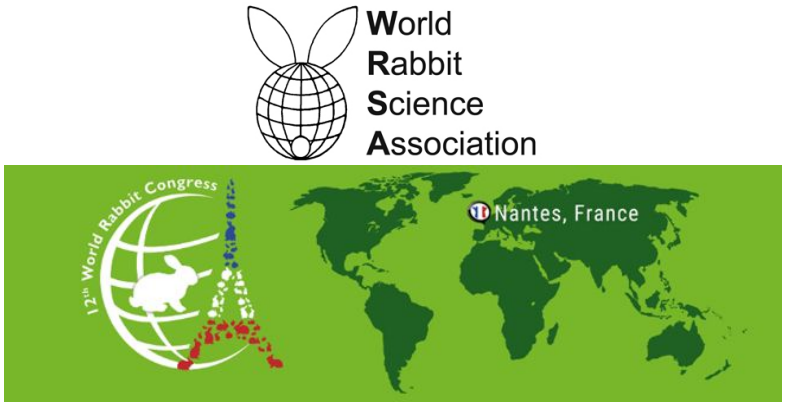

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EFFECT OF DIETARY REPLACEMENT OF SUNFLOWER OIL WITH SILKWORM (*BOMBYX MORI* L.) OIL ON THE TOTAL TRACT APPARENT DIGESTIBILITY AND NUTRITIVE VALUE IN GROWING RABBITS

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ABSTRACT

This study aimed to evaluate the effect of the dietary incorporation of silkworm (*Bombyx mori*) oil (SWO) in rabbit diets as a total replacement of sunflower oil, on the total tract apparent digestibility (TTAD) and nutritive value of experimental diets. To this purpose, twenty-four 55-days-old rabbits (Martini group hybrid) were individually housed in digestibility cages and randomly assigned to one of the two experimental groups (12 rabbits/group): the first group received a standard commercial diet containing 1.30% sunflower oil (Control), whereas the second group received a diet in which sunflower oil was replaced entirely with 1.30% silkworm oil (SWO). Rabbits had *ad libitum* access to water and feed throughout the experimental trial. The digestibility trial consisted of a seven-day adaptation period followed by a four-day collection period. The experimental diets and the collected faeces were analysed to evaluate the TTAD, and the nutritive value of the diets was then calculated. No morbidity and mortality was observed during the trial in both groups. The inclusion of SWO did not influence the TTAD of nutrients. However, SWO diet displayed a higher digestible protein (DP) to digestible energy (DE) ratio than the Control group ($P<0.001$), which resulted from the highest DP ($P=0.001$) and the lowest DE ($P=0.022$). In conclusion, SWO has provided a TTDA comparable to that of sunflower oil and can therefore replace plant-based oil in diets for growing rabbits without any adverse effect on nutrients digestibility and on the nutritive value of the diets.

Keywords: Insect, Rabbit, *In-vivo* digestibility.

INTRODUCTION

Several research have been conducted to investigate alternative feed ingredients to fulfil the nutritional requirement of the growing rabbit (Dalle Zotte *et al.*, 2018; Martins *et al.*, 2018; Gasco *et al*., 2019; Gugołek *et al*., 2019). Intensive rabbit production requires high energy diets to achieve high growth rates and simultaneously diets with high fibre to prevent the impairment of the sensitive rabbit digestive function and health (Martins *et al.*, 2018; Gasco *et al*., 2019). Therefore, insect oil could represent an environmentally friendly and nutritionally important source of lipids for the rabbit's diet, also modulating the fatty acid (FA) composition of rabbit meat (Dalle Zotte and Szendrő, 2011). To this regard, despite research is extremely scarce, a previous trial successfully tested the dietary incorporation of black soldier fly (*Hermetia illucens*) pupae (BSF) fat into growing rabbit diets and observed that overall apparent digestibility of nutrients, growth performance and meat quality traits were promising (Dalle Zotte *et al.*, 2018; Martins *et al.*, 2018). Another study testing BSF and yellow mealworm (YM) oils in rabbit diets did not reveal statistically significant differences in terms of performance, apparent digestibility and health of rabbits (Gasco *et al*., 2019). The only drawback found in all the studies mentioned above was the increase of saturated fatty acids (SFA) in the rabbit

meat, due to the peculiar FA profile of the BSF, very rich in SFA. Another potentially interesting insect source of protein and lipids for growing rabbit diets can be the silkworm (*Bombyx mori*) pupa. During processing, the silk industry generates a by-product, the silkworm pupae, which is a rich source of protein and of lipids rich in polyunsaturated fatty acids (Gugołek *et al*., 2019). This industry waste can be utilised as an alternative feed ingredient for livestock species. The dietary replacement of conventional oils with silkworm oil (SWO) could be a promising alternative as a feed energy source for growing rabbits and for increasing the omega-3 FA content of their meat. Based on these premises, the present research aimed to investigate the total tract apparent digestibility (TTAD) and nutritive value with complete dietary substitution of sunflower oil with SWO in growing rabbits.

MATERIALS AND METHODS

Animals and experimental design

The study was conducted at the facilities of a farm (Il Tramonto, Casalserugo, Padova, Italy) with which the Department of Animal Medicine, Production and Health - MAPS (University of Padova) has a scientific agreement. A total of twenty-four 55-days-old rabbits (Martini group hybrid) were individually housed in digestibility cages and randomly assigned to one of the two experimental dietary treatments (n=12 rabbits/treatment): the first group received a commercial diet containing 1.30% sunflower oil (Control), whereas the second group received a diet in which sunflower oil was completely (1.30%) replaced by silkworm (*Bombyx mori*) oil (SWO). Rabbits had *ad libitum* access to water and feed. The temperature and photoperiod inside the rabbitry were 18-19 °C and 16L:8D, respectively. The *in-vivo* digestibility trial was conducted according to the European standardised method (Perez *et al*., 1995). Rabbits were submitted to seven-days of adaptation to the experimental diets. Two rabbits per treatment were excluded at the end of the adaptation period on the basis of injuries or feed waste behaviour. The total tract apparent digestibility (TTAD) of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE), ash, crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), starch and energy, were determined, and the nutritive value of the experimental diets were calculated.

Chemical Analyses

The chemical analyses of diets and faeces were carried out following the Association of Official Analytical Chemists (2000) methods to determine DM (method no. 934.01), CP (method no. 2001.11), CF (method no. 978.10), ash (method no. 967.05) and starch (amyloglucosidase-α-amylase, method no. 996.11). The EE was determined after acid hydrolysis (EC, 1998). The energy content of diets and faeces were measured using an adiabatic bomb calorimeter (ISO, 1998). Fibre fractions: NDF, ADF, and ADL were analysed using sequential procedure according to Van Soest et al. (1991). Hemicellulose and cellulose were calculated as NDF−ADF and ADF−ADL, respectively. The chemical composition and gross energy of experimental diets are displayed in Table 1.

Table 1: Analysed chemical composition (g/kg as fed) and gross energy (MJ/kg) content of the experimental diets.

	Experimental diets	
	Control	**SWO**
Dry matter	910	912
Crude protein	173	175
Ether extract	28.5	28.6
Ash	70.8	75.2
Crude fibre	163	165
NDF	338	340
ADF	215	216
ADL	52.1	51.2
Starch	158	150
Gross energy	17.3	17.0

Statistical Analysis

The experimental data on total tract apparent digestibility of nutrients and nutritive values of diets were analysed using the GLM procedures of SAS 9.1.3 statistical analysis software for Windows (SAS Institute, 2008) with diets as a fixed effect. The experimental unit was a single animal. Least square means were obtained using the Bonferroni test, and the significance was calculated at 5% confidence level.

RESULTS AND DISCUSSION

Throughout the experimental trial, neither morbidity nor mortality was observed in rabbits. The total tract apparent digestibility (TTAD) and the nutritive value of the experimental diets are presented in Table 2. The dietary replacement of sunflower oil with SWO showed no significant effect on nutrients digestibility in growing rabbits (P>0.05). Other studies replaced conventional fat with insect fat, specifically the BSF or the YM fat, thus direct comparison is not possible. However, the result of the present research agrees with the previous findings regarding the dietary inclusion of insect fat from BSF and YM (0.75% and 1.5% inclusion level for both insects; Gasco *et al*., 2019), and from BSF (3% and 6% fat inclusion level; Martins *et al.*, 2018) for growing rabbits.

Table 2: Effect of the dietary replacement of sunflower oil either with 0% (Control) or 1.30% of silkworm (*Bombyx mori* L.) oil (SWO) on the total tract apparent digestibility (TTAD) of growing rabbits and nutritive value of the experimental diets.

	Experimental diets		RSD[1]	p-value
	Control	SWO		
N.	10	10		
Average live weight, g	2160	2154	148	0.924
Dry matter (DM) intake, g/d	141	147	17.6	0.450
Total Tract Apparent Digestibility, %:				
DM	60.3	60.2	1.77	0.980
Organic matter (OM)	61.6	61.5	1.76	0.879
Crude protein (CP)	75.2	76.3	2.37	0.299
Ether extract (EE)	76.0	74.8	3.02	0.384
Ash	44.9	46.9	2.56	0.097
Crude fibre (CF)	16.1	17.0	3.14	0.560
Neutral detergent fibre (NDF)	28.4	30.0	2.70	0.181
Acid detergent fibre (ADF)	21.1	20.6	5.59	0.840
Acid detergent lignin (ADL)	-13.7	-22.4	11.4	0.103
Hemicelluloses (NDF-ADF)	41.2	48.8	8.60	0.061
Cellulose (ADF-ADL)	28.9	28.2	5.08	0.752
Starch	98.8	98.7	0.23	0.179
Energy	61.5	60.6	1.73	0.256
Nutritive value:				
Digestible Protein (DP, g/kg)	138	146	4.42	0.001
Digestible Energy (DE, MJ/kg)	11.7	11.3	0.33	0.022
DP to DE ratio (g/MJ)	11.9	12.9	0.13	<0.0001

[1]RSD = residual standard deviation.

The nutritive value of experimental diets was affected by the inclusion level of SWO: SWO group displayed higher digestible protein (DP) (138 *vs* 146 g/kg for Control and SWO groups, respectively; P<0.001), and lower digestible energy (DE) (11.7 *vs* 11.3 MJ/kg for Control and SWO groups, respectively; P=0.022) compared to the Control group, which resulted in a higher DP to DE ratio (11.9 *vs* 12.9 g/MJ for Control and SWO groups, respectively; P<0.001). However, as the nutrients digestibility was overlapping in the two experimental groups, the observed result could simply be attributable to the chemical composition of the diets (Table 1): in fact, the protein content was numerically higher in the SWO group (173 and 175 g/kg for Control and SWO diets, respectively), while starch (158 and 150 g/kg for Control and SWO diets, respectively) and gross energy (17.3 and

17.0 MJ/kg for Control and SWO diets, respectively) contents seemed higher in the Control one. Results regarding the nutritive value of the diet of the present study are consistent with those reported by Gasco *et al*. (2018), as they observed a similar trend for the nutritive value of diets in growing rabbits when soybean oil was completely replaced with BSF fat. Differently, Martins *et al*. (2018) observed that diets supplemented with BSF fat had higher DE, thus obtaining a lower DP to DE ratio.

CONCLUSIONS

The use of oil obtained from silkworm (*Bombyx mori*) pupa in rabbit nutrition displayed comparable apparent nutrient digestibility in growing rabbits. Therefore, silkworm oil can be considered as an alternative feed ingredient for growing rabbits. Further studies should be performed to assess the impact of SWO on rabbit meat quality and sensory profile.

ACKNOWLEDGEMENTS

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EFFECT OF DIETARY REPLACEMENT OF SUNFLOWER OIL WITH SILKWORM (*BOMBYX MORI* L.) OIL ON THE TOTAL TRACT APPARENT DIGESTIBILITY AND NUTRITIVE VALUE IN GROWING RABBITS

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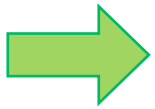
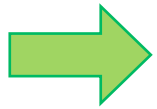
12th World Rabbit Congress, 3-5 November 2021, Nantes, France

INTRODUCTION

Why Silkworm (*Bombyx mori*) Oil?




- ✓ Silkworm pupae are energy and protein rich **by-products** of silk industry, which often represents environmental issue
- ✓ Silkworm pupae meal contains chitin, a potential anti-nutritional factor
- ✓ Silkworm pupae meal contains 1-Deoxynojirimycin (1-DNJ), an alpha-glucosidase inhibitor: prevent the degradation of complex carbohydrates into glucose
- ✓ The **oil** is free of 1-DNJ and chitin, and it is **rich in** PUFAs, especially **omega-3**, which may improve the fatty acid composition of rabbit meat
- ✓ It is not yet known whether silkworm oil can affect the energy and nutrients **digestibility** when added to rabbits diet

OBJECTIVE

To evaluate the effect of the dietary incorporation of silkworm oil (SWO) in rabbit diets as a total replacement of sunflower oil

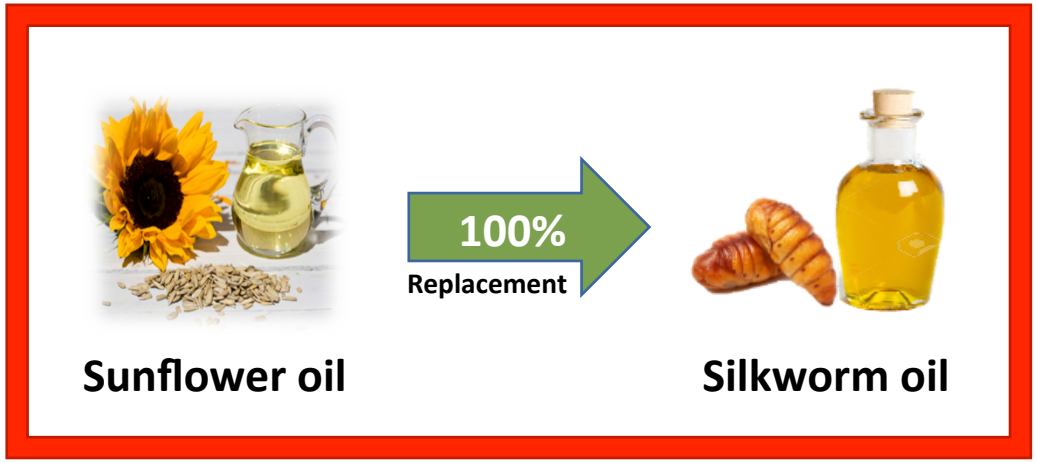
on

The total tract apparent digestibility (TTAD) and nutritive value of experimental diets

MATERIAL AND METHODS

A total of twenty four 55-days-old rabbits (Martini group hybrid) were individually housed in digestibility cages and randomly assigned to one of the two experimental groups (n=12 rabbits/treatment):

- The first group received a commercial diet containing **1.3% sunflower oil** (**Control**)
- The second group received a diet in which sunflower oil was completely (**1.3%**) replaced by silkworm oil (**SWO**)



MATERIAL AND METHODS

In vivo digestibility trial (Perez *et al.*, 1995)

Experiment Layout

- ✓ Trial duration: 11 days (7 days adaptation and 4 days sample collection)
- ✓ Feed and water: *ad libitum*
- ✓ Temperature and Photoperiod of rabbitry: 18-19 °C and 16L:8D, respectively
- ✓ Morbidity and Mortality were daily monitored

MATERIAL AND METHODS

Samples collection

- Diets
- Faeces

Chemical analyses

- DM, OM, CP, EE, Ash
- Fibre fractions
- Starch
- Energy

Computations and Statistical analysis

- Feed intake
- Total Tract Apparent digestibility (TTAD)
- Nutritive value of the experiment diets

RESULTS

CHEMICAL COMPOSITION AND GROSS ENERGY CONTENT OF THE EXPERIMENTAL DIETS

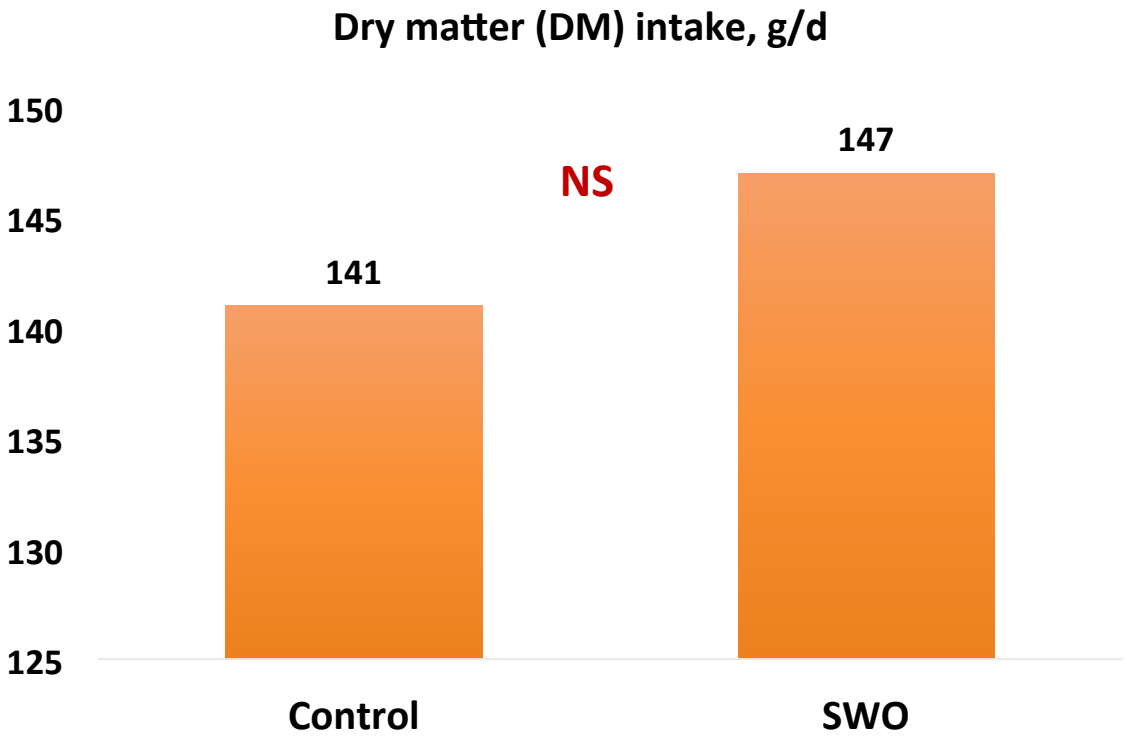
	Experimental diets	
	Control	SWO
Dry matter, %	91.8	91.6
Crude protein, %	18.6	19.0
Ether extract, %	3.72	3.97
Ash, %	7.98	8.37
Crude fibre, %	18.6	18.2
NDF, %	32.8	32.3
ADF, %	20.4	20.1
ADL, %	4.77	5.05
Starch, %	16.7	17.0
Gross energy (MJ/kg)	17.5	17.2

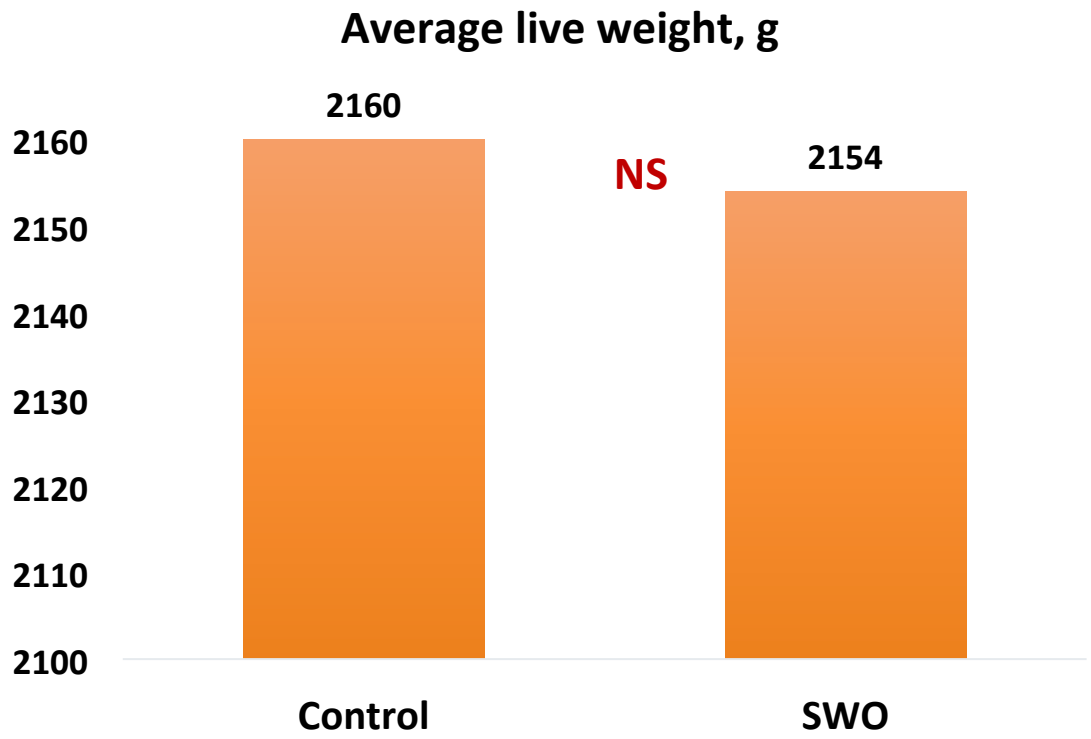
Diets were isonitrogenous and isoenergy

MAIN FATTY ACID CLASSES (% FAME) OF SILKWORM OIL (SWO) AND EXPERIMENTAL DIETS

	SWO	Experimental diets	
		Control	SWO
Total SFA	29.2	16.4	23.0
Total MUFA	33.0	28.4	25.7
Total PUFA	37.1	51.7	44.8
Total *n*-6	5.39	48.0	32.8
Total *n*-3	31.7	3.72	12.0
n-6/*n*-3	0.17	12.9	2.70

RESULTS





No, morbidity and mortality were observed during the trial period

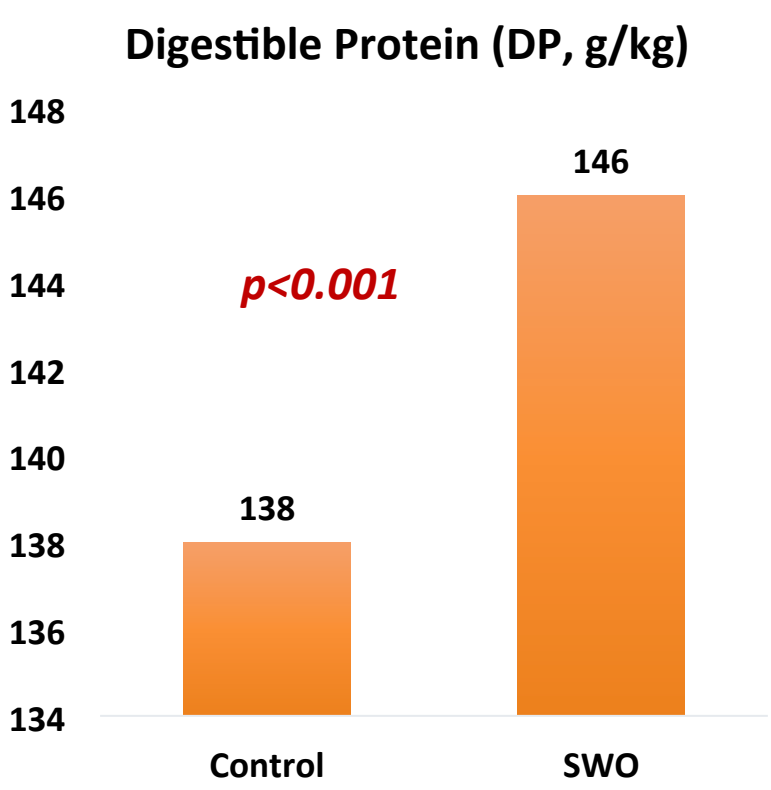
RESULTS

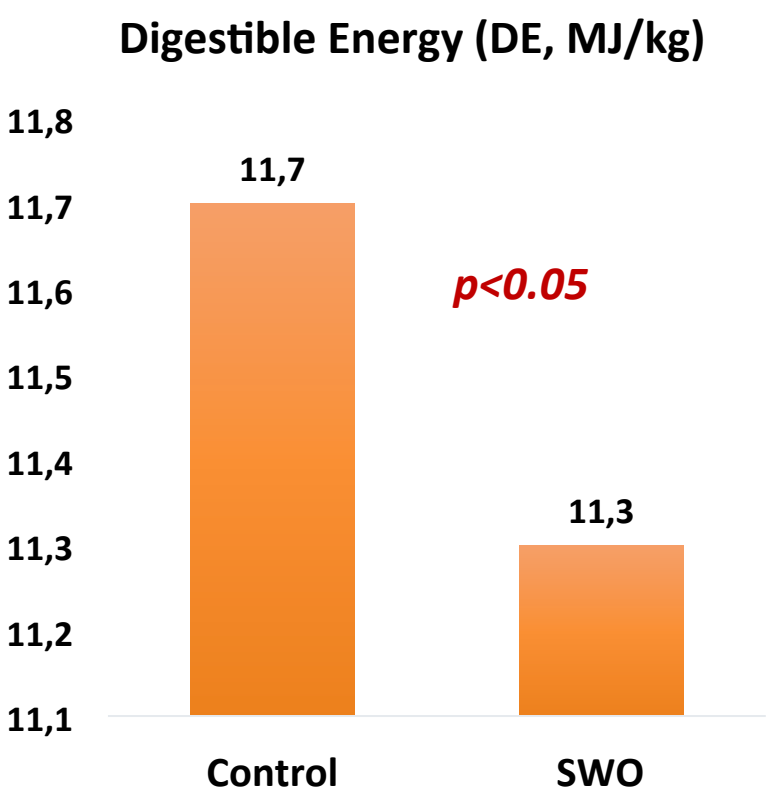
THE TOTAL TRACT APPARENT DIGESTIBILITY (TTAD, %)

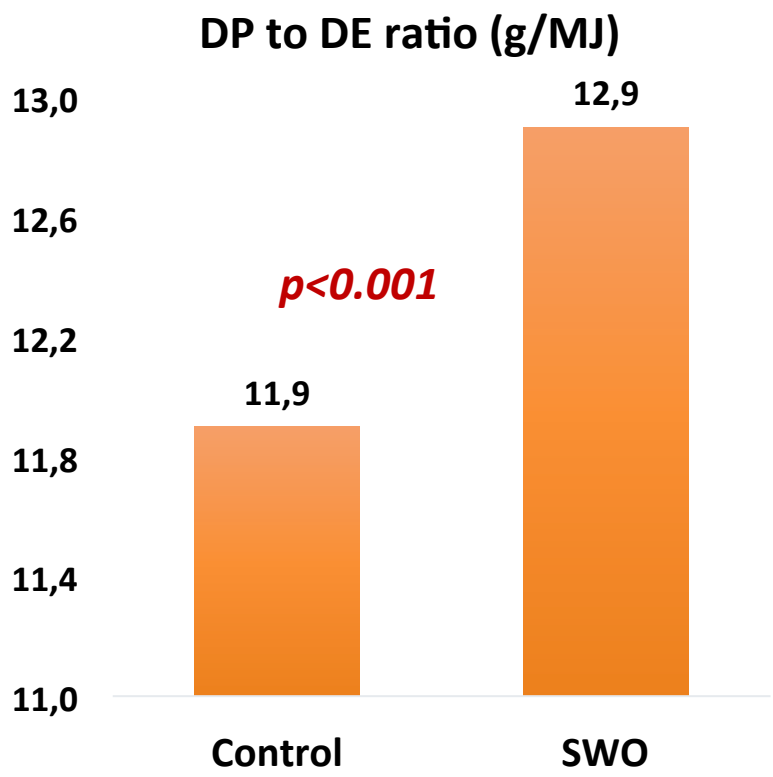
	Experimental diets		Significance
	Control	SWO	
DM	60.3	60.2	*ns*
Organic matter	61.6	61.5	*ns*
Crude protein	75.2	76.3	*ns*
Ether extract	76.0	74.8	*ns*
Ash	44.9	46.9	*ns*
Crude fibre	16.1	17.0	*ns*
NDF	28.4	30.0	*ns*
ADF	21.1	20.6	*ns*
Hemicelluloses (NDF-ADF)	41.2	48.8	*ns*
Cellulose (ADF-ADL)	28.9	28.2	*ns*
Starch	98.8	98.7	*ns*
Energy	61.5	60.6	*ns*

RESULTS

NUTRITIVE VALUE OF THE EXPERIMENTAL DIETS







CONCLUSIONS

The complete replacement of sunflower oil with silkworm (*Bombyx mori*) oil:

- No health issues were observed during the *in vivo* digestibility trial
- Displayed comparable TTAD in growing rabbits BUT modified diet nutritive value in favour of DP content
- Silkworm oil can be considered as an alternative feed ingredient for growing rabbits
- Results on growth performance and carcass traits follows (O4-2)

  The assessment of the impact of SWO on rabbit meat quality and sensory trait is in progress

Thanks!

Any questions?