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Incorporation of Two Levels of Black Soldier Fly (*Hermetia illucens* L.) Larvae Fat or Extruded Linseed in Diets of Growing Rabbits: Effects on Growth Performance and Diet Digestibility

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ABSTRACT

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The use of black soldier fly (BSF) (*Hermetia illucens* L.) larvae fat as a lipid supplement in growing rabbit's diet was evaluated at two levels of supplementation in comparison to two levels of extruded linseed. Forty-eight weaned rabbits aged 35 days were individually housed in digestibility cages and randomly allocated to one of the four diets: Linseed-Low (30 g/kg of fat from linseed), Linseed-High (60 g/kg of fat from linseed), BSF-Low (30 g/kg of BSF fat), BSF-High (60 g/kg of BSF fat). Animals had *ad libitum* access to water and feed during 5 weeks, and were slaughtered at 70 days of age. In the fourth week of the trial, faeces were collected to allow the evaluation of total tract apparent digestibility (TTAD) of the diets. Mortality, dry matter (DM) intake, average daily gain, slaughter live weight, and carcass, liver, perirenal fat, scapular fat, and digestive tract weights were not affected ($P > 0.05$) either by fat source or fat level. The TTAD of DM, organic matter, ether extract, and gross energy were lower ($P < 0.05$) in the diet containing BSF fat than in linseed diets, and the decrease observed ranged between 2.3 to 3.1 percent points. With increasing the fat inclusion level, ether extract TTAD increased ($P < 0.001$) but the cellulose TTAD decreased ($P < 0.01$). Overall, diets containing BSF fat resulted in a slightly lower TTAD than linseed diets, but this seemed not to have affected growth performance and carcass yield. In conclusion, BSF fat could be considered an alternative lipid source for growing rabbit diets highlighting similar productive results to linseed.

Keywords: black soldier fly; rabbit; insect fat; diet digestibility; fat

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Insect biomass has recently emerged as a promising protein source for the feed industry due to its high nutritional value, its biological efficiency and to the possibility of recycling organic wastes for its production (Rumpold and Schluter 2013; Sanchez-Muros et al. 2014). The black soldier fly (BSF) (*Hermetia illucens* L.) is one of the promising insect species as it is spread worldwide and has a high potential for large-scale production (Makkar et al. 2014; Surendra et al. 2016). Also, BSF larvae present high protein (38–46% of dry matter (DM)) and high fat (25–34% of DM) contents (Oonincx et al. 2015). High protein defatted insect meal preparation leads to an insect fat surplus that can be used as a supplement for animal nutrition or biodiesel production (Surendra et al. 2016). The fat extracted from BSF larvae is highly saturated, with lauric acid (12:0) usually comprising between 30–60% of the total fatty acids methyl esters – FAME (St-Hilaire et al. 2007; Spranghers et al. 2017). Lauric acid is considered to be efficiently absorbed in tissues and present antibacterial activities, particularly against Gram-positive bacteria (Dayrit 2015). It may therefore be hypothesized that BSF fat could be efficiently used as an energy source and also to have inhibitory effects on digestive microbiota intestinal fermentation. Therefore, its usefulness in rabbit nutrition needs to be evaluated.

Intensive rabbit production requires high density of metabolizable energy diets to achieve high growth rates, and simultaneously diets with high fibre content to prevent the disruption of the sensible rabbit digestive function and health (Gidenne 2003). The inclusion of fat sources in rabbit feeds allows to overcome such constraints (Gidenne et al. 2017). Several types of fat sources have been used in rabbit production diets including beef tallow, pork lard, and vegetal fat, namely soya, corn or sunflower oils, resulting in feed efficiency improvements (Fernandez-Carmona et al. 2000). Extruded linseed, rich in α-linolenic acid (18:3*n*-3), has also been used as a source of lipids to increase energy density of diets and simultaneously to enrich rabbit meat in *n*-3 polyunsaturated fatty acids (*n*-3 PUFA) (Fernandez-Carmona et al. 2000; Maertens et al. 2008). Fat sources rich in highly unsaturated fatty acids, like linolenic acid, also have a potential to disturb intestinal microbiota and fibre digestion as demonstrated in ruminants (Enjalbert et al. 2017).

The objective of this work was to compare the effects of inclusion of two fat sources, BSF larvae fat and linseed fat, in rabbit diets on feed intake, growth performance, dissectible fat and gastrointestinal organs of rabbits, as well as diet digestibility.

MATERIAL AND METHODS

Diets and animals. Four isoprotein and isofibrous diets were formulated with two different fat sources: linseed fat, added as extruded linseed (R EXTRAlín 15w3®; Reagro, Lisbon, Portugal) and black soldier fly (BSF) (*Hermetia illucens* L.) larvae fat purchased from a leading European company. Both fat sources were added at two inclusion levels: 30 g (Low) and 60 g (High) per kg DM of diet, resulting in four diets: Linseed-Low, Linseed-High, BSF-Low, BSF-High. Ingredients and nutrient composition of the experimental diets are presented in Table 1.

Forty-eight weaned commercial hybrids of rabbits Hyla 2000 (Eurolap, Gosne, France), of both sexes, at the age of 35 days were individually housed in digestibility cages and randomly allocated to one of the four diets. Rabbits had *ad libitum* access to water and feed during 5 weeks, being slaughtered when aged 70 days. During the 4th week of trial, an *in vivo* digestibility trial was conducted according to the European standardised method (Perez et al. 1995). The total tract apparent digestibility (TTAD) of dry matter (DM), organic matter, crude protein, ether extract, neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, hemicelluloses and gross energy (GE) of the experimental diets was measured. Feed intake was measured three times per week, except during the digestibility trial in which it was measured daily. Rabbits were weighed weekly.

Animals were slaughtered by cervical dislocation, then bled through carotid artery and jugular vein cut. Subsequently, liver and full digestive tract were dissected and weighed. Stomach and caecum sections were isolated and their full and empty weights measured, as well as liver, perirenal and scapular fats. Carcasses were kept for 24 h at +4°C. The pH of stomach and caecal contents was measured with a glass electrode pH meter WTW pH 522 (WTW, Germany).

Chemical analyses. Faeces were thawed and dried at 70°C for 48 h. Feed and dry faeces samples were ground through a 1 mm sieve. Dry matter

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Table 1. Ingredients, nutritional composition (g/kg as fed basis) and energy content (MJ/kg feed) of the experimental diets containing extruded linseed or black soldier fly fat (BSF)

Ingredients				
Wheat	281	246	261	211
Wheat bran	100	75	140	140
Beet pulp	100	100	100	100
Sunflower meal	75	50	105	125
Soybean meal	130	115	150	150
Wheat straw	100	100	100	100
Dehydrated alfalfa meal	100	100	100	100
Black soldier fly fat	–	–	30	60
Linseed (R extralín 15w3R)[1]	100	200	–	–
DL-Methionine	0.7	0.7	0.7	0.4
Vitamin-mineral premix[2]	2	2	2	2
NaCl	5	5	5	5
Calcium carbonate	6.5	6.5	6.5	6.5
Nutrient composition				
Dry matter	913	918	911	914
Crude protein	163	164	162	162
Ether extract	52	79	47	79
Ash	72	73	77	80
NDF	299	290	314	321
ADF	159	152	166	171
ADL	31	39	31	37
Hemicellulose[3]	141	138	148	150
Cellulose[4]	128	114	135	133
Gross Energy	17.4	18.1	17.4	18.0

NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin

[1]extruded linseeds with wheat bran and sunflower meal

[2]premix provided per kg of complete diet: vitamin A 1000 UI; vitamin D3 1500 UI; vitamin E 15 mg; vitamin K3 1.5 mg; vitamin B1 1 mg; vitamin B2 2 mg; vitamin B6 1.5 mg; vitamin B12 0.01 mg; pantothenic acid 8 mg; nicotinic acid 25 mg; biotin 0.02 mg; betaine 136.5 mg; robenidine 50 mg; Co 0.7 mg; Cu 5 mg; Fe 30 mg; I 1 mg; Mn 15 mg; Se 0.2 mg; Zn 30 mg; ethoxyquin 12.5 mg; antioxidant 12.5 mg. Note: after the raw material mixture, 1 kg of bentonite was added (to each diet, then mixed and granulated again)

[3]computed as NDF – ADF

[4]computed as ADF – ADL

content was measured by oven drying at 104°C for 24 h and ash by burning overnight at 550°C. Crude protein was measured by the Kjeldahl method (method No. 976.95; AOAC 1995). NDF, ADF, and acid detergent lignin (ADL) were analysed using a sequential procedure according to Van Soest et al. (1991). Hemicellulose and cellulose were calculated as NDF–ADF and ADF–ADL, respectively. Ether extract was measured in a Soxhlet extractor after acid hydrolysis pre-treatment (method No. 920.39; AOAC, 1995). Energy content of diets and faeces was measured using a calorimeter bomb (Parr model 1261; Paar Instrument Co., USA).

Statistical analysis. Data were analysed by a two-way ANOVA according to a 2 × 2 factorial arrangement, considering the fat source and the fat level as main factors and their interaction using the Proc GLM of SAS software (Statistical Analysis System, Version 9.3). When the F value of the interaction fat source × fat inclusion level was significant ($P < 0.05$), means were compared by the least square difference test. Mortality data were analysed following the same 2 × 2 factorial treatment arrangement as fixed effects but using the Proc GLIMMIX of SAS using the binary distribution and the logit as link function.

RESULTS AND DISCUSSION

During the 5 weeks of the trial, 10 rabbits died (3 from Linseed-Low, 2 from Linseed-High, 1 from BSF-Low and 4 from BSF-High groups) but neither the type and level of fat, nor their interaction were significantly ($P > 0.05$) associated with mortality (Table 2). Live weight (LW), daily weight gain (DWG), feed intake (FI), and feed conversion rate (FCR) of rabbits are presented in Table 2. The first period of the trial (35–49 days of age) corresponds to the most stressful post-weaning period. Live performance data indicate that rabbits were well adapted to the experimental diets since the first week. Performances of the whole period (35–70 days of age) confirmed such an initial trend, albeit with no difference among treatments. Average DWG (40 g/day) and FCR (2.89) was in line with data in literature for this rabbit hybrid and age (Gidenne et al. 2017).

Rabbit carcass, liver, perirenal and scapular fat depots weights, and carcass yield are depicted in Table 3. Carcass traits and yield did not differ among treatments, which is consistent with the lack of effects observed for live performance. Dietary fat supplementation often increases the

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Table 2. Effect of lipid source (linseed vs black soldier fly fat (BSF)) and level of inclusion (30 g/kg – Low vs 60 g/kg – High) on live performance of rabbits from 35 to 70 days of age

Source (S)	Linseed		BSF		RSD	*P*-values		
Level (L)	Low	High	Low	High		S	L	S × L
Rabbits *n*	9	10	11	8				
Mortality (%)	25	17	8	33		0.810	0.455	0.169
Live weight at 35 days (g)	1067	1048	1027	1060	98	0.678	0.846	0.438
Live weight at 70 days (SW) (g)	2395	2426	2448	2353	374	0.936	0.795	0.608
1st period (days 35–49)								
Feed intake (g/day)	98.3	97.9	103.3	107.5	15.37	0.164	0.718	0.659
Daily weight gain (g/day)	42.0	42.7	45.3	43.2	9.55	0.552	0.835	0.664
Feed conversion rate	2.43	2.33	2.31	2.58	0.38	0.631	0.492	0.158
Whole period (days 35–70)								
Feed intake (g/day)	115.8	110.5	120.4	109.4	24.63	0.826	0.319	0.720
Daily weight gain (g/day)	39.8	40.3	41.5	38.2	9.94	0.950	0.674	0.569
Feed conversion rate	2.91	2.80	2.96	2.88	0.26	0.429	0.263	0.827

RSD = residual standard deviation, SW = slaughter weight

dissectible fats of the rabbit carcass and, in general, no differences among fat sources are evident (Fernandez and Fraga 1996; Oliver et al. 1997; Pla and Cervera 1997). In the present study, higher fat inclusion level did not lead to an increase in fat depots, despite a slight increase in GE. The BSF fat is very rich in lauric acid, a medium chain fatty acid, that is readily catabolised, and it has been demonstrated that fatty acids from fats with a high rate of oxidation in animal diets are related to lower weight gains (DeLany et al. 2000). It could thus be expected that carcass fat deposition could be lower in animals fed BSF fat than in linseed fed animals. Our results were comparable to those obtained by Gondret et al. (1998), who did not report differences in rabbit fat depots when supplementing diets with coconut meal, equally rich in lauric acid, compared to sunflower oil.

According to Falcao-e-Cunha et al. (2004) a higher fat level in the diet increased the weight of the stomach with no differences in dry matter intake suggesting a longer retention time. However in this experiment neither dietary lipid source, nor inclusion level affected significantly the weight of the whole digestive tract or its main components (Table 4). Only the weight of the empty stomach showed a significant difference as a consequence of lipid source ($P < 0.006$), in favour of BSF treatment, however not confirmed when considered as percentage of slaughter weight.

Table 3. Effect of lipid source (linseed vs black soldier fly fat (BSF)) and level of inclusion (30 g/kg – Low vs 60 g/kg – High) on carcass weight and perirenal and scapular fat

Source (S)	Linseed		BSF		RSD	*P*-values		
Level (L)	Low	High	Low	High		S	L	S × L
Rabbits *n*	9	10	11	8				
Carcass weight (CW) (g)	1489	1511	1533	1427	257	0.815	0.624	0.461
Carcass yield (% SW)	62.1	61.8	62.0	60.8	2.29	0.490	0.291	0.552
Liver (g)	112	102	104	100	25.5	0.575	0.377	0.718
Liver (% CW)	6.90	7.54	7.65	6.75	1.83	0.974	0.839	0.216
Perirenal fat (% CW)	1.65	1.85	1.80	1.64	0.39	0.828	0.857	0.173
Scapular fat (% CW)	0.60	0.60	0.60	0.58	0.18	0.854	0.861	0.956

RSD = residual standard deviation, SW = slaughter weight

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Table 4. Effect of lipid source (linseed vs black soldier fly fat (BSF)) and level of inclusion (30 g/kg – Low vs 60 g/kg – High) on weight of digestive tract and its parts, and on pH value of stomach and caecum

Source (S)	Linseed		BSF		RSD	*P*-values		
Level (L)	Low	High	Low	High		S	L	S × L
Rabbits *n*	9	10	11	8				
Full digestive tract (g)	445	441	470	452	50.7	0.281	0.521	0.677
Full digestive tract (% SW)	18.8	18.4	19.0	19.5	3.07	0.515	0.994	0.677
Full stomach (g)	103	101	122	117	26.8	0.081	0.706	0.916
Empty stomach (g)	22.5	23.3	24.4	25.9	2.43	0.006	0151	0.622
Empty stomach (% SW)	0.95	0.97	1.00	1.12	0.15	0.051	0.216	0.322
Full caecum (g)	124	118	135	128	23.4	0.189	0.409	0.322
Empty caecum (g)	32.2	32.0	31.8	32.8	3.94	0.903	0.743	0.888
Empty caecum (% SW)	1.36	1.34	1.32	1.43	0.29	0.812	0.659	0.457
Stomach content (pH)	1.91	1.46	1.73	1.61	0.60	0.930	0.161	0.457
Caecum content (pH)	5.99	6.16	5.98	6.10	0.43	0.827	0.307	0.418

RSD = residual standard deviation, SW = slaughter weight

The pH measured in the stomach and caecal contents averaged 1.68 and 6.06, respectively, and were not significantly modified by the fat source or inclusion level. These values are within normal values observed in healthy rabbits as previously demonstrated (Carabano and Piquer 1998; Garcia et al. 2002).

The total tract apparent digestibility (TTAD) of the experimental diets and their nutritive value are presented in Table 5. The BSF fat inclusion decreased the TTAD of dry matter ($P < 0.001$), organic matter ($P < 0.001$), ether extract ($P < 0.001$), gross energy ($P = 0.05$) when compared to linseed fat source.

Table 5. Effect of lipid source (linseed vs black soldier fly fat (BSF)) and level of inclusion (30 g/kg – Low vs 60 g/kg – High) on total tract apparent digestibility (TTAD) coefficients and nutritive value

Source (S)	Linseed		BSF		RSD	*P*-values		
Level (L)	Low	High	Low	High		S	L	S × L
Rabbits *n*	9	10	11	8				
TTAD (%)								
Dry matter	64.1	65.2	62.2	60.9	2.05	< 0.001	0.929	0.085
Organic matter	65.6	66.7	63.9	62.6	2.01	< 0.001	0.970	0.103
Crude protein	74.7	75.8	76.1	76.9	3.82	0.327	0.459	0.900
Ether extract	86.1	90.2	82.7	88.5	2.13	< 0.001	< 0.001	0.250
NDF	24.2	24.5	22.1	21.8	4.06	0.079	0.975	0.840
ADF	14.8	13.0	11.3	11.2	4.66	0.098	0.532	0.583
Hemicellulose (NDF–ADF)	34.8	37.2	34.0	33.8	4.31	0.147	0.446	0.378
Cellulose (ADF–ADL)	18.9	11.8	15.6	12.6	4.85	0.435	0.003	0.208
Gross energy	63.8	66.1	63.1	63.0	2.47	0.022	0.172	0.139
Nutritive value								
Digestible protein (DP) (g/kg)	122	124	123	125	6.20	0.540	0.462	0.902
Digestible energy (DE) (MJ/kg)	11.1^{b}	12.0^{a}	11.0^{b}	11.3^{b}	0.43	0.013	< 0.001	0.080
DP to DE ratio (g/MJ)	11.0^{a}	10.4^{b}	11.2^{a}	11.0^{a}	0.63	< 0.001	< 0.001	0.131

RSD = residual standard deviation, NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin
^{a,b}values within a row with different superscripts differ significantly ($P < 0.05$)

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Increasing the level of dietary fat led to a significant ($P < 0.001$) increase in the TTAD of ether extract and a significant ($P < 0.01$) decrease in TTAD of cellulose.

Higher apparent digestibility of ether extract with increased dietary fat inclusion, in the quantities used in this essay, is rather common in rabbits (Maertens et al. 1986; Falcao-e-Cunha et al. 1996) and may be explained in this case by the dilution of endogenous lipid excretion, as feed intake was similar between diets.

BSF fat contains as major fatty acid, lauric acid (C12:0; 39.5% total FAME, reported by Dalle Zotte et al. (2018)), a saturated medium-chain fatty acid that is expected to be more easily digested than the long-chain fatty acids, as reported by Dayrit (2015) for coconut oil. Nevertheless, the composition of triacylglycerols and the regiospecific distribution of lauric acid within the triacylglycerols of BSF fat might differ markedly, from those of coconut oil, which may have an effect on digestibility (Dayrit 2015) and could therefore explain the decrease observed in ether extract digestibility.

Cellulose TTAD decrease at fat inclusion level increase could arise from fat inhibitory effect on microbial activity in the rabbit caecum and colon (Falcao-e-Cunha et al. 1998; Maertens 1998). A similar trend was observed by Falcao-e-Cunha et al. (2004) when sunflower oil was included in the diets of growing rabbits.

The decrease in the TTAD of DM, organic matter, and, consequently, of GE observed in BFS diets might result from the inhibitory effect of lauric acid on intestinal fermentative microbiota, as reported for ruminants (Hristov et al. 2012). In fact, it has long been proved that lauric acid displays a notable Gram-positive bacteria membrane disruption capacity (Galbraith et al. 1971) with negative impact on *in vitro* fibre digestibility in ruminants (El-Hag and Miller 1972). Linolenic acid, abundant in linseeds, also displays a high bacterial membrane disruption activity (Maia et al. 2007) but it is likely that, in our study, its concentration in the rabbit caecum was lower than that of lauric acid, as may be inferred by the highest digestibility of ether extract observed in the linseed diets compared to BSF diets.

The nutritive value of the experimental diets was substantially affected neither by lipid source, nor by lipid inclusion levels. Only Linseed-High diet differed from the other three diets, highlighting significantly higher digestible energy (DE) (12.0 vs 11.1 MJ/kg, respectively; $P < 0.05$) and, consequently, lower digestible protein (DP)/DE ratio (10.4 vs 11.1 g/MJ, respectively; $P < 0.001$), closely related to the significantly higher ether extract TTAD of Linseed-High diet. In fact, increased digestibility of ether extract will lead to an increase in the DE, and maintaining the same level of DP, DP/DE ratio decreases.

CONCLUSION

In conclusion, this study demonstrated that BSF fat seems to be an alternative energy source for rabbit diets as it did not unbalance growth performance, carcass traits, digestive tract incidences, and nutritive value of the diet. Further research to assess the impact of BSF fat on rabbit meat quality and sensory profile would be of utmost importance.

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