos negativos sobre las características de la canal (Reda et al., 2023), presentando un elevado potencial como suplemento proteico para dietas comerciales de pavos en particular y avicultura en general (Zuidhof et al., 2003).

Su empleo en dietas de cerdos está menos extendido ya que se ha utilizado con mayor frecuencia para el tratamiento del estiércol (Roffeis et al., 2015), pero también está considerada una fuente proteica alternativa en el porcino (Hong & Kim, 2022). En acuicultura se ha utilizado de forma satisfactoria, sin producir estrés fisiológico en los peces y alcanzando en muchos casos mejores rendimientos. Estos resultados hacen de estas harinas un alimento con alta calidad nutricional debido a sus ricos valores en proteínas, grasas, minerales, vitaminas del complejo B, oligoelementos y fósforo con gran aceptación por parte de los peces y crustáceos (De Leon Ramirez et al., 2018; Saleh, 2020).

C) Gusano de la harina (*Tenebrio molitor*)

El empleo de este coleóptero en su forma larvaria (Figura 2.5), es uno de los más utilizados como materia prima junto con las larvas de la mosca soldado negra. Su alto valor nutricional es comparable con la harina de soja y pescado, su nutriente más limitante es el calcio, pero este puede complementarse fácilmente en la dieta (Selaledi et al., 2020). El sector avícola industrial es uno de los sectores donde más se ha evaluado, de modo que la inclusión de sus harinas en la dieta de pollos de engorde ha beneficiado la ganancia de peso corporal y la tasa de conversión alimenticia (Sedgh-Gooya et al., 2021).

***Figura 2.5**. Larva de Tenebrio molitor*

Fuente: https://www.innovaspain.com/tenebrio-molitor-tebrio-insecto-alimentacion-humana-europa/

Se han llevado a cabo diversas investigaciones utilizando diferentes porcentajes de inclusión de las harinas de *Tenebrio molitor* como sustitutivo de harina de soja, observando que la calidad de la carne se ve favorecida con inclusiones menores, tanto en el caso de pollos de engorde (Shaviklo et al., 2021), como en el caso de perdices (Secci et al., 2018) y codornices (Secci et al., 2022).

Su utilización en cerdos destetados no muestra ningún efecto adverso en sus rendimientos, pero no se recomienda en sustituciones proteicas completas (Hong & Kim, 2022; Ko et al., 2020). En especies acuícolas no ha tenido efectos perjudiciales sobre la digestibilidad de los nutrientes y el rendimiento del crecimiento, al tiempo que se garantiza el valor nutricional (Basto et al., 2021). Sus resultados demuestran que se posicionan como buena candidata para ser una nueva fuente proteica en las producciones acuícolas sin afectar a sus rendimientos (Chemello et al., 2020).

D) Escarabajo de la cama (*Alphitobius diaperinus*)

Las larvas de este coleóptero presentan una amplia variedad de aminoácidos (Despins and Axtell, 1995). Las pupas de este insecto tienen bajo nivel de quitina y pueden contener más proteína y menos grasa que las larvas, haciendo que su empleo en forma de pupa sea una opción prometedora para la alimentación animal (Kurečka et al., 2021), aunque su uso en este campo ha sido menor que otros insectos.

El empleo de la harina del escarabajo de la cama como sustituto de la harina de soja en lechones no afectó al rendimiento del crecimiento, la composición de la canal ni a la calidad de la carne, salvo por un mayor contenido de PUFA en el tejido adiposo (Müller Richli et al., 2023). En acuicultura, como sustituto de la harina de pescado tiene efectos positivos en el crecimiento, el estado de salud y en los atributos sensoriales de la perca europea (Tran et al., 2024), a diferencia de su uso en pollitos de engorde (Despins and Axtell, 1995).

E) Grillo doméstico (*Acheta domesticus*), grillo bicolor (*Gryllus assimilis*) y grillo rayado (*Gryllodes sigillatus*)

Las tres especies de insectos pertenecen a la familia Gryllidae y, a diferencia de los insectos anteriores, no pasan por una fase larvaria al tener una metamorfosis incompleta (Figura 2.6). Su alto valor proteico les posiciona como una alternativa para incluir en las raciones de los animales, teniendo el grillo doméstico, en particular, un gran potencial debido a la digestibilidad de su materia seca, materia orgánica y proteína cruda (Kovitvadhi et al., 2019).

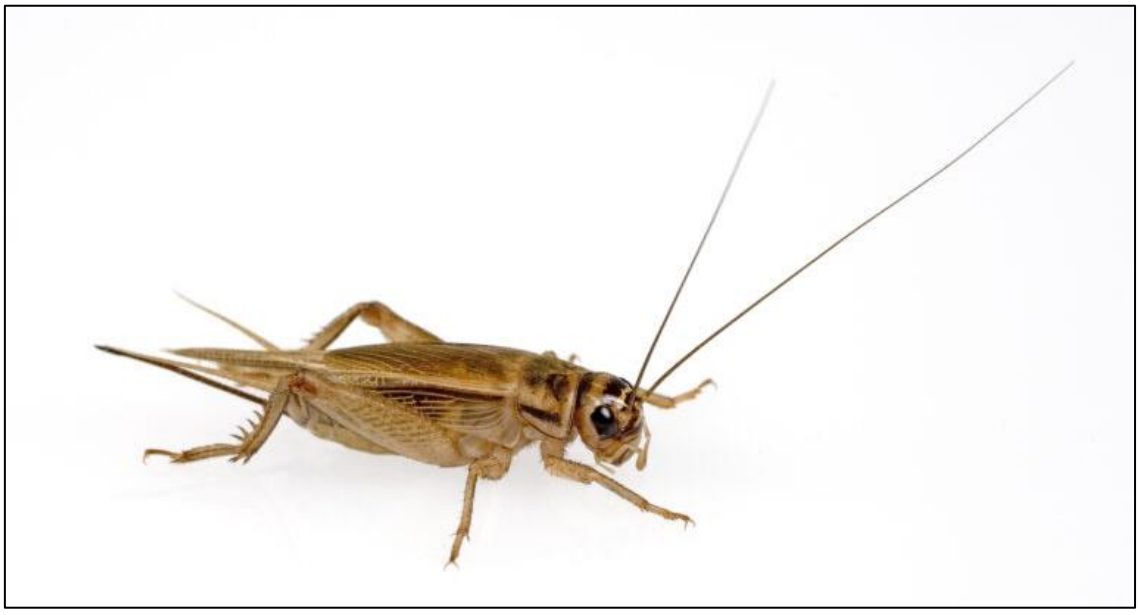

Figura 2.6. *Grillo doméstico (Acheta domesticus)*

Fuente: https://www.zooplus.es/magazine/reptiles/especies-de-reptiles/grillo-domestico-acheta-domesticus

Los diferentes estudios utilizándolo en la alimentación animal han establecido que el empleo de grillos adultos en la dieta de broilers no perjudica el crecimiento de los animales ni el sabor de la carne (Finke et al., 1985; Nakagaki et al., 1987), mejorando en otros estudios su crecimiento frente a dietas convencionales (DeFoliart et al., 1982). La harina de grillo empleada en acuicultura también revela que la sustitución parcial alcanza mejores resultados que la sustitución total de la harina de pescado para alimentar a alevines de tilapia del Nilo (Perera & Bhujel, 2021). En cerdos destetados, el grillo de campo como sustituto parcial de la harina de soja y de pescado tiene efectos beneficiosos sobre el crecimiento, morfología intestinal, la capacidad antioxidante y la microbiota intestinal (Boontiam et al., 2022).

F) Gusano de la seda (*Bombyx mori*)

Este gusano ha sido el último en incorporarse a la lista de insectos autorizados para la producción de proteínas animales transformadas destinadas a la fabricación de piensos para animales de granja (EU 1925, 2021). Las pupas presentan altos niveles de aminoácidos esenciales (Tomotake et al., 2010).

Son un recurso interesante para la alimentación animal incluyendo la acuicultura, ya que su incorporación hasta un 15% en el alimento extruido para peces no perjudica a su calidad física (Alcaraz et al., 2021). En las dietas de crecimiento para broilers, la harina de soja se puede reemplazar con este insecto sin comprometer su producción y rendimiento de sacrificio (Pietras et al., 2021; Ullah et al., 2017), mejorando incluso los índices de calidad (Zhao et al., 2023).

En cerdos de engorde, la inclusión de la harina de pupa de gusano de seda en pequeños porcentajes mejora su crecimiento, la utilización de nutrientes y la eficacia de conversión del pienso, reduciendo también el coste del pienso (Barman et al., 2021).

2.6 Factores productivos que afectan a la calidad de carne

La aceptabilidad de la carne de ave está fuertemente influenciada por su calidad (Elahi et al., 2020), que puede ser evaluada en función de diferentes atributos, como el sabor, la terneza, o la jugosidad.

La calidad de la carne de pollo puede estar influenciada por varios factores, como la genética de los animales, el sexo, aspectos nutricionales, acceso al exterior, edad al sacrificio o de procesamiento (Baéza et al., 2022; Mir et al., 2017), algunos de los cuales se describen a continuación:

2.6.1 Base animal

La demanda de los consumidores de carne de pollo ha contribuido al crecimiento de la industria avícola de carne en todo el mundo, consiguiendo animales con un ritmo de crecimiento muy elevado debido a los avances en genómica, además de la mejora en nutrición y prácticas de manejo (Devatkal et al., 2019). Este tipo de carne proviene principalmente de un sistema de producción estándar que utiliza estirpes de alta tasa de crecimiento criadas en condiciones intensivas (Baéza et al., 2022).

Sin embargo, este aumento en la eficiencia ha generado diversos problemas en la calidad de la carne y mayores costos para los productores (Mueller et al., 2023). Entre los problemas asociados a estos sistemas encontramos las deformaciones y daños en las patas de los pollos (Sanotra et al., 2001) o el aumento de casos de anomalías musculares como o la aparición del rayado blanco (*white striping*) o la pechuga leñosa (*wooden breast*). El primer caso se caracteriza por la aparición de estrías blancas paralelas a la fibra muscular en la superficie de los músculos pectorales mayores y suele aparecer junto con la pechuga leñosa, que se manifiesta en áreas visualmente duras, abultadas y pálidas, endureciendo las fibras musculares. Ambas patologías, ejercen un efecto perjudicial sobre la composición y los rasgos de calidad de la carne afectada (Petracci et al., 2013; Soglia et al., 2016).

En contraposición, se observa un aumento en el interés de los consumidores por aves criadas en sistemas alternativos, para los que el acceso al aire libre es un aspecto fundamental (Fanatico et al., 2007; Wang et al., 2009). Esta producción sostenible de carne de ave involucra la utilización de nuevas estirpes de pollitos de crecimiento más lento. Estas aves muestran variaciones en su calidad, presentando características únicas en comparación con las estirpes más comunes (Hailemariam et al., 2022; Tougan et al., 2013).

Las características de estas estirpes no solo se deben centrar en aspectos productivos, sino aspectos fundamentales para la nutrición humana (Dal Bosco et al., 2021). La comparativa entre estirpes de crecimiento lento frente las convencionales de crecimiento rápido revelaron mayores niveles de proteína y fuerza de corte y una cantidad de ácidos grasos totales y saturados menores en las primeras (Devatkal et al., 2019). Estas diferencias en la composición nutricional y las características sensoriales en la carne de pollo aparecen incluso entre genotipos alejados de las estirpes convencionales (Hailemariam et al., 2022).

Las propiedades funcionales de la carne de las estirpes de crecimiento rápido y medio parecen ser mucho más atractivas tanto para la industria como para el consumidor, ya que tiene mejores valores de despiece, menores pérdidas por goteo y cocción y mayor terneza, mientras que desde un punto de vista nutricional la carne de pollo de crecimiento lento es más saludable (Sirri et al., 2011; Valenta et al., 2022).

Por tanto, los genotipos alternativos de pollo ofrecen cualidades nutricionales únicas que son valoradas por los consumidores. Sin embargo, estas cualidades pueden no ser apreciadas por aquellos consumidores menos experimentados, cuya elección puede verse influenciada por factores culturales y por la familiaridad con los atributos sensoriales de un producto. Por ello, es crucial trabajar en la educación de los consumidores sobre estas características, lo que permitirá aprovechar un mercado prometedor al mismo tiempo que conservar razas alejadas de las líneas convencionales (Pellattiero et al., 2020).

2.6.2 Alimentación

La nutrición de las aves tiene un impacto significativo en la calidad y seguridad de su carne, dado que la respuesta al alimento suministrado está directamente relacionada con los cambios en el crecimiento del esqueleto, el músculo y el depósito de grasa (Mir et al., 2017). El sistema digestivo del pollo, como animal monogástrico, está optimizado para una rápida y eficiente digestión de los nutrientes esenciales, como proteínas, carbohidratos y grasas.

Esta capacidad permite que los nutrientes se absorban sin transformaciones significativas, facilitando su deposición en el cuerpo y contribuyendo a un crecimiento y

desarrollo eficientes, de manera que los ácidos grasos de la dieta se reflejan en los ácidos grasos de la carne. La alimentación de las aves con dietas bajas en grasas y ricas en carbohidratos disminuyen la grasa de la canal, el rendimiento de la canal y el rendimiento de la carne de pechuga (Smith et al., 2002).

La textura es un factor fundamental en la calidad de la carne de ave, ya que condiciona fuertemente su aceptación por parte del consumidor. Este factor junto con el grado de firmeza está relacionado con la cantidad de agua retenida por vía intramuscular. El color es otro de los factores con mayor importancia para los consumidores, ya que su apariencia es fundamental para su consumo. Existen una serie de factores que influyen en el mismo, como el sexo, edad, estirpe, alimentación o procesamiento; cuyos principales problemas son las manchas, palidez extrema u oscurecimiento (Mir et al., 2017). Así, las dietas bajas en energía provocan aumentos de la fuerza de corte y la reducción del amarillamiento de la carne de pollos de engorde (Hussein et al., 2020).

En relación con su calidad nutricional, la carne de pollo presenta una cantidad significativa de proteínas de alta calidad y fácil digestión y una baja porción de grasas saturadas en comparación con otras especies, recomendando su consumo en todos los grupos de edad. Esta composición nutricional puede modificarse en función de la alimentación recibida (Kralik et al., 2017)

Prueba de ello son los cambios producidos en la calidad y composición de la carne de pollo tras la inclusión de semillas de girasol en la dieta como sustitutivo parcial de la harina de soja, las cuales mejoran el color y el contenido de proteína bruta de las pechugas (Godínez-Juárez et al., 2022). La inclusión de café en polvo en la dieta de pollos también mejora la calidad de carne, incluyendo cambios en el color (Ashour et al., 2020).

La carne de pollos de crecimiento lento engordados con pastos es más rica en antioxidantes y ácidos grasos n3, a diferencia de los pollos de rápido crecimiento engordados de forma convencional (Englmaierová et al., 2020). La incorporación de algas pretratadas con enzimas para mejorar la utilización de los nutrientes de las dietas avícolas no perjudica los parámetros de calidad de carne de pollos industriales (Matshogo et al., 2021), como tampoco lo hace la incorporación de semillas de altramuz junto con harinas de insectos como sustitutivo a la harina de soja (Pietras et al., 2021).

Del mismo modo, el uso de semillas de leguminosas y harinas de colza no afecta a la mayoría de las características de la canal y la calidad de la carne, a pesar de alcanzar resultados de crecimiento inferiores frente la utilización de harina de soja (Biesek et al., 2020).

Por tanto, la incorporación de diferentes materias primas en las raciones avícolas puede no solo no perjudicar, sino incluso mejorar la calidad de la carne de los animales, siendo necesario buscar nuevas fuentes a incorporar en la dieta diferentes a las materias primas convencionales.

CAPÍTULO 3:

Performance of Slow-Growing Chickens Fed with *Tenebrio molitor* Larval Meal as a Full Replacement for Soybean Meal

Capítulo 3:

Performance of Slow-Growing Chickens Fed with *Tenebrio molitor* Larval Meal as a Full Replacement for Soybean Meal

Jaime Nieto[1], Javier Plaza[1], Javier Lara[2], José-Alfonso Abecia[3], Isabel Revilla[4], Carlos Palacios[1]

[1] Area of Animal Production, Faculty of Environmental and Agrarian Sciences, University of Salamanca, Avenida Filiberto Villalobos 119-129, 37007 Salamanca, Spain.
[2] Animal Husbandry and Animal Health Assistance, High-School Torres Villarroel, Avenida Hilario Goyenechea, 44, 37008 Salamanca, Spain.
[3] Institute of Research in Environmental Sciences of Aragón (IUCA), University of Zaragoza, Miguel Servet, 177, 50013 Zaragoza, Spain.
[4] Area of Food Technology, E.P.S. of Zamora, University of Salamanca, Avenida Requejo 33, 49022 Zamora, Spain.

Veterinary Sciences. 2022, 9(3), 131; https://doi.org/10.3390/vetsci9030131

Submission received: 16 February 2022. Revised: 3 March 2022. Accepted: 10 March 2022. Published: 12 March 2022.

Resumen

La producción avícola es una excelente fuente de carne para satisfacer la creciente demanda de proteína animal, pero los alimentos que se emplean para dicha producción deberán ser más sostenible con el alimento empleado. La harina de soja es la fuente de proteína más utilizada en la alimentación de animales no rumiantes. Sin embargo, el incremento de los precios unido a los graves problemas ambientales en relación con su producción e importación a gran escala está motivando la búsqueda de alimentos alternativos que ayuden a evitar estos problemas. El comportamiento natural de los pollos es alimentarse de insectos, posicionando las harinas de estos como una fuente alternativa y sostenible de nutrientes debido a su alto contenido proteico, teniendo permitido su uso en la dieta de aves.

Además de la sostenibilidad de la producción de las materias primas, cada vez más se demanda estirpes de pollo de crecimiento lento adaptados a sistemas alternativos con características nutricionales y organolépticas más demandadas por los consumidores.

El objetivo del ensayo fue evaluar el efecto de la sustitución total de la harina de soja por harina de larvas de *Tenebrio molitor* sobre el desarrollo de pollos de crecimiento lento, empleando dietas isoenergéticas e isoproteicas durante todo su ciclo de vida.

Un total de 128 pollos machos de crecimiento lento (*Colorield*) de un día de edad se dividieron homogéneamente en dos grupos: el grupo control (C) alimentado con harina de soja como principal fuente proteica y el grupo tenebrio (TM) alimentado con harina de larvas de *Tenebrio molitor*. Cada grupo estaba formado por 64 pollos, divididos su vez en 8 réplicas de 8 aves cada una. Desde su llegada hasta los 50 días de vida se alojaron en un alojamiento cerrado con condiciones ambientales controladas (9,79 pollos/m^2). Posteriormente y hasta el final de estudio, en el que los animales fueron sacrificados a los 95 días de vida, los pollos estuvieron en corrales con acceso al aire libre provistos con zona de refugio (0,25 pollos/m^2). Los grupos fueron alimentados con dietas isoenergéticas e isoproteicas, empleando durante todo el proceso tres dietas concentradas diferentes (F1, F2 y F3), las cuales se cambiaron cada 4 semanas.

Durante la F1, el grupo C alcanzó un peso vivo mayor respecto el grupo TM. En consecuencia, la ganancia de peso corporal también fue mayor. El consumo de pienso y agua también fue mayor para el grupo C y un índice de conversión alimenticia menor frente el grupo TM. Durante la F2 y F3, no hubo diferencias en los parámetros estudiados entre ambos grupos excepto en la ganancia de peso corporal en la F2, con mejores resultados para el grupo C. Los resultados globales de todo el periodo (1-95 días) determinaron que la ganancia de peso y los consumos de pienso y agua fueron mayores para el grupo C, pero sin diferencias en el índice de conversión ni el peso vivo.

En conclusión, la sustitución total de la harina de soja por harina de larva TM en pollos de crecimiento lento provocó una reducción del consumo y una consecuente reducción del peso en las primeras 4 semanas de vida. Durante ese periodo es más adecuada la sustitución parcial ya que después de la misma, los pollos mejoraron notablemente su desarrollo y rendimiento, alcanzando al final del ciclo (95 días) valores similares a los de los pollos alimentados con harina de soja, a pesar de comenzar de animales con menor desarrollo durante el primer mes. En estas condiciones, la harina de larvas de *Tenebrio molitor* puede ser una fuente de proteína alternativa para incluir en la dieta de pollos de crecimiento lento.

Abstract

Insect larval meal is an increasingly common protein source in poultry systems. In this study, the effect of replacing soybean meal with *Tenebrio molitor* larval meal on the performance of slow-growing chickens was assessed. A total of 128 one-day-old chickens (*Colorield*) were randomly divided into a control group (C) (n = 64), fed with soybean meal, and an experimental group (TM) (n = 64), fed with *T. molitor* larvae meal. The chicks were slaughtered after 95 days. Three different isoenergetic and isoproteic diets (F1, F2 and F3) were used for each group. The F1 diet resulted in higher body weight gain and higher feed and water intakes in group C, but a lower feed conversión ratio. Contrarily, diets F2 and F3 did not produce differences in the studied parameters between the two groups, except for body weight gain in the case of diet F2, which was highest in group C. Therefore, weight gain and feed and water intakes were significantly higher in group C, but there were no differences in feed conversion ratio or live weight. In conclusion, the total replacement of soybean meal with *T. molitor* larvae meal resulted in a reduction in feed intake and a consequent reduction in weight. During this period, partial rather than total substitution may be recommended.

Keywords: alternative poultry farming; slow-growth chicken; insect meal; *Tenebrio molitor*; alternative protein sources

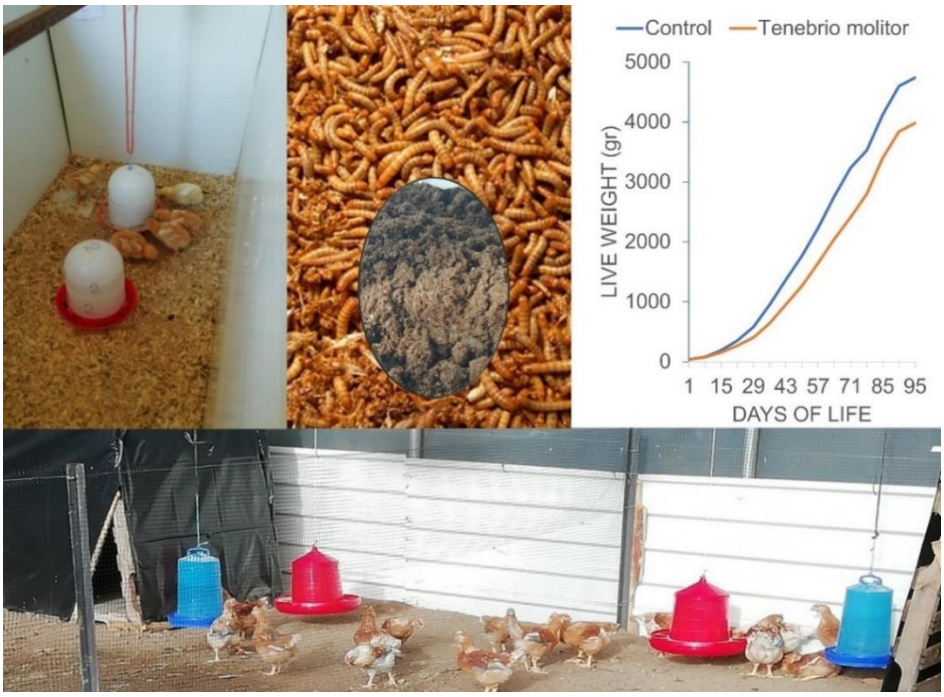

Figure 3. 1. Graphical Abstract

3.1 Introduction

Global meat consumption is increasing due to the rising incomes and changing dietary patterns of the population. Poultry production is a prime way to meet the demand for animal protein. However, it should become more sustainable regarding the environment and the type of feed used in this sector (Allegretti et al., 2018). Indeed, in modern society, the sustainability of food production and processing systems based on increased efficiency of raw material use, low pollution and reduced waste has become a priority (Gasco et al., 2020).

Soybean meal is the most widely used source of protein in non-ruminant animal feed (Olukosi et al., 2019). However, in recent years, the rising price of this raw ingredient has jeopardized poultry meat production, even more so in some developing countries (Chadd, 2008). The severe environmental problems related to soya production are also noteworthy, as it is one of the main causes of deforestation in the American continent, and in addition, it requires a very high input of water and phytosanitary products (Sánchez-Muros et al., 2014). Therefore, the use of non-soybean protein sources in poultry diets is essential to avoiding negative social, economic and ecological impacts arising from large-scale soybean imports. Currently, there is an emerging trend towards the use of alternative feeds to soybeans for poultry which are capable of achieving feed efficiency and feed quality equivalent or superior to soyabean feeds (Leiber et al., 2015; Sarmiento-García et al., 2021; Selaledi et al., 2020).

A natural behavior of chickens is feeding on different insects throughout their lives (Khan, 2018). Therefore, it is very likely that chickens would do well with insect meal as part of their dietary rations (Nascimento Filho et al., 2020). Insects are claimed to be an alternative and sustainable nutritional source for farm animals, including poultry (Kozłowski et al., 2021; Premalatha et al., 2011; Sarmiento-García et al., 2021). It is a high-protein feed (Biasato et al., 2016), which also has a considerable amount of unsaturated fats (specifically linoleic and linolenic acids), and large amounts vitamins, fiber and minerals (Huis et al., 2014; Marono et al., 2015). Furthermore, it is worth noting that insects-based diets have gained commercial interest in poultry farming since the recent EU regulation allowing the use of insect meal in monogastric diets (EU 1372, 2021). However, to know the level of sensible inclusion for this type of meal in the animal's diet is essential. Numerous studies have been conducted to elucidate the

inclusion percentage of this type of meal that optimizes chicken growth (Biasato et al., 2017, 2018; Bovera et al., 2015).

Alongside the production sustainability provided by insect meal inclusion, slow-growing chicken strains are being used more often in the meat sector (Vissers et al., 2019). Due to their growth and better adaptation to alternative production systems, the life cycles of these strains generally exceed 60 days (Allais et al., 2019; Mueller et al., 2020). Hence, although they require more time to reach market weight, they may exhibit nutritional and organoleptic characteristics that are more appreciated and demanded by consumers (Filho et al., 2021). Moreover, feed conversion of slow-growing chicken can reach even better results when using new raw materials included in their diets (Filho et al., 2021). In this context, an increasing number of insect species are used in the production of meal for feed purposes in the livestock sector. In particular, the increase in growth performance in poultry fed with meal of the coleopteran species *Tenebrio molitor*, and the changes that its inclusion generate in the immune system of the animals, have been demonstrated by several authors (Biasato et al., 2017; Sedgh-Gooya et al., 2021; Zadeh et al., 2019). However, further research is needed to determine how such inclusion affects the mechanisms for transforming food into productive yields.

The present study aimed to assess the effect of the total replacement of soybean meal as a protein source with *Tenebrio molitor* larval meal on slow growing chicken development, throughout their complete lifecycle. They were always fed isoenergetic and isoproteic diets.

3.2 Materials and Methods

3.2.1 Animals and Experimental Design

All slow-growing chickens (hereinafter named as chickens) used in the trial were treated in accordance with the principles of European Parliament and Council Directive 2003/65/EC of 22 July 2003, amending Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes. These animals were manipulated under Order ECC/566/2015, of 20 March, establishing the training requirements to be met by personnel handling animals used, bred or supplied for experimental and other scientific purposes, including teaching. The experimental protocol was approved by the Bioethics Committee of the University of Salamanca (Spain) with registration number 590, regulated by RD 53/2013, of 1 February, establishing the basic

rules applicable for the protection of animals used in experimentation and other scientific purposes, including teaching. The trial was conducted by personnel from the Animal Production area in the facilities and experimental fields of the University of Salamanca.

A total of 128 one-day-old male slow-growing chickens (*Colorield*) were used in this trial. These animals were previously vaccinated at the hatchery against Marek's disease and avian infectious bronchitis. Once in the experimental facilities, they were homogeneously divided into two groups: (1) the control group (C) with a mean weight of 39.89 ± 0.04 g that was fed soybean meal as the main protein source, and (2) the tenebrio group (TM) with a mean weight of 39.82 ± 0.10 g that was fed *Tenebrio molitor* larvae meal. Each group consisted of 64 chickens, divided in turn into eight replicates of eight birds each. The animals were raised until 95 days of age, then slaughtered in an authorized slaughterhouse in compliance with Directive 91/628/EEC on good animal welfare during transport and the slaughter procedure in Regulation 93/119/EC.

From their arrival until 50 days of life, each replicate was housed in a 1.50 × 0.60 m pen located in an enclosed housing, provided with a drinking trough and a circular feeder. The stocking rate in each pen was 9.79 chickens/m^2. First age feeders and drinkers were used for the first 21 days, being replaced later by second age ones. The floor was covered with 6 cm thick wood shavings. Temperature and humidity were controlled to maintain optimum conditions for the animals according to their age. The average temperature inside the building was 32.5 ± 1.4 °C during the first week, 28.6 ± 1.9 °C during the second week, 24.7 ± 2.1 °C during the third week and 21.5 ± 2.4 °C during the rest of the experiment. The chicks received a natural 10 h photoperiod.

From 50 days old until the end of the study, the eight chicken replicates were placed in an outdoor pen with an open-air area, with circular feeders and drinkers, and a shelter for protection, allowing them complete free movement. This shelter had a single entrance on the south side, an opening at the top to ensure ventilation and a translucent section for lighting. The stocking rate in this second stage was 0.25 chickens/m^2. The outside yard had natural soil, and it was perimetrically enclosed by a 1 m high metal sheet (buried 30 cm) and a 13 × 13 mm grid plastic mesh covering the sides and the roof to prevent predators from entering. The pens were divided with plastic mesh and the access doors were made of untreated Wood.

3.2.2 Composition of the Diets and Data Collection

Three different concentrated diets (F1, F2 and F3) were used throughout the process, which were changed every 4 weeks. For each of these three diets, the two experimental groups of chickens (C and TM) were iso-energetically and iso-proteically fed. In particular, F1 was used from day 1 to day 29, F2 from day 29 to day 57 and F3 from day 57 to day 95. Thus, following the nutritional recommendations of the National Research Council (NRC) (NRC, 1994) for medium-slow differentiated growth chickens, we attempted to meet the nutritional needs of the chickens.

The compositions of the three diets, expressed in terms of percentages of raw matter, are shown in Table 3.1.

***Table 3. 1**. Compositions of the experimental diets for the two studied groups of chickens expressed as percentages of raw matter.*

Raw matter	F1 (1-29 d)		F2 (29-57 d)		F3 (57-95 d)	
	C	TM	C	TM	C	TM
Corn	37.0	10.0	48.0	9.0	31.0	18.0
Wheat	11.0	0.0	12.0	16.8	37.0	33.5
Barley	0.0	21.0	0.0	13.2	0.0	0.0
Soybean meal	29.0	0.0	20.0	0.0	12.0	0.0
T. molitor meal	0.0	22..3	0.0	13.0	0.0	9.2
Sunflower	19.0	0.0	16.0	9.0	16.0	9.0
Oats	0.0	42.7	0.0	35.0	0.0	26.3
Vitamin-mineral premix	4.0	4.0	4.0	4.0	4.0	4.0

C: control group, TM: *T. molitor* group and d: day.

The chemical/nutritional characteristics of these diets were previously analyzed (Table 3.2) in the food analysis laboratories of the Abiomed Higiene S.L. company (Salamanca, Spain), which operates under an ISO 9001 certified quality system.

Table 3. 2. *Chemical/nutritional characteristics of the experimental diets for the two studied groups of chickens.*

Parameter	F1 (1-29 d)		F2 (29-57 d)		F3 (57-95 d)		*T. molitor* meal
	C	TM	C	TM	C	TM	
%Moisture	7.6	7.9	8.9	8.9	8.6	8.5	6.6
%Ash	6.5	6.4	6.4	6.0	5.8	5.9	4.2
%Crude fat	12.3	9.9	9.5	9.1	10.7	9.7	21.2
%Crude fiber	6.1	7.9	5.0	8.5	6.1	6.9	8.3
%Starch	35.0	38.1	41.2	37.4	42.3	40.9	2.3
%Crude protein	19.7	19.3	15.6	15.5	14.8	14.2	54.9
ME (Kcal/kgDM)	2,802.8	2,898.9	2,900.0	2,900.7	2,950.8	2,964.7	5,020.0
Mean dietary Crude Protein (%)	19.5		15.6		14.5		54.9
Mean dietary ME (Kcal/kgDM)	2,850.8		2,900.3		2,957.8		5,020.0
%Ca	1.24	1.94	1.21	1.63	1.20	1.49	3.6
%P	0.40	2.10	0.36	1.41	0.34	1.08	7.4
%SFA	1.6	2.2	1.2	1.6	1.4	1.5	5.0
%MUFA	3.0	3.7	2.3	2.8	2.4	2.8	8.4
%PUFA	7.8	4.1	6.0	4.7	7	5.4	7.9
%n-3 PUFA	0.15	0.14	0.11	0.09	0.10	0.08	0.25
%n-6 PUFA	7.6	3.9	5.9	4.7	6.8	5.3	7.6
%Trans FA (C18:1T+C18:2T+C13:3T)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
4-Hidroxiproline (mg/100gDM)	59.0	24.0	46.0	24.8	39.9	23.7	72.0
Aspartic acid + Asparagine (g/100gDM)	2.54	1.83	1.92	0.84	1.20	0.86	4.5
Glutamic acid + Glutamine (g/100gDM)	5.22	4.13	4.45	2.83	4.12	2.86	6.4
Alanine (g/100gDM)	0.91	1.40	0.90	0.92	0.95	0.99	5.0
Arginine (g/100gDM)	1.28	0.91	1.21	0.70	0.77	0.80	2.74
Cysteine (g/100gDM)	<20	<20	<20	<20	<20	<20	<20
Cystine (g/100gDM)	0.18	0.13	0.15	0.11	0.12	0.11	0.30
Phenylalanine (g/100gDM)	0.81	0.59	0.68	0.55	0.57	0.45	2.17
Glycine (g/100gDM)	1.02	0.61	0.88	0.45	0.40	0.67	2.72
Histidine (g/100gDM)	0.76	0.57	0.65	0.95	0.76	0.48	1.51
Isoleucine (g/100gDM)	0.68	0.44	0.53	0.58	0.40	0.36	2.39
Leucine (g/100gDM)	1.18	0.94	1.09	1.04	0.94	0.76	4.9
Lysine (g/100gDM)	1.44	1.23	1.28	0.97	1.01	0.69	2.80
Methionine (g/100gDM)	0.29	0.18	0.20	0.17	0.19	0.17	0.47
Proline (g/100gDM)	0.91	1.00	1.45	1.03	1.03	1.16	2.74
Serine (g/100gDM)	0.90	0.88	0.90	0.80	0.76	0.59	2.25
Tyrosine (g/100gDM)	0.58	0.69	0.46	0.81	0.34	0.80	4.1
Threonine (g/100gDM)	0.79	0.64	0.58	0.45	0.50	0.65	1.89
Tryptophan (g/100gDM)	<20	<20	<20	<20	<20	<20	<20
Valine (g/100gDM)	0.65	0.61	0.53	0.65	0.46	0.41	4.7
Hydrolyze protein (g/100gDM)	20.22	16.83	17.80	13.94	14.56	12.87	52

C: control group, TM: *T. molitor* group, d: day, ME: metabolizable energy, Ca: calcium, P: phosphorous, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n-3 PUFA: omega-3 polyunsaturated fatty acids, n-6 PUFA: omega-6 polyunsaturated fatty acids, Trans FA: trans fatty acids and DM dry matter.

The entire trial, including both the indoor and outdoor stages, lasted 95 days and was divided into three phases, according to the diets used (Table 3.1 and Table 3.2).

The diets were supplied in fine grind form. Feed and water were supplied ad libitum. The health status of the animals was monitored daily during the whole experimental period. During the 95-day experiment, the following parameters of the chickens were measured weekly: live weight (LW), feed intake (FI) and water intake (WI), by using a dynamometer and a digital balance. Subsequently, body weight gain (BWG), as an indicator of growth rate, and feed conversion ratio (FCR), which indicates the relationship between feed intake and weight gain of the animal, were calculated.

3.2.3 Statistical Analysis

Statistical processing of the data was performed using IBM-SPSS Package 26 software (IBM, Chicago, IL, USA). Each group of eight chickens (replicate) was considered a sampling unit (n = 16). Significant differences between C and TM groups for each of the studied parameters were studied by multivariate analysis of variance (MANOVA) fitted to a general linear model (GLM), taking diet and group as fix factors and the initial weights at the second and third stages as covariants, given that those weights were different for each group. Before carrying out the described statistical tests, the Kolmogorov–Smirnov test was performed to check the normality of the recorded data. The statistical significance was assessed at the 95% confidence level (α = 0.05) using Snedecor's F as the contrast statistic.

Differences in the BWG increment (%) between C and TM groups throughout the trial were analyzed using a Student's t-test for paired samples. In contrast, differences in the BWG increment (%) and in FI increment (%) between the C and TM groups for each of the three diets in the trial (F1, F2 and F3) were analyzed using a Student's t-test for independent samples.

All the results are expressed as means and standard errors of the mean (SEM).

3.3 Results

3.3.1 Productive Performance

Throughout the experiment, the chicks remained healthy, and no deaths were observed in any of the groups. The results during this feeding period (Table 3.3) show that the C group reached a significantly higher LW ($p < 0.01$) than the TM group. Consequently, the BWG during the first 29 days of life was also significantly higher ($p < 0.01$) in the chickens of group C than in those of group TM.

Those results are directly related to those obtained for FI, in which BWG was likewise significantly higher ($p < 0.01$) for the C group than for the TM group. In addition, the chickens in group C consumed significantly more water than those in group TM. Finally, regarding the FCR, significant differences were also found between the two groups ($p < 0.01$), with the highest value coming from the TM group.

Weekly analysis of LW revealed significant differences ($p < 0.01$) between the two groups after 15 days of life (Table 3.4), and these differences were maintained until the end of this first period. The C group chickens reached the highest weights. These results are in agreement with those shown in Table 3.3. As for BWG, significant differences ($p < 0.01$) were found between groups in the 2nd, 3rd and 4th weeks. Again, higher values in all cases were reached by group C. The FI was significantly higher for group C during the 1st and 4th weeks ($p < 0.05$, $p < 0.01$, respectively). In contrast, WI showed significant differences ($p < 0.01$) throughout the entire phase, the higher values in all cases being for the C group. For the FCR, the opposite situation to the FI occurred, since significant differences ($p < 0.01$) were obtained between groups in the 2nd and 3rd weeks, the values being higher in both cases in group C.

The results during this diet (Table 3.3) revealed no differences between the two groups in relation to LW, FI, WI and FCR. Nevertheless, at the end of this period, chickens in group C reached a higher weight and had higher feed and water consumption than those in group TM. Conversely, BWG was significantly higher ($p < 0.05$) in the chickens of group C compared to those of group TM. The weekly results (Table 3.4) did not show any significant difference between groups in any of the variables studied during this period.

The results for diet F3 (Table 3.3) did not reveal significant differences in any of the studied variables. However, group C reached higher values for all of them. Weekly analysis (Table 3.4) showed that group C obtained significantly higher LW values ($p < 0.05$) than group TM at 64, 78 and 92 days of life. Similarly, FI was significantly higher ($p < 0.05$) at the 9th week in the C group than in the TM group, which analogously occurred at the 10th week for WI ($p < 0.01$). By contrast, BWG and FCR did not present differences in any of the weeks of this last period.

Table 3. 3. *Total production performance parameters of the two studied chicken groups (C and TM) for the three different diets (mean±SEM).*

Parameter	F1 (1-29 d)			F2 (29-57 d)			F3 (57-95 d)			Total (1-95 d)		
	C	TM	Sig.	C	TM	Sig.	C	TM	Sig.	C	TM	Sig.
LW[1]	583.6±13.6	407.5±14.8	**	2229.0±45.3	1643.3±49.2	NS	4739.0±73.5	3981.5±124.4	NS	4739.0±73.5	3981.5±124.4	NS
BWG	19.4±0.5	13.1±0.5	**	60.2±1.9	44.3±2.1	*	66.0±1.4	63.8±1.2	NS	49.99±0.78	43.8±1.2	**
FI	1059.4±41.6	858.8±47.0	**	3749.9±71.7	2915.7±78.6	NS	8865.0±41.1	8182.8±193.8	NS	13,674.30±198.56	11,957.4±282.6	*
WI	2004.3±48.8	1339.4±43.5	**	6934.2±178.1	4623.6±118.5	NS	14,813.5±618.5	12,014.1±54.1	NS	23,751.88±601.13	17,977.1±427.6	**
FCR	2.0±0.1	2.3±0.1	**	2.3±0.0	2.4±0.1	NS	3.5±0.0	3.5±0.1	NS	2.9±0.1	3.0±0.1	NS

NS, * or **: non-significant or significant at $p < 0.05$ or 0.01, respectively; SEM: standard error of the mean, C: control group, TM: tenebrio group, Sig.: significance, d: days, LW: live weight, BWG: body weight gain, FI: feed intake, WI: water intake and FCR: feed conversion ratio; [1] LW was punctually measured on the last day of the week, coinciding with the end of each period, i.e., on days 29, 57 and 95.

Table 3. 4*. Weekly production performance parameters of the two studied chicken groups (C and TM) for the three different diets (mean ± SEM).*

		Indoor stage									Outdoor stage					
		F1 (0-29 d)					F2 (29-57)				F3 (57-95)					
P	G	0 w (0-1 d)	1st w (1-8 d)	2nd w (8-15 d)	3rd w (15-22 d)	4th w (22-29 d)	5th w (29-36 d)	6th w (36-43 d)	7th w (43-50 d)	8th w (50-57 d)	9th w (57-64 d)	10th w (64-71 d)	11th w (71-78 d)	12th w (78-85 d)	13th w (85-92 d)	14th w (92-95 d)
	C	39.9±0.08	83.8±2.61	191.5±5.13	351.8±11.75	583.6±13.6	949.4±17.2	1,368.6±31.7	1,772.7±35.0	2,229.0±45.3	2,756.7±51.6	3,237.0±53.2	3,493.5±66.5	4,125.0±70.0	4,598.4±73.5	4,739.0±73.5
W[1]	TM	39.8±0.17	83.3±2.91	156.9±4.4	268.7±9.2	407.5±14.8	643.6±18.3	956.9±23.8	1,267.3±25.5	1,643.2±49.2	2,047.5±59.5	2,408.2±68.9	2,803.0±78.2	3,403.0±97.7	3,841.7±111.7	3,981.5±124.4
	Sig.	NS	NS	**	**	**	NS	NS	NS	NS	*	NS	*	NS	*	NS
	C	-	6.3±0.4	15.4±0.6	22.9±1.0	33.1±1.3	52.3±1.0	59.9±3.4	57.7±4.1	67.8±2.2	75.4±2.1	68.6±2.1	36.6±5.5	85.1±2.6	67.6±2.5	46.8±3.5
BWG	TM	-	5.5±0.4	11.2±0.4	16.0±0.9	19.8±1.0	33.7±1.1	44.8±1.8	44.3±2.3	59.1±3.0	57.7±2.5	51.5±2.7	56.4±3.0	85.7±4.6	62.7±4.0	46.6±6.1
	Sig.	-	NS	**	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	C	-	11.5±0.9	28.1±1.5	41.1±1.2	70.7±3.8	111.3±2.0	134.9±3.7	122.3±3.7	167.2±0.2	209.1±1.8	233.4±2.1	212.2±0.3	260.8±3.3	250.3±1.1	234.8±4.6
FI	TM	-	9.0±0.6	28.5±1.4	40.1±3.0	45.1±2.7	76.4±1.5	101.6±3.5	97.6±2.4	140.9±2.0	169.6±0.7	205.0±2.9	207.8±12.2	248.2±1.2	241.5±7.1	225.9±17.1
	Sig.	-	*	NS	NS	**	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
	C	-	21.5±0.6	47.9±1.4	83.6±2.7	133.4±3.4	219.3±9.7	231.3±6.5	242.0±7.4	298.0±14.4	410.0±15.7	410.3±21.2	301.3±19.4	409.8±7.8	411.7±18.5	404.0±13.2
WI	TM	-	14.7±1.3	29.4±1.0	55.2±2.3	92.0±5.1	113.0±6.5	161.2±5.0	165.9±3.9	220.4±3.2	287.6±10.9	328.4±2.3	266.4±8.6	349.0±10.6	330.1±6.0	361.0±20.8
	Sig.	-	**	**	**	**	NS	NS	NS	NS	NS	**	NS	NS	NS	NS
	C	-	1.8±0.1	1.8±0.1	1.8±0.1	2.2±0.2	2.1±0.1	2.3±0.1	2.2±0.1	2.5±0.1	2.8±0.0	3.4±0.0	5.1±0.3	3.1±0.2	3.7±0.2	5.0±0.1
FCR	TM	-	1.7±0.1	2.5±0.1	2.5±0.1	2.3±0.1	2.3±0.1	2.2±0.0	2.2±0.1	2.4±0.1	2.9±0.1	4.0±0.5	3.8±0.4	2.9±0.1	3.9±0.3	4.9±0.3
	Sig.	-	NS	**	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, * or **: non-significant or significant at $p < 0.05$ or 0.01, respectively; SEM: standard error of the mean, P: parameter, G: group, C: control group, TM: tenebrio group, Sig.: significance, w: week, d: days, LW: live weight, BWG: body weight gain, FI: feed intake, WI: water intake and FCR: feed conversion ratio; [1] LW was punctually measured the last day of each week, i.e., on days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85 and 95.In general, during the whole experimental period, BWG was significantly higher ($p < 0.01$) for chickens of group C. Furthermore, FI and WI were also significantly higher ($p < 0.05$ and $p < 0.01$, respectively) in group C. However, in both LW and FCR there were no significant differences between the two groups, albeit the LW deviation was higher in the TM group (Figure 3.2).

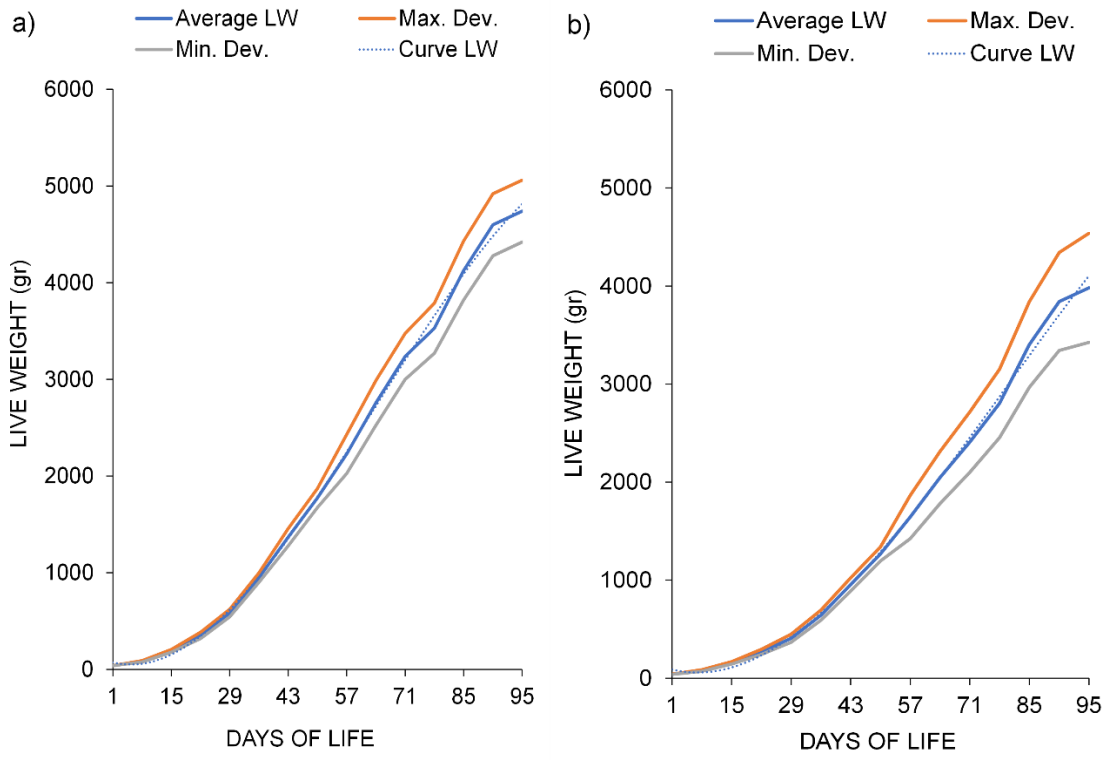

Figure 3. 2. *LW curves for the two studied groups (C, Control group and TM, Tenebrio group) throughout the growing period (95 days). (a) Group C and (b) group TM. LW: live weight, Max Dev.: maximum live weight deviation and Min. Dev.: minimum live weight deviation.*

3.3.2 Growth and Feed Intake Increases

Chicken growth was highest in the early stages of life (Figure 3.3). The BWG between successive periods was significantly different ($p < 0.01$) throughout the trial. Analyzing each group separately, the greatest BWG corresponded to the period between days 8 and 15, the greater increase being for group C than group TM (128.45% vs. 100.36%, respectively). After 36 days, coinciding with having had less *T. molitor* content in the rations, the weight increase was lower than in the previous stages, though the increase was higher for the TM group.

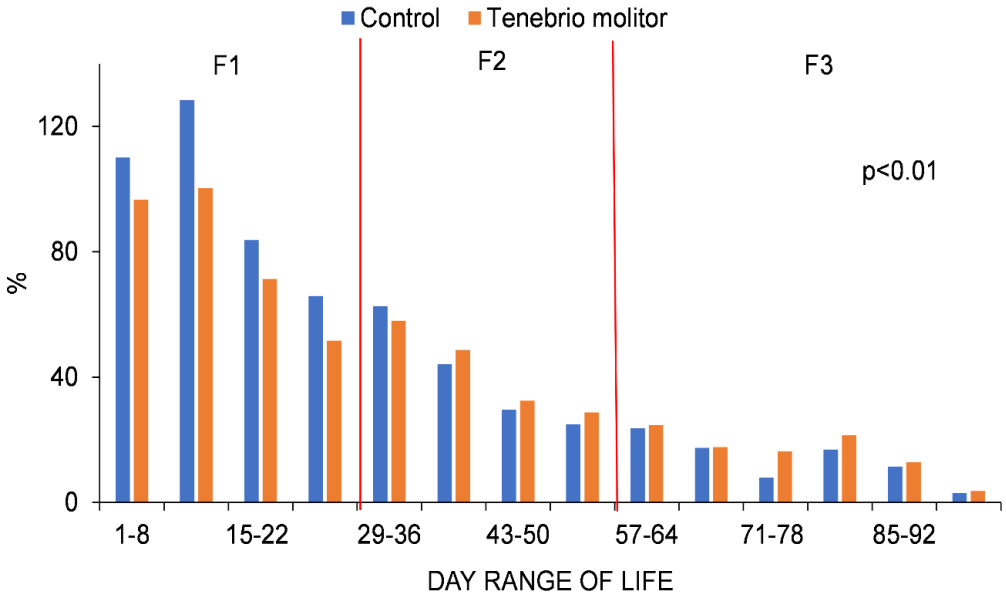

Figure 3. 3. *BWG (Body Weight Gain) percentage increase in each studied group (C and TM) between two consecutive weeks throughout the whole study period.*

The greatest differences in BWG for a specific period between the two groups were found in the mid-term days of the trial (Figure 3.4). Significant results were found from 15 days of life to the end of the trial ($p < 0.01$), during which time group C reached higher weights. The greatest difference in BWG between the two groups occurred at 36 days, when the weight of group C exceeded the weight of group TM by 32.21%. At the end of the trial, the differences between groups decreased.

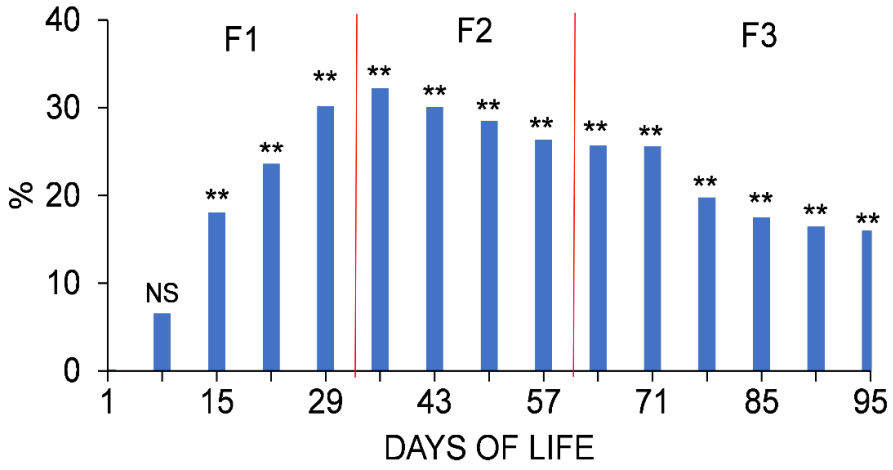

Figure 3. 4*. BWG percentage increase in the C group over the TM group throughout the whole study period. NS or **: non-significant or significant at p < 0.01, respectively.*

Significant differences ($p < 0.01$) in the FI increase between the two groups (Figure 3.5) occurred from the 4th to 10th weeks. The greatest difference between the two groups occurred in the 4th week, group C being the one that exceeded the TM group by 36.20%. From day 29, i.e., when they changed from the F1 to the F2 diet, differences were still significative but diminished.

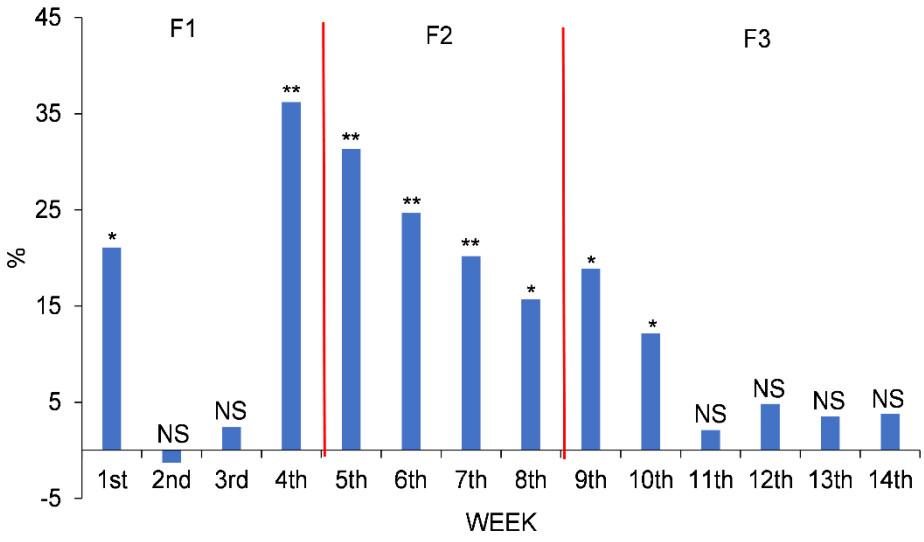

Figure 3.5. *FI percentage increase in the C group over the TM group throughout the whole study period. NS, * or **: non-significant or significant at p < 0.05 or 0.01, respectively.*

3.4 Discussion

There are relatively few studies involving *Tenebrio molitor* meal for slow-growing chickens for 95 days, as the growth cycle is usually shorter (approximately 60 days) (Bovera et al., 2016; Nascimento Filho et al., 2021). The results obtained in this work are partially in agreement with those found by Benzertiha et al. (2020), who determined that the addition of insect meal during the first 35 days of life of chickens improved their BWG and FI, but increased their FCR.

During the period of F1 intake, there was a reduction in FI and a lower final LW. In the compositions of the diets, non-important differences were observed, except for a lower fat content in the TM group. However, the amino acid contents were very similar in both groups, which did not explain the obtained differences. Therefore, it could be considered that the proportion of *Tenebrio molitor* used during the first days of life may affect the total digestibility of the diet.

During the feeding period of the second and third diets (F2 and F3), no differences were found in growth parameters except for BWG in F2. These results coincide with those exposed by Biasato et al. (2016), who pointed out that the inclusion of insect meal did not negatively affect chicken performance, supporting the feasibility of its inclusion (Bellezza Oddon et al., 2021). Between 30 and 62 days, Bovera et al. (2015) found no differences in FI in chickens when meal from this same insect species was used to replace soybean meal in isoproteic and isoenergetic diets, which is in line with our results regarding FI. Furthermore, the results of this work also coincided with those of Biasato et al. (2016), who claimed that *T. molitor* meal inclusion did not affect productive performance of chickens by either the 43rd or the 97th day of life.

In the middle and final periods of the trial, no differences in LW were found, contrary to the results of Biasato et al. (2018), who found that chicken LW improved when *T. molitor* larval meal was included. Throughout the entire trial, no differences in BWG or FI were found, agreeing with the results of Leiber et al. (2015), in which FI and BWG were equal for both chickens that received insect meal and those that did not over a period of 7 to 82 days. The overall FCR of the trial showed no differences between groups. This is in agreement with the results described by Biasato et al. (2016), in which the inclusion of *T. molitor* meal in the period from 43 to 97 days did not affect the FCR.

During the first 4 weeks, the FCR exhibited differences between the two groups. There were higher values for the TM group, which is consistent with the results of Benzertiha et al., (2020), whose work indicated that the inclusion of *Tenebrio molitor* negatively affected the FCR during the first 35 days. According to Bovera et al. (2016), FCR improved in chickens fed *Tenebrio molitor* from 30 to 62 days; in the present study, no differences in F2 were found. Even so, better results in both groups were recorded than those obtained by other authors (Bovera et al., 2016).

The results of this work demonstrate that, despite the fact that during the first month the performance of the chickens in the TM group was lower, at the end of the growth cycle it was equivalent to that of the C group. Therefore, starting from smaller chickens in the first month, the substitution of soybean meal by *Tenebrio molitor* meal improves the productive performance of the animals in the later stages (Benzertiha et al., 2020).

Consequently, this insect meal is suggested as a feasible alternative to replace soybean in poultry diets (Selaledi et al., 2020). This is also supported by the conclusions elucidated by other authors (Kim et al., 2020), who obtained better performance in chickens that were fed insect meal. For Leiber et al. (2015), the LW of chickens fed insect meal was also higher. In other experiments conducted with fattening poultry, increasing the inclusion of *T. molitor* in quail diets could improve LW and FCR (Zadeh et al., 2019).

High amounts of insect meal in early life stages can be detrimental to chickens, as it reduces FI and LW (Atteh & Ologbenla, 1993; Bamgbose, 1999). Thus, some authors have obtained better results by making partial substitutions, suggesting that the optimal ratio of soybean meal to insect meal substitution depends on the age of the animals (Awoniyi et al., 2003). For Biasato et al. (2017), progressively increasing *Tenebrio molitor* content in the diet during the first 25 days impaired FI and FCR of chickens. This conclusion is supported by other studies, which concluded that the inclusion of *T. molitor* meal in chickens diets can improve LW and FI, albeit it could negatively affect FCR if inclusion levels are not adequate (Biasato et al., 2018).

The alternative feed tested could partially replace soybean feeds in chicken diets, but full substitution would not be appropriate (Leiber et al., 2015). In this context, Ravindran and Blair (1993) noted that the chitin contained in the insect exoskeleton is hard to digest by chickens. This situation is even more acute in the early stages of chickens' lives, in which their digestive system is still in an immature state, and in addition, there is a tendency to increase the insect meal intake due to the high protein requirements at this stage of growth. Therefore, in the first month of life, soybean should continue to be part of the diets of chickens, its substitution being feasible 5th week of life onwards. This fact reaffirms the idea of Olkowski (2018), who tried to replace soybean meal with lupin meal in a chicken diet, concluding that it could be carried out from the end of 4 weeks onwards, because the application of high levels of yellow lupin seed in the initial rations produced a negative effect on bird performance. The results of the present study also show that the initial differences in growth between the two groups decreased as the trial progressed, coinciding with a lower inclusion of insect meal in the diet.

3.5 Conclusions

In this work, we tried to assess the effect of replacing soybean meal as the main protein intake with *Tenebrio molitor* larvae meal in isoproteic and isoenergetic diets for slow-growing chickens. The results suggest that such a high percentage of insect meal should not be included in the first days of life of the animals (up to 29 days).

Therefore, a partial substitution would be more appropriate during this first period, since after it, the chickens improved notably their development and performance, reaching at the end of the cycle (95 days) values similar to those of chickens fed soybean meal, despite starting from animals with less development during the first month. Under these conditions, *T. molitor* larval meal can be an alternative protein source to include in the diets of slow-growing chickens.

CAPÍTULO 4:

Use of *Acheta domesticus* meal as a full soybean substitute in the feeding of slow-growing chicks

Capítulo 4:

Use of *Acheta domesticus* meal as a full soybean substitute in the feeding of slow-growing chicks

Jaime Nieto[1], Javier Plaza[1], Javier Lara[2], José-Alfonso Abecia[3], Isabel Revilla[4], Carlos Palacios[1]

[1] Area of Animal Production, Faculty of Environmental and Agricultural Sciences, University of Salamanca, 37007 Salamanca, Spain.
[2] Animal Husbandry and Animal Health Assistance Educational Program, High-School Torres Villarroel, 37008 Salamanca, Spain.
[3] Institute of Research in Environmental Sciences of Aragón (IUCA), University of Zaragoza, 50013 Zaragoza, Spain.
[4] Area of Food Technology, E.P.S. of Zamora, University of Salamanca, 49022 Zamora, Spain.

Poultry Science. 2023, 102(4), 102503; https://doi.org/10.1016/j.psj.2023.102503

Received 18 October 2022, Accepted 10 January 2023, Available online 16 January 2023, Version of Record 3 February 2023.

Resumen

En los últimos años se ha visto una creciente reticencia frente a determinadas materias primas convencionales utilizadas en la industria de pienso en general, y de la harina de soja en particular, provocada por su baja sostenibilidad ambiental, lo que ha puesto de manifiesto la necesidad de buscar fuentes alternativas de proteínas para la alimentación animal.

La harina de insectos se considera un alimento rico en proteínas y ambientalmente sostenible, ya que su producción está asociada a bajos requerimientos de tierra y agua, baja huella de carbono y un modelo de economía circular, posicionándose como un posible sustituto a la harina de soja. Además, la regulación europea ha permitido recientemente el uso de diferentes harinas de insectos entre las que se encuentra el grillo doméstico (*Acheta domesticus*). Los consumidores están cada vez más concienciados con la producción sostenible y el bienestar animal, aumentando así el uso de estirpes de crecimiento lento, con ciclos de producción más largos y una mayor adaptabilidad a sistemas de producción alternativos.

El objetivo de este trabajo fue evaluar el efecto de la harina de grillo (*Acheta domesticus*) cuando se usa como sustituto total de la harina de soja sobre los rendimientos de pollos de crecimiento lento durante los primeros 95 días de vida.

Un total de 128 pollitos de crecimiento lento machos (*Colorield*) de un día de edad se separaron de forma aleatoria en 16 corrales (unidades experimentales) de 8 pollos cada uno. La mitad de los corrales fueron alimentados con harina de soja como principal fuente proteica, conformando el grupo control (C) y la otra mitad fue alimentada con harina de *Acheta domesticus* como reemplazo total a la harina de soja (grupo AD). El ensayo finalizó cuando los animales alcanzaron los 95 días de vida, momento en el que se realizó el sacrificio.

Durante los primeros 45 días, los animales se alojaron en corrales interiores con condiciones de temperatura controladas, con una densidad animal de 0,10 m^2/pollito. Posteriormente y hasta el final del ensayo todos los animales fueron trasladados a una instalación al aire libre dividida en dieciséis corrales provistos de refugio con una densidad animal de 0,25 m^2/pollo. A lo largo del ensayo se emplearon tres dietas diferentes (F1, F2 y F3) que fueron cambiando cada mes. Para cada una de estas tres dietas, los dos grupos experimentales de pollos (C y AD) fueron alimentados isoenergéticamente e isoproteicamente.

Al final de la dieta F1, el grupo C alcanzó un peso vivo mayor que el grupo AD, derivando también en una mayor ganancia de peso corporal. Los consumos de pienso y agua también fueron mayores en el grupo C y su índice de conversión alimenticia fue menor frente el grupo AD. Sin embargo, al final de la dieta F2, las diferencias entre los grupos C y AD sólo se observaron para el consumo de agua, con valores más elevados para los primeros En el caso de la dieta F3, el consumo de pienso presentó diferencias significativas entre grupos, siendo los pollos del grupo AD los que consumieron mayor cantidad de alimento. Los resultados generales del ensayo (1-95 días) no mostraron diferencias entre grupos para ninguno de los parámetros analizados.

En conclusión, los resultados obtenidos sugirieron que no es apropiado incluir niveles elevados de harina de insectos en la dieta de los polluelos durante las primeras 4 semanas de vida. En su lugar, se podría recomendar una sustitución parcial por harina de soja, ya que cantidades elevadas de harina de insectos parecen ser perjudiciales para los pollitos jóvenes.

Por tanto, en estas condiciones, la harina de *Acheta domesticus* puede ser una fuente proteica interesante para incluir en pollitos de lento crecimiento como sustituto parcial de la harina de soja durante el primer mes de vida de las aves y como sustituto total a partir de ese momento hasta el final del ciclo productivo.

Abstract

Sustainable poultry meat production involves the use of slow-growing chick strains and the utilization of new protein sources as an alternative to the current monopoly of soybean meal. In this scenario, a study was conducted to assess the effect of replacing soybean meal with domestic cricket (*Acheta domesticus*) meal on the developing cycle of slow-growing chicks. To this end, a total of 128 one-day-old male chicks (*Colorield*) were randomly assigned into 16 experimental units, each consisting of 8 chicks, which in turn were grouped into 2 groups fed isoproteic and isoenergetic diets in which the protein source differed: the control group (C) fed soybean meal and the Acheta group (AD) fed *Acheta domesticus* insect meal as the main protein source. Chicks were slaughtered at 95 d of age. Three different diets (F1, F2 and F3) were used for each experimental group according to the nutritional needs of the birds during their growth. The F1 diet (1–29 d) resulted in higher feed and water intake and higher body weight gain for group C, but a lower feed conversion rate. On the contrary, during F2 (29–60 d) no differences in productive performances were observed between the 2 groups, except for a higher water intake for group C. Finally, during the period corresponding to diet F3 (60–95 d) there were only differences in feed intake, which was higher for the AD group. In conclusion, the substitution of soybean meal for *Acheta domesticus* meal caused a decrease in feed intake during the first month and consequently a lower body weight. During the first 4 weeks of life of the chicks, a partial replacement of soybean meal may be recommended, since high inclusions of *A. domesticus* meal in this period seem to be detrimental in young chicks. Given the absence of relevant differences in productive performances between both groups, it could be concluded that the use of *A. domesticus* cricket meal can be a potential protein alternative to soybean meal.

Keywords: *Acheta domesticus*; alternative poultry farmin high-protein sourc; insect meal; slow-growing chick

4.1 Introduction

The world's population is exponentially increasing, estimated at 9.7 billion inhabitants in 2050 (Newbold, 2017), and along with it the consumption of resources from the primary sector. This population growth, together with dietary changes toward greater consumption of meat products, has encouraged poultry meat to become an alternative to meet this food demand (Allegretti et al., 2018).

Besides, the increasing reluctance against certain conventional raw materials used in the feed industry, triggered by their negative environmental sustainability, highlights the need to look for new alternative protein sources for animal feed (Abro et al., 2020). Particularly worrying is the case of soybean as the main protein source in animal feed (Vauterin et al., 2021), since its cultivation is one of the main causes of deforestation worldwide, it is associated with an enormous water consumption, an indiscriminate use of pesticides and chemical synthesis products, as well as an irreparable loss of biodiversity, given the monopoly of the transgenic varieties used (Sánchez-Muros et al., 2014). This scenario has motivated a growing interest in nonconventional feed ingredients that are more efficient in terms of production performance and environmental sustainability (de Souza et al., 2021), especially those that represent an alternative protein source, the price, and availability of which may jeopardize poultry meat production (Nalunga et al., 2021).

Among the alternative sources of animal feed, insect meal is considered a high protein and environmentally sustainable food, since its production is associated with low land and water requirements, low carbon footprint and a circular economy model (Abro et al., 2020). Insect-based protein is rapidly emerging as an alternative food source both as a potential substitute for soy-based protein for use in animal feed and for direct human consumption (Vauterin et al., 2021). The use of insect meal is even more interesting in certain animal husbandry systems. Particularly, it is considered a potential substitute for fishmeal and soybean meal in the diets of conventional poultry systems (Barroso et al., 2014; Khan, 2018; Nieto et al., 2022), due to its high protein content, its rapid production owing to the short life cycle of insects and its relative ease of production (Perera & Bhujel, 2021).

Besides, consumers are increasingly demanding greater transparency in the production chain, in which achieving a sustainable, healthy product that also complies with animal welfare has become a priority objective (Font-i-Furnols & Guerrero, 2022). For this reason, slow-growing chick strains are more and more used in the meat poultry sector, with longer production cycles and a greater adaptability to alternative production systems (Allais et al., 2019; Vissers et al., 2019). Although these strains need more time to reach market weight, their feed conversion might become more efficient when using new ingredients included in their diets, plus presenting nutritional characteristics more appreciated by consumers (Filho et al., 2021).

Furthermore, given that the natural behavior of chicks is to feed on different insects throughout their lives (Khan, 2018), strong preferences of chicks for diets composed of insect meal offered simultaneously with other ingredients have already been revealed (Nascimento Filho et al., 2020). Therefore, it could be expected that the use of insect meal in their diet would have a positive impact on both the productive performance of chickens and the nutritional quality of their meat. In the scientific literature, some experiences have been reported on the use of more common insect meals in the market, such as the *Tenebrio molitor* or the *Hermetia illucens* (Biasato et al., 2017; de Marco et al., 2015; de Souza et al., 2021), in shorter production cycles of approximately 2 mo, typical of broilers (Bovera et al., 2016; Nascimento Filho et al., 2021)).

In addition, European regulation recently allowed the use of Acheta domesticus as an ingredient in monogastric diets, together with *Herrmetia illucens*, *Musca domestica, Tenebrio molitor, Alphitobius diaperinus, Gryllodes sigillatus*, and *Gryllus assimilis*. Considering the above the main objective of this work was to assess the effect of cricket (*Acheta domesticus*) meal made from insects in the early stages of adulthood (incomplete metamorphosis) on the performance of slow-growing chicks during 95 d of life, acting as a total substitute for soybean meal. Three different diets were used throughout the life of the animals, monitoring their growth along the trial.

4.2 Material and Methods

4.2.1 Chick management and experimental design

The experiments were conducted in accordance with the principles and specific guidelines presented in the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 4th edition, 2020, avoiding unnecessary discomfort to the animals.

Particularly, all slow-growing chicks used in the trial were treated in accordance with the principles of European Parliament and Council Directive 2003/65/EC of 22 July 2003, amending Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes. These animals were manipulated under Order ECC/566/2015, of 20 March, establishing the training requirements to be met by personnel handling animals used, bred or supplied for experimental and other scientific purposes, including teaching. The experimental protocol was approved by the Bioethics Committee of the University of Salamanca (Spain), with registration number 0000590 regulated by RD 53/2013, of 1 February, establishing the basic rules applicable for the protection of animals used in experimentation and other scientific purposes, including teaching. The trial was conducted by qualified personnel from the Animal Production area in the facilities and experimental fields of the University of Salamanca.

A total of 128 *Colorield*-strain one-day-old male slow-growing chicks (hereinafter named as chicks), vaccinated in the hatchery facilities against Marek´s disease and avian infectious bronchitis, were used in this experiment. After their reception, these chicks were randomly separated into 16 different experimental units ($n = 8$ chicks each) on the basis of maintaining a homogeneous initial life weight among them. In turn, eight of the 16 experimental units were randomly selected to comprise the control group (C) (mean live weight of 36.0±0.8 g), that was fed soybean meal as the main protein source, and the remaining eight units formed the Acheta group (AD) (mean live weight of 36.5±0.6 g), whose main protein intake was *Acheta domesticus* meal, as a total replacement of soybean meal. All the chicks were raised until 95 days of age and then slaughtered in an authorized slaughterhouse in compliance with Council Regulation (EC) No. 1/2005 of 22 December 2004 on the protection of animals during transport and related operations and amending Directives 64/432/EEC and 93/119/EC and Regulation (EC) No. 1255/97 and with Council Regulation (EC) No. 1099/2009 of 24 September 2009 on the protection of animals at the time of slaughter.

During the first 45 days of life, each experimental unit was housed in a 1.50 × 0.60 m^2 indoor pen (0.10 m^2/chick) with first age feeders and drinkers until day 20, which were replaced by second age ones from that day onwards. The floor of the pens was cover with 7 cm thick layer of wood shavings. Temperature and humidity were progressively fitted to the optimum life conditions of the chicks in each moment of the experiment. Specifically, the average temperature inside the barn was 31.9±1.8 ºC during the first

week, 29.1±2.1 ºC the second week, 25.4±1.7 ºC the third week and 20.8±2.7 ºC for the rest of the trial. Overall, the chicks were subjected to natural daily photoperiods (10-11 hours).

From the 46th day of life (approximately half of the cycle of the chicks) until the end of the trial, the 128 chicks were moved to an outdoor facility divided into sixteen 8.2 × 4 m^2 pens (0.25 m^2/chick) with plastic mesh, where they had uninterrupted freedom of movement. The soil of the outside yard was completely natural, neither treated nor cultivated, and it was perimetrically enclosed by a 1 m high metal sheet, which was buried 30 cm, and a 13 × 13 mm grid plastic mesh covering roof and sides to prevent predators from entering. Moreover, these pens counted with circular feeders and drinkers and a wooden shelter with a single entrance on the south side (0.5 × 0.5 m^2), and an upper opening to ensure a good ventilation and a translucent section for lighting.

4.2.2 Composition of the experimental diets and data treatment

Three concentrated diets (F1, F2 and F3) were used throughout the whole experiment, feeding the chicks for about a month. Thus, F1 was used from day 1 to day 29, F2 from day 29 to day 60 and F3 from day 60 to day 95. The proportions of the raw materials used in the composition of these diets are shown in Table 4.1. Regardless the diet used, both groups of chicks (C and AD) were iso-energetically and iso-proteically fed throughout the whole experiment. Thus, in order to meet the nutritional requirements of the birds, the recommendations of the National Research Council (NRC, 1994) for medium-slow differentiated growth chicks were followed. Moreover, prior to fed these diets to the chicks, they were chemically and nutritionally analyzed (Table 4.2) in the food analysis laboratories of the Abiomed Higiene S.L. company (Salamanca, Spain), who operates under an ISO 9001 quality system.

***Table 4. 1**. Composition of the experimental diets for two groups of chicks (% ingredients), fed diets including either soybean meal (group C) or Acheta domesticus insect meal (group AD) as the main protein source.*

Ingredients	C[1]			AD[2]		
	F1[3]	F2[4]	F3[5]	F1[3]	F2[4]	F3[5]
Corn	37.0	48.0	31.0	0.0	9.0	16.0
Wheat	11.0	12.0	37.0	12.0	20.0	38.0
Barley	0.0	0.0	0.0	32.0	16.0	0.0
Soybean meal	29.0	20.0	12.0	0.0	0.0	0.0
A. domesticus meal	0.0	0.0	0.0	17.0	10.0	7.3
Sunflower	19.0	16.0	16.0	0.0	11.0	8.7
Oats	0.0	0.0	0.0	35.0	30.0	26.0
Vitamin-mineral premix[6]	4.0	4.0	4.0	4.0	4.0	4.0

[1] C: control group.
[2] AD: *Acheta domesticus* group.
[3] F1: 1-29 days.
[4] F2: 29-60 days.
[5] F3: 60-95 days.
[6] Vitamin-mineral prefix includes in percentage: Methionine 0.21; Calcium 20.75; Phosphorus 5.58; Sodium 6.38. Includes per kg of diet: Vitamin D3 100490 UI, Vitamin E 398 UI, Vitamin A 251230 UI, Betaine anhydrous 4002.84 mg. Anti-caking agents: bentonite 10%; sepiolite 3340.2 mg/kg. Trace element premix includes per kg of diet Cu (sulfate II pentahydrate), 335 mg; Fe (sulfate II monohydrate), 1324 mg; Mn (oxide), 3336 mg, Zn (oxide), 2835; I (calcium iodate anhydrous), 51.07 mg; Se (sodium selenite), 10.10 mg.

Table 4. 2. *Chemical/nutritional characteristics of the experimental diets for two groups of chicks fed diets including either soybean meal (group C) or Acheta domesticus insect meal (group AD) as the main protein source.*

Item	F1 (1-29 d)		F2 (20-60 d)		F3 (60-95 d)		Soybean meal	*A. Domesticus* meal
	C[1]	AD[1]	C	AD	C	AD		
%Moisture	10.1	9.1	10.7	9.3	10.2	9.7	12.1	4.4
%Ashes	6.1	6.4	4.8	6.3	5.6	5.9	6.2	4.1
%Crude fat	12.1	8.4	9.8	12.4	6.7	10.7	1.9	22.6
%Crude fiber	6.0	8.3	5.3	8.2	4.7	7.4	5.9	6.9
%Starch	35.8	33.8	45.1	37.1	45.5	41.5	0.1	1.7
%Crude protein	19.8	19.3	15.7	15.2	14.6	14.8	44.0	59.5
ME[2] (Kcal/kgDM)	2,802.8	2,897.3	2,900.0	2,966.2	2,950.8	2,978.3	1,890.0	5,817.0
Mean dietary crude protein (%)	19.6		15.5		14.7		44.0	59.5
Mean dietary ME (Kcal/kgDM)	2,850.0		2,933.1		2,964.6		1,890.0	5,817.0
%Ca[3]	1.24	1.19	1.21	1.19	1.20	1.18	0.29	0.25
%P[3]	0.40	0.61	0.36	0.50	0.34	0.43	0.61	2.13
%SFA[4]	1.6	2.7	1.3	2.4	0.9	2.1	0.6	5.3
%MUFA[4]	7.9	3.1	6.2	7.6	4.0	6.3	0.9	8.9
%PUFA[4]	2.6	2.6	2.3	2.3	1.9	2.3	2.6	8.5
%n-3 PUFA[4]	0.17	0.09	0.13	0.08	0.11	0.08	0.31	0.27
%n-6 PUFA[4]	2.4	2.5	2.2	2.3	1.79	2.18	2.27	8.20
%Trans FA[5] (C18:1T+C18:2T+C13:3T)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Aspartic acid + Asparagine (g/100gDM)	1.78	1.02	1.62	0.85	1.26	1.10	4.84	3.60
Glutamic acid + Glutamine (g/100gDM)	3.09	2.36	3.05	3.14	3.01	2.36	7.56	6.30
Alanine (g/100gDM)	1.19	1.26	0.77	0.87	0.77	0.84	1.89	5.0
Arginine (g/100gDM)	1.37	0.82	1.36	0.91	1.04	0.85	3.18	3.40
Cysteine (g/100gDM)	<20	<20	<20	<20	<20	<20	<20	<20
Cystine (g/100gDM)	0.11	0.11	0.13	0.19	0.14	0.13	0.70	0.24
Phenylalanine (g/100gDM)	0.91	0.51	0.76	0.50	0.68	0.55	2.19	1.62
Glycine (g/100gDM)	1.33	1.22	1.19	1.05	0.79	1.03	1.89	2.68
Histidine (g/100gDM)	0.81	0.44	0.65	0.47	0.52	0.57	1.17	1.46
Isoleucine (g/100gDM)	0.74	0.46	0.59	0.41	0.51	0.48	1.96	2.03
Leucine (g/100gDM)	1.50	0.88	1.25	0.82	0.89	0.73	3.29	4.20
Lysine (g/100gDM)	1.16	0.85	1.08	0.59	0.83	0.65	2.68	2.83
Methionine (g/100gDM)	0.20	0.17	0.19	0.16	0.19	0.18	0.59	0.69
Proline (g/100gDM)	1.18	1.09	1.12	0.73	1.07	0.86	2.16	3.70
Serine (g/100gDM)	1.18	0.75	1.02	0.65	0.90	0.84	2.11	1.70
Tyrosine (g/100gDM)	0.56	0.46	0.43	0.36	0.36	0.44	1.73	4.40
Threonine (g/100gDM)	0.76	0.59	0.63	0.46	0.55	0.55	1.72	2.01
Tryptophan (g/100gDM)	<20	<20	<20	<20	<20	<20	<20	<20
Valine (g/100gDM)	0.82	0.62	0.64	0.59	0.59	0.63	2.07	3.70

[1] C: control group, AD: *Acheta domesticus* group.
[2] ME: metabolizable energy.
[3] Ca: calcium, P: phosphorous.
[4] SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n-3 PUFA: omega-3 polyunsaturated fatty acid, n-6 PUFA: omega-6 polyunsaturated fatty acid.
[5] Trans FA: trans fatty acid, DM: dry matter.

Both water and feed were supplied *ad libitum*, being the latter provided to the chicks in find grind form. Although the health status of the birds was daily monitored, the following variables were weekly measured during the whole trial, according to the procedure indicated in (Nieto et al., 2022): live body weight (LBW), body weight gain (BWG), feed intake (FI), water intake (WI) and feed conversion ratio (FCR).

4.2.3 Statistical analysis

As aforementioned, 16 experimental units randomly assigned to two different groups were considered in this trial. In order to assess the potential significant differences between C and AD groups for each of the studied variables, a multivariate analysis of variance (MANOVA) fitted to a general linear model (GLM) was use, taking diet and group as fix factors. The initial weight at the beginning of the second and third stages (days 29 and 60, respectively) were included in the model as covariates, given the heterogeneity of those weights between groups in these specific moments of the trial. Prior to this procedure, the Kolmogorov–Smirnov test was performed to check the normality of the recorded data. The statistical significance was assessed at the 95% confidence level ($a = 0.05$) using Snedecor's F as the contrast statistic.

Regarding the assessment of the possible existence of differences in the LBW increment (%) between C and AD groups throughout the trial, a Student's t-test for paired samples was conducted. Contrary, differences in the BWG increment (%) between the C and AD groups for each of the three diets in the trial (F1, F2 and F3) were analyzed using a Student's t-test for independent samples.

All the statistical processing of the data was performed using IBM-SPSS Package 26 software (IBM, Chicago, IL, USA). Results were expressed as mean and pooled standard error of the mean (SEM).

4.3 Results

4.3.1 Productive performance

Overall, the results suggested similar performances between both experimental groups (C and AD) in all the variables studied throughout the trial, with no significant differences between groups (Table 4.3). However, there were differences between the performance of the groups within each of the stages of the experiment, corresponding to each of the diets supplied (F1, F2 and F3).

Table 4. 3. *Total production performance parameters of two chicken groups fed diets including either soybean meal (group C) or Acheta domesticus insect meal (group AD) as the main protein source for the three different diets offered (mean ± SEM[1]).*

Variable	Group	F1 (1-29 d)	F2 (29-60 d)	F3 (60-95 d)	Total (1-95 d)
LBW[3,4] (g)	C[2]	353.71	1,084.74	2,484.26	2,484.26
	AD[2]	291.41	960.25	2,121.05	2,121.05
	SEM[1]	9.80	32.35	72.09	72.09
	P-value	0.000	0.762	0.763	0.763
BWG[3] (g)	C	11.35	24.10	41.31	25.02
	AD	9.10	21.58	33.17	22.18
	SEM[1]	0.35	1.06	1.28	1.18
	P-value	0.000	0.948	0.255	0.617
FI[3] (g)	C	896.90	2,499.40	4,502.85	7,899.15
	AD	766.33	2,826.10	5,276.97	8,869.40
	SEM[1]	22.21	158.36	237.81	346.72
	P-value	0.001	0.581	0.030	0.459
WI[3] (mm)	C	1,245.20	4,005.01	9,305.92	14,556.13
	AD	1,040.87	3,277.89	7,323.75	11,642.51
	SEM[1]	35.04	211.91	621.71	873.91
	P-value	0.001	0.010	0.259	0.189
FCR[3]	C	2.83	3.35	3.32	3.39
	AD	3.01	4.24	4.55	4.26
	SEM[1]	0.04	0.31	0.39	0.30
	P-value	0.043	0.274	0.207	0.097

[1] SEM: pooled standard error of the mean.
[2] C: control group, AD: *Acheta domesticus* group.
[3] BWG, body weight gain; FCR, feed conversion ratio; FI, feed intake; LBW, live body weight; WI, water intake.
[4] LBW was punctually measured on the last day of each period, i.e., on days 29, 60, and 95.

At the end of the stage corresponding to the F1 diet, group C reached a higher LBW than group AD (P=0.000). Consequently, the BWG in this first period was also higher in the chicks of group C compared to those of group AD (P=0.000). Regarding intakes, FI and WI were higher for group C (P=0.001;P=0.001, Table 4.3). Differences were also observed for the FCR, being higher for the AD group (P=0.043). However, at the end of the diet F2, differences between C and AD groups were only observed for the WI (P=0.010), with higher values for the former (Table 4.3). In the case of the diet F3, FI presented significant differences between groups (P=0.030), being the chicks of group AD the ones that consumed a higher amount of feed.

The weekly analysis (Table 4.4) revealed differences in LBW and BWG among the experimental groups (C and AD) within the period of the F1 diet, particularly between weeks 2 and 4 (LBW: $P_{2w} = 0.000; P_{3w} = 0.000; P_{4w} = 0.000$; BWG: $P_{2w} = 0.000; P_{3w} = 0.013; P_{4w} = 0.001$), with higher values for group C. Furthermore, in this first period, group C chicks showed higher FI from weeks 1 to 3 ($P_{1w} = 0.046; P_{2w} =$

$0.000; P_{3w} = 0.000$) than the chicks from group AD. As for the WI, group C chicks consumed more water than the group AD chicks during the second and fourth weeks ($P_{2w} = 0.001; P_{4w} = 0.000;$). However, the FCR was higher for group C during the first week, but lower during the second and fourth weeks than the FCR of the AD group ($P_{1w} = 0.034; P_{2w} = 0.003; P_{4w} = 0.000$). During the F2 diet period, differences between experimental groups were found regarding for LBW and WI (Table 4.4). Specifically, the chicks of group C were heavier than the chicks of group AD at the end of the eighth week ($P_{8w} = 0.003$), which corresponded to the measure taken on day 53. During the ninth week, chicks from group C consumed more water than the ones from group AD ($P_{9w} = 0.021$). Contrary to the results found within the previous diets, during the period of the diet F3·there were not differences between the two experimental groups regarding growing performance (Table 4.4), albeit differences in the WI were exhibited in the last week of the trial ($P_{14w} = 0.028$), with a higher consume, again, for the chicks of group C.

Table 4. 4. *Weekly production performance parameters of the chicken groups fed diets including either soybean meal (group C) or Acheta domesticus insect meal (group AD) as the main protein source. for the three different diets offered (mean ± SEM[1]).*

Variable	Group	Indoor stage							Outdoor stage						
		F1 (1-29 d)				F2 (29-60 d)					F3 (60-95 d)				
		1 w (1-8 d)	2 w (8-15 d)	3 w (15-22 d)	4 w (22-29 d)	5 w (29-36 d)	6 w (36-43 d)	7 w[5] (43-46 d)	8 w (46-53 d)	9 w (53-60 d)	10 w (60-67 d)	11 w (67-74 d)	12 w (74-81 d)	13 w (81-88 d)	14 w (88-95 d)
LBW[3,4] (g)	C[2]	60.84	113.29	207.66	353.71	518.82	699.26	776.58	906.32	1,084.74	1,339.21	1,628.95	1,950.26	2,242.89	2,484.26
	AD[2]	61.95	95.08	174.78	291.41	426.17	569.31	630.66	768.25	960.25	1,162.75	1,409.00	1,656.25	1,899.00	2,121.05
	SEM[1]	0.86	2.73	5.34	9.80	13.73	18.81	21.11	28.39	32.35	43.01	51.74	58.36	65.98	72.09
	P-value	0.478	0.000	0.000	0.000	0.095	0.134	0.191	0.003	0.762	0.650	0.702	0.785	0.807	0.763
BWG[3,4] (g/day)	C	3.55	7.49	13.48	20.87	23.59	25.78	25.78	22.07	23.36	36.35	41.39	45.90	41.80	34.48
	AD	3.63	4.73	11.39	16.66	19.25	20.45	20.10	21.23	27.43	28.93	35.18	35.32	34.68	31.72
	SEM[1]	0.15	0.39	0.43	0.72	0.67	0.83	0.85	1.25	3.26	2.20	1.47	1.58	1.56	1.39
	P-value	0.479	0.000	0.013	0.001	0.094	0.506	0.506	0.983	0.059	0.650	0.851	0.254	0.949	0.519
FI[3,4] (g)	C	21.55	34.15	35.94	36.50	58.21	74.02	80.18	82.50	107.97	92.37	120.88	137.97	144.16	147.90
	AD	18.63	25.75	27.90	37.19	54.11	68.10	71.71	94.50	156.29	150.89	126.07	138.90	161.97	176.04
	SEM[1]	0.71	1.24	1.24	0.75	1.16	1.50	1.79	7.93	19.55	23.74	10.31	2.31	6.58	11.92
	P-value	0.046	0.000	0.000	0.761	0.152	0.302	0.348	0.789	0.285	0.243	0.078	0.726	0.234	0.466
WI[3,4] (mm)	C	22.42	32.14	52.98	70.35	97.92	107.82	132.26	128.50	181.22	179.17	216.00	267.65	296.92	369.69
	AD	22.61	26.82	47.38	51.88	67.57	82.17	92.49	113.61	165.29	166.79	194.36	207.18	205.18	272.75
	SEM[1]	0.38	1.01	1.69	2.85	4.75	3.79	6.02	10.17	4.78	10.56	8.77	19.95	29.26	28.06
	P-value	0.789	0.001	0.124	0.000	0.750	0.415	0.050	0.114	0.021	0.887	0.520	0.317	0.261	0.028
FCR[3,4]	C	6.13	4.58	2.68	1.75	2.47	2.88	3.12	3.60	4.35	2.60	3.09	3.18	3.63	4.61
	AD	5.27	5.52	2.48	2.25	2.81	3.35	3.52	4.50	5.79	5.21	3.56	3.94	4.71	5.53
	SEM[1]	0.26	0.18	0.07	0.07	0.06	0.09	0.08	0.34	0.50	0.93	0.32	0.26	0.40	0.41
	P-value	0.034	0.003	0.173	0.000	0.207	0.852	0.690	0.694	0.146	0.175	0.260	0.393	0.420	0.582

[1] SEM: pooled standard error of the mean.
[2] C: control group, AD: *Acheta domesticus* group.
[3] LBW: live body weight, BWG: body weight gain, FI: feed intake, WI: water intake, FCR: feed conversion ratio.
[4] LBW was punctually measured on the last day of each period, i.e., on days 8, 15, 22, 29, 36, 43, 46, 53, 60, 67, 74, 81, 88, and 95.
[5] 7 w (43-46 d) is composed only of four days since it is the point in which the chicks were carried to the outdoor stage.

4.3.2 Assessment of the growth and the feed intake

The results suggested that the LBW curves of both experimental groups fit almost perfectly to grade two polynomial trend line (Figure 4.1), based on the R^2 values. Furthermore, from these results can be stated that the chicks from group AD grew slower than the chicks from group C from the second week until the end of the trial. The LBW increase of the birds, expressed as a percentage, was different among consecutive weeks ($P = 0.001$) throughout the whole experiment, being higher in the first stages of their life (Figure 4.2). The separate analysis of each group revealed that the greatest increase in LBW for group C corresponded to the second week; in contrast to the AD group that occurred in the days of the third week. Differences were found in the growth rate increase, expressed in terms of BWG, between the C and AD groups from the second week of life to the end of the trial (Figure 4.3), except at week 9, with higher increases for the C group compared to the AD group. The largest difference between the two groups occurred at week 6 of life (green rectangle of Figure 4.3).

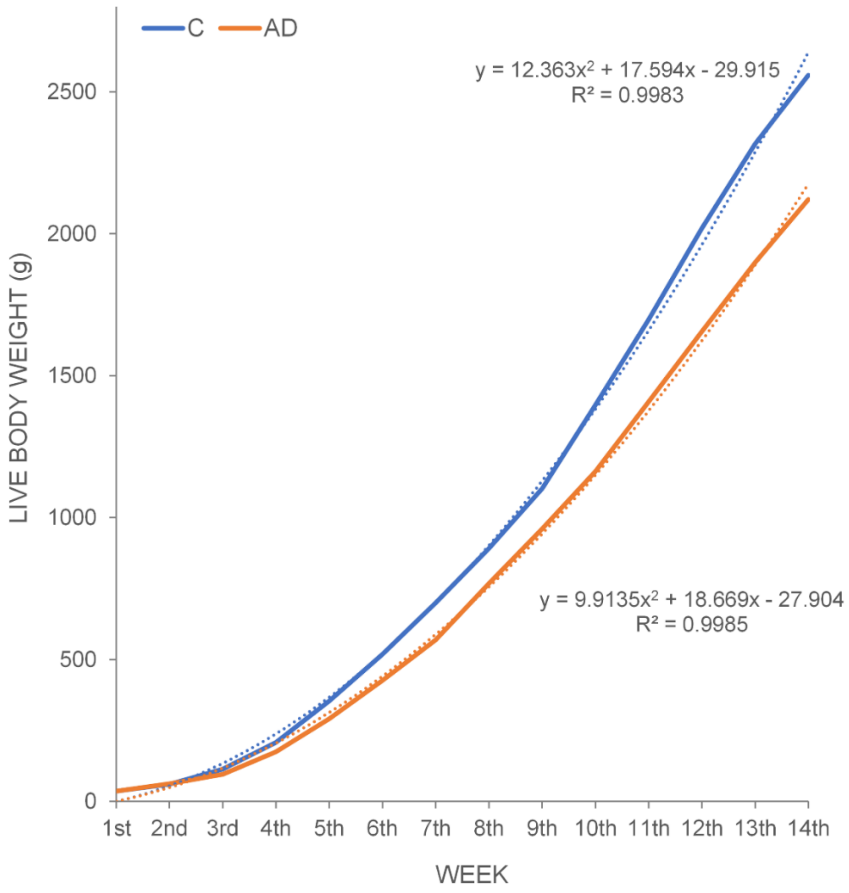

Figure 4. 1. LBW curves for two groups of chicks fed diets that included soybean meal (group C) or Acheta domesticus insect meal (group AD) as the main protein source throughout the growing period (14 wk). Discontinuous lines reflect degree 2 polynomial trend lines.

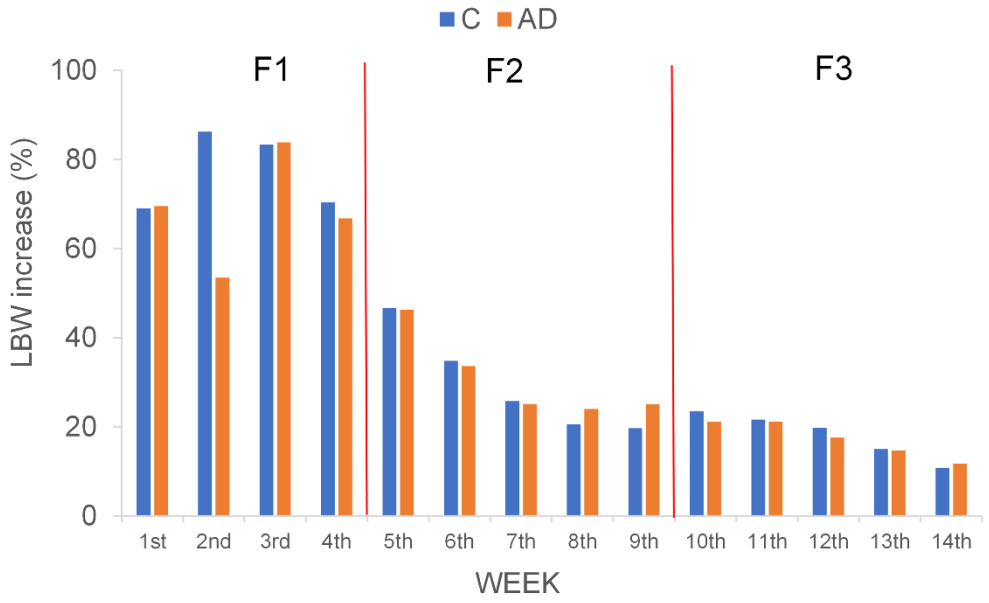

Figure 4. 2. LBW increase (%) for two groups of chicks fed diets that included soybean meal (group C) or Acheta domesticus insect meal (group AD) as the main protein source between two consecutive weeks over the entire studied period.

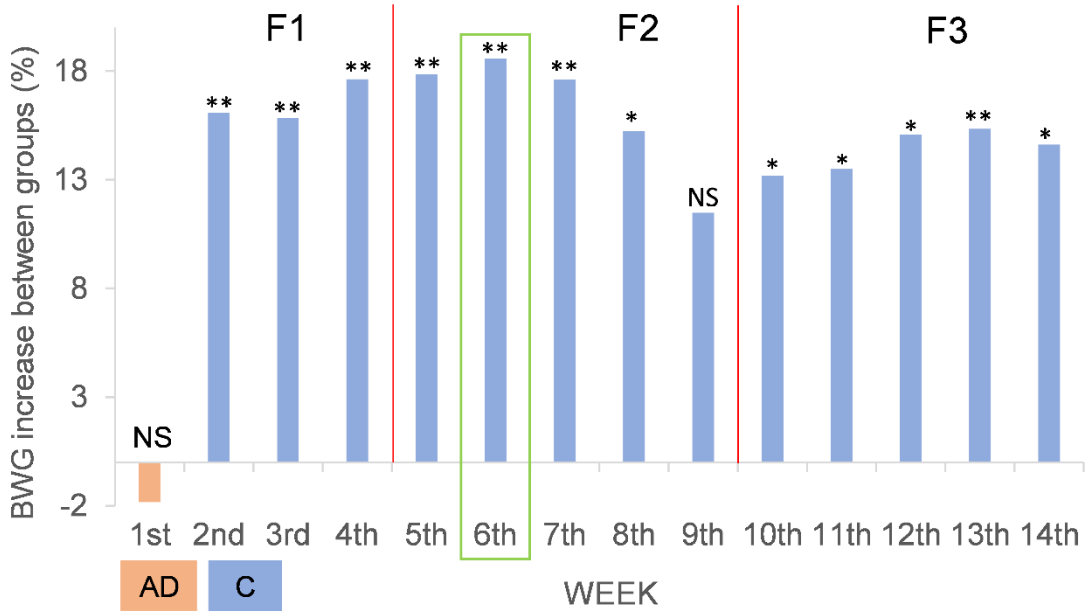

*Figure 4. 3. BWG increase (%) for two groups of chicks fed diets that included soybean meal (group C) relative to the group fed Acheta domesticus insect meal (group AD) as the main protein source over the entire period studied. NS, * or **: non significant or significant at P < 0.05 or 0.01, respectively.*

4.4 Discussion

The results obtained in this study revealed that the inclusion of a high percentage of insect meal in the diet of slow-growing chicks during their first month of life negatively affects their productive performance, contrary to what occurs from the second month onward.

These results agree with those of Nieto et al. (2022) in their work with *Tenebrio molitor* meal, but contrast with those found by Nakagaki et al. (1987), who showed no difference in the BWG of the birds caused by the substitution of soybean meal for cricket meal during the first 14 days of life.

During the period corresponding to the F1 diet, there was a decrease in feed intake by the AD group, along with a lower final weight. The feeding of both experimental groups was slightly different in terms of fat, calcium and fiber content, the first two being lower in the diet of the AD group, while fiber content was higher. Some authors (Makkar et al., 2014) also found that diets containing insect meal as a protein source are often deficient in calcium. Possibly, the proportion of *Acheta domesticus* during their first days of life may affect their digestibility, as Kovitvadhi et al. (2019) found, proving that crude protein from the meal of different crickets (including *Acheta domesticus*) showed a worse *in vitro* digestibility than soybean and fish meal.

During the feeding of diets F2 and F3 there were hardly any differences in the productive performance of the chicks, except in the case of water consumption in F2 and feed consumption in F3. These results coincide with those described by Finke et al. (1985), in which the inclusion of crickets in broiler diets to replace soybean meal showed no differences in final weights or in the feed/weight gain ratio, corroborating the feasibility of their use. In the case of the work of Bovera et al. (2015), the use of insects in isoproteic and isoenergetic diets as a substitute for soybean did not affect consumption during the second month of life of chickens. Biasato et al. (2016) did not find differences in feed conversion after the inclusion of insect meal in broilers between 43 and 97 days of age. Similarly, Leiber et al. (2015) found no differences in feed intake or body weight gain between broilers that consumed insect and those that did not between 7 and 82 days of age. Therefore, the substitution of soybean meal by insect meal appears to be a valuable feed management with good production rates in alternative poultry systems (Benzertiha et al., 2020; DeFoliart et al., 1982; Khan et al., 2018; Kim et al., 2020; Wang et al., 2008).

In the light of these results, it could be stated that including high concentrations of house cricket meal (>10%) in the diet of chickens during the first stages of their life might be detrimental to the birds, since it disinhibits the chickens to consume the feed and, therefore, negatively affects their BWG (Atteh and Ologbenla, 1993; Bamgbosei, 1999).

In fact, Benzertiha et al. (2020) found a worse FCR in those chicks that consumed insect meal in high concentrations during the first 35 days of life. In the same line, Perera and Bhujel (2021) used cricket meal to replace fish meal in *Oreochromis niloticus* diets, obtaining better results in growth with partial substitutions than with total substitution. Similarly, Wang et al., (2005) argued that the use of cricket meal in isoproteic and isonergic poultry diets was more productive and beneficial at low inclusion levels. This results could be extrapolated to other insect species, as it is the case of *Tenebrio molitor* larval meal (Biasato et al., 2017, 2018; Nieto et al., 2022).

Therefore, insect meals could partially replace soybean products in poultry diets without affecting feed efficiency (Leiber et al., 2015). However, it has been shown that total substitution at early stages is inappropriate given the production losses involved (Nieto et al., 2022). This could be explained by the hard outer chitin layer of insects, which is difficult to digest by domestic birds (Ravindran & Blair, 1993), especially in the early stages of life when the digestive system of chicks is very immature. Therefore, soybean meal (or other alternative protein source) should continue to be part of the diets of the chicks in the first stage, being its total substitution feasible from the first four weeks of life, as proposed in the work of Olkowski, (2018). In addition, the results obtained in the trial showed that the initial differences between groups (C and AD) decreased as the trial progressed, i.e., as the content of the insect in the diet decreased, ultimately improving the performances of the chicks that consumed *Acheta domesticus* meal.

In conclusion, this study assessed the effect of totally replacing soybean meal with *Acheta domesticus* meal as the main protein source in isoprotein and isoenergetic diets of slow-growing male *Colorield* chicks during their entire productive cycle. The results obtained suggested that it is not appropriate to include high levels of insect meal in the diet of chicks during the first four weeks of life. Instead, a partial substitution of soybean meal could be recommended, since high amounts of insect meal seem to be detrimental in young chicks.

Therefore, under these conditions, *Acheta domesticus* meal may be an interesting protein source to include in slow-growing chicks as a partial substitute for soybean meal during the first month of birds' life and as a full replacement from that time onwards until the end of the productive cycle.

CAPÍTULO 5:

Carcass traits and meat quality assessment of two slow-growing chicks strains fed *Acheta domesticus* larval meal

Capítulo 5:

Carcass traits and meat quality assessment of two slow-growing chicks strains fed *Acheta domesticus* larval meal

Jaime Nieto[1], Javier Plaza[1], Miriam Hernández-Jiménez[2], Isabel Revilla[2], Carlos Palacios[1]

[1] Area of Animal Production, Faculty of Environmental and Agricultural Sciences, University of Salamanca, Salamanca 37007, Spain.
[2] Area of Food Technology, E.P.S. of Zamora, University of Salamanca, Zamora 49022, Spain.

Revista: Poultry Science. 2024, 103(6), 103684

https://doi.org/10.1016/j.psj.2024.103684

Received 4 January 2024, Accepted 18 March 2024, Available online 21 March 2024, Version of Record 3 April 2024.

Resumen

En la actualidad, existe un interés creciente por parte de los consumidores en las aves criadas en sistemas alternativos con acceso al exterior, asociando este manejo con una mayor calidad del producto. Estos sistemas suelen utilizar estirpes de pollos de crecimiento lento las cuales han mostrado diferencias en las características nutricionales y organolépticas en comparación con las estirpes convencionales. Los sistemas alternativos presentan una baja disponibilidad de fuentes proteicas, cuya producción es limitada y, en el caso de la soja, poco respetuosa con el medio ambiente.

Los insectos emergen como una alternativa prometedora aprobada por la UE para su uso en las raciones avícolas, pero existe un conocimiento limitado de su influencia sobre la calidad y la canal de pollos de crecimiento lento. La certificación de muestras de carne de sistemas de cría alternativos que utilizan nuevas fuentes de proteínas con estirpes diferentes en comparación con la producción industrial es fundamental para garantizar el cumplimiento de los estándares de calidad, siendo necesario un sistema capaz de caracterizar y discriminar dichas muestras.

El objetivo de este ensayo fue evaluar las características de la canal y la calidad de la carne de dos líneas de pollo de crecimiento lento alimentadas con harina de larvas de *Acheta domesticus* como sustituto total de la harina de soja y probar el potencial de la Espectroscopia de Inflarrojo Cercano (NIRS) para discriminar muestras de carne según la estirpe y la dieta utilizadas.

Se utilizaron un total de 256 pollitos machos de un día de edad de 2 estirpes de crecimiento lento: la mitad de las aves (128) pertenecían a la estire Rojo (R) y los otros 128 pertenecían a la cepa Cuello desnudo (CD). Los pollitos de cada estirpe se dividieron aleatoriamente en 16 réplicas de 8 pollos cada una. Dentro de cada estipe, las 16 réplicas se dividieron en 2 grupos experimentales distintos (n = 64 pollos, divididos en 8 réplicas de 8 pollos cada una). Los grupos de control para las estripe Roja (R-C) y cuello desnudo (CD-C) se alimentaron con harina de soja como principal fuente proteica. Por el contrario, lotes experimentales de la estirpe Roja (R-AD) y cuello desnudo (CD-AD) fueron alimentados con harina de *Acheta domesticus*. La crianza de los animales tuvo una duración de 95 días de vida, coincidiendo con el momento del sacrificio. A lo largo de todo el experimento, se emplearon 3 alimentos concentrados (F1, F2 y F3) que cambiaron cada mes.

Se seleccionaron 8 pollos por tratamiento (N=32) para la evaluación de las características de la canal y la calidad de carne. Se empleó espectroscopía de reflectancia en el infrarrojo cercano (NIRS) para clasificar la carne en función del alimento y la estirpe del pollo y posteriormente se analizaron las características físicas (pH, jugo exprimible, color, textura y pérdidas por cocción) y químicas de la carne (cenizas, grasa, proteína y humedad), además del perfil de ácidos grasos.

Los resultados mostraron que aplicando el método RMS-X residuals a los espectros NIR registrados directamente sobre la pechuga, y pretratados con una segunda derivada y una corrección de la dispersión mediante el método SNV (2,4,4,1) se consiguió clasificar correctamente el 100% de las muestras en los cuatro grupos objetivo. En cuanto a las características de la canal, los pollos del grupo R-C tuvieron mayor peso en el momento del sacrificio, mientras que los pollos alimentados con harina de *Acheta domesticus* mostraron valores más bajos para todos los parámetros de las características de la canal, excepto para el rendimiento de la canal fría, independientemente de la estirpe utilizada.

Por otro lado, las pérdidas por cocción y el pH fueron mayores para los pollos alimentados con harina de grillo, al igual que ocurrió con la humedad. El tratamiento experimental influyó en el jugo exprimible, presentando mayor retención de agua la estirpe R, y en la fuerza de corte, de modo que el tratamiento CD-C mostró mayor dureza.

Sin embargo, el contenido de proteínas fue menor en la carne de los pollos que consumieron harina de grillo. El contenido de grasa mostró valores más bajos para los pollos de la estirpe CD. Hubo diferencias mínimas en el perfil de ácidos grasos, con valores más bajos de C18:3n-3 para los pollos alimentados con harina de grillo. Por lo tanto, incluir harina de *Acheta domesticus* en las dietas de estirpes de pollitos de crecimiento lento es una alternativa viable para reemplazar la harina de soja, proporcionando características de calidad de carne comparables a las obtenidas en sistemas convencionales.

Abstract

Presently, there has been a noticeable rise in the consumption of poultry meat within the general population, particularly focusing on poultry sourced from alternative rearing systems as opposed to intensive ones. This study evaluated the impact of incorporating house cricket (*Acheta domesticus,* AD) larvae meal into the diet of two slow-growing chicken strains on their carcass traits and meat quality. A total of 256 one-day-old male chicks were utilized, with 128 belonging to the Red (R) strain and 128 to the Naked Neck (NN) strain. Chicks from each strain were randomly assigned to 16 replicates of eight chicks each. Eight replicates were allocated to two distinct treatments ($n = 64$ chicks/treatment) based on the diet employed: the control treatments for the Red (R-C) and Naked Neck (NN-C) strains were fed soybean meal, while the other two treatments were fed AD meal (R-AD and NN-AD). Three different rations were used throughout the growth cycle of the animals, tailored to meet the nutritional needs of the birds. All chickens were slaughtered at 95 days of age, and eight chickens per treatment were randomly chosen to assess carcass traits and meat quality. Near-infrared reflectance spectroscopy (NIRS) was employed to classify meat based on the feed and the chick strain, achieving a correct discrimination of 100% of the samples. The carcass traits exhibited significant alterations due to the inclusion of insect larval meal in the diet, resulting in reduced values across all parameters for chickens consuming AD meal, irrespective of genotype. The pH and cooking losses were generally higher for chicks fed cricket meal, as occurred with moisture. However, protein content was lower in the meat of chicks consuming cricket meal. Fat content showed lower values for NN chickens. There were minimal differences in the fatty acid profile, with lower C18:3n-3 values for chickens fed cricket meal. Therefore, including *Acheta domesticus* meal in the diets of slow-growing chick strains is a viable alternative to replace soybean meal. It provides meat quality characteristics comparable to those obtained in conventional systems.

Keywords: *Acheta domesticus;* alternative protein source; meat quality; slow-growing chicken strains; alternative poultry systems.

5.1 Introduction

The surging consumer demand for chicken meat has spurred rapid global growth in the poultry meat industry, featuring animals with exceptionally high growth rates (Devatkal et al., 2019), contrary to their natural evolutionary development. The perceived quality of a meat product plays a pivotal role in its acceptance (Elahi et al., 2020). This is evidenced by the increasing demand for poultry meat sourced from chickens raised under alternative management systems, where they have continuous access to external yards (Jeni et al., 2021).

Moreover, these alternative models typically employ slow-growing chicken strains, thereby honoring the natural growth cycle of the birds (Nieto et al., 2023a). This practice leads to notable nutritional and organoleptic quality disparities compared to industrial chicken strains (Jaturasitha et al., 2008; Hailemariam et al., 2022).

Alternative poultry production systems are currently grappling with a significant global challenge: the limited availability of protein sources for animal feed (Sarmiento-García et al., 2021). Adding to this challenge, there has been a noteworthy decrease in suitable agricultural production areas in recent years (Murawska et al., 2021). As traditional feed resources dwindle, the imperative to identify new protein sources intensifies (Elahi et al., 2020), fueled by escalating social and ecological concerns regarding food security and the environmental sustainability concerns associated with soybean meal as the sole protein source in poultry feed (Altmann et al., 2020; Leiber et al., 2015).

Insects, being naturally part of the poultry diet, represent high-protein resources (Sarmiento-García et al., 2021). Consequently, their utilization as a supplementary feed resource is considered highly appropriate in animal nutrition (Hamani et al., 2022). Insects emerge as a promising, valuable, and EU-approved ingredient for poultry feed (EU 1372, 2021), presenting a viable alternative to conventional feeds such as soybean meal (Cullere et al., 2019; Secci et al., 2022). However, despite ongoing research into the inclusion of insects in poultry diets in recent years (Bovera et al., 2015), there remains limited understanding regarding its impact on the meat and carcass quality of slow-growing chicks. This is largely due to the fact that previous studies have primarily focused on broiler meat (Mlaga et al., 2022; Murawska et al., 2021; Shaviklo et al., 2021).

Certifying meat samples from alternative rearing systems that utilize new protein sources and different strains compared to industrial production becomes a crucial task for ensuring traceability and guaranteeing consumers adherence to quality standards (Serva et al., 2023). This underscores the need for a system capable of characterizing and discriminating such samples. Near-infrared spectroscopy (NIRS) emerges as a valuable tool, not only for controlling the quality of meat from poultry fed diverse diets (Berzaghi et al., 2005), but also as an objective and rapid method for authenticating different species (Cozzolino & Murray, 2004), capable of assessing the quality of different poultry strains (Abeni & Bergoglio, 2001). This technology for sample discrimination is highly efficient, rapid, non-destructive, and non-contaminating (Hernández-Jiménez et al., 2021).

Motivated by these concerns, this study evaluated the carcass traits and meat quality of two slow-growing chicken strains fed with *Acheta domesticus* larvae meal as a total substitute for soybean meal. We opted to utilize this orthoptera species for several reasons: 1) It is authorized by the EU for use as a protein source in poultry feeding, ensuring compliance with regulatory standards; 2) the farm producing this species is located in close proximity to our experimental facilities, thereby enhancing the sustainability of the process by minimizing transportation distance; and 3) compared to other species such as *Hermetia illucens* and *Tenebrio molitor*, there is relatively less research conducted on the inclusion of this species in poultry diets, making it an area worthy of investigation. The study also tested the potential of NIRS to discriminate meat samples based on the strain and diet used.

5.2 Material and Methods

5.2.1 Ethics statement

The experiments were conducted in strict adherence to the principles and specific guidelines outlined in the Guide for the Care and Use of Agricultural Animals in Research and Teaching, 4th edition, 2020, ensuring the avoidance of unnecessary discomfort to the animals. Specifically, all slow-growing chicks utilized in the trial were handled in compliance with the principles laid out in the European Parliament and Council Directive 2003/65/EC of 22 July 2003, amending Directive 86/609/EEC concerning the protection of animals used for experimental and other scientific purposes. These animals were manipulated under Order ECC/566/2015, dated 20 March, which establishes the training requirements for personnel handling animals used, bred, or supplied for experimental and

other scientific purposes, including teaching. The experimental protocol received approval from the Bioethics Committee of the University of Salamanca (Spain), with registration number 0000590, regulated by RD 53/2013, dated 1 February, establishing the fundamental rules applicable to the protection of animals used in experimentation and other scientific purposes, including teaching. The trial was conducted by qualified personnel from the Animal Production area in the facilities and experimental fields of the University of Salamanca.

5.2.2 Experimental design and farming specifications

A total of 256 one-day-old male chicks (Santa Isabel Farm, Córdoba, Spain) from two different slow-growing strains were employed in this trial, all having received prior vaccination against Marek's disease and avian infectious bronchitis at the hatchery facilities. Half of the birds, comprising 128 chicks, belonged to the Red (R) strain, which was developed through crossbreeding of local breeds, while the other 128 belonged to the Naked Neck (NN) strain, known for its high tolerance to elevated temperatures.

The chicks from each strain were randomly assigned to 16 replicates, each consisting of 8 chicks. Within each strain, the 16 replicates were further divided into 2 distinct experimental groups, hereafter referred to as treatments (n = 64 chicks and 8 replicated pens/treatment). The control treatments for the Red (R-C, mean live weight of 36.9 ± 1.3 g) and Naked Neck (NN-C, mean live weight of 35.1 ± 0.7 g) strains involved feeding soybean meal as the primary protein source. In contrast, the other 2 treatments were fed *Acheta domesticus* (AD) larvae meal as the protein raw material (R-AD, mean live weight of 36.2 ± 1.0 g, and NN-AD, mean live weight of 36.9 ± 0.7 g).

Throughout the entire experiment, 3 concentrated feeds (F1, F2, and F3) were employed to fulfill the nutritional needs of the chicks at each developmental stage based on the recommendations of the National Research Council (NRC, 1994) for medium-low differentiated growth chicks. Each feed was administered to the chicks for approximately 4 wk. Precisely, F1 was utilized from d 1 to d 29, F2 from d 29 to d 60, and F3 from d 60 to d 95. The composition of these feeds, expressed as a percentage of ingredients, is detailed in Table 5.1.

Table 5. 1. *Composition of the diets for the four experimental treatments (g/Kg ingredients), fed diets including either soybean meal (treatments R-C and NN-C) or Acheta domesticus insect meal (treatments R-AD and NN-AD) as the main protein source. These diets align with those utilized in the study by Nieto, et al. (2023a).*

	R-C[1]/NN-C[2]			R-AD[3]/NN-AD[4]		
Ingredients	F1[5]	F2[6]	F3[7]	F1[5]	F2[6]	F3[7]
Corn	370	480	310	0	90	160
Wheat	110	120	370	120	200	380
Barley	0	0	0	320	160	0
Soybean meal	290	200	120	0	0	0
AD meal	0	0	0	170	100	73
Sunflower	190	160	160	0	110	87
Oats	0	0	0	350	300	260
Vitamin-mineral premix[8]	40	40	40	40	40	40

[1] R-C: red control group.
[2] NN-C: naked neck control group.
[3] R-AD: red *Acheta domesticus* group.
[4] NN-AD: naked neck *Acheta domesticus* group.
[5] F1: 1−29 d.
[6] F2: 29−60 d.
[7] F3: 60−95 d.
[8] Vitamin-mineral prefix includes in percentage: Methionine 0.21; Calcium 20.75; Phosphorus 5.58; Sodium 6.38. Includes per kg of diet: Vitamin D3 100490 UI, Vitamin E 398 UI, Vitamin A 25,1230 UI, Betaine anhydrous 4,002.84 mg. Anti-caking agents: bentonite 10%; sepiolite 3,340.2 mg/kg. Trace element premix includes per kg of diet Cu (sulfate II pentahydrate), 335 mg; Fe (sulfate II monohydrate), 1,324 mg; Mn (oxide), 3,336 mg, Zn (oxide), 2,835; I (calcium iodate anhydrous), 51.07 mg; Se (sodium selenite), 10.10 mg.

Furthermore, these feeds underwent chemical and nutritional analysis, along with the soybean and cricket meals analyzed separately, in the food analysis laboratories of Abiomed Higiene S.L. company (Salamanca, Spain). The moisture content was determined by drying the samples at 105°C until reaching a constant weight. Crude fat was extracted using the Soxhlet extraction method. Crude fiber was assessed through acid and basic hydrolysis. Crude protein content was determined using the Kjeldahl method and a subsequent conversion utilizing a correction factor of 6.25. Ash content was measured through calcination at 550°C. Fatty acid analysis was conducted using gas chromatography, while amino acid analysis was performed using liquid chromatography. The laboratory operates under an ISO 9001 certified quality system. The results of these analyses are presented in Table 5.2. Furthermore, acknowledging that the last feed consumed by the chicks before slaughter, namely the F3 feed, exerts the most direct impact on carcass and meat quality outcomes, the comprehensive fatty acid profile of this feed is outlined in Table 5.3.

Table 5. 2. *Chemical/nutritional characteristics of the diets for the 4 experimental treatments, fed diets including either soybean meal (treatments R-C and NN-C) or Acheta domesticus insect meal (treatments R-AD and NN-AD) as the main protein source (expressed in grams/100 grams). These diets align with those utilized in the study by Nieto, et al. (2023a).*

Item	F1 (1-29 d)		F2 (29-60 d)		F3 (60-95 d)		Soybean meal	*A. domesticus* meal
	R-C[1] NN-C[1]	R-AD[1] NN-AD[1]	R-C NN-C	R-AD NN-AD	R-C NN-C	R-AD NN-AD		
Moisture	10.1	9.10	10.7	9.30	10.2	9.70	12.1	4.40
Ashes	6.10	6.40	4.80	6.30	5.60	5.90	6.20	4.10
Crude fat	12.1	8.40	9.80	12.4	6.70	10.7	1.90	22.6
Crude fiber	6.00	8.30	5.30	8.20	4.70	7.40	5.90	6.90
Starch	35.8	33.8	45.1	37.1	45.5	41.5	0.100	1.70
Crude protein	19.8	19.3	15.7	15.2	14.6	14.8	44.0	59.5
ME[2] (Kcal/kg)	2,803	2,897	2,900	2,966	2,951	2,978	1,890	5,817
Mean dietary Crude Protein	19.6		15.5		14.7		44.0	59.5
Mean dietary ME (Kcal/kg)	2,850		2,933		2,965		1,890	5,817
Ca[3]	1.24	1.19	1.21	1.19	1.20	1.18	0.290	0.250
P[3]	0.400	0.610	0.360	0.500	0.340	0.430	0.610	2.13
Ca/P[3]	3.10	3.11	3.36	2.38	3.53	2.74	0.475	0.117
SFA[4]	1.60	2.70	1.30	2.40	0.900	2.10	0.600	5.30
MUFA[4]	7.90	3.10	6.20	7.60	4.00	6.30	0.900	8.90
PUFA[4]	2.60	2.60	2.30	2.30	1.90	2.30	2.60	8.50
n-3 PUFA[4]	0.170	0.090	0.130	0.080	0.110	0.080	0.310	0.270
n-6 PUFA[4]	2.40	2.50	2.20	2.30	1.79	2.18	2.27	8.20
n-6/n-3 PUFA[4]	14.1	27.8	16.9	28.8	16.3	27.3	7.32	30.4
Trans FA[4] (C18:1T+C18:2T+C13:3T)	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050	<0.050
Lysine[5]	1.16	0.850	1.08	0.590	0.830	0.650	2.68	2.83
Methionine[5]	0.200	0.170	0.190	0.160	0.190	0.180	0.590	0.690

[1] R-C: Red Control group, NN-C: Naked Neck Control group, R-AD: Red *Acheta domesticus* group, NN-AD: Naked Neck *Acheta domesticus* group.
[2] ME: metabolizable energy, expressed in Kcal/kg of dry matter.
[3] Ca: calcium, P: phosphorous, expressed in g/100 g.
[4] SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n-3 PUFA: omega-3 polyunsaturated fatty acid, n-6 PUFA: omega-6 polyunsaturated fatty acid, Trans FA: trans fatty acid. Expressed in g/100 grams total fatty acids.
[5] Expressed in g/100 g of dry matter.

***Table 5. 3**. Fatty acids composition (as g/100 g of fatty acid methyl esters) of the F3 (60–95 d) experimental feed for the 4 studied treatments of chicks, fed diets including either soybean meal (treatments R-C and NN-C) or Acheta domesticus insect meal (treatmentR-AD and NN-AD) as the main protein source.*

Fatty acid	R-C[1] NN-C[1]	R-AD[1] NN-AD[1]
C12:0	0.040	0.080
C14:0	0.170	0.360
C15:0	0.290	0.930
C16:0	9.01	12.2
C16:1n-7	0.080	0.170
C16:1	0.210	0.400
C17:0	0.070	0.070
C17:1	0.020	0.030
C18:0	3.73	3.54
C18:1n-9	0.150	0.190
C18:1	54.6	53.7
C18:1n-7	0.840	0.780
C18:2n-6t	0	0.020
C18:2n-6	26.5	22.9
C20:0	0.380	0.240
C18:3n-6	0.010	0.030
C18:3n-3	1.28	1.34
C20:2n-6	0.040	0.120
C22:0	0.800	0.480
C20:3n-6	0.010	0.040
C22:1n-9	0.030	0.050
C20:3n-3	0.030	0.080
C20:4n-6	0.030	0.010
C22:2n-6	0	0.330
C24:0	0.320	0.210
C20:5n-3-epa	0.010	0.060
C24:1n-9	0.090	0.120
C22:6n-3	0.080	0.020
C22:5n-3	1.17	1.55
Total SFA[2]	14.8	18.1
Total MUFA[2]	56.0	55.5
Total PUFA[2]	29.2	26.5
n-3 PUFA[2]	2.57	3.05
n-6 PUFA[2]	26.6	23.5
n-6/n-3 PUFA[2]	10.4	7.75

[1] R-C: Red Control group, NN-C: Naked Neck Control group, R-AD: Red *Acheta domesticus* group, NN-AD: Naked Neck *Acheta domesticus* group.
[2] SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n-3 PUFA: omega-3 polyunsaturated fatty acid, n-6 PUFA: omega-6 polyunsaturated fatty acid.

The health status of the birds was monitored daily through direct observation of their behavior, with particular attention given to the potential emergence of any pathological symptoms. The feed was provided as milled meal throughout the entire trial, and both feed and water were supplied *ad libitum*.

The chicks were initially reared in an enclosed facility for the first 45 d. Particularly, each experimental unit was housed in a 1.50 × 0.60 m^2 indoor pen (0.10 m^2/ chick) equipped with first age feeders and drinkers until d 20, after which they were replaced by second age ones. The floor of the pens was covered with a 7 cm thick layer of wood shavings. Temperature and humidity were adjusted progressively to optimize the living conditions of the chicks throughout the experiment. In detail, the average temperature inside the barn was maintained at 31.9 ± 1.8 ºC during the first week, 29.1 ± 2.1 ºC during the second week, 25.4 ± 1.7 ºC during the third week, and 20.8 ± 2.7 ºC for the remainder of the trial. Overall, the chicks were exposed to natural daily photoperiods of 10 to 11 h.

From the 46th day of life until the end of the trial (95th day), the chickens were transferred to an outdoor facility comprising thirty-two 8.2 ± 4 m^2 pens (0.25 m^2/chick) with plastic mesh, allowing them unrestricted freedom of movement. The soil of the outdoor yard was entirely natural, untreated, and uncultivated. It was enclosed by a 1 m high metal sheet, buried 30 cm deep, and surrounded by a 13 x 13 mm grid plastic mesh covering the roof and sides to prevent predator entry. Furthermore, these pens were equipped with circular feeders and drinkers, along with a wooden shelter featuring a single entrance on the south side (0.5 x 0.5 m^2). The shelter also had an upper opening to facilitate ventilation and a translucent section to provide lighting.

5.2.3 Slaughtering specifications, carcass obtainment and processing to obtain meat cuts

The rearing of the birds concluded at 95 d of age. Then, 1 chicken from each pen, that is, 8 chickens per treatment (N = 32 samples) were randomly selected for the assessment of carcass traits and meat quality. These selected chicks were transported and humanely slaughtered in a nearby certified slaughterhouse, following the guidelines outlined in Council Regulation (EC) No. 1/2005 of 22 December 2004, which details the protection of animals during transport and related operations. This regulation amends Directives 64/432/EEC and 93/119/EC, as well as Regulation (EC) No. 1255/97. The entire process was conducted in accordance with Council Regulation (EC) No. 1099/2009 of 24 September 2009, addressing the protection of animals at the time of slaughter. The chicks were weighed prior to slaughter. Following slaughter, the chicks were plucked, weighed again and then eviscerated. Afterwards, the head was separated by making a cut between the occipital bone of the skull and the first cervical vertebra.

Similarly, the legs were removed, with the cut performed at the junction with the thigh, specifically between the tarsometatarsal bone and the tibiotarsus. Individual weights were recorded for both the head and legs. Once again, their weight was measured to obtain the hot carcass weight. Twenty-four h postslaughter, the carcasses were transported to the Food Technology Laboratory at the University of Salamanca where they underwent a final weighing to establish the cold carcass weight at 4ºC, which was utilized to calculate carcass yield in relation to the slaughter weight. Furthermore, in these facilities the analysis of carcass traits and meat quality were also conducted.

Following this, the wings were removed along with the breast, including the keel bone, and their combined weight was recorded as the forequarter. The breast was then individually weighed, excluding the keel bone and skin. Furthermore, the hindquarter, comprising the thighs and drumsticks, was weighed, and the thighs were recorded separately.

5.2.4 Samples preparation for analysis and analytical procedures

The analysis was conducted on boneless, skinless breast meat obtained from 32 chicken samples, with eight samples per treatment. These samples had been stored at 4°C for 48 h. Following this storage period, the breasts were halved. The fresh left half breast underwent NIRS spectra recording, followed by color evaluation, pH measurement, and expressible juice determination. Subsequently, instrumental texture analysis was performed on these samples. Concurrently, the right half breasts were vacuum-packed and frozen at -18°C until chemical analysis was carried out. Twenty-four h before chemical analysis, the frozen samples were gradually thawed at 4°C. The samples were then minced and homogenized before undergoing chemical analysis, which included determining moisture, ash, crude protein, total fat, and fatty acid profile.

5.2.5 NIR spectrometry.

A Foss NIRSystem 5000 (Foss NIRSystems, Silver Spring, MD) was employed to capture NIR spectra using a standard fiber optic probe (210/210 beam) of regular Bundle type remote reflectance, with a ceramic plate serving as a reference. This probe, connected to the equipment by 1.5 meters of optical fiber (Ref. No. R6539-A), features a 5 cm x 5 cm quartz window applied to the breast surface at 3 different points. The radiation originating from diffuse scattering is detected by reflectance receivers (4 PbS elements) positioned at a 45° angle to the breast surface.

The spectral range utilized is 1,100 to 2,000 nm. Spectra were recorded at 2 nm intervals, amounting to 451 data points for each spectrum. For each sample, 32 sweeps were recorded for both the reference and the samples. The spectra were stored as the reciprocal logarithm of the reflectance log (1/R), with R indicating reflectance (Hernández-Jiménez et al., 2020; Revilla et al., 2020).

5.2.6 Physical characteristics

In the meat, various physical characteristics were analyzed, including pH, expressible juice, color, texture, and cooking losses. Meat color was measured after 1 h blooming at 4°C on the skinless breast surface using a MiniScan XE Plus Spectrophotometer (Hunter Lab, Reston, VA) with a 25 mm measuring head and diffuse/8° optical geometry. CIELab parameters, specifically lightness (L*), redness (a*), and yellowness (b*), as per the Commission Internationale de l'Eclairage standards (CIE, 1976), were determined under the CIE illuminant D65 and 10º standard observer conditions. Three random readings were performed on each sample on the center of the piece.

The samples were tempered at room temperature for approximately 2 hours until they reached 18°C and then pH was measured using a Crison pHMeter Basic 20 (Crison, Barcelona, Spain) equipped with a penetration electrode at three different points on the raw breasts at a depth of 10 mm into the meat at 3 different points in the center of the piece. The water holding capacity of the meat was determined using the expressible juice method through compression, a modification of the Grau and Hamm (1953) method as described by Pla and Apolinar (2000). Three replicates were analyzed for each sample.

To calculate cooking losses, each sample, that is, half breast, was initially weighed (M1) and placed individually in a polyethylene bag. The samples were then cooked in a water bath at 75ºC until the center of the pieces reached 70ºC. The meat temperature was monitored using a K-type high-temperature probe (Omega, Laval, QC, CA). Once cooked, the samples were cooled in running water at room temperature (20ºC) and subsequently reweighed (M2). Cooking losses were calculated following Eq.1. This analysis was conducted as a single instance.

$$Cooking\ losses\ (\%) = \frac{M1\ -\ M2}{M1}\ x\ 100 \qquad (1)$$

Texture analysis was conducted following the method developed by Szczesniak (1987). The same samples used in the cooking loss calculation were utilized, following the methods described by Revilla and Vivar-Quintana (2006), with four prisms per sample cut into pieces measuring 1 x 1 x 3 cm (height x width x length) parallel to the direction of muscle fibers. Shear strength was measured using the Texture Analyser TA-XT2i (Texture Analyser®, Stable Micro Systems, Surrey, UK) equipped with a Warner-Bratzler blade. Each sample was placed in a transverse position to the cutting probe at a speed of 1 mm/s. The maximum shear force (WBSF) was recorded, and all determinations were conducted in triplicate.

5.2.7 Chemical analysis

One day prior to the chemical analyses, the frozen samples were gradually thawed at 4 ºC. Ash content was determined by muffle incineration according to ISO 936 (ISO, 1998), moisture content was determined by oven drying according to ISO 1443 (ISO, 1973) and intramuscular fat (ether extractable) was determined according to the Soxhelt method (AOAC, 2006). Protein analysis was performed using the Kjeldahl method by the Abiomed Higiene SL laboratory (Salamanca, Spain), which adheres to an ISO 9001 certified quality system. All chemical analyses were carried out in duplicate.

The fatty acid profile was determined through gas chromatography, involving the processes of extraction, esterification, separation, identification, and quantification of fatty acids (Schettino et al., 2011). Total lipid extraction utilized a combination of mechanical and organic solvent extraction, following the method outlined by (Folch et al., 1957). The fatty acids of all the samples were methylated following the procedure outlined by Murrieta et al. (2003) and analyzed by gas chromatography, as per the method proposed by (Lurueña-Martínez et al., 2010). The analysis was conducted using a GC 6890 N gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a 7,683 automatic injector from the same company and a 100 m × 0.25 mm × 0.20 µm silica capillary column SP-2560 (Supelco, Inc., Bellefonte, PA). The temperature program of the equipment was as follows: it started at 150ºC and increased by 1ºC/min until reaching 165ºC, then increased by 0.20ºC/min until reaching 167ºC, and finally increased by 1.50ºC/min until reaching 225ºC. One microliter was injected into the chromatograph in splitless mode, and the injector (FID) temperature was maintained at 250ºC. Helium served as the carrier gas at a flow rate of 1 mL/min.

The identification of different fatty acids was based on retention time and compared with a standard mixture of 37 Supelco FAME components (47885-U Supelco, Sigma-Aldrich, Germany) injected at the beginning of each sequence. Fatty acid content was calculated from the peak areas of the chromatograms and expressed as g/100 g of total fatty acid methyl esters.

5.2.8 Statistical analysis

Statistical processing of the data was performed using IBM-SPSS Package 28 software (IBM, Chicago, IL), except for NIRS analysis results. Initially, the normality of the recorded data was assessed through the Kolmogorov-Smirnov test.

Overall significant differences among the 4 experimental treatments (R-C, R-AD, NN-C, and NN-AD) for each carcass traits and meat quality parameter were tested using a univariate analysis of variance. For this statistical procedure, a total of 32 sampling units were employed, corresponding to one slaughtered chick per treatment (n = 8 sampling units per treatment). Different homogeneous subsets were identified by applying Tukey's honest significant difference test (Tukey, 1949).

Subsequently, in order to gain further insight into the effects of the different strains and diets employed and their potential interactions, a complete factorial general linear model was selected (Eq. 2).

$$\mu = g^{-1}(X\mathrm{B}) \tag{2}$$

Where μ is the expected value, XB is the linear predictor, i.e., a linear combination of unknown B parameters, and g is the link function (Nelder & Wedderburn, 1972). In this model, strain and diet, were treated as fixed factors. Means and standard error of the mean were calculated for all the variables, and statistical significance was assessed at a 95% confidence level ($\alpha = 0.05$), using Snedecor's F as the contrast statistic.

The collection of spectra and discriminant analysis of the samples based on diet and strain used in the trial were conducted using the residual mean squares method (RMS-X residuals) through the Win ISI 4.10 software (Infrasoft International, State College, PA). The software was installed on a Hewlett-Packard Pentium III computer, and the entire NIR spectrum was utilized, following the methodology described by Sarmiento-García et al. (2021). The selection of the best mathematical treatment was based on achieving the highest percentage of correctly classified samples.

5.3 Results and Discussion

5.3.1 NIRS analysis

Discriminant analysis of the entire NIR spectrum among the four experimental treatments was conducted using the RMS-X Residuals method. Spectral pretreatment was employed to correct for potential scattering effects in the spectrum, including multiplicative scatter correction (MSC), standard normal variant (SNV), DeTrend (DT), and SNV-DT. Furthermore, these treatments were combined with various mathematical treatments involving derivatives and smoothing procedures, in order to avoid band overlap and baseline shifts. The various mathematical treatments were encoded with four digits (a, b, c, d), where: a represents the order of the derivative, b represents the number of points at which the derivative is carried out, c represents the number of points at which the first smoothing is conducted, and d represents the number of points at which the second smoothing is conducted.

The best treatment is determined by the one that yields a higher percentage of correctly classified samples. In this case, SNV 2,4,4,1 successfully discriminated 100% of the samples based on the type of feed received by the animals and the strains used. This same mathematical treatment was found to be the most suitable for distinguishing samples based on the percentage of *Calliphora* sp. included in the diet (Sarmiento-García et al., 2021). Therefore, it can be considered the most appropriate mathematical procedure for differentiating the type of diet in poultry meat. Figure 5.1 displays the spectra of each of the experimental treatments.

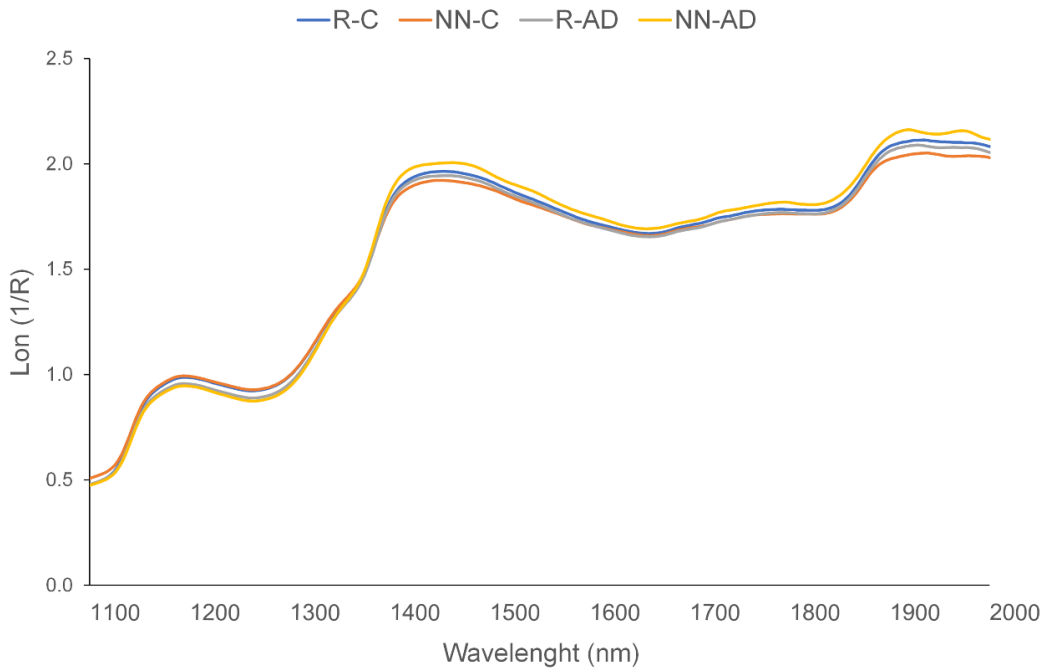

Figure 5. 1. *NIRS spectra of poultry meat from the different experimental treatments of slow-growing chicks fed diets including either soybean meal (treatments R-C and NN-C) or Acheta domesticus insect meal (treatments R-AD and NN-AD) as the main protein source, and slaughtered at 95 d of age. R-C: Red Control group, NN-C: Naked Neck Control group, R-AD: Red Acheta domesticus group, NN-AD: Naked Neck Acheta domesticus group.*

The most significant differences in the recorded spectra are observed between 1,200–1,300, 1,400–1,500, and 1,900–2,000 nm. The absorbance in the range between 1,200 and 1,300 nm corresponds to the second stretching overtone and the deformation of the C-H bond in fat. The absorbance at 1,410 and 1,420 can be associated with the first stretching overtone of the O-H bond in water. However, in the 1,400–1,500 range, the absorbance from the first stretching overtone of N-H and C-H bonds from proteins can also be observed. Lastly, the absorbance of asymmetric stretching of N-H, the second overtone of C=O, and O-H bonds from protein and water can be observed in the 1,900–2,000 nm interval (Barbin et al., 2014; Cozzolino & Murray, 2004). Furthermore, the NN-AD group exhibited higher moisture content and the highest absorbances in the ranges 1,400–1,500 and 1,900–2,000 nm, while the NN-C group showed elevated fat content and increased absorbance in the range 1,200–1,300 nm.

The discriminative capability of NIRS technology based on the diet is consistent with findings observed by Berzaghi et al. (2005) and Sarmiento-García et al. (2021), who achieved 100% classification of breast meat samples from laying hens and slow-growing chickens, respectively, subjected to different diets.

Similarly, NIR spectroscopy has been effective in classifying other products based on the diet used, such as meat from Iberian pigs (Hernández-Jiménez et al., 2021) or sheep milk (Revilla et al., 2017).

5.3.2 Carcass traits assessment

The slaughter weight and the carcass characteristics of the different experimental treatments are presented in Table 5.4. Notably, significant differences ($p = 0.008$) in slaughter weight were observed among the treatments, attributed to both strain variation and feed composition. Specifically, Red chickens consuming soybean meal as their protein source (R-C treatment) demonstrated the highest growth rates. The lower slaughter weight of chickens fed cricket meal aligns with findings from prior studies involving the incorporation of various insect meals in slow-growing chickens (Nieto et al., 2022, 2023a). Furthermore, all studied carcass characteristics exhibited significant differences ($p < 0.005$) across treatments, except for cold carcass performance (Table 5.4), where dietary composition emerged as the primary determinant in all instances In particular, chickens fed *Acheta domesticus* meal showed lower values for all studied carcass traits parameters, contrasting with findings from other studies where the partial substitution of soybean meal with *Tenebrio molitor* at various proportions did not influence carcass traits (Sedgh-Gooya et al., 2021), and complete substitution similarly had no effect on carcass characteristics (Bovera et al., 2016). This finding may be attributed to the generally lower in vitro absorption capacity for chicks in rations that incorporate insect meal (Nieto, et al., 2023b), necessitating an additional protein input. Genotype did not significantly influence any of the studied carcass parameters.

***Table 5. 4**. Slaughter weight (g) and weights of several carcass quality parameters (g) and cold carcass yield (g/kg) of the different experimental treatments of slow-growing chickens fed diets including either soybean meal (treatments R-C and NN-C) or Acheta domesticus insect meal (treatments R-AD and NN-AD) as the main protein source, and slaughtered at 95 d of age.*[1]

Parameter	Treatment				SEM[3]	P-value			
	R-C[2]	NN-C[2]	R-AD[2]	NN-AD[2]		T[4]	D[4]	S[4]	D×S
Slaughter weight	2,563[a]	2,479[a]	2,331[ab]	1,858[b]	89.7	0.008	0.005	0.044	0.143
Dead-plucked chicken	2,215[a]	2,177[a]	2,034[ab]	1,619[b]	79.2	0.011	0.007	0.068	0.121
Chicken head	97.8[a]	82.0[ab]	80.8[ab]	61.8[b]	4.18	0.007	0.007	0.011	0.783
Chicken legs	102[a]	99.8[a]	101[a]	81.0[b]	2.92	0.013	0.034	0.025	0.064
Hot carcass	1,746[a]	1,729[a]	1,578[ab]	1,226[b]	67.9	0.006	0.003	0.069	0.096
Cold carcass	1,624[a]	1,590[a]	1,460[ab]	1,134[b]	64.9	0.011	0.006	0.077	0.141
Chicken forequarters	456[a]	457[a]	409[ab]	321[b]	17.6	0.003	0.002	0.077	0.072
Boneless chicken breast	259[a]	264[a]	222[ab]	167[b]	12.3	0.004	0.001	0.141	0.086
Chicken hindquarters	765[a]	741[a]	674[ab]	519[b]	33.9	0.023	0.010	0.109	0.230
Chicken thighs	265[a]	263[a]	240[ab]	184[b]	11.5	0.021	0.011	0.124	0.149
Cold carcass yield	63.4	64.1	62.5	60.8	0.560	0.195	0.072	0.625	0.269

Within a row, means followed by the same letter are not significantly different (P > 0.05) according to Tukey's test.

[1] Each value is the mean of eight replicates (chickens).
[2] R-C: Red Control group, NN-C: Naked Neck Control group, R-AD: Red *Acheta domesticus* group, NN-AD: Naked Neck *Acheta domesticus* group.
[3] SEM: pooled standard error of the mean.
[4] T: Treatment, D: diet, S: strain.

5.3.3 Meat quality analysis

a) Technological quality

The results from the physical analyses, which assess the technological quality of the meat, including color, pH, expressible juice, cooking losses, and WBSF, are detailed in Table 5.5. Color was the only physical parameter in which no significant differences were observed among treatments, suggesting no discernible influence from either the diet or the strain. However, pH levels and cooking losses were notably impacted by the diet ($p = 0.041$), with elevated values observed in the meat of chickens fed *A. domesticus* meal. Popova et al. (2020) suggested that the augmented content of SFA in some insect larval meal compared to soybean meal might be responsible for the higher pH values in the meat of the chickens consuming cricket meal. Moreover, expressible juice and Warner-Bratzler shear force (WBSF) were also distinctly influenced by the experimental treatment.

Regarding the former parameter, it appears that meat from Red chickens exhibited higher juiciness, although this observation was not significantly associated with either the diet or the genotype. This fact might be related to the histological structure of the meat (Murawska et al., 2021). Furthermore, it was observed that the meat from chickens in the NN-C treatment displayed significantly greater toughness compared to the other treatments, which might be directly related with its lower juiciness and cooking losses (Mir et al., 2017) These findings are consistent with the results of similar studies where it was observed that the substitution of soybean meal by *T. molitor* (Bovera et al., 2016) or *H. ilucens* (Leiber et al., 2015; Popova et al., 2020) in broiler diets caused an increase in pH and cooking losses.

Table 5. 5. *Technological meat characteristics of the different experimental treatments of slow-growing chicks fed diets including either soybean meal (treatments R-C and NN-C) or Acheta domesticus insect meal (treatments R-AD and NN-AD) as the main protein source and slaughtered at 95 d of age*[1]

Parameter	Treatment				SEM[3]	P-value			
	R-C[2]	NN-C[2]	R-AD[2]	NN-AD[2]		T[4]	D[4]	S[4]	D×S
L*[5]	61.7	62.1	62.1	64.9	0.590	0.188	0.343	0.745	0.386
a*[5]	8.84	8.71	8.36	8.43	0.240	0.885	0.301	0.713	0.753
b*[5]	17.5	16.4	14.8	16.2	0.390	0.125	0.054	0.935	0.294
pH	5.50^{c}	5.55^{bc}	5.58^{ab}	5.64^{a}	0.010	0.000	0.041	0.266	0.926
Expressible juice (µL)	12.0^{ab}	11.4^{b}	13.5^{a}	11.7^{ab}	0.270	0.029	0.064	0.145	0.481
Cooking losses (%)	10.0^{ab}	8.87^{b}	12.0^{ab}	12.4^{a}	0.510	0.047	0.005	0.650	0.342
WBSF[5] (N)	2,960^{ab}	4,307^{a}	3,849^{ab}	2,764^{b}	200	0.012	0.859	0.769	0.449

Within a row, means followed by the same letter are not significantly different (P > 0.05) according to Tukey's test.

[1] Each value is the mean of eight replicates (chicks), in which the variables were measured in triplicate.
[2] R-C: Red Control group, NN-C: Naked Neck Control group, R-AD: Red *Acheta domesticus* group, NN-AD: Naked Neck *Acheta domesticus* group.
[3]SEM: pooled standard error of the mean.
[4]T: Treatment, D: diet, S: strain.
[5] L* = lightness; a* = redness; b* = yellowness; WBSF = Warner-Braztler shear force.

b) Proximate composition

The results of the chemical analysis, including moisture, ash, total fat, and crude protein percentages, are presented in Table 5.6. The strain did not exhibit a significant effect on the moisture content, while the diet led to differences ($p = 0.023$), with higher moisture values observed for the meat of chickens fed *Acheta domesticus.* The ash percentage was not significantly affected by either diet or strain.

However, total fat content was influenced by the strain used ($p = 0.007$) but not by the diet, with lower fat values for meat from NN strain chickens. The crude protein level of meat varied according to the diet used ($p = 0.009$) but without differences between strains, with higher values for meat from chickens fed soybean meal (R-C and NN-C). These observations align with the differences recorded by the NIRS spectra among the study treatments for fat, moisture, and protein contents (Figure 5.1).

Table 5. 6. *Chemical proximal meat characteristics (%) of the different experimental treatments of slow-growing chicks fed diets including either soybean meal (treatments R-C and NN-C) or Acheta domesticus insect meal (treatments R-AD and NN-AD) as the main protein source, and slaughtered at 95 d of age.*[1]

	Treatment				SEM[3]	P-value			
Parameter	R-C[2]	NN-C[2]	R-AD[2]	NN-AD[2]		T[4]	D[4]	S[4]	D×S
Moisture	73.4	73.3	74.1	74.0	0.210	0.055	0.023	0.792	0.890
Ash	1.24[b]	1.27[ab]	1.23[b]	1.40[a]	0.020	0.013	0.901	0.176	0.471
Total fat	1.38[ab]	0.68[b]	1.60[a]	1.44[a]	0.110	0.009	0.224	0.007	0.629
Crude protein	26.5	26.5	25.5	25.5	0.210	0.083	0.009	0.837	0.787

Within a row, means followed by the same letter are not significantly different (P > 0.05) according to Tukey's test.

[1] Each value is the mean of eight replicates (chicks), in which the variables were measured in duplicate.
[2] R-C: Red Control group, NN-C: Naked Neck Control group, R-AD: Red *Acheta domesticus* group, NN-AD: Naked Neck *Acheta domesticus* group.
[3] SEM: pooled standard error of the mean.
[4] T: Treatment, D: diet, S: strain.

The absence of differences in proximal meat composition caused by strain, except for total fat, is consistent with the results of the study by Fanatico et al. (2007), where different chicken genotypes showed similar chemical composition except for total fat percentage, with values in the slow-growing strain similar to those obtained in this study. Mueller et al. (2020) also found no differences in moisture caused by the strain used but found lower fat values in the meat of slow-growing chicks. Abeni and Bergoglio (2001) also found no differences in moisture and crude protein when using different chicken strains to produce commercial hybrids, although they did report differences in ash content without affecting fat.

The results obtained by Devatkal et al. (2019) also found no differences in the percentages of moisture, ash, and total fat when comparing the meats of different chicken genotypes with different growth rates, although they did observe differences in crude protein content, with higher levels for slow-growing chicks, but with these values being lower than those obtained in the present study.

In contrast, in the work of Hailemariam et al. (2022), variations were found in the percentages of moisture, crude protein, and ash when using different genotypes, obtaining values similar to our results with the *Improved Horro* and *Indigenous* genotypes.

The results obtained according to the diet utilized partially coincide with several studies in which the use of *Hermetia illucens* in broilers did not affect any of the parameters studied (Altmann et al., 2020; Cullere et al., 2019; Schiavone, et al., 2017), nor did it do so in ducks (Gariglio et al., 2021) or quail (Cullere et al., 2018). The absence of differences in fat is refuted by Mlaga et al. (2022), where the use of black soldier fly in broiler diets caused an increase in fat, as it also caused an increase in protein and a reduction in moisture content. The use of *Tenebrio molitor* in the diet of partridges and chickens also did not cause differences in the proximal composition of their meat (Secci et al., 2018; Shaviklo et al., 2021), nor did *Calliphora* sp. in slow-growing chicks (Sarmiento-García et al., 2021). This reduction in protein content in breasts of chickens fed *Acheta domesticus* may be due to the lower protein digestibility of the insect meal versus soybean meal (Nieto et al., 2023b).

c) Fatty Acid profile

The fatty acid profiles of chicken meat from the various experimental treatments are detailed in Table 5.7. A total of 19 different fatty acids were identified and quantified, with the most prevalent being oleic acid (C18:1), linoleic acid (C18:2n-6), palmitic acid (C16:0), stearic acid (C18:0), and palmitoleic acid (C16:1). These findings directly align with the fatty acid composition of the feed utilized in the poultry rations (Table 5.3). The higher concentrations of MUFA compared to PUFA observed in the feed are reflected in a similar pattern in the chickens' meat.

***Table 5.** 7. Fatty acids composition (as g/100 g of fatty acid methyl esters) of the meat of the different experimental treatments of slow-growing chicks fed diets including either soybean meal (treatments R-C and NN-C) or Acheta domesticus insect meal (treatments R-AD and NN-AD) as the main protein source, and slaughtered at 95 d of age.*[1]

	Treatment				SEM[3]	P-value			
Fatty acid	R-C[2]	NN-C[2]	R-AD[2]	NN-AD[2]		T[4]	D[4]	S[4]	D×S
C14:0	0.660	0.720	0.70 0	0.640	0.030	0.810	0.828	0.969	0.363
C14:1n-5	0.190	0.110	0.140	0.10 0	0.020	0.589	0.517	0.254	0.691
C16:0	17.3	17.7	17.0	18.1	0.470	0.821	0.946	0.447	0.719
C16:1n-7	0.960	1.04	1.02	0.870	0.040	0.532	0.557	0.672	0.244
C16:1	3.86	2.68	3.04	2.00	0.300	0.160	0.209	0.071	0.906
C17:0	0.080	0.060	0.130	0.140	0.020	0.239	0.049	0.897	0.600
C18:0	4.48	5.00	4.61	5.20	0.220	0.644	0.734	0.250	0.941
C18:1	50.5	46.0	49.9	46.7	1.43	0.663	0.981	0.221	0.833
C18:1n-7	1.63	1.54	1.42	1.35	0.050	0.257	0.073	0.458	0.934
C18:2n-6	16.3	18.3	17.5	19.5	0.720	0.446	0.433	0.183	0.996
C20:0	0.040	0	0.070	0.070	0.010	0.192	0.051	0.335	0.460
C18:3n-6	0.250	0.120	0.270	0.180	0.030	0.180	0.394	0.040	0.709
C18:3n-3	1.07ab	1.10a	0.720b	0.750ab	0.050	0.010	0.001	0.740	0.955
C20:2n-6	0.060	0.240	0.090	0.080	0.040	0.418	0.374	0.274	0.218
C20:3n-6	0.190	0.200	0.200	0.230	0.030	0.959	0.735	0.784	0.805
C22:1n-9	1.98	4.00	2.56	3.36	0.540	0.668	0.980	0.234	0.602
C20:4n-6	0.110	0.070	0.120	0.080	0.020	0.873	0.901	0.443	0.928
C22:6n-3	0.350	1.05	0.490	0.500	0.100	0.156	0.312	0.089	0.095
C22:5n-3	0.120	0.140	0.090	0.170	0.030	0.723	0.955	0.380	0.620
Total SFA[5]	22.5	23.5	22.5	24.1	0.580	0.680	0.794	0.301	0.767
Total MUFA[5]	59.1	55.3	58.1	54.4	1.14	0.434	0.676	0.132	0.992
Total PUFA[5]	18.4	21.2	19.5	21.5	0.760	0.469	0.674	0.142	0.803
n-3 PUFA[5]	1.54	2.29	1.36	1.42	0.130	0.101	0.055	0.127	0.191
n-6 PUFA[5]	16.9	18.9	18.1	20.1	0.730	0.475	0.438	0.199	0.974

Within a row, means followed by the same letter are not significantly different (P > 0.05) according to Tukey's test.

[1] Each value is the mean of eight replicates (chicks), in which the variables were measured in duplicate.
[2] R-C: Red Control group, NN-C: Naked Neck Control group, R-AD: Red *Acheta domesticus* group, NN-AD: Naked Neck *Acheta domesticus* group.
[3] SEM: pooled standard error of the mean.
[4] T: Treatment, D: diet, S: strain.
[5] SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n-3 PUFA: omega-3 polyunsaturated fatty acid, n-6 PUFA: omega-6 polyunsaturated fatty acid.

In general, the utilization of different slow-growing chicken strains and the substitution of soybean meal with house cricket meal had minimal impact on the fatty acid profile of the meat. Significant differences were only observed in α-linolenic acid (C18:3n-3) among treatments, with higher values of this fatty acid found in the meat from chickens fed soybean meal, i.e., R-C and NN-C.

This finding may be attributed to the chitin content of the cricket larvae, which could potentially inhibit the absorption of lipidic molecules (Ndiritu et al., 2023). Presently, there are limited studies comparing the fatty acid profiles of meat from animals of different strains. Jaturasitha et al. (2008) identified the most prevalent fatty acids in chicken meat as C16:0, C18:0, C18:1, and C18:2n6 for four genotypes adapted to extensive farming, closely resembling the findings of this study, although C18:3n6 was not detected. Similarly, Devatkal et al. (2019) did not observe C18:3n6 in their comparative analysis of meat from slow-growing chickens and commercial broilers, with minimal differences between them. Additionally, there were no distinctions in the cumulative levels of SFA, MUFA, PUFA, n-3 PUFA, or n-6 PUFA.

While the use of insect-based raw materials in animal feed is an emerging area of research, there is currently limited information available on the impact of house cricket meal in poultry production. A review of other studies involving insects in poultry diets indicates that the use of *Tenebrio molitor* in broilers did not result in differences in SFA, MUFA, n-3 PUFA, and n-6 PUFA concentrations, with researchers considering it a promising source of insect protein for the poultry feed industry (Dabbou et al., 2020; Loponte et al., 2019). However, Kierończyk et al. (2018) found no difference in C17:0 levels, but the inclusion of *T. molitor* altered SFA, MUFA, and PUFA levels. The use of *T. molitor* in the feeding of partridges caused a decrease in the concentration of C17:0 without altering the value of C18:3n-6 (Secci et al., 2018). The results obtained by Sarmiento-García et al. (2021) after the inclusion of *Calliphora* sp. in slow-growing chickens align with our results, showing no significant differences for most of the analyzed fatty acids.

Schiavone et al. (2019) reported that the use of black soldier fly in chicken feed did not cause changes in SFA nor in n3 and n6 PUFA. The reduction of C18:3n-3 after including cricket meal is consistent with results from other studies (Cullere et al., 2018; Mlaga et al., 2022; Schiavone et al., 2017).

In other poultry meat species, the use of *Tenebrio molitor* did not generate a difference in the SFA of partridge meat (Secci et al., 2018), but it did for *Hermetia illucens* when included in the diet of quail (Cullere et al., 2018). The effects of the ingestion of this fly species by ducks in their meat partially align with our results, as it also decreased the C18:3n-3 value without altering the overall fatty acids profile (Gariglio et al., 2021).

Therefore, the addition of cricket meal in chicken diets barely caused modifications in the fatty acid profile of the meat, making this raw material a suitable alternative protein source to include in poultry rations.

In conclusion, the findings of this study suggest that substituting soybean meal with *Acheta domesticus* meal in the diet of various slow-growing chicken strains led to a reduction in the weight of different carcass traits across all genotypes. However, despite variations in pH, cooking losses, moisture, and crude protein content, overall meat quality remained satisfactory. Conversely, the choice of slow-growing chicken strain had a discernible impact on the total fat content of the meat. Furthermore, NIR spectroscopy effectively classified all samples based on diet and strain, confirming its utility in differentiating meat according to genotype and feeding system.

Therefore, these findings position *Acheta domesticus* meal as a potential alternative protein source to soybean meal in the feeding of slow-growing chicks, as meat quality is comparable to those obtained in conventional diets.

CAPÍTULO 6:

Substitution of soybean meal for yellow mealworm meal in the diet of slow-growing chickens provides comparable carcass traits and meat quality

Capítulo 6:

Substitution of soybean meal for yellow mealworm meal in the diet of slow-growing chickens provides comparable carcass traits and meat quality

Jaime Nieto[1], Javier Plaza[1] Miriam Hernández-Jiménez[2], Isabel Revilla[2], Carlos Palacios[1]

[1] Area of Animal Production, University of Salamanca, Avenida Filiberto Villalobos 119, Faculty of Agricultural and Environmental Sciences, 37007, Salamanca, Spain.
[2] Area of Food Technology, University of Salamanca, Avenida Requejo 33, Superior Polytechnic School of Zamora, 49022, Zamora, Spain.

British Poultry Science. 2024. Accepted: 26 May 2024.

Resumen

La creciente demanda proteica de una población en continuo crecimiento posiciona a la carne de ave como una fuente de proteína fácilmente disponible y rentable. La producción de estos animales está ligada a un alto consumo de harina de soja, la cual lleva asociada graves preocupaciones ambientales en su cultivo, de seguridad alimentaria y fluctuaciones en sus precios. Estos han motivado la búsqueda de alternativas proteicas, posicionando a las harinas de insectos como un alimento prometedor como sustituto a las materias primas convencionales, debido a su producción más sostenible. Además, cabe señalar que la nutrición de las aves tiene un impacto significativo en la calidad de la carne y posteriormente en su compra, siendo necesario profundizar en el impacto de los cambios alimenticios sobre sobre dicha calidad.

También crece el interés por las estirpes de pollos de crecimiento lento adaptadas a sistemas de producción alternativos que aumenten el bienestar animal, para los que los conocimientos sobre la calidad de su carne incluyendo la repercusión de los cambios en su dieta es limitado. Además, la discriminación de este tipo de carne es crucial para su verificación y clasificación frente otras muestras.

El objetivo de este estudió fue evaluar los efectos de la inclusión de harina de larvas de *Tenebrio molitor* como remplazo parcial y total a la harina de soja sobre las características de la canal y los parámetros de calidad de carne de pechuga en pollos de engorde de crecimiento lento. Además, se empleó la metodología NIRS para la posible discriminación de las muestras en función de la dieta suministrada.

Un total de 256 pollos machos de crecimiento lento de un día de edad (*Coloryeld*) se dividieron aleatoriamente en 32 unidades experimentales de 8 pollos cada una, repartidas a su vez en 4 tratamientos dietéticos distintos: un grupo control (C) alimentado con harina de soja como principal fuente proteica y tres tratamientos experimentales en los que la harina de soja se sustituyó por harinas de larva de *Tenebrio molitor* al 50%, 75% y 100 % (T1, T2 y T3). La crianza finalizó con el sacrificio a los 92 días. Durante el ensayo se utilizaron tres dietas concentradas diferentes a lo largo del ciclo de vida de los pollos (F1, F2 y F3), cambiándose cada 4 semanas. Tras el sacrificio se seleccionaron aleatoriamente 8 pollos por cada tratamiento en los que se evaluaron las características de canal y calidad de carne, además del registro de los espectros NIRS.

Los pollos del grupo C alcanzaron mejores resultados productivos frente el resto de los grupos (peso vivo, ganancia de peso e índice de conversión). A pesar de ello, apenas hubo diferencia en los parámetros de canal, excepto en el peso de la cabeza y los muslos con valores más elevados en el grupo C.

La carne de los pollos en los que se sustituyó parcialmente la harina de soja por harina de *Tenebrio molitor* (tratamientos T1 y T2) presentaba un menor enrojecimiento, mayor jugosidad y menor dureza. Estas mejoras pueden atribuirse a un mayor contenido de humedad y a un menor porcentaje de proteínas. El contenido de cenizas fue mayor para el grupo T3 frente el resto de los tratamientos.

La inclusión de insectos mejoró el perfil de ácidos grasos, lo que se tradujo en un mayor contenido de ácidos grasos monoinsaturados y un menor contenido de ácidos grasos poliinsaturados n-6, mientras que el contenido de ácidos grasos saturados se mantuvo similar.

La sustitución completa de la harina de soja por harina de *T. molitor* no altera los parámetros fisicoquímicos salvo un mayor contenido en cenizas y ácidos grasos monoinsaturados.

El sistema NIRS clasificó eficazmente todas las muestras según el tipo de pienso ingerido, demostrando la utilidad de esta herramienta para la diferenciación de la carne en función de factores dietéticos. En conclusión, estos resultados arrojan luz sobre el potencial de la harina de *Tenebrio molitor* como fuente proteica alternativa adecuada para la alimentación de pollos de crecimiento lento, ya que no afecta negativamente a las características físico-químicas de la carne.

Abstract

1. This study investigates the effects of incorporating yellow mealworm (*Tenebrio molitor*) larval meal as a partial and/or complete substitute for soybean meal on carcass and meat quality in slow-growing chickens.

2. A total of 256 one-day-old male chicks (*Colorield*) were randomly allocated to 32 experimental units (eight chickens each). These units were further evenly distributed among four treatments (n=8): the control treatment (C), where soybean (SB) meal was the sole protein source, and three experimental treatments, denoted as T1, T2, and T3, in which SB meal was replaced by *Tenebrio molitor* (TM) larval meal at levels of 50%, 75%, and 100%, respectively. Three different feeds (F1, F2, and F3) were used for each treatment. All chickens were slaughtered at 92 days of age, with eight animals per treatment randomly selected to assess carcass and meat quality. Near-infrared reflectance spectroscopy (NIR) was employed to classify meat based on the feed consumed.

3. Carcass traits did not exhibit significant differences between treatments, except for head and thigh weight, which were higher in treatment C (P <0.01). In terms of physicochemical characteristics, treatment T2 showed a lower degree of yellowness (P <0.05), while expressible juice and cooking losses were lower in treatments T1 and T2 (P <0.01). Both treatments T1 and T2 had lower shear forces (P <0.01), higher moisture content (P <0.01), and lower total protein values (P <0.05) compared to treatments C and T3, with the latter having the highest ash content (P <0.01). Chickens consuming insects had higher monounsaturated fatty acid (MUFA) levels and lower polyunsaturated fatty acid (PUFA) and n-6 acids PUFA (P <0.01).

4. The substitution of soybean meal with *Tenebrio molitor* meal represents a protein alternative for slow-growing chickens that provides carcass and meat quality comparable to that of a conventional diet typically incorporating plant-protein sources.

Keywords: Carcass quality, Feed efficiency, Meat analysis, Nutrition, Protein alternative, Slaughter, Welfare

6.1 Introduction

The rising global demand for high-quality protein sources, coupled with diminishing available land suitable for traditional livestock farming, poses a significant challenge (Murawska et al., 2021). Within this context, poultry meat emerges as a readily available and cost-effective protein source for human consumption (Elahi et al., 2020). However, the growing appetite for meat is intrinsically linked to the increasing reliance on soybean meal, particularly in poultry production, where soybean constitutes the primary protein source in bird feed. Soybean meal is known not only for its high protein content but also for its susceptibility to price fluctuations associated with changes in climatic conditions and social factors in its production regions. These challenges, in addition to the environmental and food security concerns surrounding soybean cultivation, necessitate the quest for alternative, economically viable protein sources (Selaledi et al., 2020). Exploring such new sources could help mitigate the adverse social and ecological impacts of extensive soybean imports from overseas (Leiber et al., 2015).

Insects are an integral part of poultry nutrition (Sarmiento-García et al., 2021) and are emerging as a promising ingredient in poultry diets, offering an alternative to conventional feeds (Cullere et al. 2019; Secci et al. 2022; Nieto et al. 2022). This interest has grown substantially following recent EU regulations that permit the use of insect meal in monogastric diets (EU 1372, 2021). The inclusion of insects in poultry diets contributes to more sustainable production, given their low land and water requirements, low carbon footprint, and their role in the circular economy by converting organic waste into high-quality feed ingredients (Abro et al., 2020). Furthermore, it's worth noting that poultry nutrition significantly influences meat quality and, consequently, consumer purchase decisions (Elahi et al., 2020; Mir et al., 2017). Therefore, it is crucial to delve deeper into understanding the impact of dietary changes on meat quality.

There's also growing interest among both producers and consumers in using slow-growing chicken strains that are adapted to alternative production systems emphasizing animal welfare (Vissers et al., 2019). These birds have longer life cycles, resulting in different nutritional and sensory characteristics that are often more appreciated and demanded by consumers (Filho et al., 2021). However, there is limited knowledge about meat quality and the implications of dietary changes, such as the inclusion of insect meal, in these strains (Cullere et al. 2018; Mueller et al. 2020).

The characterization and differentiation of meat samples from more sustainable production systems is crucial for verification. In this regard, near-infrared reflectance spectroscopy (NIRS) has proven to be a valuable tool in quality control for poultry meat, enabling sample classification based on the birds' diets while maintaining a contamination-free process (Berzaghi et al., 2005). Its non-invasive nature and simplicity in terms of sample preparation make it a convenient technology for operators (Hernández-Jiménez et al., 2021).

In light of the aforementioned arguments, this study hypothesizes that the partial and/or total substitution of soybean meal with yellow mealworm meal in the feeding regimen for slow-growing chickens can enhance their carcass and meat quality. Consequently, the primary objective of this research is to evaluate the impact of incorporating yellow mealworm (*Tenebrio molitor*) larval meal as a partial and/or complete replacement for soybean meal on carcass attributes and meat quality parameters in slow-growing chickens. Additionally, NIR methodology will be employed to potentially differentiate samples based on the dietary feed. This study builds upon the work of Nieto et al. (2022), which assessed the effects of a complete replacement of soybean meal with yellow mealworm meal in the diet of slow-growing chickens. The novelty of the present study lies in two fundamental aspects: firstly, exploring partial substitutions of soybean meal with *T. molitor* meal, following the insights from prior research; and secondly, investigating the changes brought about by this dietary modification not only on productivity but also on carcass characteristics and meat quality.

6.2 Material and Methods

The slow-growing chicks used in the trial were treated in accordance with the principles of European Parliament and Council Directive 2003/65/EC of 22 July 2003, amending Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes. The experimental protocol was approved by the Bioethics Committee of the University of Salamanca (Spain), with registration number 0000590.

6.2.1 Experimental design, chickens management and housing

In this trial, a total of 256 one-day-old male slow-growing chicks of the *Colorield* strain, previously vaccinated against Marek´s disease and H/120 avian infectious bronchitis at the hatchery, were utilized. The chicks were randomly distributed into 32

experimental units of eight chicks each, with careful consideration given to ensuring their initial weight homogeneity. Subsequently, these 32 experimental units were randomly assigned to four distinct dietary treatments (n=8 units each): the control treatment (C) (mean live weight, MLW, of 39.89±0.04 g), where soybean (SB) meal served as the exclusive protein source, and three experimental treatments, labelled as T1 (MLW, 41.63 ±0,24 g), T2 (MLW, 41.83 ±0,47 g), and T3 (MLW, 39.82 ±0,10 g), in which SB meal was substituted by *Tenebrio molitor* (TM) larval meal at rates of 50%, 75%, and 100%, respectively. These TM larval meal incorporations align with previous studies that have investigated the partial and complete substitution of soybean meal with *Hermetia illucens* larvae meal (Schiavone et al. 2017; Cullere et al. 2019).

The rearing of the chickens was carried out in two distinct stages: the first took place in an enclosed facility and lasted from the beginning of the experiment until the animals were 45 days old, while the second was conducted in outdoor housing from the 46th day of life until the animals were 92 days old, when they were slaughter. Comprehensive details regarding the rearing conditions and housing specifics for both phases of the experiment are available in Nieto et al. (2022), with the noteworthy distinction that this experiment featured twice as many pens compared to the aforementioned article.

6.2.2 Ration Composition and data collection and processing

During the trial, three distinct concentrated feeds (F1, F2, and F3) were administered in four-week intervals for each of the feed treatments. These feeds were adjusted to meet the specific requirements of each stage in the chickens' life cycle. F1 was used from day 1 to day 29, F2 from day 29 to day 57, and F3 from day 57 to day 92. The experimental feeds were meticulously formulated to align with the nutritional needs of medium-slow growth chickens, following the nutritional guidelines of the National Research Council (NRC, 1994). These feeds were different for each experimental treatment. The compositions of these feeds, expressed g/kg of dry matter (DM), are shown in Table 6.1.

Prior to supplying these feeds to the chicks, they underwent chemical and nutritional analyses, as presented in Table 6.2. These analyses were conducted in the food analysis laboratories of Abiomed Higiene S.L., a company based in Salamanca, Spain, that adheres to an ISO 9001 quality system.

Furthermore, soybean and yellow mealworm meals underwent individual analyses. Recognizing that the last feed consumed by the chickens before slaughter, i.e., the F3 feed, has the most direct impact on carcass and meat quality outcomes, the detailed fatty acid profile of this feed is provided in Table 6.3.

***Table 6. 1**. Composition of the experimental feeds (F1, F2 and F3) for the four studied treatments of slow-growing chickens (C, T1, T2 and T3), which were fed feeds including either soybean meal and/or yellow mealworm insect meal as the main protein source. Composition of C and T3 feeds coincide with the ones used in Nieto et al. (2022).*

Ingredients (g/kg of DM)	F1 (1-29 d)				F2 (29-57 d)				F3 (57-92 d)			
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3
Corn	370	210	130	100	480	370	170	90	310	298	258	180
Wheat	110	350	157	00	120	310	150	168	370	340	350	335
Barley	0	50.0	50.0	210	0	100	200	132	0	0	0	0
SB[1] meal	290	111	56.0	00	200	115	32.0	0	120	66.0	43.0	0
YM[1] meal	00	112	167	223	0	65.0	98.0	130	0	46.0	69.0	92.0
Sunflower	190	0	0	00	160	0	90.0	90.0	160	90.0	90.0	90.0
Oats	0	50.0	350	427	0	0	170	350	0	120	150	263
Peas	0	77.0	50.0	00	0	0	50.0	0	0	0	0	0
Vitamin-mineral premix[2]	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Total feed	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

[1] SB = soybean; YM = yellow mealworm.
[2] Fatty acid and vitamin-mineral prefix includes (per kg): Methionine 2.10 g; Calcium 207.50 g; Phosphorus 55.80 g; Sodium 63.80 g; Choleocalciferol 2.51 mg; Tocopherol Acetate 292.65 mg; Retinyl acetate 86.42 mg; Betaine anhydrous 4,002.84 mg. Anti-caking agents: bentonite 100 g/Kg; sepiolite 3,340.2 mg/kg. Trace element premix includes per kg: Cu (sulphate II pentahydrate), 335 mg; Fe (sulphate II monohydrate), 1,324 mg; Mn (oxide), 3,336 mg, Zn (oxide), 2,835; I (calcium iodate anhydrous), 51.07 mg; Se (sodium selenite), 10.10 mg.

Table 6. 2. *Chemical-nutritional composition of the experimental feeds (F1, F2 and F3) for the four studied treatments of chickens (C, T1, T2 and T3) and of the soybean and yellow mealworm meals separately, expressed as g/kg of dry matter, except for metabolizable energy (ME), which is expressed as MJ/kg of dry matter. Composition of C and T3 feeds coincide with the ones used in Nieto et al. (2022).*

Nutrient level4	F1 (1-29 d)1				F2 (29-57 d)1				F3 (57-92 d)1				SB2	YW3
	C	T1	T2	T3	C	T1	T2	T3	C	T1	T2	T3		
Moisture	76.0	103	94.0	79.0	89.0	105	102	89.0	86.0	114	115	85.0	121	66.0
Ashes	65.0	69.0	68.0	64.0	64.0	62.0	58.0	60.0	58.0	53.0	55.0	59.0	62.0	42.0
Crude fat	123	77.0	103	99.0	95.0	74.0	69.0	91.0	107	64.0	75.0	97.0	19.0	212
Neutral detergent fibre	61.0	82.0	90.0	79.0	50.0	67.0	71.0	85.0	61.0	67.0	65.0	69.0	59.0	83.0
Starch	350	337	314	381	412	432	411	374	423	449	385	409	1.00	23.0
Crude protein	200	194	198	199	166	161	163	163	148	142	145.7	143	440	549
ME	11.7	12.1	11.9	12.1	12.1	12.1	12.3	12.1	12.4	12.4	12.5	12.4	7.91	21.0
Ca	12.4	16.1	17.8	19.4	12.1	14.3	15.3	16.3	12.0	13.5	14.2	14.9	2.90	36
Total P	4.00	14.2	17.6	21.0	3.60	9.00	11.1	14.1	3.40	7.20	9.00	10.8	6.10	74.1
SFA	16.0	14.0	17.0	22.0	12.0	12.0	12.0	16.0	14.0	10.0	12.0	15.0	6.00	50.0
MUFA	30.0	40.0	62.0	37.0	23.0	21.0	21.0	28.0	24.0	17.0	20.0	28.0	9.00	84.0
PUFA	78.0	22.0	24.0	41.0	60.0	41.0	37.0	47.0	70.0	37.0	43.0	54.0	26.0	79.0
n-3 PUFA	1.50	0.900	0.900	1.40	1.10	0.800	0.900	0.900	1.00	0.600	0.800	0.800	3.10	2.50
n-6 PUFA	76.0	21.2	23.5	39.0	59.0	40.0	36.0	47.0	68.0	37.0	42.0	53.0	22.7	76.0
Aspartic acid + Asparagine	25.4	15.6	14.3	18.3	19.2	11.4	14.4	8.40	12.0	10.5	8.30	8.60	48.4	45.0
Glutamic acid + Glutamine	52.2	26.0	25.8	41.3	44.5	27.1	28.4	28.3	41.2	20.8	22.5	28.6	75.6	64.0
Alanine	9.10	11.3	<0.200	14.0	9.00	9.10	9.30	9.20	9.50	6.50	7.00	9.90	18.9	50.0
Arginine	12.8	14.6	12.1	9.10	12.1	8.20	8.50	7.00	7.70	7.90	7.10	8.00	31.8	27.4
Cystine	1.80	1.20	1.30	1.30	1.50	1.20	0.900	1.10	1.20	0.900	1.30	1.10	7.00	3.00
Phenylalanine	8.10	6.50	9.00	5.90	6.80	7.90	7.50	5.50	5.70	5.40	4.80	4.50	21.9	21.7
Glycine	10.2	9.40	8.10	6.10	8.80	7.20	6.50	4.50	4.00	5.30	6.60	6.70	18.9	27.2
Histidine	7.60	5.00	6.00	5.70	6.50	2.60	3.20	9.50	7.60	2.20	3.60	4.80	11.7	15.1
Isoleucine	6.80	5.40	7.20	4.40	5.30	5.10	6.30	5.80	4.00	3.70	3.90	3.60	19.6	23.9
Lysine	14.4	10.1	8.90	12.3	12.8	9.20	6.80	9.70	10.1	4.90	5.80	6.90	26.8	28.0
Methionine	2.90	2.20	5.50	1.80	2.00	2.10	1.80	1.70	1.90	2.30	1.90	1.70	5.90	4.70
Proline	9.10	11.1	14.2	10.0	14.5	13.7	11.3	10.3	10.3	9.20	10.0	11.6	21.6	27.4
Serine	9.00	8.60	8.20	8.80	9.00	6.60	6.40	8.00	7.60	4.80	5.10	5.90	21.1	22.5
Tyrosine	5.80	8.10	11.3	6.90	4.60	8.30	7.10	8.10	3.40	5.10	6.50	8.00	17.3	41.0
Threonine	7.90	6.30	6.90	6.40	5.80	5.40	5.90	4.50	5.00	4.90	4.00	6.50	17.2	18.9
Valine	6.50	10.2	13.8	6.10	5.30	9.40	10.1	6.50	4.60	5.90	7.00	4.10	20.7	47.0

[1] d = days; [2] SB = soybean meal; [3] YM = yellow mealworm larvae meal; [4] Ca = calcium; P = phosphorous; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-3 PUFA = omega-3 polyunsaturated fatty acid; n-6 PUFA = omega-6 polyunsaturated fatty acid; Trans FA = trans fatty acid.

Table 6. 3. *Fatty acids composition (as g/kg of fatty acids) of the F3 experimental feed for the four studied treatments of chickens (C, T1, T2 and T3).*

Fatty acid[1]	C	T1	T2	T3
C12:0	0.435	2.21	2.29	2.28
C14:0	1.67	8.61	10.7	18.4
C15:0	2.90	4.08	1.83	1.98
C16:0	90.1	113	118	108
C16:1n-7	0.770	2.15	2.69	3.97
C16:1	2.08	3.90	4.62	6.16
C17:0	0.670	0.825	0.920	0.865
C17:1	0.215	0.760	1.00	1.24
C18:0	37.3	31.5	29.2	31.1
C18:1n-9	1.48	2.10	1.47	0.0
C18:1	265	249	254	190
C18:1n-7	8.44	6.50	6.08	7.35
C18:2n-6t	0.0	0.460	0.350	0.435
C18:2n-6	546	551	538	569
C20:0	3.81	2.57	2.29	2.21
C18:3n-6	0.120	0.110	0.100	0.180
C18:3n-3	12.8	5.92	11.8	10.1
C20:2n-6	0.390	0.280	0.330	0.415
C22:0	8.00	4.40	3.80	5.40
C20:3n-6	0.090	0.395	0.615	0.485
C22:1n-9	0.280	0.380	0.325	0.525
C20:3n-3	0.250	0.150	0.130	0.240
C20:4n-6	0.320	0.230	0.200	0.180
C22:2n-6	0.0	0.145	0.180	0.185
C24:0	3.20	2.17	1.91	2.25
C20:5n-3-epa	0.105	0.515	1.025	0.330
C24:1n-9	0.930	2.37	2.61	3.45
C22:6n-3	0.790	1.28	1.30	2.37
C22:5n-3	11.7	3.56	2.89	30.5
Total SFA	148	169	171	173
Total MUFA	279	267	272	213
Total PUFA	573	564	557	614
n-3 PUFA	25.7	11.4	17.2	43.5
n-6 PUFA	547	553	540	571

[1] SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-3 PUFA = omega-3 polyunsaturated fatty acid; n-6 PUFA = omega-6 polyunsaturated fatty acid.

Both water and feed were available to the chickens ad libitum, with feed being provided as milled meal throughout the entire trial. Additionally, the health status of the birds was monitored on a daily basis.

Throughout the entire experiment, the live weight (LW) and feed intake (FI) were measured on a weekly basis using a dynamometer and a digital scale. Furthermore, the body weight gain (BWG) and feed conversion ratio (FCR) were calculated accordingly.

6.2.3 Poultry slaughter and carcass assessment

Ninety-two days following the commencement of the trial, eight chickens were randomly selected from each of the experimental units (n = 32) and humanely slaughtered in a certified slaughterhouse in accordance with Council Regulation (EC) No. 1/2005 of 22 December 2004, which outlines the protection of animals during transport and related operations and amends Directives 64/432/EEC and 93/119/EC, as well as Regulation (EC) No. 1255/97. The process was also carried out in compliance with Council Regulation (EC) No. 1099/2009 of 24 September 2009, which addresses the protection of animals at the time of slaughter. Following slaughter, the chickens were promptly transported to the Food Technology Laboratory at the University of Salamanca for the analysis of carcass characteristics and meat quality.

Prior to slaughter, the chickens were weighed to determine their live weight. Subsequently, post-slaughter, the chickens were plucked and weighed again. The head was then separated by making a cut between the occipital bone of the skull and the first cervical vertebra, and the legs were also removed, with the cut performed at the junction with the thigh, specifically between the tarsometatarsal bone and the tibiotarsus. Weights were recorded for both the head and legs separately. The chickens were then eviscerated, and their weight was once again measured to obtain the hot carcass weight. Twenty-four hours after slaughter, the carcasses were weighed a final time to establish the cold carcass weight, which was used to calculate carcass yield in relation to the live weight at the time of slaughter. Subsequently, the wings were removed along with the breast, including the keel bone, and their combined weight was recorded as the forequarter. The breast was then weighed separately, excluding the keel bone and skin. Additionally, the hindquarter, comprising the thighs and drumsticks, was weighed, with the thighs being recorded separately.

6.2.4 Poultry meat quality analysis

For meat quality analyses, the breast meat of the chickens, skinless and boneless, was kept at 4ºC for 48 hours following slaughter. At this point, NIRS and physical analysis, including colour and instrumental texture measurements, were conducted. Afterward, the samples were vacuum-packed and frozen at -18ºC until chemical analysis was performed.

a) NIRS analysis

NIRS spectroscopy, being a non-destructive method, was the initial analysis performed before meat physicochemical assessments. The spectra were recorded using the Foss NIR System 5000 (Foss NIRSystems, Silver Spring, MD, USA) with a 1.5 m 210/210 remote reflectance bundle fibre-optic probe (Ref no. R6539-A). This probe featured a quartz register window of 5 cm × 5 cm and measured reflectance in the spectral range between 1100 and 2000 nm, using a ceramic plate as a reference. The measurement was conducted by directly applying the fibre optic probe to the meat surface. The diffuse reflectance signal of the NIR spectrum is represented as log(1/R), with R indicating reflectance.

b) Physical characteristics

Among the physical characteristics analysed in the meat, pH, expressible juice, colour, texture, and cooking losses were assessed. Meat pH was measured at 18ºC using a Crison pHMeter Basic 20 (Crison, Barcelona, Spain) equipped with a penetration electrode. Water holding capacity, expressed as expressible juice, was determined following the method proposed by Grau and Hamm (1953). Meat colour was measured using a MiniScan XE Plus Spectrophotometer (Hunter Lab, Reston, VA, USA). CIELab parameters, i.e., lightness (L*), redness (a*) and yellowness (b*), (CIE, 1977) were determined for the CIE illuminant D65 and 10º standard observer conditions. To calculate cooking losses, each sample was first weighed and packed individually in a polyethylene bag. They were cooked in a water bath at 75ºC until reaching a centre piece temperature of 70ºC. Meat temperature was monitored with a K-type high temperature probe (Omega, Laval, QC, CA). Subsequently, the cooked samples were cooled in tap water at room temperature and reweighed. Texture was analysed according to the method developed by Szczesniak (1987). The same samples used in the cooking loss calculation were employed, cutting four prisms per sample into pieces measuring 1 x 1 x 3 cm (height x width x length) parallel to the direction of muscle fibres. Shear strength was measured using the Texture Analyser TA-XT2i (Texture Analyser®, Stable Micro Systems, Surrey, UK) equipped with a Warner-Bratzler blade, placing each sample in a transverse position to the cutting probe at a speed of 1 mm/s. The maximum shear force (WBSF) was recorded. All determinations were conducted in triplicate.

c) Chemical analysis

One day prior to the chemical analysis, the frozen samples were gradually thawed at 4ºC. Moisture, ash and fat content were determined by AOAC-approved methods (procedure identification numbers 930.15, 985.01 and 996.06, respectively) (AOAC, 2006). Fat and ash were reported as a percentage of dry matter (DM). Protein analysis was performed using the Kjeldahl method by the Abiomed Higiene SL laboratory (Salamanca, Spain), which adheres to an ISO 9001 certified quality system. All chemical analyses were carried out in duplicate.

The fatty acid profile was determined through gas chromatography, involving extraction, esterification, separation, identification, and quantification of fatty acids (Schettino et al., 2011). Total lipid extraction combined mechanical and organic solvent extraction, following the method described by Folch et al. (1957). Subsequent methylation was conducted as described by Lurueña-Martínez et al. (2010). The analysis used a GC 6890 N gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a 7683 automatic injector from the same company. A 100 m x 0.25 mm x 0.20 µm fused silica capillary column SP-2560 (Supelco, Inc, Bellefonte, PA, USA) was used. Different fatty acids were identified based on retention times, employing a mixture of fatty acid standards (47885-U, Supelco, Sigma-Aldrich, Germany). Fatty acid contents were calculated using chromatogram peak areas and expressed as g per 100 g of total fatty acid methyl esters.

6.2.5 Statistical análisis

Data analysis was conducted using IBM-SPSS Package 28 software (IBM, Chicago, IL, USA), with the exception of the NIRS analysis results. First, the Kolmogorov-Smirnov test was performed to check the normality of the data recorded. Statistical significance was assessed at the 95% confidence level (a = 0.05) using Snedecor's F as the contrast statistic. In this statistical procedure, a total of 32 sampling units, corresponding to one slaughtered chicken per experimental unit, were utilized (n = 8 chickens per treatment). Potential significant differences among the experimental treatments (C, T1, T2 and T3) attributed to the inclusion ratio of SB/YM meals in the different feeds (F1, F2, and F3) for slow-growing chickens, were evaluated employing the SPSS General Linear Model (GLM) procedure (Eq. 1), with treatment and feed serving as fixed factors.

$$\mu = g^{-1}(X\mathrm{B}) \quad (1)$$

Where μ is the expected value, XB is the linear predictor, i.e., a linear combination of unknown B parameters, and g is the link function (Nelder & Wedderburn, 1972).

The live weight at slaughter was introduced as a covariate in the GLM to mitigate the influence of potential differences stemming from heterogeneity in this variable. In cases where significant differences were detected, means were compared using Tukey's test (Tukey, 1949).

Discriminant analysis of the samples according to feed was carried out using the residual mean square method (RMS-X residuals) with the Win ISI WinISI 4.10 software (Infrasoft International, State College, PA, USA). This analysis utilized the entire NIR spectrum, following the method previously described by Sarmiento-García et al. (2021). The selection of the most effective mathematical treatment for distinguishing between the samples was based on the highest percentage of correctly classified samples.

6.3 Results

6.3.1 Growth performance assessment

Throughout the trial, no mortalities were recorded in any of the experimental treatments. The growth performance results (Table 6.4) indicated that chickens in treatment C achieved significantly higher live weight (LW) and body weight gain (BWG) compared to the other experimental treatments (P <0.01). Furthermore, treatment C also displayed significantly higher feed intake (FI), followed by treatment T1, and, in the last place, treatments T2 and T3 (P <0.05). In terms of feed conversion ratio (FCR), the significantly lowest value was observed in chickens from treatment C, with treatment T2 exhibiting the highest FCR (P <0.01).

Table 6. 4. *Growth performances of slow-growing chickens fed different proportions of soybean meal and/or yellow mealworm meal and slaughtered at 92 days of age*[1].

Variable[4]	C	T1	T2	T3	PSEM[2]	P-value[3]
LW (g)	4,598[a]	3,801[b]	3,422 [b]	3,842 [b]	86.9	<0.010
FI (g)	12,970[a]	11,546[ab]	11,054[b]	11,280[b]	298.2	<0.050
BWG (g/day)	50.1[a]	41.3[b]	37.2[b]	41.8[b]	0.96	<0.010
FCR	2.85[c]	3.07[b]	3.27[a]	2.97[b]	0.059	<0.010

[1] Each value is the mean of eight replicates.
[2] PSEM = pooled standard error of the mean.
[3] [a-b-c] Values within a row with different superscripts differ significantly at P < 0.05.
[4] LW = live weight prior to slaughter (92 days old); FI = total feed intake; BWG = body weight gain; FCR = feed conversion ratio.

6.3.2 Carcass quality assessment

Carcass characteristics of 92-day-old chickens fed with varying proportions of yellow mealworm larvae meal are presented in Table 6.5. Treatment C achieved significantly higher values for both head and thigh weights in comparison to the other treatments. In the case of the other parameters related to carcass quality, no significant impact of the different feeds used was observed (P <0.01).

Table 6. 5. *Weights of several carcass quality parameters (g) and cold carcass yield (g/kg) of slow-growing chickens fed different proportions of soybean meal and/or yellow mealworm meal and slaughtered at 92 days of age*[1].

Variable	C	T1	T2	T3	PSEM[2]	P-value[3]
Dead-plucked	4,275	3,176	3,046	3,377	130.6	0.420
Head	120[a]	85.0[b]	79.4[b]	68.3[b]	5.04	<0.010
Legs	116	107	103	106	2.0	0.760
Hot carcass	3,426	2,760	2,504	2,797	108.5	0.580
Cold carcass	3,355	2,691	2,418	2,725	108.0	0.800
Forequarters	1,333	957	919	1,038	46.6	0.980
Boneless breast	766	507	480	529	27.3	0.760
Hindquarters	1,504	1,086	1,050	1,143	47.4	0.230
Thighs	516[a]	365[b]	349[b]	395[b]	16.3	<0.010
Cold carcass yield	730	708	707	709	3.5	0.960

[1] Each value is the mean of eight replicates.
[2] PSEM = pooled standard error of the mean.
[3] [a-b-c] Values within a row with different superscripts differ significantly at P < 0.05.

6.3.3 Meat quality analysis

The results of the physical analyses, including colour, pH, expressible juice, cooking losses, and shear force, are presented in Table 6.6. Neither L* nor pH exhibited significant differences among the experimental treatments studied. However, the colour component a* reached significantly higher values in treatments C and T3 when compared to treatments T1 and T2, with the former two treatments displaying more reddish hues (P <0.01). Similarly, the b* component was significantly higher in treatment T3, followed by treatment C and then T1, with T2 displaying the lowest values for this parameter (P <0.05).

***Table 6. 6**. Physical meat quality characteristics of slow-growing chickens fed different proportions of soybean meal and/or yellow mealworm meal and slaughtered at 92 days of age[1].*

Variable[4]	C	T1	T2	T3	PSEM[2]	P-value[3]
L*	56.9	58.7	59.0	57.9	0.28	0.050
a*	8.77[a]	6.15[b]	6.10[b]	8.21[a]	0.174	<0.010
b*	11.5[ab]	11.3[ab]	10.7[b]	12.2[a]	0.17	<0.050
pH	5.64	5.66	5.68	5.62	0.007	0.060
Expressible juice (µL)	11.5[a]	10.0[b]	10.0[b]	10.4[ab]	0.18	<0.010
Cooking losses (g/kg)	129[a]	89.6[b]	97.2[b]	135[a]	3.8	<0.010
WBSF (N)	3,426[a]	2,271[ab]	1,934[b]	2,267[ab]	149.0	<0.010

[1] Each value is the mean of eight replicates, in which the variables were measured in triplicate.
[2] PSEM = pooled standard error of the mean.
[3] [a-b-c] Values within a row with different superscripts differ significantly at P < 0.05.
[4] L* = lightness; a* = redness; b* = yellowness; WBSF = Warner-Braztler shear force.

Water holding capacity, as measured through expressible juice, showed significantly higher values in treatment C, followed by treatment T3 (P <0.01). Cooking losses were also significantly higher in treatments C and T3 (P <0.01). Moreover, the meat from the chickens in treatment C displayed significantly higher shear force resistance, whereas the meat from chickens in treatment T2 had the lowest shear force resistance (P <0.01).

The results of the chemical analyses, which include moisture, ash, total fat, and protein, are presented in Table 6.7. Moisture content was significantly higher in the meat of birds from treatments T1 and T2, followed by treatment T3, and, finally, treatment C (P <0.01). Ash content was significantly higher in treatment T3 (P <0.01). In contrast, protein content was significantly higher in birds from treatment C, followed by treatment T3, with treatments T1 and T2 showing the lowest protein values (P <0.05). Regarding the levels of total fat, no statistically significant differences were observed among the experimental treatments.

***Table 6. 7**. Chemical meat quality characteristics of slow-growing chickens, expressed as g/kg of dry matter, fed different proportions of soybean meal and/or yellow mealworm meal and slaughtered at 92 days of age[1].*

Parameter	C	T1	T2	T3	PSEM[2]	P-value[3]
Moisture	723[b]	736[a]	734[a]	723[ab]	1.3	<0.010
Ash	12.9[b]	12.5[b]	12.3[b]	14.6[a]	0.23	<0.010
Total fat	13.7	17.9	13.7	11.8	1.12	0.370
Crude protein	278[a]	260[b]	260[b]	272[ab]	2.4	<0.050

[1] Each value is the mean of eight replicates, in which the variables were measured in duplicate.
[2] PSEM = pooled standard error of the mean.
[3] [a-b-c] Values within a row with different superscripts differ significantly at P < 0.05.

As for the fatty acid profile (Table 6.8), the most abundant fatty acid was linoleic acid (C18:2n-6), followed by oleic acid (C18:1), palmitic acid (C16:0), and stearic acid (C18:0). Additionally, C14:0 and C14:1n-5 fatty acids reached significantly higher levels in the meat of chickens that consumed insect meal, compared to treatment C (P <0.01). C15:0 reached significantly higher values in treatment T2, followed by T1, T3, and C treatments (P <0.01). Palmitic acid (C16:0) was found in higher concentrations in the meat of chickens from the T1 treatment, followed by T2, with treatments C and T3 displaying lower values (P <0.01). Palmitoleic acid (C16:1) exhibited similar trends, with even lower values for treatment C compared to T3 (P <0.01). Trans-vaccenic acid (C18:1n-7) levels were also significantly higher in the T1 treatment, followed by T2 and C, while the T3 treatment had lower concentrations (P <0.05). In contrast, stearic acid (C18:0) levels were higher in treatment C, followed by treatments T1 and T3, while treatment T2 had lower values (P <0.01). In the case of linoleic acid (C18:2n-6), it was found in significantly higher concentrations in treatments C and T3, followed by T2 and T1 (P <0.01). C20:2n-6 was higher in treatment C, followed by treatments T2 and T1 (P <0.01). The remaining fatty acids did not exhibit statistically significant differences among the experimental treatments.

In general, the sum of monounsaturated fatty acids was significantly higher in the treatments that consumed insects (T1, T2, and T3) compared to treatment C (P <0.01). Conversely, the total sum of polyunsaturated fatty acids and n-6 acids reached higher levels in treatment C, followed by treatments T3 and T2, with treatment T1 recording the lowest levels (P <0.01).

Table 6. 8. *Fatty acids (as g/kg of fatty acids) of slow-growing chickens fed different proportions of soybean meal and/or yellow mealworm meal and slaughtered at 92 days of age*[1].

Fatty acid[4]	C	T1	T2	T3	PSEM[2]	P-value[3]
C14:0	4.90^{b}	9.60^{a}	10.6^{a}	11.7^{a}	0.660	<0.010
C14:1n-5	0.210^{b}	1.43^{a}	1.33^{a}	0.930^{ab}	0.1190	<0.010
C15:0	0.420^{c}	1.11^{ab}	1.16^{a}	0.590^{bc}	0.0810	<0.010
C16:0	157^{b}	180^{a}	168^{ab}	159^{b}	2.6	<0.010
C16:1n-7	6.90	9.30	8.90	7.50	0.580	0.440
C16:1	15.4^{c}	31.0^{a}	25.9^{ab}	23.7^{b}	1.17	<0.010
C17:0	1.00	1.60	1.60	1.00	0.110	0.060
C17:1	2.40	2.00	3.20	2.20	0.920	0.980
C18:0	61.7^{a}	51.5^{ab}	46.7^{b}	50.7^{ab}	1.72	<0.010
C18:1	294	317	302	314	4.3	0.190
C18:1n-7	12.1^{ab}	13.8^{a}	12.5^{ab}	10.1^{b}	0.40	<0.050
C18:2n-6	400^{a}	341^{b}	369^{ab}	380^{a}	5.7	<0.010
C20:0	0.770	0.470	0.760	0.250	0.1240	0.340
C18:3n-6	3.10	3.30	3.50	2.70	0.220	0.650
C18:3n-3	8.30	7.40	8.60	10.4	0.580	0.340
C20:2n-6	2.90^{a}	1.90^{ab}	2.30^{ab}	1.50^{b}	0.140	<0.010
C20:3n-6	3.40	2.80	2.90	2.20	0.440	0.810
C22:1n-9	17.1	19.1	22.7	16.8	1.29	0.370
C20:4n-6	0.730	0.570	0.760	0.760	0.1800	0.990
C22:6n-3	7.40	4.90	5.90	3.50	0.840	0.340
C22:5n-3	1.30	1.00	2.00	1.00	0.220	0.380
Total SFA	226	244	229	223	3.1	0.120
Total MUFA	348^{b}	393^{a}	377^{a}	375^{a}	3.9	<0.010
Total PUFA	427^{a}	363^{b}	395^{ab}	402^{ab}	6.0	<0.010
n-3 PUFA	17.0	13.3	16.5	14.8	1.07	0.660
n-6 PUFA	410^{a}	349^{b}	378^{ab}	387^{ab}	5.8	<0.010

[1] Each value is the mean of eight replicates.
[2] PSEM = pooled standard error of the mean.
[3] $^{a\text{-}b\text{-}c}$ Values within a row with different superscripts differ significantly at $P < 0.05$.
[4] SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; n-3 PUFA = omega-3 polyunsaturated fatty acid; n-6 PUFA = omega-6 polyunsaturated fatty acid.

6.3.4 NIRS analysis

Discriminant analysis of the entire NIR spectrum between the four experimental treatments was performed using the RMS-X Residuals method. This analysis involved spectral pretreatment to correct for potential scattering effects in the spectrum. Various scattering treatments, including multiplicative scatter correction (MSC), standard normal variant (SNV), DeTrend (DT), and SNV-DT, were applied. Additionally, these treatments were combined with various mathematical treatments based on derivatives and smoothing procedures. The optimal treatment was determined based on the highest percentage of correctly classified samples. In this case, SNV 2,4,4,1 successfully discriminated 100% of the samples based on the type of feed the animals received.

The Figure 6.1 displays the raw spectra of each of the analysed treatments, with particular emphasis on treatment T3, which did not receive soybean meal, placed above those that did include soybean in their feed.

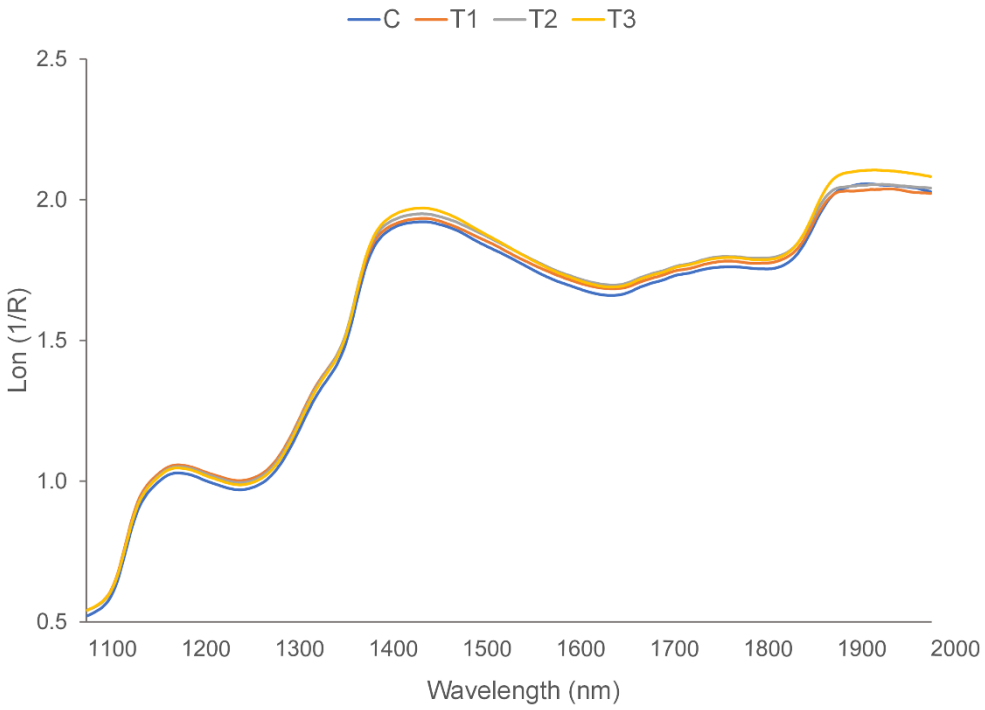

Figure 6. 1. *NIRS spectra of poultry meat from slow-growing chickens fed with different percentages of Tenebrio molitor meal inclusión.*

6.4 Discussion

In the current study, the partial replacement of dietary soybean meal with yellow mealworm meal showed slight effects on carcass characteristics and meat quality in slow-growing chickens. The results obtained in this study are noteworthy because previous research has focused either on industrial broiler strains (Shaviklo et al. 2021; Schiavone et al. 2019), or has emphasized the impact of including yellow mealworm (YM) meal only on productivity (Nieto et al., 2022).

6.4.1 Growth performance assessment

Our results align with previous works that observed a decrease in live weight (LW) at slaughter, as well as reduced body weight gain (BWG) and feed intake (FI) for *Hermetia ilucens* (Murawska et al., 2021), as also observed for *Calliphora* sp. (Sarmiento-García et al. 2021).

On the contrary, other authors claim that the inclusion of mealworm in the diet of broilers did not cause a significant effect on most of the growth traits of the birds (Bovera et al., 2016; Elahi et al., 2020).

6.4.2 Carcass quality assessment

There were no significant effects of the diet on most carcass traits, which aligns with the absent of differences in the final weight between treatment C and the experimental treatment found in a previous study (Nieto et al. 2022). Notably, chickens in treatment T1 achieved comparable productive performances to the other treatments, even when fed a diet with lower fat and protein content. This suggests a potential optimal threshold for the maximum utilization of protein from yellow mealworm meal, potentially reducing the economic cost of the feed.

Our results coincided with those described by other authors (Bovera et al., 2016; Sedgh-Gooya et al., 2021), who found that, in spite of the proportion, the inclusion of yellow mealworm (*T. molitor*) in chickens' diets did not have a significant effect on most carcass traits, as also observed for quail carcass and breast performance (Secci, et al., 2022). Similarly, the incorporation of different percentages of insects in chick diets did not significantly affect their primary performance and quality characteristics (Awoniyi et al. 2003) although the control treatment did achieve higher weights (Pietras et al. (2021).

6.4.3 Meat quality analysis

The pH results from our study were consistent with findings reported by other researchers who observed no significant differences in pH values following the inclusion of various percentages of mealworm (*T. molitor*) in broiler diets (Elahi et al., 2020) or common bluebottle fly (*Calliphora* sp.) in the diet of slow-growing chickens (Sarmiento-García et al., 2021).

Redder meats were obtained from chickens fed exclusively soybean meal as a protein source (treatment C) or yellow mealworm meal (treatment T3), compared to those chickens that consumed both raw materials in the same diet (treatments T1 and T2). These findings were consistent with those of Zadeh et al. (2019), who found that meat became redder as the percentage of insect inclusion in the diet of quails increased, while also reducing yellowness without affecting brightness. Murawska et al. (2021) reported that the partial and total replacement of soybean meal with black soldier fly larvae meal in broilers resulted in redder meat.

The values for expressible juice and cooking losses were observed to be higher in birds fed exclusively with insect meal (treatment T3) or soybean meal (treatment C) in comparison to those fed a combination of both ingredients (treatments T1 and T2). This suggests a lower water holding capacity in the latter group, resulting in reduced juiciness. This result concurred with trials that found no differences in juiciness when black soldier fly meal was included in chick feeds (Cullere et al. 2019; Altmann et al. 2020).

The treatments that exhibited significantly lower cooking losses, i.e., T1 and T2, also displayed reduced values for Warner-Braztler shear force (WBSF), while treatment C exhibited the highest values for this parameter. These findings are in line with those reported by Secci et al. (2022), who observed a decrease in meat toughness when using yellow mealworm in various proportions in the diet of quail.

Regarding nutritional composition, our study found no significant differences in fat content, which is consistent with the majority of prior research (Secci et al. 2018; Cullere et al. 2019; Altmann et al. 2020; Mlaga et al. 2022). These studies also did not identify any differences in protein content, in agreement with our results for treatments C and T3. However, treatments T1 and T2 exhibited significantly lower protein contents. The slight differences in protein content in the feeds of treatments T1 and T2 may indicate an influence of the nutritional composition of the feed rather than the inclusion of yellow mealworm itself, as the nutritional composition of the diet can significantly impact the quality of the birds (Mir et al., 2017).

Approximately 90% of the fatty acids detected in the breast of the studied chickens were represented by C16:0, C18:0, C18:1, and C18:2n-6, regardless of the feed consumed. The results for myristic acid (C14:0) demonstrated a progressive increase in its concentration as the percentage of yellow mealworm inclusion in the diet increased (Table 8). This observation aligns with the findings in the F3 experimental feed and is consistent with results from previous studies due to the higher concentration of this fatty acid in insects (Loponte et al., 2019). Similarly, the use of *T. molitor* in partridge feed (Secci et al., 2018), and of *H. illucens* in chick (Schiavone et al. 2017, 2019; Cullere et al. 2019; Altmann et al. 2020) and duck (Gariglio et al., 2021) diets, resulted in increased concentrations of C14:0. Treatments T1 and T2 exhibited the highest values for pentadecanoic acid (C15:0), myristoleic (C14:1n-5), and palmitic (C16:0) acids, which corresponded to the observations in the F3 feed for both treatments.

Additionally, a significant increase in palmitoleic acid (C16:1) was noted in feeds incorporating yellow mealworm, which correlated with the progressive increase of this fatty acid as the proportion of yellow mealworm in the diet increased. This phenomenon had also been observed in other studies in which black soldier fly was included in broiler diets (Schiavone et al., 2019; Cullere et al., 2019; Altmann et al., 2020) as well as when *Calliphora* sp. was used in slow-growing chickens, which was associated with the higher content of this fatty acid in the insect. The concentration of stearic acid (C18:0) did not exhibit significant differences between treatments C and T3.

Significant differences were observed in total MUFA values, with higher levels in the experimental treatments including yellow mealworm larval meal. For Kierończyk et al. (2018), the use of *T. molitor* also increased total MUFA, as occurred in other trials where *H. illucen* was used in broilers (Altmann et al., 2020; Mlaga et al., 2022).

In general, linoleic acid (C18:2n-6) was the predominant constituent in the meat. Lower values were obtained for the treatments in which the insect was partially included in their feeds (T1 and T2). This fatty acid was also the most abundant in the last feed consumed by the chickens, showing slight differences between treatments. Nevertheless, the sum of polyunsaturated fatty acids (ΣPUFA) in the F3 feed exhibited a similar trend to what was observed in the meat, with treatments C and T3 having lower values of PUFA. In other trials conducted with black soldier fly, it was found that the highest levels of linoleic acid and ΣPUFA were in the feed that did not contain insect (Schiavone et al. 2017, 2019; Cullere et al. 2019; Altmann et al. 2020), as occurred in the present study for PUFA. The results obtained for Cis-11,14-Eicosadienoic acid (C20:2n-6) agreed with those described by Cullere et al. (2019), where values were higher for the breasts of chickens fed soybean and a partial substitution with *H. illucens vs* its total substitution.

The results concerning the sum of saturated fatty acids (ΣSFA) aligned with other studies where no differences were observed after the inclusion of yellow mealworm (Loponte et al., 2019), or black soldier fly (Schiavone et al. 2019) in broiler diets. The significant increase in MUFA in the meat of the chickens fed with *T. molitor* was inversely correlated with the lower values of oleic acid observed in the feed received by those birds. As for the sum of PUFA, the meat of the T1 and T2 insect-fed animals presented a decrease in this group of fatty acids, which was related to the lower amount of it observed in the feed.

The results related to the amount of n-3 acids (Σ n-3 PUFA) were consistent with those obtained by Gariglio et al. (2021) and Sarmiento-García et al. (2021), who found no differences when including *H. illucens* in duck diets and *Calliphora* sp. in slow-growing chick diets, respectively. The n-6 acids (Σn-6 PUFA) values were significantly lower in the treatments where *T. molitor* was added to the feed, which can be attributed to the progressive decrease in this group of fatty acids as the proportion of yellow mealworm in the feed increase. This finding is in agreement with the results of Schiavone et al. (2017, 2019) when using black soldier fly larvae meal in broiler diets.

Overall, the results of this study were consistent with other research where the incorporation of yellow mealworm did not result in an increase in SFA levels but did lead to an increase in MUFA and a slight decrease in n-6 PUFA, confirming that its inclusion positively influences the fatty acid profile of meat, a crucial consideration for modern consumers (Kierończyk et al., 2018).

6.4.4 NIRS analysis

The results from the discriminant analysis of the NIR spectra align with the findings of Berzaghi et al (2005), who accurately classified 100% of poultry breast samples on the basis of their feeding regime. Similarly, Sarmiento-García et al. (2021) successfully discriminated breast samples from slow-growing chickens fed varying levels of *Calliphora* sp.

In the NIR spectra recorded in this work, more pronounced differences between the four treatments were observed in specific regions of the spectrum, including the ranges between 1160-1300 nm, 1400-1850 nm and 1900-2000 nm. The first range is associated with the absorption of C-H, C-H2, C-H3 and HC=CH bonds, corresponding to fat (Barbin et al., 2014b), and can be correlated with the significant differences in MUFA and PUFA content among the treatments. The second range is associated with O-H, C-H and N-H bonds related to water, fats and proteins (Cozzolino & Murray, 2004). In this case, the overlapping of the bands made it challenging to establish correlations with the analytical results obtained. The third range is linked to various bonds such as O-H, C=O, N-H, mainly associated with proteins and wáter (Barbin et al., 2014). It should be noted that treatments T1 and T2 showed significantly different moisture and protein contents compared to treatments C and T3.

In conclusion, the findings of this study indicate that the partial and/or total substitution of soybean meal with *Tenebrio molitor* (yellow mealworm) meal resulted in a slight reduction in growth performance, as reflected in live weight (LW) and body weight gain (BWG), but did not have adverse effects on the main carcass characteristics. In fact, feeds with lower fat and protein content, incorporating insect meal, achieved comparable performance.

The meat of chickens in which soybean meal was partially replaced by *T. molitor* (treatments T1 and T2) exhibited characteristics such as reduced redness, increased juiciness, and improved tenderness. These improvements can be attributed to higher moisture content and lower protein percentage. Furthermore, the fatty acid profile was enhanced by the inclusion of insects, resulting in higher monounsaturated fatty acid content and lower n-6 acids polyunsaturated fatty acid (n-6 PUFA) content, while saturated fatty acid (SFA) content remained similar. When soybean meal was entirely replaced by *T. molitor*, no significant differences were observed in physicochemical quality parameters, except for higher ash and monounsaturated fatty acid (MUFA) contents.

The near-infrared spectroscopy (NIR) system effectively classified all samples according to the type of feed ingested, highlighting its utility as a valuable tool for meat differentiation based on dietary factors.

These results shed light on the potential of yellow mealworm meal as a suitable alternative protein source for feeding slow-growing chickens, as it does not adversely affect the physical characteristics of the meat.

CAPÍTULO 7:

Conclusiones

Capítulo 7: Conclusiones

La presente tesis se ha centrado en evaluar el efecto de la harina de gusano amarillo (*Tenebrio molitor*) y grillo doméstico (*Acheta domesticus*) como sustitutivo de la harina de soja, tanto en los rendimientos productivos como en las características de la canal y la calidad de carne en pollos de engorde de crecimiento lento. En este capítulo se recogen las conclusiones particulares de cada uno de los trabajos presentados, así como la conclusión general de dicha Tesis Doctoral.

Conclusiones particulares

- La sustitución completa de la harina de soja por harina de larvas de *Tenebrio molitor* o por harina de *Acheta domesticus* en pollos de engorde de crecimiento lento resultó en una reducción del consumo de alimento y de agua y la consiguiente reducción del peso durante el primer mes, con índices de conversión alimenticia más altos. Durante el segundo y tercer mes no hubo diferencias en los rendimientos de estos.
- La sustitución de la harina de soja por harina de *Acheta domesticus* en la dieta de varias razas de pollos de crecimiento lento condujo a una reducción en el peso de diferentes características de la canal en todos los genotipos. Sin embargo, a pesar de las variaciones en el pH, las mayores pérdidas por cocción, mayor contenido de humedad y menor de proteína total, las diferencias fueron pequeñas por lo que la calidad general de la carne se mantuvo en niveles satisfactorios. Por su parte, la elección de una estirpe de pollo de crecimiento lento tuvo un impacto significativo en el contenido total de grasa de la carne. La espectroscopia NIRS clasificó eficazmente todas las muestras según la dieta y la estirpe, lo que confirma su utilidad para diferenciar la carne según el genotipo y el sistema de alimentación.
- La sustitución parcial y/o total de la harina de soja por harina de *Tenebrio molitor* dio lugar a una ligera reducción del rendimiento del crecimiento, pero no tuvo efectos adversos en las principales características de la canal. La carne de los pollos en los que se sustituyó parcialmente la harina de soja por harina del insecto presentó menor enrojecimiento, mayor jugosidad y mejor terneza, observándose también que no se produjeron cambios significativos en los parámetros de composición para la sustitución total de soja por harina de insecto.

La inclusión de esta harina, además, mejoró el perfil de ácidos grasos ya que se aumentó el contenido de ácidos grasos monoinsaturados, junto con un menor contenido de ácidos grasos poliinsaturados n6. La tecnología NIRS clasificó eficazmente todas las muestras según el tipo de pienso ingerido.

Conclusiones generales

La sustitución de la harina de *Tenebrio molitor* o *Acheta domesticus* por harina de soja en pollos de engorde de crecimiento lento en dietas isoproteicas e isoenergéticas, no es apropiado que dicha inclusión sea en niveles elevados durante las primeras 4 semanas de vida de los pollitos, siendo más recomendable una sustitución parcial. Después del primer mes puede realizarse la sustitución completa, ya que los pollos mejoran notablemente su desarrollo y rendimiento alcanzando al final del ciclo valores similares a los de los pollos alimentados con harina de soja, a pesar de comenzar con un menor desarrollo durante el primer mes. Por tanto, el uso de la harina de *Tenebrio molitor* y *Acheta domesticus* son una gran fuente de proteína alternativa frente a la harina de soja para incluir en la dieta de pollos de crecimiento lento, dada la ausencia de diferencias relevantes en los desempeños productivos finales.

Además, su uso no afecta de forma negativa las características de la carne, siendo comparable a la obtenida con dietas convencionales. La espectroscopia de infrarrojo cercano (NIR) es capaz de clasificar eficazmente todas las muestras según el tipo de pienso ingerido (control vs harina de insecto) y la estirpe de crecimiento lento empleada, lo que pone de relieve su utilidad como herramienta valiosa para la diferenciación de la carne en función de factores dietéticos y genéticos.

Conclusions

This thesis has focused on evaluating the effect of yellow worm meal (*Tenebrio molitor)* and domestic cricket (*Acheta domesticus*) as a substitute for soybean meal, both on production performance and on carcass characteristics and meat quality in slow-growing broilers. This chapter presents the specific conclusions of each of the studies presented, as well as the general conclusion of this thesis.

Specific conclusions

- The complete replacement of soybean meal with *Tenebrio molitor* larval meal or *Acheta domesticus* meal in slow-growing broilers resulted in reduced feed and water consumption and consequently in weight loss during the first month, with higher feed conversion ratios. During the second and third months, there was no difference in broiler performance.
- Replacing soybean meal with *Acheta domesticus* meal in the diets of several slow-growing broiler breeds reduced the weight of several carcass traits in all genotypes. However, despite variations in pH, higher cooking losses, higher moisture content, and lower total protein, the differences were small and overall meat quality remained satisfactory. The choice of a slow-growing strain of chicken had a significant effect on the total fat content of the meat. NIRS spectroscopy effectively classified all samples according to diet and strain, confirming its usefulness in differentiating meat according to genotype and feeding system.
- Partial and/or total replacement of soybean meal with *Tenebrio molitor* meal resulted in a slight reduction in growth performance but had no adverse effect on the main carcass characteristics. The meat of chickens in which soybean meal was partially replaced with insect meal showed less redness, more juiciness, and better tenderness. It was also observed that there were no significant changes in the compositional parameters when soybean meal was completely replaced with insect meal.

  The inclusion of insect meal also improved the fatty acid profile by increasing the monounsaturated fatty acid content and decreasing the n-6 polyunsaturated fatty acid content. The near-infrared spectroscopy (NIR) system effectively classified all samples according to the type of feed consumed.

Overall conclusions

The replacement of *Tenebrio molitor* or *Acheta domesticus* meal for soybean meal in slow-growing broilers on isoproteic and isoenergetic diets is not suitable at high levels during the first 4 weeks of the chicks' lives. A partial replacement is more advisable during this period. After the first month, complete substitution can be carried out, as the chicks show significant improvement in development and performance, reaching values similar to those of chicks fed with soybean meal by the end of the cycle, despite starting with lower development during the first month. Therefore, the use of *Tenebrio molitor* and *Acheta domesticus* meal is an excellent alternative protein source to soybean meal in the diet of slow-growing broilers, as there are no relevant differences in final production performance.

Furthermore, their use does not negatively affect meat characteristics, which are comparable to those obtained with conventional diets. The near-infrared spectroscopy (NIR) system was able to efficiently classify all samples according to the type of diet consumed (control vs. insect meal) and the slow-growing strain used, highlighting its usefulness as a valuable tool for differentiating meat based on dietary and genetic factors.

.

CAPÍTULO 8:

Líneas futuras de investigación

Capítulo 8: Líneas futuras de investigación

Tras la realización del presente trabajo se pueden destacar como puntos fuertes que las investigaciones realizadas, tanto en la evaluación de los rendimientos como de la calidad de la carne, se ha abordado de manera sistemática y rigurosa, empleando un número suficiente de animales y réplicas en todos los parámetros analizados. Además, se ha evaluado la sustitución de la harina de soja por las materias primas propuestas, augurando un futuro prometedor para ser utilizadas en el sector avícola.

Los ensayos se han llevado a cabo en un sistema de producción en expansión, como son los distintos sistemas alternativos que emplean pollos de crecimiento lento. Se ha analizado la calidad de carne de los animales estudiados usando métodos de referencia aceptados internacionalmente lo que permite la comparación de resultados. También se ha procedido a optimizar las condiciones de registro y análisis de los espectros NIR de manera que se ha conseguido clasificar las diferentes muestras analizadas, mostrando el potencial de esta técnica para este propósito

Para finalizar, se exponen las posibles líneas futuras de investigación que surgen tras la realización de los trabajos presentados para cumplir los objetivos planteados al inicio. Estas serían:

- Profundizar en la digestibilidad de las harinas de insectos: a pesar de posicionarse como una clara alternativa a las harinas de soja por su alto contenido proteico, los resultados de esta tesis aconsejan no incluir altos niveles de insectos en las primeras semanas de vida de los animales. Además, los niveles proteicos en la dieta no se traducen de forma similar en la proteína de la carne. Los trabajos de digestibilidad *in vitro* han mostrado una menor digestibilidad de las harinas de insectos frente a la harina de soja. Por tanto, se debe seguir trabajando en esta línea para profundizar en el conocimiento relacionado con dicha digestibilidad (también *in vivo*) y si es posible mejorar los niveles de asimilación proteicos para que se traduzcan en mejores rendimientos y calidad de carne.

- Uso de otras especies de insectos en avicultura alternativa: las investigaciones realizadas con otras harinas de insectos se centran en pollos criados en condiciones intensivas (broiler). Por tanto, se abre la puerta a profundizar el estudio de su uso en sistemas alternativos empleando pollos de crecimiento lento, con ciclos de producción más largos y con acceso al exterior.
- A pesar de tener limitado el uso de especies de insectos permitidos en la alimentación de monogástricos, puede evaluarse el efecto de otras especies ampliamente utilizadas en otros campos, de cara a una posible inclusión en la lista de especies permitidas actualmente. Ejemplo de ello puede ser el uso de lombrices u otros insectos empleados ampliamente en la agricultura (en el control de plagas), alimentación animal, pesca, cosmética o biocombustibles.
- Uso de insectos en razas autóctonas: estas razas con mayor rusticidad y grado de adaptación a la crianza al aire libre pueden ser grandes candidatas para utilizar harina de insectos en su dieta. Sus menores rendimientos productivos en comparación con las estirpes empleadas actualmente generan una baja utilización comercial, pudiendo revertir su situación en caso de que los insectos produzcan características diferentes en su carne.

CAPÍTULO 9:

Otras contribuciones científicas

Capítulo 9: Otras contribuciones científicas

Además de los artículos recogidos en la presente Tesis Doctoral, las investigaciones realizadas también han generado otras contribuciones científicas en forma de comunicaciones a congresos, jornadas o artículos divulgativos, que se presentan a continuación,

- **Uso de harina de *Tenebrio molitor* en pollos ecológicos: índices de conversión y consumos de pienso y agua.**
  Congreso: AIDA (2021), XIX Jornadas sobre Producción Animal. Zaragoza, España. 01/06/2021-02/06/2021. Asociación Interprofesional para el Desarrollo Agrario, pp. 55. ISBN. 978-84-09-30674-9.
  Jaime Nieto; Ainhoa Sarmiento-García; Javier Plaza; Javier Lara; Alfonso Abecia; Isabel Revilla; Carlos Palacios.

- **Uso de harina de *Tenebrio molitor* en pollos ecológicos: peso y velocidad de crecimiento.**
  Congreso: AIDA (2021), XIX Jornadas sobre Producción Animal. Zaragoza, España.
  01/06/2021-02/06/2021. Asociación Interprofesional para el Desarrollo Agrario, pp. 100. ISBN 978-84-09-30674-9.
  Jaime Nieto; Ainhoa Sarmiento-García; Javier Plaza; Javier Lara; Alfonso Abecia; Isabel Revilla; Carlos Palacios.

- **Efecto de la sustitución de la harina de soja por harina de larva de *Tenebrio molitor* en la velocidad de crecimiento de parámetros anatómicos de pollos de crecimiento lento en producción ecológica**.
  Congreso: XXIX Jornadas Técnicas SEAE: Una salud, un planeta, un mundo rural diverso.
  28/06/2021-01/07/2021. Sociedad Española de Agricultura Ecológica, pp.200-205. ISBN 978-84-949844-3-3.
  Jaime Nieto; Javier Plaza; Alfonso Abecia; Isabel Revilla; Carlos Palacios.

- **Efecto de la sustitución de la harina de soja por harina de larva de *Tenebrio molitor* en la calidad de la carne de pollos de crecimiento lento en producción ecológica.**

  Jornada: III Jornadas de Jóvenes Investigadores en Ciencia y Tecnología Químicas. Salamanca, Castilla y León, España. 15/07/2021-16/07/2021.

- **Consumption preference of feed including *Tenebrio molitor* flour in slow-growing**

  Chickens.

  Congreso: 72nd Annual Meeting of the European Federation of Animal Science. Davos, Suiza. 30/08/2021-03/09/2021.

  "Book of Abstracts of the 72nd Annual Meeting of the European Federation of Animal Science". Poster 19, pp. 151 - 151. ISBN 1382-6077; 978-90-8686-918-3.

  DOI: 10.3920/978-90-8686-918-3.

  Jaime Nieto; Javier Plaza; Alfonso Abecia; Isabel Revilla; Carlos Palacios.

- **Efecto de la climatología en el consumo de pienso, agua e índice de conversión de pollos de crecimiento lento en producción ecológica durante la crianza al aire libre.**

  Congreso: XIV Congreso SEAE 2022: "Soberanía alimentaria, emergencia climática" Palma de Mallorca, España. 25/04/2022-27/04/2022. Sociedad Española de Agricultura Ecológica, pp. 141 – 141.

  Jaime Nieto; Carlos Palacios; Javier Plaza; Alfonso Abecia; Isabel Revilla.

- **Crecimiento y desarrollo morfológico de tres estirpes diferentes de pollos de engorde en crecimiento lento ecológicos durante 95 días.**

  Congreso: XIV Congreso SEAE 2022: "Soberanía alimentaria, emergencia climática" Palma de Mallorca, España. 25/04/2022-27/04/2022. Sociedad Española de Agricultura Ecológica, pp. 142 – 142.

  Carlos Palacios; Jaime Nieto; Javier Plaza; Alfonso Abecia; Isabel Revilla.

- **Performance of slow-growing broilers fed *Acheta domesticus* meal during the first four weeks of life.**

  Congreso: 73rd Annual Meeting of the European Federation of Animal Science. Oporto, Portugal. 05/09/2022-07/09/2022.

  "Book of Abstracts of the 73rd Annual Meeting of the European Federation of Animal Science". Session 07, pp. 176 - 176. Wageningen Academic Publishers, ISBN 978-90-8686-385-3.

  Jaime Nieto; Javier Plaza; Alfonso Abecia; Isabel Revilla; Carlos Palacios Riocerezo.

- **Uso de harina de *Tenebrio molitor* en el desarrollo de pollos ecológicos durante los 50 primeros días de vida.**

  Artículo de divulgación: Mundo Ganadero. Septiembre-Octubre/308, pp. 42 - 44. (España): 14/11/2022. ISSN 0214-9192.

  Jaime Nieto; Javier Plaza; Javier Lara; Alfonso Abecia; Isabel Revilla; Carlos Palacios.

- **In vitro digestibility in organic chick diets incorporating insects.**

  Congreso: European Organic Congress. Córdoba, España. 26/09/2023-28/09/2023.

  Jaime Nieto; Leticia Merchán; Isabel Revilla; Javier Plaza; Alfonso Abecia; Manuel González-Ronquillo; Carlos Palacios.

Referencias bibliográficas

Referencias bibliográficas

Abbas, Y., & Ahmad, A. (2018). Impact of processing on nutritional and antinutritional factors of legumes: A review. *Annals. Food Science and Technology*, 1–17.

Abeni, F., & Bergoglio, G. (2001). Characterization of different strains of broiler chicken by carcass measurements, chemical and physical parameters and NIRS on breast muscle. *Meat Science*, *57*(2), 133–137. https://doi.org/10.1016/S0309-1740(00)00084-X

Abro, Z., Kassie, M., Tanga, C., Beesigamukama, D., & Diiro, G. (2020). Socio-economic and environmental implications of replacing conventional poultry feed with insect-based feed in Kenya. *Journal of Cleaner Production*, *265*, 121871. https://doi.org/10.1016/j.jclepro.2020.121871

Alcaraz, R., Mita, G. I., Hernández-Contreras, A., & Hernández, M. D. (2021). Physical properties of extruded fish feed containing silkworm (*Bombyx mori*) pupae meal. *Journal of Insects as Food and Feed*, *7*(4), 449–456. https://doi.org/10.3920/JIFF2020.0106

Allais, S., Hennequet-Antier, C., Berri, C., Salles, L., Demeure, O., & Le Bihan-Duval, E. (2019). Mapping of QTL for chicken body weight, carcass composition, and meat quality traits in a slow-growing line. *Poultry Science*, *98*(5), 1960–1967. https://doi.org/10.3382/ps/pey549

Allegretti, G., Talamini, E., Schmidt, V., Bogorni, P. C., & Ortega, E. (2018). Insect as feed: an emergy assessment of insect meal as a sustainable protein source for the Brazilian poultry industry. *Journal of Cleaner Production*, *171*, 403–412. https://doi.org/10.1016/j.jclepro.2017.09.244

Altmann, B. A., Wigger, R., Ciulu, M., & Mörlein, D. (2020). The effect of insect or microalga alternative protein feeds on broiler meat quality. *Journal of the Science of Food and Agriculture*, *100*(11), 4292–4302. https://doi.org/10.1002/JSFA.10473

AOAC. (2006). *Official Methods of Analysis Association; Association of Official Analytical Chemist, Ed.; 18th ed.; Washington DC.*

Ashour, E. A., El-Hack, M. E. A., Shafi, M. E., Alghamdi, W. Y., Taha, A. E., Swelum, A. A., Tufarelli, V., Mulla, Z. S., El-Ghareeb, W. R., & El-Saadony, M. T. (2020). Impacts of Green Coffee Powder Supplementation on Growth Performance, Carcass Characteristics, Blood Indices, Meat Quality and Gut Microbial Load in Broilers. *Agriculture*, *10*(10), 457. https://doi.org/10.3390/agriculture10100457

Atteh, J. O., & Ologbenla, F. D. (1993). Replacement of fish meal with maggots in broiler diets: effects on performance and nutrient retention. *Nigerian Journal of Animal Production*, *20*(1), 44–49. https://doi.org/10.51791/njap.v20i.2100

Awoniyi, T. A. M., Aletor, V. A., & Aina, J. M. (2003). Performance of Broiler - Chickens Fed on Maggot Meal in Place of Fishmeal. *International Journal of Poultry Science*, *2*(4), 271–274. https://doi.org/10.3923/ijps.2003.271.274

Baéza, E., Guillier, L., & Petracci, M. (2022). Review: Production factors affecting poultry carcass and meat quality attributes. *Animal*, *16*, 100331. https://doi.org/10.1016/j.animal.2021.100331

Baker, B. P., Green, T. A., & Loker, A. J. (2020). Biological control and integrated pest management in organic and conventional systems. *Biological Control*, *140*, 104095. https://doi.org/10.1016/j.biocontrol.2019.104095

Bamgbose, A. (1999). Utilization of maggot-meal in cockerel diets. *Indian Journal of Animal Sciences*, *69*(12), 1056–1058.

Barbin, D. F., de Souza Madureira Felicio, A. L., Sun, D.-W., Nixdorf, S. L., & Hirooka, E. Y. (2014). Application of infrared spectral techniques on quality and compositional attributes of coffee: An overview. *Food Research International*, *61*, 23–32. https://doi.org/10.1016/j.foodres.2014.01.005

Barman, K., Banik, S., Thomas, R., Kumar, S., Das, A. K., Dutta, K., & Rajkhowa, S. (2021). Effect of partial replacement of protein supplement with silkworm (*Bombyx mori* L.) pupa meal on production performances in crossbred (HS × GH) grower pigs. *The Indian Journal of Animal Sciences*, *90*(11), 1519–1523. https://doi.org/10.56093/ijans.v90i11.111563

Barroso, F. G., de Haro, C., Sánchez-Muros, M.-J., Venegas, E., Martínez-Sánchez, A., & Pérez-Bañón, C. (2014). The potential of various insect species for use as food

for fish.*Aquaculture*, *422–423*,193–201. https://doi.org/10.1016/j.aquaculture.2013.12.024

Basto, A., Calduch-Giner, J., Oliveira, B., Petit, L., Sá, T., Maia, M. R. G., Fonseca, S. C., Matos, E., Pérez-Sánchez, J., & Valente, L. M. P. (2021). The Use of Defatted *Tenebrio molitor* Larvae Meal as a Main Protein Source Is Supported in European Sea Bass (*Dicentrarchus labrax*) by Data on Growth Performance, Lipid Metabolism, and Flesh Quality. *Frontiers in Physiology*, *12*. https://doi.org/10.3389/fphys.2021.659567

Bbosa, T., Tamale Ndagire, C., Muzira Mukisa, I., Fiaboe, K. K. M., & Nakimbugwe, D. (2019). Nutritional Characteristics of Selected Insects in Uganda for Use as Alternative Protein Sources in Food and Feed. *Journal of Insect Science*, *19*(6). https://doi.org/10.1093/jisesa/iez124

Belhadj Slimen, I., Yerou, H., Ben Larbi, M., M'Hamdi, N., & Najar, T. (2023). Insects as an alternative protein source for poultry nutrition: a review. *Frontiers in Veterinary Science*, *10*. https://doi.org/10.3389/fvets.2023.1200031

Bellezza Oddon, S., Biasato, I., Imarisio, A., Pipan, M., Dekleva, D., Colombino, E., Capucchio, M. T., Meneguz, M., Bergagna, S., Barbero, R., Gariglio, M., Dabbou, S., Fiorilla, E., Gasco, L., & Schiavone, A. (2021). Black soldier fly and yellow mealworm live larvae for broiler chickens: Effects on bird performance and health status. *Journal of Animal Physiology and Animal Nutrition*, *105*(S1), 10–18. https://doi.org/10.1111/JPN.13567

Benzertiha, A., Kierończyk, B., Kołodziejski, P., Pruszyńska–Oszmałek, E., Rawski, M., Józefiak, D., & Józefiak, A. (2020). *Tenebrio molitor* and *Zophobas morio* full-fat meals as functional feed additives affect broiler chickens' growth performance and immune system traits. *Poultry Science*, *99*(1), 196–206. https://doi.org/10.3382/ps/pez450

Berzaghi, P., Dalle Zotte, A., Jansson, L. M., & Andrighetto, I. (2005). Near-infrared reflectance spectroscopy as a method to predict chemical composition of breast meat and discriminate between different n-3 feeding sources. *Poultry Science*, *84*(1), 128–136. https://doi.org/10.1093/PS/84.1.128

Biasato, I., De Marco, M., Rotolo, L., Renna, M., Lussiana, C., Dabbou, S., Capucchio, M. T., Biasibetti, E., Costa, P., Gai, F., Pozzo, L., Dezzutto, D., Bergagna, S.,

Martínez, S., Tarantola, M., Gasco, L., & Schiavone, A. (2016). Effects of dietary *Tenebrio molitor* meal inclusion in free-range chickens. *Journal of Animal Physiology and Animal Nutrition*, *100*(6), 1104–1112. https://doi.org/10.1111/jpn.12487

Biasato, I., Gasco, L., De Marco, M., Renna, M., Rotolo, L., Dabbou, S., Capucchio, M. T., Biasibetti, E., Tarantola, M., Bianchi, C., Cavallarin, L., Gai, F., Pozzo, L., Dezzutto, D., Bergagna, S., & Schiavone, A. (2017). Effects of yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for female broiler chickens: implications for animal health and gut histology. *Animal Feed Science and Technology*, *234*, 253–263. https://doi.org/10.1016/j.anifeedsci.2017.09.014

Biasato, I., Gasco, L., De Marco, M., Renna, M., Rotolo, L., Dabbou, S., Capucchio, M. T., Biasibetti, E., Tarantola, M., Sterpone, L., Cavallarin, L., Gai, F., Pozzo, L., Bergagna, S., Dezzutto, D., Zoccarato, I., & Schiavone, A. (2018). Yellow mealworm larvae (*Tenebrio molitor*) inclusion in diets for male broiler chickens: effects on growth performance, gut morphology, and histological findings. *Poultry Science*, *97*(2), 540–548. https://doi.org/10.3382/ps/pex308

Biesek, J., Kuźniacka, J., Banaszak, M., Kaczmarek, S., Adamski, M., Rutkowski, A., Zmudzińska, A., Perz, K., & Hejdysz, M. (2020). Growth Performance and Carcass Quality in Broiler Chickens Fed on Legume Seeds and Rapeseed Meal. *Animals*, *10*(5), 846. https://doi.org/10.3390/ani10050846

Bonny, S. (2008). Genetically modified glyphosate-tolerant soybean in the USA: adoption factors, impacts and prospects. A review. *Agronomy for Sustainable Development*, *28*(1), 21–32. https://doi.org/10.1051/agro:2007044

Boontiam, W., Hong, J., Kitipongpysan, S., & Wattanachai, S. (2022). Full-fat field cricket (*Gryllus bimaculatus*) as a substitute for fish meal and soybean meal for weaning piglets: effects on growth performance, intestinal health, and redox status. *Journal of Animal Science*, *100*(4). https://doi.org/10.1093/jas/skac080

Bovera, F., Loponte, R., Marono, S., Piccolo, G., Parisi, G., Iaconisi, V., Gasco, L., & Nizza, A. (2016). Use of *Tenebrio molitor* larvae meal as protein source in broiler diet: Effect on growth performance, nutrient digestibility, and carcass and meat traits. *Journal of Animal Science*, *94*(2), 639–647. https://doi.org/10.2527/jas.2015-9201

Bovera, F., Piccolo, G., Gasco, L., Marono, S., Loponte, R., Vassalotti, G., Mastellone, V., Lombardi, P., Attia, Y. A., & Nizza, A. (2015). Yellow mealworm larvae (*Tenebrio molitor* , L.) as a possible alternative to soybean meal in broiler diets. *British Poultry Science*, *56*(5),1–7. https://doi.org/10.1080/00071668.2015.1080815

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Research International*, *43*(2), 414–431. https://doi.org/10.1016/j.foodres.2009.09.003

Chadd, S. (2008). Future trends and developments in poultry nutrition. *Poultry in the 21st Century: Avian Influenza and Beyond. UN Food and Agriculture Organisation (FAO) International Poultry Conference. Bangkok, Thailand*.

Chemello, G., Renna, M., Caimi, C., Guerreiro, I., Oliva-Teles, A., Enes, P., Biasato, I., Schiavone, A., Gai, F., & Gasco, L. (2020). Partially Defatted *Tenebrio molitor* Larva Meal in Diets for Grow-Out Rainbow Trout, Oncorhynchus mykiss (Walbaum): Effects on Growth Performance, Diet Digestibility and Metabolic Responses. *Animals*, *10*(2), 229. https://doi.org/10.3390/ani10020229

CIE, O. J. (1976). Official recommendations on uniform color space, color difference equations and metric color terms. *CIE Publication*, *15*, 1976.

CIE, O. J. (1977). CIE recommendations on uniform color spaces, color-difference equations, and metric color terms. *Color Research & Application*, *2*(1), 5–6. https://doi.org/10.1002/J.1520-6378.1977.TB00102.X

Cozzolino, D., & Murray, I. (2004). Identification of animal meat muscles by visible and near infrared reflectance spectroscopy. *LWT - Food Science and Technology*, *37*(4), 447–452. https://doi.org/10.1016/J.LWT.2003.10.013

Cullere, M., Schiavone, A., Dabbou, S., Gasco, L., & Dalle Zotte, A. (2019). Meat Quality and Sensory Traits of Finisher Broiler Chickens Fed with Black Soldier Fly (*Hermetia Illucens* L.) Larvae Fat as Alternative Fat Source. *Animals*, *9*(4), 140. https://doi.org/10.3390/ani9040140

Cullere, M., Tasoniero, G., Giaccone, V., Acuti, G., Marangon, A., & Dalle Zotte, A. (2018). Black soldier fly as dietary protein source for broiler quails: meat proximate composition, fatty acid and amino acid profile, oxidative status and sensory traits. *Animal*, *12*(3), 640–647. https://doi.org/10.1017/S1751731117001860

Dabbou, S., Gasco, L., Lussiana, C., Brugiapaglia, A., Biasato, I., Renna, M., Cavallarin, L., Gai, F., & Schiavone, A. (2020). Yellow mealworm (*Tenebrio molitor* L.) larvae inclusion in diets for free-range chickens: effects on meat quality and fatty acid profile. *Renewable Agriculture and Food Systems*, *35*(5), 571–578. https://doi.org/10.1017/S1742170519000206

Dal Bosco, A., Mattioli, S., Cartoni Mancinelli, A., Cotozzolo, E., & Castellini, C. (2021). Extensive Rearing Systems in Poultry Production: The Right Chicken for the Right Farming System. A Review of Twenty Years of Scientific Research in Perugia University, Italy. *Animals*, *11*(5), 1281. https://doi.org/10.3390/ani11051281

de Castro, R. J. S., Ohara, A., Aguilar, J. G. dos S., & Domingues, M. A. F. (2018). Nutritional, functional and biological properties of insect proteins: Processes for obtaining, consumption and future challenges. In *Trends in Food Science and Technology* (Vol. 76, pp. 82–89). Elsevier. https://doi.org/10.1016/j.tifs.2018.04.006

De Leon Ramirez, J., Garcia Trejo, J. F., Sosa Ferreyra, C., Martinez Ramos, S., & Bottini Cedeno, B. (2018). Fly larvae (*Musca domestica*) as a protein alternative in the feeding of Macrobrachium tenellum. *Latin American Journal of Aquatic Research*, *46*(3), 599–603. https://doi.org/10.3856/vol46-issue3-fulltext-14

De Marco, M., Martínez, S., Hernandez, F., Madrid, J., Gai, F., Rotolo, L., Belforti, M., Bergero, D., Katz, H., Dabbou, S., Kovitvadhi, A., Zoccarato, I., Gasco, L., & Schiavone, A. (2015). Nutritional value of two insect larval meals (*Tenebrio molitor* and *Hermetia illucens*) for broiler chickens: Apparent nutrient digestibility, apparent ileal amino acid digestibility and apparent metabolizable energy. *Animal Feed ScienceandTechnology*,*209*,211–218. https://doi.org/10.1016/j.anifeedsci.2015.08.006

de Souza, J., Andronicos, N. M., Kolakshyapati, M., Hilliar, M., Sibanda, T. Z., Andrew, N. R., Swick, R. A., Wilkinson, S., & Ruhnke, I. (2021). Black soldier fly larvae in broiler diets improve broiler performance and modulate the immune system. *Animal Nutrition*, *7*(3), 695–706. https://doi.org/10.1016/j.aninu.2020.08.014

DeFoliart, G. R., Finke, M. D., & Sunde, M. L. (1982). Potential Value of the Mormon Cricket (Orthoptera: Tettigoniidae) Harvested as a High-Protein Feed for Poultry. *Journal of Economic Entomology*, *75*(5), 848–852. https://doi.org/10.1093/JEE/75.5.848

Despins, J. L., & Axtell, R. C. (1995). Feeding Behavior and Growth of Broiler Chicks Fed Larvae of the Darkling Beetle, *Alphitobius diaperinus*. *Poultry Science*, *74*(2), 331–336. https://doi.org/10.3382/ps.0740331

Devatkal, S. K., Naveena, B. M., & Kotaiah, T. (2019). Quality, composition, and consumer evaluation of meat from slow-growing broilers relative to commercial broilers. *Poultry Science*, *98*(11), 6177–6186. https://doi.org/10.3382/ps/pez344

EC 43. (2007). *Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production*.

EFSA. (2015). Risk profile related to production and consumption of insects as food and feed. *EFSA Journal*, *13*(10), 4257. https://doi.org/10.2903/j.efsa.2015.4257

Elahi, U., Wang, J., Ma, Y., Wu, S., Wu, J., Qi, G., & Zhang, H. (2020). Evaluation of Yellow Mealworm Meal as a Protein Feedstuff in the Diet of Broiler Chicks. *Animals*, *10*(2), 224. https://doi.org/10.3390/ani10020224

Englmaierová, M., Skřivan, M., Taubner, T., & Skřivanová, V. (2020). Performance and Meat Quality of Dual-Purpose Cockerels of Dominant Genotype Reared on Pasture. *Animals*, *10*(3), 387. https://doi.org/10.3390/ani10030387

EU 848. (2018). *Regulation (EU) 2018/848 of the European Parliament and of the Council of 30 May 2018 on organic production and labelling of organic products and repealing Council Regulation (EC) No 834/2007*. https://eur-lex.europa.eu/legal-content/ES/TXT/PDF/?uri=CELEX:32018R0848&from=EN

EU 893. (2017). *Commission Regulation (EU) 2017/893 of 24 May 2017 amending Annexes I and IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council and Annexes X, XIV and XV to Commission Regulation (EU) No 142/2011 as regards the provisions on processed animal protein (Text with EEA relevance.)*.

EU 1372. (2021). *Commission Regulation (EU) 2021/1372 of 17 August 2021 amending Annex IV to Regulation (EC) No 999/2001 of the European Parliament and of the Council as regards the prohibition to feed non-ruminant farmed animals, other than fur animals, with protein derived from animals*.

EU 1925. (2021). *Commission Regulation (EU) 2021/1925 of 5 November 2021 amending certain Annexes to Regulation (EU) No 142/2011 as regards the*

requirements for placing on the market of certain insect products and the adaptation of a containment method.

Fanatico, A. C., Pillai, P. B., Emmert, J. L., & Owens, C. M. (2007). Meat Quality of Slow- and Fast-Growing Chicken Genotypes Fed Low-Nutrient or Standard Diets and Raised Indoors or with Outdoor Access. *Poultry Science*, *86*(10), 2245–2255. https://doi.org/10.1093/ps/86.10.2245

Fehlenberg, V., Baumann, M., Gasparri, N. I., Piquer-Rodriguez, M., Gavier-Pizarro, G., & Kuemmerle, T. (2017). The role of soybean production as an underlying driver of deforestation in the South American Chaco. *Global Environmental Change*, *45*, 24–34. https://doi.org/10.1016/j.gloenvcha.2017.05.001

Filho, I. C. P., Broch, J., Eyng, C., Silva, I. M., Souza, C., Avila, A. S., Castilha, L. D., Cirilo, E. H., Tesser, G. L. S., & Nunes, R. V. (2021). Effects of feeding dried brewers grains to slow-growing broiler chickens. *Livestock Science*, *250*, 104561. https://doi.org/10.1016/j.livsci.2021.104561

Finke, M. D., Sunde, M. L., & Defoliart, G. R. (1985). An Evaluation of the Protein Quality of Mormon Crickets (*Anabrus simplex* Haldeman) When Used as a High Protein Feedstuff for Poultry. *Poultry Science*, *64*(4), 708–712. https://doi.org/10.3382/ps.0640708

Flachowsky, G., Meyer, U., & Südekum, K.-H. (2017). Land Use for Edible Protein of Animal Origin—A Review. *Animals*, *7*(12), 25. https://doi.org/10.3390/ani7030025

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, *226*(1), 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Font-i-Furnols, M., & Guerrero, L. (2022). Spanish perspective on meat consumption and consumer attitudes. *Meat Science*, *191*, 108874. https://doi.org/10.1016/j.meatsci.2022.108874

Garcia, M. A., & Altieri, M. A. (2005). Transgenic Crops: Implications for Biodiversity and Sustainable Agriculture. *Bulletin of Science, Technology & Society*, *25*(4), 335–353. https://doi.org/10.1177/0270467605277293

Gariglio, M., Dabbou, S., Gai, F., Trocino, A., Xiccato, G., Holodova, M., Gresakova, L., Nery, J., Oddon, S. B., Biasato, I., Gasco, L., & Schiavone, A. (2021). Black soldier

fly larva in Muscovy duck diets: effects on duck growth, carcass property, and meat quality. *Poultry Science*, *100*(9), 101303. https://doi.org/10.1016/j.psj.2021.101303

Gasco, L., Acuti, G., Bani, P., Dalle Zotte, A., Danieli, P. P., De Angelis, A., Fortina, R., Marino, R., Parisi, G., Piccolo, G., Pinotti, L., Prandini, A., Schiavone, A., Terova, G., Tulli, F., & Roncarati, A. (2020). Insect and fish by-products as sustainable alternatives to conventional animal proteins in animal nutrition. *Italian Journal of Animal Science*, *19*(1), 360–372. https://doi.org/10.1080/1828051X.2020.1743209

Godínez-Juárez, B., Hernández-Mendo, O., Pro-Martínez, A., Hernández-Sánchez, D., Leyva-Ruelas, G., Martínez-Martínez, U., & Zarate-Contreras, D. (2022). Partially Dehulled Sunflower Seeds in Diets for Grazing Chickens: Effect in Meat Quality. *Brazilian Journal of Poultry Science*, *24*(4). https://doi.org/10.1590/1806-9061-2021-1573

Grau, R., & Hamm, R. (1953). Eine einfache Methode zur Bestimmung der Wasserbindung im Muskel. *Die Naturwissenschaften*, *40*(1), 29–30. https://doi.org/10.1007/BF00595734

Hailemariam, A., Esatu, W., Abegaz, S., Urge, M., Assefa, G., & Dessie, T. (2022). Nutritional composition and sensory characteristics of breast meat from different chickens.*Applied Food Research*, *2*(2), 100233. https://doi.org/10.1016/j.afres.2022.100233

Hall, H. N., Masey O'Neill, H. V., Scholey, D., Burton, E., Dickinson, M., & Fitches, E. C. (2018). Amino acid digestibility of larval meal (*Musca domestica*) for broiler chickens. *Poultry Science*, *97*(4), 1290–1297. https://doi.org/10.3382/ps/pex433

Hamani, B., Moula, N., Taffa, A. G., Leyo, I. H., Mahamadou, C., Detilleux, J., & Van, Q. C. D. (2022). Effect of housefly (*Musca domestica*) larvae on the growth performance and carcass characteristics of local chickens in Niger. *Veterinary World*, 1738–1748. https://doi.org/10.14202/vetworld.2022.1738-1748

Hernández-Jiménez, M., Hernández-Ramos, P., Martínez-Martín, I., Vivar-Quintana, A. M., González-Martín, I., & Revilla, I. (2020). Comparison of artificial neural networks and multiple regression tools applied to near infrared spectroscopy for predicting sensory properties of products from quality labels. *Microchemical Journal*, *159*(August), 105459. https://doi.org/10.1016/j.microc.2020.105459

Hernández-Jiménez, M., Revilla, I., Arce, L., Cardador, M. J., Ríos-Reina, R., González-Martín, I., & Vivar-Quintana, A. M. (2021). Authentication of the montanera period on carcasses of Iberian pigs by using analytical techniques and chemometric analyses. *Animals*, *11*(9), 2671. https://doi.org/10.3390/ani11092671

Hong, J., & Kim, Y. Y. (2022). Insect as feed ingredients for pigs. *Animal Bioscience*, *35*(2), 347–355. https://doi.org/10.5713/ab.21.0475

Hovhannisyan, S. V., & Grigoryan, K. A. (2016). The main problems and features of the global and local meat production. *Annals of Agrarian Science*, *14*(4), 315–318. https://doi.org/10.1016/j.aasci.2016.09.008

Hubbard. (2024). *Machos de crecimiento lento. Gama Premium*. Consultado el 18 de Mayo de 2024. https://www.hubbardbreeders.com/es/

Huis, A. van, Itterbeeck, J. van, Klunder, H., Mertens, E., Halloran, A., Muir, G., & Vantomme, P. (2014). Edible insects: future prospects for food and feed security. *FAO Forestry Paper*, 171–178.

Hussein, E. O. S., Suliman, G. M., Alowaimer, A. N., Ahmed, S. H., Abd El-Hack, M. E., Taha, A. E., & Swelum, A. A. (2020). Growth, carcass characteristics, and meat quality of broilers fed a low-energy diet supplemented with a multienzyme preparation. *Poultry Science*, *99*(4), 1988–1994. https://doi.org/10.1016/j.psj.2019.09.007

Ipema, A. F., Bokkers, E. A. M., Gerrits, W. J. J., Kemp, B., & Bolhuis, J. E. (2021). Providing live black soldier fly larvae (*Hermetia illucens*) improves welfare while maintaining performance of piglets post-weaning. *Scientific Reports*, *11*(1), 7371. https://doi.org/10.1038/s41598-021-86765-3

ISO. (1973). Determination of total fat content, ISO 1443:1973 standard. In International Organization for Standarization (Ed.), *International standards meat and meat products*.

ISO. (1998). Determination of ash content, ISO 936:1998 standard. In International Organization for Standardization (Ed.), *International standards meat and meat products*.

Jaturasitha, S., Srikanchai, T., Kreuzer, M., & Wicke, M. (2008). Differences in carcass and meat characteristics between chicken indigenous to Northern Thailand (Black-

Boned and Thai Native) and imported extensive breeds (Bresse and Rhode Island Red). *Poultry Science*, *87*(1), 160–169. https://doi.org/10.3382/ps.2006-00398

Jeni, R. El, Dittoe, D. K., Olson, E. G., Lourenco, J., Seidel, D. S., Ricke, S. C., & Callaway, T. R. (2021). An overview of health challenges in alternative poultry production systems. *Poultry Science*, *100*(7), 101173. https://doi.org/10.1016/J.PSJ.2021.101173

Karlsson, J. O., Parodi, A., van Zanten, H. H. E., Hansson, P.-A., & Röös, E. (2020). Halting European Union soybean feed imports favours ruminants over pigs and poultry. *Nature Food*, *2*(1), 38–46. https://doi.org/10.1038/s43016-020-00203-7

Khan, M., Chand, N., Khan, S., Naz, S., Alrefaei, A. F., Almutairi, M. H., Sifa, D., & Khan, R. U. (2023). Partial and total replacement of soybean meal with maggot larvae meal in broiler chicken diets: Impact on growth performance, nutrients digestibility, haemotological parameters and intestinal histomorphology. *Livestock Science*, *277*, 105355. https://doi.org/10.1016/j.livsci.2023.105355

Khan, S. H. (2018). Recent advances in role of insects as alternative protein source in poultry nutrition. *Journal of Applied Animal Research*, *46*(1), 1144–1157. https://doi.org/10.1080/09712119.2018.1474743

Kierończyk, B., Rawski, M., Józefiak, A., Mazurkiewicz, J., Świątkiewicz, S., Siwek, M., Bednarczyk, M., Szumacher-Strabel, M., Cieślak, A., Benzertiha, A., & Józefiak, D. (2018). Effects of replacing soybean oil with selected insect fats on broilers. *Animal Feed Science and Technology*, *240*, 170–183. https://doi.org/10.1016/j.anifeedsci.2018.04.002

Kim, S. W., Less, J. F., Wang, L., Yan, T., Kiron, V., Kaushik, S. J., & Lei, X. G. (2019). Meeting Global Feed Protein Demand: Challenge, Opportunity, and Strategy. *Annual Review of Animal Biosciences*, *7*(1), 221–243. https://doi.org/10.1146/annurev-animal-030117-014838

Kim, Y. B., Kim, D.-H., Jeong, S.-B., Lee, J.-W., Kim, T.-H., Lee, H.-G., & Lee, K.-W. (2020). Black soldier fly larvae oil as an alternative fat source in broiler nutrition. *Poultry Science*, *99*(6), 3133–3143. https://doi.org/10.1016/j.psj.2020.01.018

Ko, H., Kim, Y., & Kim, J. (2020). The produced mealworm meal through organic wastes as a sustainable protein source for weanling pigs. *Journal of Animal Science and Technology*, *62*(3), 365–373. https://doi.org/10.5187/jast.2020.62.3.365

Kovitvadhi, A., Chundang, P., Luapan, J., Amarapitak, P., Sriyaphai, P., Buahom, R., Cham-iam, T., Leelehapongsathon, K., Tirawattanawanich, C., & Thongprajukaew, K. (2019). Cricket powder as an alternative protein source for broilers based on in vitro digestibility. *Journal of Insects as Food and Feed*, *5*(3), 185–191. https://doi.org/10.3920/JIFF2018.0043

Kozłowski, K., Ognik, K., Stępniowska, A., Juśkiewicz, J., Zduńczyk, Z., Kierończyk, B., Benzertiha, A., Józefiak, D., & Jankowski, J. (2021). Growth performance, immune status and intestinal fermentative processes of young turkeys fed diet with additive of full fat meals from *Tenebrio molitor* and *Hermetia illucens*. *Animal Feed Science and Technology*, *278*, 114994. https://doi.org/10.1016/j.anifeedsci.2021.114994

Kralik, G., Kralik, Z., Grčević, M., & Hanžek, D. (2017). Quality of Chicken Meat. In B. Yücel & T. Taşkin (Eds.), *Animal Husbandry and Nutrition*. IntechOpen. https://doi.org/10.5772/intechopen.72865

Kulma, M., Kouřimská, L., Plachý, V., Božik, M., Adámková, A., & Vrabec, V. (2019). Effect of sex on the nutritional value of house cricket, *Acheta domestica* L. *Food Chemistry*, *272*, 267–272. https://doi.org/10.1016/j.foodchem.2018.08.049

Kurečka, M., Kulma, M., Petříčková, D., Plachý, V., & Kouřimská, L. (2021). Larvae and pupae of *Alphitobius diaperinus* as promising protein alternatives. *European Food Research and Technology*, *247*(10), 2527–2532. https://doi.org/10.1007/s00217-021-03807-w

Leiber, F., Gelencsér, T., Stamer, A., Amsler, Z., Wohlfahrt, J., Früh, B., & Maurer, V. (2015). Insect and legume-based protein sources to replace soybean cake in an organic broiler diet: Effects on growth performance and physical meat quality. *Renewable Agriculture and Food Systems*, *32*(1), 21–27. https://doi.org/10.1017/S1742170515000496

Lombardo, L., & Zelasco, S. (2016). Biotech Approaches to Overcome the Limitations of Using Transgenic Plants in Organic Farming. *Sustainability*, *8*(5), 497. https://doi.org/10.3390/su8050497

Loponte, R., Bovera, F., Piccolo, G., Gasco, L., Secci, G., Iaconisi, V., & Parisi, G. (2019). Fatty acid profile of lipids and caeca volatile fatty acid production of broilers fed a full fat meal from *Tenebrio molitor* larvae. *Italian Journal of Animal Science*, *18*(1), 168–173. https://doi.org/10.1080/1828051X.2018.1502053

Lurueña-Martínez, M. A., Palacios, C., Vivar-Quintana, A. M., & Revilla, I. (2010). Effect of the addition of calcium soap to ewes' diet on fatty acid composition of ewe milk and subcutaneous fat of suckling lambs reared on ewe milk. *Meat Science*, *84*(4), 677–683. https://doi.org/10.1016/j.meatsci.2009.11.002

Makkar, H. P. S., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, *197*, 1–33. https://doi.org/10.1016/J.ANIFEEDSCI.2014.07.008

Marono, S., Piccolo, G., Loponte, R., Meo, C. Di, Attia, Y. A., Nizza, A., & Bovera, F. (2015). In Vitro Crude Protein Digestibility of *Tenebrio Molitor* and *Hermetia Illucens* Insect Meals and its Correlation with Chemical Composition Traits. *Italian Journal of Animal Science*, *14*(3), 338–343. https://doi.org/10.4081/IJAS.2015.3889

Matshogo, T. Ben, Mnisi, C. M., & Mlambo, V. (2021). Effect of Pre-Treating Dietary Green Seaweed with Proteolytic and Fibrolytic Enzymes on Physiological and Meat Quality Parameters of Broiler Chickens. *Foods*, *10*(8), 1862. https://doi.org/10.3390/foods10081862

Mir, N. A., Rafiq, A., Kumar, F., Singh, V., & Shukla, V. (2017). Determinants of broiler chicken meat quality and factors affecting them: a review. *Journal of Food Science and Technology*, *54*(10), 2997–3009. https://doi.org/10.1007/s13197-017-2789-z

Mlaga, K. G., Agboka, K., Attivi, K., Tona, K., & Osseyi, E. (2022). Assessment of the chemical characteristics and nutritional quality of meat from broiler chicken fed black soldier fly (*Hermetia illucens*) larvae meal. *Heliyon*, e11718. https://doi.org/10.1016/j.heliyon.2022.e11718

Mohan, K., Rajan, D. K., Muralisankar, T., Ganesan, A. R., Sathishkumar, P., & Revathi, N. (2022). Use of black soldier fly (*Hermetia illucens* L.) larvae meal in aquafeeds for a sustainable aquaculture industry: A review of past and future needs. *Aquaculture*, *553*, 738095. https://doi.org/10.1016/j.aquaculture.2022.738095

Mueller, A. J., Maynard, C. J., Jackson, A. R., Mauromoustakos, A., Kidd, M. T., Rochell, S. J., Caldas-Cueva, J. P., Sun, X., Giampietro-Ganeco, A., & Owens, C. M. (2023). Assessment of meat quality attributes of four commercial broiler strains processed at various market weights. *Poultry Science*, *102*(5), 102571. https://doi.org/10.1016/j.psj.2023.102571

Mueller, S., Taddei, L., Albiker, D., Kreuzer, M., Siegrist, M., Messikommer, R. E., & Gangnat, I. D. M. (2020). Growth, carcass, and meat quality of 2 dual-purpose chickens and a layer hybrid grown for 67 or 84 D compared with slow-growing broilers. *Journal of Applied Poultry Research*, *29*(1), 185–196. https://doi.org/10.1016/j.japr.2019.10.005

Müller Richli, M., Weinlaender, F., Wallner, M., Pöllinger-Zierler, B., Kern, J., & Scheeder, M. R. L. (2023). Effect of feeding *Alphitobius diaperinus* meal on fattening performance and meat quality of growing-finishing pigs. *Journal of Applied Animal Research*, *51*(1), 204–211. https://doi.org/10.1080/09712119.2023.2176311

Murawska, D., Daszkiewicz, T., Sobotka, W., Gesek, M., Witkowska, D., Matusevičius, P., & Bakuła, T. (2021). Partial and total replacement of soybean meal with full-fat black soldier fly (*Hermetia illucens* L.) larvae meal in broiler chicken diets: impact on growth performance, carcass quality and meat quality. *Animals*, *11*(9), 2715. https://doi.org/10.3390/ani11092715

Murrieta, C. M., Hess, B. W., & Rule, D. C. (2003). Comparison of acidic and alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid. *Meat Science*, *65*(1), 523–529. https://doi.org/10.1016/S0309-1740(02)00244-9

Nakagaki, B. J., Sunde, M. L., & Defoliart, G. R. (1987). Protein Quality of the House Cricket, *Acheta domesticus*, When Fed to Broiler Chicks. *Poultry Science*, *66*(8), 1367–1371. https://doi.org/10.3382/ps.0661367

Nalunga, A., Komakech, A. J., Jjagwe, J., Magala, H., & Lederer, J. (2021). Growth characteristics and meat quality of broiler chickens fed earthworm meal from Eudrilus eugeniae as a protein source. *Livestock Science*, *245*, 104394. https://doi.org/10.1016/j.livsci.2021.104394

Nascimento Filho, M. A., Pereira, R. T., de Oliveira, A. B. S., Suckeveris, D., Burin Junior, A. M., Mastrangelo, T. de A., da Costa, D. V., & Menten, J. F. M. (2020). Cafeteria-Type Feeding of Chickens Indicates a Preference for Insect (*Tenebrio molitor*) Larvae Meal. *Animals*, *10*(4), 627. https://doi.org/10.3390/ANI10040627

Nascimento Filho, M. A., Pereira, R. T., Oliveira, A. B. S., Suckeveris, D., Burin Junior, A. M., Soares, C. A. P., & Menten, J. F. M. (2021). Nutritional value of *Tenebrio molitor* larvae meal for broiler chickens: metabolizable energy and standardized ileal amino acid digestibility. *Journal of Applied Poultry Research*, *30*(1), 100102. https://doi.org/10.1016/j.japr.2020.10.001

Ndiritu, A., Kinyuru, J., Onyango, A., & Kipkoech, C. (2023). Functional and microstructural characteristics of chitin extracted from field cricket, house cricket, and black soldier fly cocoons. *Journal of Food Measurement and Characterization*, *17*(6), 5903–5912. https://doi.org/10.1007/S11694-023-02086-1/FIGURES/2

Nelder, J. A., & Wedderburn, R. W. M. (1972). Generalized linear models. *Journal of the Royal Statistical Society: Series A (General)*, *135*(3), 370–384. https://doi.org/https://doi.org/10.2307/2344614

Newbold, B. (2017). Population Growth. In *The International Encyclopedia of Geography*. https://doi.org/10.1002/9781118786352.wbieg0043

Nieto, J., Plaza, J., Lara, J., Abecia, J.-A., Revilla, I., & Palacios, C. (2023a). Use of *Acheta domesticus* meal as a full soybean substitute in the feeding of slow-growing chicks. *Poultry Science*, *102*(4), 102503. https://doi.org/10.1016/j.psj.2023.102503

Nieto, J., Merchán, L., Revilla, I., Plaza, J., Abecia, J. A., González-Ronquillo, M., & Palacios, C. (2023b). In vitro digestibility in organic chick diets incorporating insects. *European Organic Congress*.

Nieto, J., Plaza, J., Lara, J., Abecia, J.-A., Revilla, I., & Palacios, C. (2022). Performance of Slow-Growing Chickens Fed with *Tenebrio molitor* Larval Meal as a Full Replacement for Soybean Meal. *Veterinary Sciences*, *9*(3), 131. https://doi.org/10.3390/vetsci9030131

Nikmaram, N., Leong, S. Y., Koubaa, M., Zhu, Z., Barba, F. J., Greiner, R., Oey, I., & Roohinejad, S. (2017). Effect of extrusion on the anti-nutritional factors of food

products: An overview. *Food Control*, *79*, 62–73. https://doi.org/10.1016/j.foodcont.2017.03.027

NRC. (1994). *Nutrient requirements of poultry* (National Research Council, Ed.; The Nation). https://doi.org/10.17226/2114

Olkowski, B. (2018). Feeding high lupine based diets for broiler chickens: Effect of soybean meal substitution with yellow lupine meal at various time points of growth cycle. *Livestock Science*, *218*, 114–118. https://doi.org/10.1016/j.livsci.2018.10.017

Olukosi, O. A., Walker, R. L., & Houdijk, J. G. M. (2019). Evaluation of the nutritive value of legume alternatives to soybean meal for broiler chickens. *Poultry Science*, *98*(11), 5778–5788. https://doi.org/10.3382/ps/pez374

Palacios, C. (2021). *Producción sostenible en avicultura*. ISBN 978-84-18020-88-9.

Pellattiero, E., Tasoniero, G., Cullere, M., Gleeson, E., Baldan, G., Contiero, B., & Dalle Zotte, A. (2020). Are Meat Quality Traits and Sensory Attributes in Favor of Slow-Growing Chickens? *Animals*, *10*(6), 960. https://doi.org/10.3390/ani10060960

Perera, A. D., & Bhujel, R. C. (2021). Field cricket (*Gryllus bimaculatus*) meal (FCM) to replace fishmeal in the diets for sex reversal and nursing of Nile tilapia (*Oreochromis niloticus*) fry. *Aquaculture Research*, *52*(10), 4946–4958. https://doi.org/10.1111/are.15328

Petracci, M., Mudalal, S., Bonfiglio, A., & Cavani, C. (2013). Occurrence of white striping under commercial conditions and its impact on breast meat quality in broiler chickens. *Poultry Science*, *92*(6), 1670–1675. https://doi.org/10.3382/ps.2012-03001

Pietras, M., Orczewska-Dudek, S., Szczurek, W., & Pieszka, M. (2021). Effect of dietary lupine seeds (*Lupinus luteus* L.) and different insect larvae meals as protein sources in broiler chicken diet on growth performance, carcass, and meat quality. *Livestock Science*, *250*, 104537. https://doi.org/10.1016/j.livsci.2021.104537

Pimentel, D., Doughty, R., Carothers, C., Lamberson, S., Bora, N., & Lee, K. (2002). Energy inputs in crop production in developing and developed countries. *Food Security and Environmental Quality in the Developing World*, *2*(1), 129–151.

Pla, M., & Apolinar, R. (2000). The filter-paper press as method for measuring water holding capacity of rabbit meat. *7 Th World Rabbit Congress*, *A*, 659–662.

Popova, T., Petkov, E., & Ignatova, M. (2020). Effect of Black Soldier Fly (*Hermetia illucens*) meals on the meat quality in broilers. *Agricultural and Food Science*, *29*(3), 177–188. https://doi.org/10.23986/AFSCI.88098

Premalatha, M., Abbasi, T., Abbasi, T., & Abbasi, S. A. (2011). Energy-efficient food production to reduce global warming and ecodegradation: The use of edible insects. *Renewable and Sustainable Energy Reviews*, *15*(9), 4357–4360. https://doi.org/10.1016/j.rser.2011.07.115

Ravindran, V., & Blair, R. (1993). Feed resources for poultry production in Asia and the Pacific. III. Animal protein sources. *World's Poultry Science Journal*, *49*(3), 219–235. https://doi.org/10.1079/WPS19930020

Reda, A. A., Hussien, F. Z., Gizaw, T. T., & Beza, G. M. (2023). Research Note: Effects of partial replacement of noug seed (*Guizotia abyssinica*) cake with maggot meal in dual-purpose chicken. *Poultry Science*, *102*(7), 102760. https://doi.org/10.1016/j.psj.2023.102760

Revilla, I., Escuredo, O., González-Martín, M. I., & Palacios, C. (2017). Fatty acids and fat-soluble vitamins in ewe's milk predicted by near infrared reflectance spectroscopy. Determination of seasonality. *Food Chemistry*, *214*, 468–477. https://doi.org/10.1016/j.foodchem.2016.07.078

Revilla, I., & Vivar-Quintana, A. M. (2006). Effect of breed and ageing time on meat quality and sensory attributes of veal calves of the "Ternera de Aliste" Quality Label. *Meat Science*, *73*(2), 189–195. https://doi.org/10.1016/j.meatsci.2005.11.009

Revilla, I., Vivar-Quintana, A. M., González-Martín, M. I., Hernández-Jiménez, M., Martínez-Martín, I., & Hernández-Ramos, P. (2020). NIR spectroscopy for discriminating and predicting the sensory profile of dry-cured beef "Cecina." *Sensors*, *20*(23), 6892. https://doi.org/10.3390/s20236892

Ribeiro, J. C., Lima, R. C., Maia, M. R. G., Almeida, A. A., Fonseca, A. J. M., Cabrita, A. R. J., & Cunha, L. M. (2019). Impact of defatting freeze-dried edible crickets (*Acheta domesticus* and *Gryllodes sigillatus*) on the nutritive value, overall liking

and sensory profile of cereal bars. *LWT*, *113*, 108335. https://doi.org/10.1016/j.lwt.2019.108335

Richards, P. D., Myers, R. J., Swinton, S. M., & Walker, R. T. (2012). Exchange rates, soybean supply response, and deforestation in South America. *Global Environmental Change*, *22*(2), 454–462. https://doi.org/10.1016/j.gloenvcha.2012.01.004

Roffeis, M., Muys, B., Almeida, J., Mathijs, E., Achten, W. M. J., Pastor, B., Velásquez, Y., Martinez-Sanchez, A. I., & Rojo, S. (2015). Pig manure treatment with housefly (*Musca domestica*) rearing – an environmental life cycle assessment. *Journal of Insects as Food and Feed*, *1*(3), 195–214. https://doi.org/10.3920/JIFF2014.0021

Ruiz-Mirazo, J. (2011). *Environmental benefits of extensive livestock farming: wildfire prevention and beyond*.

Sajid, Q. U. A., Asghar, M. U., Tariq, H., Wilk, M., & Płatek, A. (2023). Insect Meal as an Alternative to Protein Concentrates in Poultry Nutrition with Future Perspectives (An Updated Review). *Agriculture*, *13*(6), 1239. https://doi.org/10.3390/agriculture13061239

Saleh, H. H. E. (2020). Review on using of housefly maggots (*Musca domestica*) in fish diets. *Journal of Zoological Research*, *2*(4). https://doi.org/10.30564/jzr.v2i4.2190

Sánchez-Muros, M.-J., Barroso, F. G., & Manzano-Agugliaro, F. (2014). Insect meal as renewable source of food for animal feeding: a review. *Journal of Cleaner Production*, *65*, 16–27. https://doi.org/10.1016/j.jclepro.2013.11.068

Sanotra, G. S., Lund, J. D., Ersøll, A. K., Petersen, J. S., & Vestergaard, K. S. (2001). Monitoring leg problems in broilers: a survey of commercial broiler production in Denmark. *World's Poultry Science Journal*, *57*(1), 55–69. https://doi.org/10.1079/WPS20010006

Sarmiento-García, A., Palacios, C., González-Martín, I., & Revilla, I. (2021). Evaluation of the Production Performance and the Meat Quality of Chickens Reared in Organic System. As Affected by the Inclusion of *Calliphora sp*. in the Diet. *Animals*, *11*(2), 324. https://doi.org/10.3390/ani11020324

Schettino, B., Pérez, J., Gutiérrez, R., León, S., Faure, R., & Escobar, A. (2011). Analysis of robustness in the determination of fatty acids by gas chromatography on goat milk. *Rev. Salud Animal*, *33*(2), 83–89.

Schiavone, A., Cullere, M., De Marco, M., Meneguz, M., Biasato, I., Bergagna, S., Dezzutto, D., Gai, F., Dabbou, S., Gasco, L., & Dalle Zotte, A. (2017). Partial or total replacement of soybean oil by black soldier fly larvae (*Hermetia illucens* L.) fat in broiler diets: effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. *Italian Journal of Animal Science*, *16*(1), 93–100. https://doi.org/10.1080/1828051X.2016.1249968

Schiavone, A., Dabbou, S., Petracci, M., Zampiga, M., Sirri, F., Biasato, I., Gai, F., & Gasco, L. (2019). Black soldier fly defatted meal as a dietary protein source for broiler chickens: effects on carcass traits, breast meat quality and safety. *Animal*, *13*(10), 2397–2405. https://doi.org/10.1017/S1751731119000685

Secci, G., Dabbou, S., de Medeiros, A. C. L., Addeo, N. F., Atallah, E., Parisi, G., Moniello, G., & Bovera, F. (2022). Low dietary inclusion levels of *Tenebrio molitor* larva meal slightly modify growth performance, carcass and meat traits of Japanese quail (*Coturnix japonica*). *Journal of the Science of Food and Agriculture*, *102*(14), 6578–6585. https://doi.org/10.1002/jsfa.12023

Secci, G., Moniello, G., Gasco, L., Bovera, F., & Parisi, G. (2018). Barbary partridge meat quality as affected by *Hermetia illucens* and *Tenebrio molitor* larva meals in feeds. *Food Research International*, *112*, 291–298. https://doi.org/10.1016/j.foodres.2018.06.045

Sedgh-Gooya, S., Torki, M., Darbemamieh, M., Khamisabadi, H., Karimi Torshizi, M. A., & Abdolmohamadi, A. (2021). Yellow mealworm, *Tenebrio molito*r (Col: Tenebrionidae), larvae powder as dietary protein sources for broiler chickens: Effects on growth performance, carcass traits, selected intestinal microbiota and blood parameters. *Journal of Animal Physiology and Animal Nutrition*, *105*(1), 119–128. https://doi.org/10.1111/jpn.13434

Selaledi, L., Mbajiorgu, C. A., & Mabelebele, M. (2020). The use of yellow mealworm (*T. molitor*) as alternative source of protein in poultry diets: a review. *Tropical Animal Health and Production*, *52*(1), 7–16. https://doi.org/10.1007/S11250-019-02033-7/TABLES/5

Serva, L., Marchesini, G., Cullere, M., Ricci, R., & Dalle Zotte, A. (2023). Testing two NIRs instruments to predict chicken breast meat quality and exploiting machine learning approaches to discriminate among genotypes and presence of myopathies. *Food Control*, *144*, 109391. https://doi.org/10.1016/J.FOODCONT.2022.109391

Shaviklo, A. R., Alizadeh-Ghamsari, A. H., & Hosseini, S. A. (2021). Sensory attributes and meat quality of broiler chickens fed with mealworm (*Tenebrio molitor*). *Journal of Food Science and Technology*, *58*(12), 4587–4597. https://doi.org/10.1007/s13197-020-04946-w

Siegford, J. M., Powers, W., & Grimes-Casey, H. G. (2008). Environmental Aspects of Ethical Animal Production. *Poultry Science*, *87*(2), 380–386. https://doi.org/10.3382/ps.2007-00351

Sirri, F., Castellini, C., Bianchi, M., Petracci, M., Meluzzi, A., & Franchini, A. (2011). Effect of fast-, medium- and slow-growing strains on meat quality of chickens reared under the organic farming method. *Animal*, *5*(2), 312–319. https://doi.org/10.1017/S175173111000176X

Smith, D., Lyon, C., & Lyon, B. (2002). The effect of age, dietary carbohydrate source, and feed withdrawal on broiler breast fillet color. *Poultry Science*, *81*(10), 1584–1588. https://doi.org/10.1093/ps/81.10.1584

Soares Araújo, R. R., dos Santos Benfica, T. A. R., Ferraz, V. P., & Moreira Santos, E. (2019). Nutritional composition of insects *Gryllus assimilis* and *Zophobas morio*: Potential foods harvested in Brazil. *Journal of Food Composition and Analysis*, *76*, 22–26. https://doi.org/https://doi.org/10.1016/j.jfca.2018.11.005

Soglia, F., Mudalal, S., Babini, E., Di Nunzio, M., Mazzoni, M., Sirri, F., Cavani, C., & Petracci, M. (2016). Histology, composition, and quality traits of chicken Pectoralis major muscle affected by wooden breast abnormality. *Poultry Science*, *95*(3), 651–659. https://doi.org/10.3382/ps/pev353

Soisontes, S., Freund, F., & Laquai, V. (2023). The future of protein feed: a case study of sustainable substitutes in the German livestock industry. *Environment, Development and Sustainability*. https://doi.org/10.1007/s10668-023-03676-1

Sundrum, A. (2001). Organic livestock farming. *Livestock Production Science*, *67*(3), 207–215. https://doi.org/10.1016/S0301-6226(00)00188-3

Szczesniak, A. S. (1987). Correlating sensory with instrumental texture measurements—An overview of recent developments. *Journal of Texture Studies*, *18*(1), 1–15. https://doi.org/10.1111/j.1745-4603.1987.tb00566.x

Szendrő, K., Nagy, M. Z., & Tóth, K. (2020). Consumer Acceptance of Meat from Animals Reared on Insect Meal as Feed. *Animals*, *10*(8), 1312. https://doi.org/10.3390/ani10081312

te Pas, M. F. W., Veldkamp, T., de Haas, Y., Bannink, A., & Ellen, E. D. (2021). Adaptation of Livestock to New Diets Using Feed Components without Competition with Human Edible Protein Sources—A Review of the Possibilities and Recommendations. *Animals*, *11*(8), 2293. https://doi.org/10.3390/ani11082293

Thornton, P. K. (2010). Livestock production: recent trends, future prospects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1554), 2853–2867. https://doi.org/10.1098/rstb.2010.0134

Tomotake, H., Katagiri, M., & Yamato, M. (2010). Silkworm Pupae (*Bombyx mori*) Are New Sources of High Quality Protein and Lipid. *Journal of Nutritional Science and Vitaminology*, *56*(6), 446–448. https://doi.org/10.3177/jnsv.56.446

Tougan, P. U., Dahouda, M., Salifou, C. F. A., Ahounou, S. G. A., Kpodekon, M. T., Mensah, G. A., Thewis, A., & Karim, I. Y. A. (2013). Conversion of chicken muscle to meat and factors affecting chicken meat quality: a review. *International Journal of Agronomy and Agricultural Research*, *3*(8), 1–20. https://doi.org/10.6084/M9.FIGSHARE.1476821

Tran, H. Q., von Siebenthal, E. W., Luce, J.-B., Nguyen, T. T., Stejskal, V., Weinlaender, F., & Janssens, T. (2024). A novel protein source from lesser mealworm (*Alphitobius diaperinus*) larvae meal for European perch (*Perca fluviatilis*): Investigation on pellet characteristics, production performance, serum biochemistry, digestibility, histology, sensory and trait of fillet, and environmental indices. *Aquaculture*, *581*, 740460. https://doi.org/10.1016/j.aquaculture.2023.740460

Tukey, J. W. (1949). Comparing individual means in the analysis of variance. *Biometrics*, *5*(2), 99–114. https://doi.org/10.2307/3001913

Ullah, R., Khan, S., Hafeez, A., Sultan, A., Khan, N. A., Chand, N., & Ahmad, N. (2017). Silkworm (*Bombyx mori*) Meal as Alternate Protein Ingredient in Broiler Finisher

Ration. *Pakistan Journal of Zoology*, *49*(4), 1463–1470. https://doi.org/10.17582/journal.pjz/2017.49.4.1463.1470

Valenta, J., Chodová, D., Tůmová, E., & Ketta, M. (2022). Carcass characteristics and breast meat quality in fast-, medium- and slow-growing chickens. *Czech Journal of Animal Science*, *67*(7), 286–294. https://doi.org/10.17221/91/2022-CJAS

Vauterin, A., Steiner, B., Sillman, J., & Kahiluoto, H. (2021). The potential of insect protein to reduce food-based carbon footprints in Europe: The case of broiler meat production. *Journal of Cleaner Production*, *320*, 128799. https://doi.org/10.1016/j.jclepro.2021.128799

Veldkamp, T., & Bosch, G. (2015). Insects: A protein-rich feed ingredient in pig and poultry diets. *Animal Frontiers*, *5*(2), 45–50. https://doi.org/10.2527/AF.2015-0019

Vida, V., & Szakály, Z. (2024). Analysis of consumer behaviour in the european poultry meat market. *Applied Studies in Agribusiness and Commerce*, *17*(1). https://doi.org/10.19041/APSTRACT/2023/1/2

Vissers, L. S. M., de Jong, I. C., van Horne, P. L. M., & Saatkamp, H. W. (2019). Global Prospects of the Cost-Efficiency of Broiler Welfare in Middle-Segment Production Systems. *Animals 2019, Vol. 9, Page 473*, *9*(7), 473. https://doi.org/10.3390/ANI9070473

Wahyono, N. D., & Utami, M. M. D. (2018). A Review of the Poultry Meat Production Industry for Food Safety in Indonesia. *Journal of Physics: Conference Series*, *953*, 012125. https://doi.org/10.1088/1742-6596/953/1/012125

Wang, D., Bai, Y. Y., Li, J. H., & Zhang, C. X. (2008). Nutritional value of the field cricket (*Gryllus testaceus* walker). *Insect Science*, *11*(4), 275–283. https://doi.org/10.1111/J.1744-7917.2004.TB00424.X

Wang, D., Shao, W. Z., Chuan, X. Z., Yao, Y. B., Shi, H. A., & Ying, N. X. (2005). Evaluation on nutritional value of field crickets as a poultry feedstuff. *Asian-Australasian Journal of Animal Sciences*, *18*(5), 667–670. https://doi.org/10.5713/AJAS.2005.667

Wang, K. H., Shi, S. R., Dou, T. C., & Sun, H. J. (2009). Effect of a free-range raising system on growth performance, carcass yield, and meat quality of slow-growing

chicken. *Poultry Science*, *88*(10), 2219–2223. https://doi.org/10.3382/ps.2008-00423

Wang, Y.-S., & Shelomi, M. (2017). Review of Black Soldier Fly (*Hermetia illucens*) as Animal Feed and Human Food. *Foods*, *6*(10), 91. https://doi.org/10.3390/foods6100091

Yu, M., Li, Z., Chen, W., Rong, T., Wang, G., Li, J., & Ma, X. (2019). Use of *Hermetia illucens* larvae as a dietary protein source: Effects on growth performance, carcass traits, and meat quality in finishing pigs. *Meat Science*, *158*, 107837. https://doi.org/10.1016/j.meatsci.2019.05.008

Zadeh, Z. S., Kheiri, F., & Faghani, M. (2019). Use of yellow mealworm (T*enebrio molitor*) as a protein source on growth performance, carcass traits, meat quality and intestinal morphology of Japanese quails (*Coturnix japonica*). *Veterinary and Animal Science*, *8*, 100066. https://doi.org/10.1016/j.vas.2019.100066

Zhao, X., Kuang, Z., Yang, J., Bilal, M., & Yang, R. (2023). Effect of Fermented Silkworm (*Bombyx mori*) Pupae on Growth Performance, Immune Index and Meat Quality in Lingnan Yellow Broiler Chickens. *Pakistan Journal of Zoology*, *55*(4). https://doi.org/10.17582/journal.pjz/20220217030205

Zou, X., Liu, M., Li, X., Pan, F., Wu, X., Fang, X., Zhou, F., Peng, W., & Tian, W. (2024). Applications of insect nutrition resources in animal production. *Journal of Agriculture and Food Research*, *15*, 100966. https://doi.org/10.1016/j.jafr.2024.100966

Zuidhof, M. J., Molnar, C. L., Morley, F. M., Wray, T. L., Robinson, F. E., Khan, B. A., Al-Ani, L., & Goonewardene, L. A. (2003). Nutritive value of house fly (*Musca domestica*) larvae as a feed supplement for turkey poults. *Animal Feed Science and Technology*, *105*(1–4), 225–230. https://doi.org/10.1016/S0377-8401(03)00004-X