INSECT *VS* LINSEED OIL IN DIETS OF GROWING-FATTENING RABBITS: EFFECT ON FATTY ACID COMPOSITION AND OXIDATIVE STABILITY IN HIND LEG MEAT

Cátia Martins[1], Marco Cullere[2], Diogo Ribeiro[1], Susana P. Alves[3], Rui J. B. Bessa[3], João Pedro Bengala Freire[1], Luísa Falcão-e-Cunha[1], Antonella Dalle Zotte[2*]

[1]DCEB, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal;
[2]Department of Animal Medicine, Production and Health - MAPS, University of Padova, Legnaro (PD), Italy;
[3]CIISA, Faculdade de Medicina Veterinária, Universidade de Lisboa, Avenida da Universidade Técnica,1300-477 Lisboa, Portugal

*antonella.dallezotte@unipd.it

Abstract – This was the first experiment testing insect oil from *Hermetia illucens* in the diet for growing rabbits. The trial was designed to assess the effect of two different oil sources, insect oil or linseed oil, at two inclusion levels in the diet of growing rabbits on fatty acid (FA) profile and oxidative stability in hind leg meat. Four isoprotein and isofibrous diets were formulated with two oil sources (oil from *Hermetia illucens* or linseed oil) at two inclusion levels (3 or 6%). Rabbits (12×4) were individually caged and experimental diets were fed *ad libitum* from weaning to 70 days of age, then slaughtered. The hind legs were excised 24 h *post mortem* from carcass and deboned. The fatty acid profile of hind leg meat was evaluated, and the meat oxidative stability was assessed after 5 days of chilling and 6 weeks of freezing using the thiobarbituric acid (TBA) method. The meat of rabbits fed with insect oil diet was characterized by higher saturated fatty acids and lower unsaturated fatty acids content compared to that of rabbits fed with linseed oil diet. All the FA-based nutritional indexes of meat were more favorable for linseed diet ($P<0.001$). The oxidative stability of meat was not affected ($P>0.05$) by lipid source or level after 5 days of chilling, but after 6 weeks of frozen storage, meat from animals fed insect oil presented lower ($P<0.001$) TBA values than meat from animals fed insect oil. This study showed that if the FA profile of *Hemetia illucens* oil can be improved, it would be a promising feed ingredient for growing rabbits. Consequently, further research on this topic is required.

Key Words – Insect oil, Rabbit meat, Fatty acids

I. INTRODUCTION

Currently, there has been increasing interest in the use of insects as food in general, and particularly as feed ingredient for monogastric animals. This because there is the urgent need for alternative and sustainable feed ingredients to partly replace the conventional feedstuffs such as soybean meal and soybean oil [1]. The protein of insects has a high biological value [2], and thus can be a potential replacement of soybean meal. Lipids, are also a major component of insects and are produced during protein isolation technologies, thus being another feed ingredient for potential application in animal farming. Lipid content and composition of insects vary according to their species and life stage, although the FA profile presents certain uniformity, with high amounts of unsaturated FA found relative to saturated fatty acid (SFA) [3]. Among insects, *Hermetia illucens* is a Diptera of the Stratiomydae family which was indicated as one of the most promising insects for industrial feed production in the Western world [4]. The interest of people in reducing the amount of fat in food products has been followed by the increased concern with fat quality of food. In fact, some specific fatty acids (FA) have been associated with several positive health effects, while others or their imbalance could have negative effects [5].

Fatty acid composition of meat can be manipulated through changes in animal diet, particularly in monogastric. In rabbits, different dietary fat sources had been used to modify the FA composition of meat [6;7]. However, the FA profile of the intramuscular fat does not entirely reflect the FA composition of the diet [8].

Rabbit meat usually presents excellent nutritive and dietetic properties and may be considered as functional food [9]. Nevertheless, it is important to confirm that these properties are maintained with the inclusion of new dietary fats sources, such as insect oil.

The utilization of linseed oil in rabbit diets, rich in linolenic acid, contributes to increase omega-3 FA in rabbit meat, which can improve the nutritional value of rabbit meat but can have negative effects on the meat oxidative stability during conservation.

The aim of this study was to test for the first time the inclusion of *Hermetia illucens* oil in the diet for growing rabbits and to compare it with the linseed oil. With this purpose, the effect of a dietary inclusion (3 or 6%) of either insect or linseed oils on the chemical composition, FA profile and oxidative stability of rabbit meat were studied.

II. MATERIALS AND METHODS

2.1. Animals and experimental design

The rabbits were weaned at 5 week of age and divided in four homogenous groups (by live weight), of twelve animals each. The rabbits were individually housed and fed *ad libitum* the experimental diets until 10 weeks of age. Four isoprotein and isofibrous diets were formulated incorporating 3 or 6% of insect oil (extracted from *Hermetia illucens* larvae) and 3 or 6% of linseed oil. The chemical composition and FA classes of experimental diets are shown in Table 1.

Table 1. Chemical composition (g/kg DM) and fatty acids profile (% of total FAME) of experimental diets

Lipid source (S)	Insect oil		Linseed oil	
Inclusion level (L)	30 g/kg	60 g/kg	30 g/kg	60 g/kg
Chemical composition:				
Dry matter	91.1	91.4	91.3	91.8
Crude Protein	17.8	17.7	17.8	17.9
Ether extract	5.2	8.6	5.7	8.6
NDF	34.5	35.1	32.8	31.6
FA classes:				
ΣSFA	46.8	56.0	16.1	13.6
ΣMUFA	14.7	13.7	19.7	20.4
ΣPUFA	38.5	30.3	64.2	66.0
18:2 *n*-6	34.5	27.0	30.9	24.5
18:3 *n*-3	2.9	3.7	33.2	41.5

2.2. Slaughter, carcass dissection and meat sampling

At the end of the experimental period, the rabbits were slaughtered. The hind legs were excised 24 h *post mortem* from carcass and deboned. One of the hind legs was used for the determination of lipid oxidation after 5 days chilling at +4 °C and the other after 6 weeks after slaughter (frozen storage at -20 ºC until lipid oxidation analysis). The last was also used for FA analysis.

2.3. Chemical analyses

The feed samples were analyzed for dry matter content which was measured by oven drying at 104 ºC for 24 h. Crude protein was measured by the [10], NDF according to [11] and ether extract was measured in a Soxhlet extractor.

2.4. Fatty acid analysis

Boneless rabbit leg meat was ground and approximately 25 g were collected and stored at -20 ºC until freeze-drying. For the FA analysis, freeze-dried samples were individually weighed (approximately 0.15 g) into glass tubes, then 1 ml of internal standard (C19:0, 1 mg/ml in hexane) and 1 ml of toluene were added, followed by the addition of 2 ml of sodium methoxide in methanol (0.5 M). Tubes were kept at 50 ºC for 10 min in a water bath. Then, 3 ml of 5% HCl methanolic solution were added and the sample was kept in a water bath at 80 ºC for 10 min. After cooling to room temperature, FA were extracted with 4 ml of hexane; the hexane was evaporated under nitrogen and the final residue dissolved in 1 ml of hexane. Vials were stored at -20 ºC until gas chromatography (GC) analysis. The lipid extraction of diets and that of raw materials was performed according to [12]. Fatty acids in meat and diets were quantified using a Shimadzu GC-2010 Plus chromatograph (Shimadzu, Kyoto, Japan) with flame ionization detection (GC-FID), equipped with a SP-2560 GC column (100 m, 0.25 mm ID, 0.20 µm f.t.). Quantification of FA was performed using the chromatographic peak area according to the internal standard method. The FA composition was expressed as % of total FAME. The average amount of each FA was used to calculate the sum of the total saturated (SFA), total monounsaturated (MUFA) and total polyunsaturated fatty acids (PUFA). The saturation (SFA/PUFA: S/P),

atherogenic (AI) and thrombogenic (TI) indexes were calculated according to [13] using the equations presented by [14].

2.5. Lipid oxidation of meat

The meat lipid oxidation was evaluated according to the method of [15], in which the thiobarbituric acid (TBA) value represented the content of malondialdehyde (MDA). Briefly, 15 g of ground leg meat with 30 ml of extracting solution (7.5% trichloroacetic acid in water, 0.1% propyl gallate and 0.1% ethylenediaminetetraacetic acid) were homogenized at 13500 rpm for 2 min with an Ultra-Turrax homogenizer T-25 (Ika, Janke & Kundel, Germany). The homogenate was filtered through Whatman N° 93 filter paper, and then, 5 ml clear filtrate were used for the TBA reaction after the addition of 5 ml of 0.02M TBA water solution (total volume of 10 ml). Samples were then heated in boiling water for 40 minutes and, after cooling, the absorbance was measured by a spectrophotometer (Hitachi U-2001, Tokyo, Japan) set at 530 mn. The TBA value was calculated from a standard curve determined by using TEP standard (1,1,3,3 tetraethoxypropane) and was expressed as mg MDA/kg of meat.

2.7. Statistical Analysis

Data were analyzed by analysis of variance, according to a 2×2 factorial arrangement, considering the lipid source, the lipid level and their interaction as independent variables [16]. When the F value of the interaction oil source x oil level was significant (P<0.05) means were compared by the least square difference test.

III. RESULTS AND DISCUSSION

Table 2 shows the effect of lipid source and level on slaughter weight and general FA composition of rabbits' hind leg meat. The FA composition of meat was largely affected by the lipid source and level of inclusion. The SFA were higher (P<0.001) and the PUFA were lower (P<0.001) in meat from rabbits fed insect oil than in meat from rabbits fed linseed oil and such differences were accentuated (P<0.01) by increasing the dietary inclusion level of oil. The MUFA were lower (P<0.001) in meat from rabbits fed insect oil than in meat from rabbits fed linseed oil and increasing the level of both oil the MUFA proportion in meat decreased (P=0.003).

Table 2. Effect of lipid source (S) and level (L) on rabbits slaughter weight (SW, kg), fatty acid composition (FA, % of total FAME) and nutritional indexes of their hind leg meat

Lipid source (S)	Insect oil		Linseed oil		RSD[1]	Significance		
Inclusion level (L)	30 g/kg	60 g/kg	30 g/kg	60 g/kg		S	L	SxL
N.	11	8	9	10				
SW, kg	2.47	2.38	2.40	2.44	0.38	ns	ns	ns
FA classes:								
ΣSFA	43.2^{a}	45.3^{b}	36.3^{c}	31.3^{d}	2.16	***	ns	***
ΣMUFA	22.5	19.6	25.8	23.9	2.26	***	**	ns
ΣPUFA	34.3^{a}	35.1^{a}	37.9^{b}	44.7^{c}	8.10	***	***	**
FA indexes:								
n-6/*n*-3	14.6	14.4	2.06	1.31	1.03	***	ns	ns
S/P	0.70^{a}	0.73^{a}	0.55^{b}	0.44^{c}	0.06	***	*	**
AI	0.79^{b}	0.93^{a}	0.48^{c}	0.37^{d}	0.10	***	ns	***
TI	1.18^{a}	1.21^{a}	0.55^{b}	0.36^{c}	0.09	***	**	***

[1]RSD: Residual standard deviation.
*,**,***: Means within row with different superscripts differ for P≤0.05, P≤0.01 and P≤0.001, respectively.

Table 3. Effect of lipid source (S) and level (L) on the oxidative status of rabbits hind leg meat, analyzed after 5 days of chilling and after 6 weeks of frozen storage

Lipid source (S)	Insect oil		Linseed oil		RSD[1]	Significance		
Inclusion level (L)	30 g/kg	60 g/kg	30 g/kg	60 g/kg		S	L	SxL
N.	11	8	9	10				
TBA[2] (mg MDA /kg meat):								
5 days	0.19	0.37	0.32	0.37	0.17	ns	*	ns
6 weeks	0.57^{a}	0.51^{a}	1.24^{a}	1.67^{b}	1.27	***	*	**

[1]RSD: Residual standard deviation.
[2]TBA: thiobarbituric acid (MDA value representing the content of malondialdehyde).
*,**,***: Means within row with different superscripts differ for P≤0.05, P≤0.01 and P≤0.001, respectively.

Consistently with the FA composition of meat, the *n*-6/*n*-3 ratio and the AI and TI indexes of meat

were reduced (P<0.001) with linseed oil feeding. The *n*-6/*n*-3 ratio of hind leg meat from rabbits fed insect oil were about twice the 7.02±3.62 average value of 14 studies referred in the bibliography [9], by making the different indexes come close to red meat values (Ulbricht & Southgate, 1991).
No effects of lipid source and level were found in the oxidative status of meat measured after 5 days of chilling (Table 3). However, when oxidative stability was evaluated after 6 weeks of frozen storage, the linseed oil increased (P<0.001) the MDA content of meat compared to insect oil, being thus indicative of a larger oxidative damage of meat. Increasing the linseed oil inclusion level, but not the insect oil, exacerbated this pattern. Thus, increased PUFA content in meat resulted in higher values of MDA in long storage time, which is consistent with higher lipid oxidation and reduced shelf-life [17].

IV. CONCLUSION

This study showed that if the FA profile of *Hermetia illucens* oil can be improved, i.e. through the modulation of the rearing substrate, it would be a promising feed ingredient for growing rabbits: further research on this topic is thus necessary. As well established in literature, the supplementation with linseed oil confirmed to effectively improve the *n*-6/*n*-3 ratio as well the atherogenic and thrombogenic indexes of rabbit meat, however reducing its oxidative stability.

ACKNOWLEDGEMENT

The research was funded by Padova University funds (Ex60%, 60A08-7341/15).

REFERENCES

1. FAO. (2014). Insects to feed the World. 1st International Conference, 14-17 May 2014, Wageningen (Ede), The Netherlands.
2. Yi, L. Y., Lakemond, C. M. M., Sagis, L. M. C., Eisner-Schadler, V., van Huis, A. & van Boekel, M. A. J. S. (2013). Extraction and characterisation of protein fractions from five insect species. Food Chemistry 141: 3341–3348.
3. Tzompa-Sosa, D. A, Yi, L., van Valenberg, H. J. F., van Boekel, M. A. J. & Lakemond, C. M. M. (2014). Insect lipid profile: aqueous versus organic solvent-based extraction methods. Food Research International 62: 1087-1094.
4. Veldkamp, T. & Bosch, G. (2015). Insects: a protein-rich feed ingredient in pig and poultry diets. Animal Frontiers 5: 45-50.
5. FAO. (2010). Fats and fatty acids in human nutrition (FAO report of an expert consultation). FAO, Rome. http://foris.fao.org/preview/25553-0ece4cb94ac52f9a25af77ca5cfba7a8c.pdf
6. Cobos, A., Cambero, M. I., Ordonez, J. A. & De La Hoz, L. (1993). Effect of fat enriched diets on rabbit meat fatty acid composition. Journal of the Science of Food and Agriculture 62: 83-88.
7. Cavani, C., Zucchi, P., Minelli, G., Tolomelli, B., Cabrini, L. & Bergami, R. (l996). Effect of whole soybeans on growth performance and body fat composition in rabbits. In: Proceedings of the 6th World Rabbit Congress (pp. 127-133), 9-12 July 1996, Toulouse France.
8. Dalle Zotte A. (2002). Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. Livestock Production Science 75: 11-32.
9. Dalle Zotte A. & Szendrő Zs. (2011). The role of rabbit meat as functional food. Meat Science 88: 319-331.
10. AOAC. 2000. Official Methods of Analysis, 17th ed. Association of Official Analytical Chemists, Arlington, VA, USA.
11. Van Soest, P. J., Robertson, J. B. & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. Journal Dairy Science 74: 3583-3591.
12. Palmquist, D. L. & Jenkins, T. C. (2003). Challenges with fats and fatty acid methods. Journal of Animal Science 8: 3250–3254.
13. Ulbrich, T. L. & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. Lancet 338: 985-992.
14. Peiretti, P. G., Mussa, P. P., Prola, L. & Meineri G. (2007). Use of different levels of false flax (*Camelina sativa* L.) seed in diet for fattening rabbits. Livestock Science 107: 192-198.
15. Lemon, D. (1975). An improved TBA Test for Rancidity. New Series Circular (Bligh, E.G., Halifax, Nova Scotia), n° 51, 4.
16. SAS. (1991). SAS Systems for Linear Models, third ed. SAS Institute Inc., Cary, NC.
17. Tres, A., Magrinya, R., Bou, F., Guardiola, F., Nuchi, C. D. & Codony, R. (2014). Impact of the oxidative quality of fish oils in feeds on the composition and oxidative stability of chicken and rabbit meat. Animal Feed Science and Technology 196: 76-87.