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Improved Contribution of Local Feed to Support 100% Organic Feed Supply to Pigs and Poultry

Ileal digestibility of amino acids in novel organic protein feedstuffs for pigs: Black soldier fly larvae meal (*Hermetia illucens*)

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Uusien luomuvalkuaisrehujen aminohappojen sulavuus sioilla: Mustasotilaskärpäsen toukkajauho (*Hermetia illucens*)

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Tiivistelmä

Tutkimuksen tavoitteena oli määrittää uuden, luonnonmukaisesti tuotetun eläinperäisen rehuaineen, mustasotilaskärpäsen toukista valmistetun jauhon (*Hermetia illucens*) aminohappojen standardoitu ohutsuolisulavuus kasvavilla porsailla. Toukkajauhon käyttö sikojen ruokinnassa ei toistaiseksi ole sallittua, mutta käytön mahdollisuutta tullaan käsittelemään EU-lainsäädännössä.

Sveitsistä (FiBL Research Institute of Organic Agriculture) tuli kaksi toukkaerää, joista ensimmäisessä oli erotettu rasvaa mekaanisesti, toisessa heksaaniuutolla. Kokeessa oli 40 porsasta, 17 imisää ja 23 leikkoa, jotka painoivat kokeen alussa keskimäärin 17 kg. Porsaat olivat duroc-, maatiais- ja yorkshirerotujen risteytyksiä. Totutusjaksoilla siat saivat luomuporsasrehua vapaasti porsimiskarsinassa (koejakso 0) ja samaa luomurehua siirryttyään lihasikalaan kahden porsaan karsinoihin (koejakso 1). Aminohappojen ohutsuolisulavuuden määritystä varten (koejakso 2) siat siirrettiin tärkkelyspohjaiselle rehulle. Koeruokintoja oli viisi: 1) vähäproteiininen rehu aminohappojen endogeenisen perustason erityksen määritystä varten, 2) rehu, jossa oli erän 1toukkajauhoa 10,2 %, 3) rehu, jossa oli erän 1 toukkajauhoa 20,4 %, 4) rehu, jossa oli erän 2 toukkajauhoa 9,3 % ja 5) rehu, jossa oli erän 2 toukkajauhoa 18,6 % rehun kuiva-aineessa (KA). Ryhmien 2–5 rehuissa oli valkuaisen lähteenä myös heravalkuaisjauhetta (WPC) 22,85 % rehun kuiva-aineessa. Jokaisessa ryhmässä oli 8 porsasta pariruokinnalla. Teurastus tapahtui porrastetusti ja ohutsuolen sisältöä kerättiin 50 – 60 cm:n matkalta aminohappojen ohutsuolisulavuuden määrittämistä varten. Maksa, munuaiset ja tyhjä mahalaukku punnittiin ja mahahaavojen esiintyminen arvioitiin.

Toukkajauho, josta rasvaa oli poistettu mekaanisesti (erä 1), sisälsi 629 g raakavalkuaista, 185 g raakarasvaa ja 51 g tuhkaa/kg KA. Vastaavat arvot toukkajauholle, josta rasvaa oli poistettu heksaaniuutolla (erä 2), olivat 705 g, 90 g ja 53 g/kg KA. Ensimmäisen erän toukkajauhossa oli 31,7 g lysiiniä, 12,0 g metioniinia, 3,5 g kystiiniä ja 39,6 g/kg KA valiinia. Vastaavat arvot toisen erän toukkajauholle olivat 37,8 g, 14,1 g, 3,7 g ja 44,2 g/kg KA.

Toukkaerä, toukkajauhon lisäystaso tai porsaiden sukupuoli eivät vaikuttaneet dieettien aminohappojen näennäiseen ohutsuolisulavuuteen.

Mekaanisesti erotetun (erä1) toukkajauhon näennäiset (AID) ja standardoidut (SID) aminohappojen ohutsuolisulavuudet olivat suurempia verrattuna heksaaniuutettuun (erä 2) toukkajauhoon. Välttämättömien aminohappojen AID oli ensimmäisessä toukkajauhoerässä 79,3 – 93,2 % ja toisessa toukkajauhoerässä 61,2 – 79,9 %. Välttämättömien aminohappojen SID oli ensimmäisessä toukkajauhoerässä 81,3 – 94,8 % ja toisessa toukkajauhoerässä 64,0 – 81,8 %. Lysiinin standardoitu ohutsuolisulavuus oli ensimmäisessä toukkajauhoerässä 81,3 %, metioniinin 90,7 %, kystiinin 49,8 %, treoniinin 82,5 % ja valiinin 92,9 %. Vastaavat sulavuudet toisessa toukkajauhoerässä olivat 77,2 %, 81,8 %, -10,8 %, 64,0 % ja 73,6 %.

Lähes kaikilla (87,5 %:lla) vähäproteiinista rehua saaneilla porsailla oli vakava mahahaava, joka todennäköisesti aiheuttaa kipua porsaalle. Toukkajauhoa saaneista porsaista 75 – 100 %:lla ei ollut mahahaavaa, tai mahan limakalvomuutokset olivat vain vähäisiä. Vain alle kolmasosalla toukkaryhmien porsaista oli vakavia mahahaavoja, eikä yhtään pahimmanlaatuista mahahaava havaittu. Porsaiden maksan ja munuaisten paino ja munuaisten paino suhteessa elopainoon kasvoivat, kun toukkajauhoa lisättiin rehuun.

Tulokset osoittavat, että rasvan erotusmenetelmä vaikuttaa mustasotilaskärpäsen toukista tehdyn jauhon aminohappojen sulavuuteen, sillä heksaaniuutetun toukkajauhon sulavuusarvot olivat huonommat kuin mekaanisesti erotetun toukkajauhon. Mustasotilaskärpäsen toukkajauho sisältää runsaasti hyvin sulavia aminohappoja, joten sen avulla voisi parantaa luomuporsasrehujen aminohappotasapainoa. Toukkajauho voisi monipuolistaa valkuaisen lähteitä sikojen luomuruokinnassa, mutta sen tuotannon taloudellisia näkökulmia tulisi selvittää. Ohutsuolisulavuuden määrityksessä käytetyt hienojakoiset, tärkkelyspohjaiset rehut, erityisesti aminohappojen endogeenisen erityksen määrityksessä käytetty vähäproteiininen rehu, aiheuttivat porsaille mahahaavoja. Tutkimusmenetelmiä tulisi kehittää niin, että niistä aiheutuisi mahdollisimman vähän haittoja eläinten terveydelle.

Avainsanat:

Sika

Porsas

Sulavuus

Ohutsuolisulavuus

Aminohapot

Mustasotilaskärpänen

Hermetia illucens

Toukka

Ileal digestibility of amino acids in novel organic protein feedstuffs: Black soldier fly larvae meal (*Hermetia illucens*)

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Abstract

The objective of this study was to determine the standardised ileal digestibility (SID) of amino acids in organically produced black soldier fly larvae (*Hermeti illucens*) meal in growing piglets. The use of *Hermetia* meal in pig feeding is not allowed for the time being, but feed legislation in the EU concerning the use of *Hermetia* meal for pigs is in progress.

Two batches of *Hermetia* meal arrived from Switzerland (FiBL Research Institute of Organic Agriculture). In batch 1, fat was extracted by mechanical extraction and in batch 2 hexane extraction was used.The experiment was carried out with a total of 40 growing piglets, 17 gilts and 23 barrows, with the initial body weight of ca. 17 kg. The piglets were distributed in experimental groups from litters of Finnish Landrace or Finnish Yorkshire x Finnish Landrace sows inseminated with mixed semen from Duroc and Norwegian Landrace crossbred boars. Piglets were first fed in the farrowing pen with organic feed for piglets (period 0). The piglets received the same diet when they were moved to the fattening unit (2piglets/pen) (period 1). Diets were switched to starch based diets for the determination of the SID of amino acids. There were five dietary treatments: 1) low-protein diet to determine basal endogenous losses of amino acids, 2) diet with batch 1 *Hermetia* meal 10.2%, 3) diet with batch 1 *Hermetia* meal 20.4%, 4) diet with batch 2 *Hermetia* meal 9.3%, and 5) diet with batch 2 *Hermetia* meal 18.6% (of diet DM). Diets in groups 2–5 contained also 22.85% (of diet DM) whey protein concentrate (WPC) as a protein source. There were 8 pigs per treatment in pair feeding. At the end of the trial, 3.5 h after the morning feeding, the piglets were stunned by bolt pistol, bled and ileal digesta was collected for digestibility determination. Liver, kidneys and empty stomach was weighed and stomach was visually estimated for gastric ulcers.

Hermetia meal batch 1(mechanical fat extraction) contained 629 g crude protein, 185 g crude fat and 51 g ash per kg DM. Corresponding values for *Hermetia* meal in batch 2 (hexane fat extraction) were 705 g, 90 g and 53 g/kg DM, respectively. There was 31.7 g of lysine, 12.0 g of methionine, 3.5 g of cystine and 39.6 g of valine per kg diet DM in *Hermetia* meal batch 1. Corresponding values for *Hermetia* meal batch 2 were 37.8 g, 14.1 g, 3.7 g and 44.2 g/kg DM, respectively.

There were no differences in the apparent ileal digestibility (AID) of amino acids in the experimental diets between the *Hermetia* meal inclusion levels or batches. The sex of the piglets did not affect the AID of amino acids in the experimental diets.

The AID and the SID of the amino acids was higher in *Hermetia* meal batch 1 compared to *Hermetia* meal batch 2. The AID of essential amino acids varied between 79.3–93.2% in batch 1 and 61.2–79.9% in batch 2. The SID of essential amino acids varied between 81.3–94.8% in batch 1 and 64.0–81.8% in batch 2. The SID of lysine in *Hermetia* meal batch 1 was 81.3%, methionine 90.7%, cystine 49.8%, threonine 82.5% and valine 92.9%. Corresponding values for *Hermetia* meal batch 2 were 77.2%, 81.8%, -10.8%, 64.0% and 73.6%.

Most of the piglets (87.5%) fed low-protein diet had severe gastric lesions in the oesophageal area (grades 2 and 3) which are expected to cause pain and reduce the welfare of the piglets. In diets with *Hermetia* meal 75–100% of the piglets had no gastric lesions or the lesions were only minor. Severe gastric lesions were found in less than one third of the piglets fed with *Hermetia* meal and no grade 3 lesions were found. Feeding *Hermetia* meal to piglets increased the size of liver and kidneys and the proportion of kidneys in relation to live weight.

Results indicate that the fat extraction method in *Hermetia* meal affects the AID and the SID of amino acids, as the digestibility values were lower in hexane extracted *Hermetia* meal compared to mechanically extracted *Hermetia* meal. *Hermetia* meal provides highly digestible amino acids, which can improve the amino acid balance in organic feeds for piglets. *Hermetia* meal could diversify the protein supply for organic pig production, but the economic aspects of the production of *Hermetia* meal for pig feeding need to be explored. The fine-grained starch based feeds, especially the low-protein feed used for the determination of the basal endogenous losses of amino acids, caused gastric ulcers for the piglets. The research methods should be developed to minimize the disadvantages to animal welfare.

Keywords:

Pig

Piglets

Digestibility

Apparent ileal digestibility

Standardised ileal digestibility

Amino acids

Black soldier fly

Hermetia illucens

Larvae

Table of Contents

1 Introduction

1.1 Background

Currently, there are many challenges in food production: growing world population, over consuming, climate changes and running out of non-renewable natural resources. Meat consumption is increasing, which increases the demand for new protein sources in livestock feeding. Insects are already used in feeding pets like iguanas and birds and insects are used as human food in many countries. Legislation does not yet allow feeding livestock with insect based processed animal protein (PAP). Insect fat anyhow, is allowed to be fed to non-ruminants. There is no specific section for "insect meal" in Catalogue of Feed Materials (EC 68/2013) although there is listing for "whole or parts of terrestrial invertebrates" suggesting the use of insect derived protein may be possible. There are still many regulations to be met if insects would be used as feed. These regulations concern for example maximum permitted levels of contaminants such as heavy metals, and processing practises for turning insects to PAP. But due to BSE outbreak regulations (EC 999/2001) prohibited all PAP (hydrolysed protein is exception) from being used in animal feed. Exception was also made in 2013 for fishmeal (non-ruminant PAP use for farmed fish species intended for human consumption). This regulation concerns slaughterhouse processing and because insects do not have similar slaughtering processes, insect PAP is forbidden. Insects reared for the production of PAP would consider being "farmed animals" and with current legislation this causes problems concerning the material the insects are grown on. Waste products from bioethanol production (wheat protein and barley hulls) are found in the Catalogue of feed materials (EC 68/2013), and could therefore be used in insect feeding. Manure on the other hand is in the category of "not allowed to be used as feed for farm animals". Housefly larvaes can reduce manure substrate mass by 60% over a 10 day period, which would be a benefit for the environment as well. Vegetable and domestic waste and manure would be ecologically feasible waste to use in the feeding of insects (Koeleman 2014). Using insect PAP for livestock feeding is currently under discussion in EU.

Both house fly pupae and soldier fly larvae contain high quality protein which will support normal growth of chicks. Nearly half of the larvae is crude protein and one third is fat. Some of the crude protein in larvae is in the form of chitin, which is unavailable for the monogastric animals (Partanen 2012). Soldier fly larvae contain only 70% as much protein as house fly pupae, but soldier fly larvae contain 265% as much fat as house fly pupae. This appears to be helpful in converting animal manure into quality feedstuff. Soldier fly is also easier to harvest than house fly or pupae due to its size (Newton et al. 1977). Newton et al. (1977) suggest that *Hermetia* meal is suitable ingredient in swine diets, especially because of its amino acid, ether extract and calcium content. They suggest that the inclusion level of 33% which they used might be too high, and that *Hermetia* meal could be better utilized when used at lower inclusion levels for pigs.

1.2 Objectives

The objective of this study was to determine the standardised ileal digestibility (SID) of amino acids in black soldier fly (*Hermetia illucens*) meal in growing piglets.

2 Materials and methods

2.1 Test feedstuffs

The SID of amino acids in new organic protein feedstuff of animal origin, black soldier fly (*Hermetia illucens*) meal, in growing piglets was studied. Two batches of *Hermetia* meal arrived from FiBL (Research Institute of Organic Agriculture, Switzerland) in April 2013. Salmonella test was done before the experiment and the result was negative. The photograph of dry *Hermetia* meal is presented in Appendix III. In batch 1, fat was extracted by mechanical extraction and in batch 2 hexane extraction was used. The second batch was not premilled for fat extraction, so it was ground at MTT's laboratory through 2mm sieve. The chemical and mineral composition of *Hermetia* meal analysed for the ICOPP database of organic feedstuffs is presented in Tables 1–3 (Kyntäjä et al. 2014).

Table 1. Chemical composition of *Hermetia* pupae and meal.

		Hermetia pupae ´MBM´[1] dried, full fat	*Hermetia* meal ´MBM´[1] defatted	*Hermetia* pupae ´CHO´[2] dried, full fat	*Hermetia* meal ´CHO´[2] defatted	*Hermetia* pupae ´kitchen waste´[3] full fat	*Hermetia* meal ´kitchen waste´[3] defatted
Dry matter	%	91.8	92.5	86.5	89.5	88.0	90.3
Ash	g/kg DM	124	168	40	60	88	137
Crude protein	g/kg DM	446	627	482	710	405	659
Crude fat	g/kg DM	342	43	396	41	410	20
Crude fibre	g/kg DM	102	130	112	183	112	172
Sugars	g/kg DM	8	11	8	12	10	15
NDF	g/kg DM	191	259	212	325	151	235
ADF	g/kg DM	88	93	101	199	91	142
Lignin	g/kg DM	18	0	50	29	13	23
ADF-N[4]	g/kg DM	7	10	8	13	7	12

[1]MBM = grown on meat and bone meal

[2]CHO = grown on carbohydrate rich material

[3]Kitchen waste = grown on kitchen waste

[41]ADF-N = The nitrogen in this form may be unavailable to the animal.

There were no large differences in crude protein (CP) content between the *Hermetia* grown on different materials. When full fat samples were compared, the *Hermetia* grown on carbohydrate rich material had the highest crude protein content, and the *Hermetia* grown on kitchen waste had the lowest content of crude protein. There was more crude protein in defatted samples and the defatted *Hermetia* grown on carbohydrate rich material had the highest crude protein content, and the defatted *Hermetia* grown on meat and bone meal had the lowest content of crude protein.

Analyses of crude fibre, NDF, ADF, ADF-N and lignin (Table 1) do not necessarily describe correctly the cell wall content in insects, because instead of cellulose, the main cell wall material in insects is chitin. However, cellulose and chitin have very similar molecular structures, except that cellulose contains hydroxyl groups and chitin contains acetamides at the C2 position of the monomers. They have different physicochemical properties, such as different solubility in diverse solvents, but similar functions. Main function is to support cell and body surfaces. Chitin strengthens fungal cell walls and exoskeletons of arthropods, whereas cellulose strengthens the

cell wall of plant cells (Merzendorfer 2006). The ADF-N analyse was done in order to find out how much of the total nitrogen is protein and how much of the nitrogen comes from chitin. According to Finke (2007), ADF fraction in insects contains significant amount of amino acids (9.3–32.7%) of the ADF (by weight). Thus using ADF as a measure of chitin in insects results in an overestimation of chitin content of insects. *Hermetia* does not contain much of the sulphur containing amino acids (Table 2).

Table 2. Chemical composition of *Hermetia* pupae and meal.

		Hermetia pupae ´MBM´[1] dried, full fat	*Hermetia* meal ´MBM´[1] defatted	*Hermetia* pupae ´CHO´[2] dried, full fat	*Hermetia* meal ´CHO´[2] defatted	*Hermetia* pupae ´kitchen waste´[3] full fat	*Hermetia* meal ´kitchen waste´[3] defatted
Amino acids							
Essential							
Arginine	g/16 g N	4.8	4.9	4.5	4.7	5.2	4.9
Histidine	g/16 g N	2.8	2.9	2.7	2.9	3.0	3.0
Isoleucine	g/16 g N	4.3	4.4	4.2	4.4	4.5	4.3
Leucine	g/16 g N	6.9	7.0	6.8	7.1	7.3	6.9
Lysine	g/16 g N	5.1	5.3	4.9	5.1	5.7	5.4
Methionine	g/16 g N	2.0	1.9	1.8	1.8	2.0	1.8
Phenylalanine	g/16 g N	4.1	4.2	3.8	4.0	4.1	4.1
Threonine	g/16 g N	3.9	3.9	3.7	3.9	4.0	3.9
Valine	g/16 g N	6.0	6.2	6.0	6.4	6.3	6.2
Non-essential							
Alanine	g/16 g N	5.9	6.0	5.9	6.2	6.3	6.2
Aspartic acid	g/16 g N	9.4	9.8	8.7	9.2	9.9	9.5
Cystine	g/16 g N	0.5	0.5	0.5	0.5	0.5	0.5
Glutamic acid	g/16 g N	9.7	10.1	9.1	9.8	10.7	10.5
Glycine	g/16 g N	5.9	6.1	6.0	6.4	6.5	6.3
Proline	g/16 g N	5.3	5.4	5.4	5.7	5.8	5.7
Serine	g/16 g N	3.9	4.1	3.9	4.2	4.3	4.3
Tyrosine	g/16 g N	5.9	6.3	5.9	6.3	6.9	6.8
In vitro ileal digestibility							
DM	%	85.0	78.7	82.9	71.9	87.2	77.8
N	%	78.4	78.4	77.0	74.9	81.7	80.5
In vitro total tract digestibility							
OM	%	84.8	77.4	82.8	73.8	86.8	77.4

[1]MBM = grown on meat and bone meal

[2]CHO = grown on carbohydrate rich material

[3]Kitchen waste = grown on kitchen waste

According to Finke (2007), house fly pupaes and black soldier fly larvaes contain significant amounts of calsium in their cuticle, compared to other insects. The material on which the *Hermetia* is grown can affect the mineral content of *Hermetia*. In full fat samples (Table 3) the calsium content is much lower in *Hermetia* grown on carbohydrate rich material compared to *Hermetia* grown on meat and bone meal and kitchen waste (6.3, 37.1 and 24.0 g/kg DM, respectively). Also the content of iron is the lowest in *Hermetia* grown on carbohydrate rich material (Table 3).

Table 3. Mineral composition of *Hermetia* pupae and meal.

		Hermetia pupae ´MBM´[1] dried, full fat	*Hermetia* meal ´MBM´[1] defatted	*Hermetia* pupae ´CHO´[2] dried, full fat	*Hermetia* meal ´CHO´[2] defatted	*Hermetia* pupae ´kitchen waste´[3] full fat	*Hermetia* meal ´kitchen waste´[3] defatted
Minerals							
Calcium	g/kg DM	37.1	52.9	6.3	10.1	24.0	38.4
Magnesium	g/kg DM	2.7	3.6	2.3	3.5	2.3	3.5
Phosphorus	g/kg DM	6.7	8.9	5.6	8.2	4.8	6.9
Sulphur	g/kg DM	2.9	4.0	3.0	4.6	2.7	4.3
Potassium	g/kg DM	6.9	7.7	7.1	8.6	6.5	8.2
Sodium	g/kg DM	0.8	0.9	1.3	1.5	1.0	1.3
Fe	mg/kg DM	97.8	172.4	74.4	118.9	155.6	302.1
Cu	mg/kg DM	9.3	13.7	9.5	14.7	8.4	13.3
Zinc	mg/kg DM	67.2	95.7	78.4	125.9	69.5	111.3
Manganese	mg/kg DM	128.0	171.7	162.6	242.0	195.4	319.7

[1]MBM = grown on meat and bone meal

[2]CHO = grown on carbohydrate rich material

[3]Kitchen waste = grown on kitchen waste

2.2 Animals and housing

The experiment was carried out with a total of 40 growing piglets, 17 gilts and 23 barrows, with the initial body weight of ca.17.2 kg (std. 1.96). The piglets were distributed in experimental groups from litters of Finnish Landrace or Finnish Yorkshire x Finnish Landrace sows inseminated with mixed semen from Duroc x Norwegian Landrace crossbred boars. These sows were in an organic feeding experiment and the piglets were weaned at the age of 40–49 days. Sows were taken away and the piglets stayed in their farrowing pen for 4 days. After that the piglets were moved to the fattening unit. In the fattening unit the piglets were housed 2 piglets per pen in pens of 0.8 m x 2.49 m. The pens had 1.63 m² of concrete floor and 0.77 m² of slatted metal floor (dunging area). The pen walls were made of concrete and there were vertical metal bars to separate pens at the dunging area. Piglets had a nose-to nose-contact with each other at the dunging area. Feed was given twice daily and water was available *ad libitum* from drinking nipples. Wood shavings were used as bedding material.

2.3 Experimental treatments

A total of 40 piglets were randomly distributed to pens (2 piglets / pen) and the pens were randomly allotted to five dietary treatments (Table 4). Piglets in the same pen were not from the same litter. The experiment was in three periods, and there where different diets and different objectives in these periods, but the same animals continued from start to finish. The purpose of period 0 was adaptation after weaning. Period 1 was used for adaptation to the new environment and new pen mate. The feed was the same organic feed as offered before. The purpose of period 2 was to determine the SID of amino acids in *Hermetia* meal by regression method. It was taken into accoutn that part of the nitrogen in insects is adhered in chitin and may not be available to the animal. The ADF-N was subtracted from the total nitrogen of the *Hermetia* meal and the diets were planned to contain the same amount of protein that was not originated from the ADF-N.

Table 4. The experimental treatments for the determination of the SID of amino acids (period 2) in organically produced *Hermetia illucens* meal (feed amounts are given on DM basis).

Group	Period 0 4 d	Period 1 4-5 d	Period 2 7 d (+ 2 d transition period)	Piglets
1 – Low-protein	Organic diet	Organic diet	Starch based, low-protein diet	8
2 - *Hermetia* meal Batch 1, Level 1	Organic diet	Organic diet	89.8% starch based diet with 22.85% WPC + 10.2% *Hermetia* meal batch 1	8
3 - *Hermetia* meal Batch 1, Level 2	Organic diet	Organic diet	79.6% starch based diet with 22.85%WPC + 20.4% *Hermetia* meal batch 1	8
4 - *Hermetia* meal Batch 2, Level 1	Organic diet	Organic diet	90.7% starch based diet with 22.85% WPC + 9.3% *Hermetia* meal batch 2	8
5 - *Hermetia* meal Batch 2, Level 2	Organic diet	Organic diet	81.4% starch based diet with 22.85% WPC + 18.6% *Hermetia* meal batch 2	8

WPC = whey protein concentrate
Batch 1 = mechanical fat extraction
Batch 2 = hexane fat extraction

2.4 Diets and feeding

During the periods 0 and 1 piglets received organically produced feed for piglets. Dietary ingredients were oats (10%), wheat (32.3%), barley (10%), rapeseed expeller (11.3%), peas (24%), concentrate (12%) (RehuX-concentrate), and some minerals and salt. The starch based low-protein diet in group 1 in period 2 was similar to the low-protein diet that was used in the digestibility trial of the ICOPP project to determine the SID of amino acids in organic grass silage for pigs. The starch based diet contained the same raw materials as the starch based low-protein diet but the targeted CP content was 160 g/kg DM. This was achieved by increasing the amount of whey protein concentrate (WPC) and decreasing the amount of barley starch and some other minor ingredients (Table 5). A portion of the starch based diet was replaced by *Hermetia* meal from either batch 1 or batch 2 according to the experimental group (Table 3.) The CP intake was still 160 g/kg DM. Starch based diet and *Hermetia* meal where weighed separately and mixed before feeding. Titanium dioxide was used as an indigestible marker in both of the period 2 feeds (3 g/kg DM).

Table 5. Dietary ingredients of the experimental diets.

	Period 2	
Dietary ingredients, g/kg:	Starch based, low-protein diet	Starch based diet[1]
Barley starch	767.4	620.1
WPC75 [2]	50.0	228.5
Sugar	80	80
Cellulose	30	30
Rapeseed oil	35	15
Monocalciumfosfate	16.1	13.2
Limestone	14.5	6.2
Mineral-vitamin mixture [3]	4	4
Titanium dioxide	3	3

[1] Used with *Hermetia* meal according to experimental group, see Table 3:

In group 2: 898 g/kg DM starch based diet + 102 g/kg DM *Hermetia* meal batch 1

In group 3: 796 g/kg DM starch based diet + 204 g/kg DM *Hermetia* meal batch 1

In group 4: 907g/kg DM starch based diet + 93 g/kg DM *Hermetia* meal batch 2

In group 5: 814 g/kg DM starch based diet + 186 g/kg DM *Hermetia* meal batch 2

[2]WPC = Whey protein concentrate

[3] The organic mineral-vitamin mixture Sika-Hiven supplied per kg of feed: 0.44 g of Ca, 0.26 g of P, 0.18 g of digestible P, 40 mg of Mg, 36 mg of Na, 5.6 mg of Fe, 0.4 mg of Cu, 4 mg of Mn, 8.8 mg of Zn, 48 µg of I, 40 µg of Se, 1200 IU of vitamin A, 200 IU of vitamin D_3, 10 mg of vitamin E 3a700, 9.08 mg as α-tocopherol, 60 µg of vitamin K, 0.2 mg of vitamin B_1, 0.4 mg of vitamin B_2, 0.3 mg of vitamin B_6, 10 µg of vitamin B_{12}, 0.18 mg of biotin, 2.4 mg of niacin, 0.12 mg of folic acid, and 1.6 mg of pantothenic acid.

The piglets were given 100 g DM per kg metabolic body weight ($W^{0.75}$), based on the initial body weight at the beginning of period 1. The daily allowance was increased by 200 g DM at the beginning of period 2. The piglets were fed twice daily. Basal feed and *Hermetia* meal were hand-weighed on daily basis and the daily ration was given to piglets in the afternoon (15:00) and morning (07:00) feeding. Water was added on top of feed.

The piglets were gradually switched to starch based diets of period 2 at the end of period 1. This transition lasted for 2 days. Period 2 lasted for 7 days. Feeding was graded at the last morning and the piglets were slaughtered at the end of trial, 3.5 h after the morning feeding for the collection of ileal digesta. The feeding in periods 0, 1 and 2 and the experimental procedures are summarised in Table 6. All animals had exactly the same procedures, but for half of the animals the period 1 lasted for five days and for half of the animals it lasted for four days. Reason for this was that it was not possible to slaughter 30 piglets at the same day. Consequently, first 10 animals started the trial one week earlier than the others and were slaughtered at 3th of June (Table 6), 20 animals were slaughtered at 10th of June and the rest of the animals were slaughtered one day after that. Sub-samples of feeds and experimental feedstuffs were collected during the last 3 days of period 2, pooled per treatment for the analyses of proximate composition, amino acids and markers.

Table 6. The experimental procedures of periods 0, 1 and 2.

Day		Period	Diets and procedures	Unit	piglets/ pen	Date
1	Fri	Period 0, adaptation to dry feeding	Organic diet, weighing of the piglets was done day before	farrowing pen	litter	17.5.2013
2	Sat	Period 0, adaptation to dry feeding	Organic diet	farrowing pen	litter	18.5.2013
3	Sun	Period 0, adaptation to dry feeding	Organic diet	farrowing pen	litter	19.5.2013
4	Mon	Period 0, adaptation to dry feeding	Organic diet, weighing of the piglets	farrowing pen	litter	20.5.2013
5	Tue	Period 1, adaptation to fattening unit and pen mate	Organic diet	fattening unit	2piglets/ pen	21.5.2013
6	Wed	Period 1, adaptation to fattening unit and pen mate	Organic diet	fattening unit	2piglets/ pen	22.5.2013
7	Thu	Period 1, adaptation to fattening unit and pen mate	Organic diet	fattening unit	2piglets/ pen	23.5.2013
8	Fri	Period 1, adaptation to fattening unit and pen mate	Organic diet	fattening unit	2piglets/ pen	24.5.2013
9	Sat	Gradual change to period 2 feeding	Starch based low-protein diet for the group 1 and	fattening unit	2piglets/ pen	25.5.2013
10	Sun	Gradual change to period 2 feeding	starch based diet with WPC + *Hermetia* meal for groups 2-5	fattening unit	2piglets/ pen	26.5.2013
11	Mon	Period 2, adaptation to starch based diets	Weighing of the piglets and increasing of daily ratio	fattening unit	2piglets/ pen	27.5.2013
12	Tue	Period 2, adaptation to starch based diets		fattening unit	2piglets/ pen	28.5.2013
13	Wed	Period 2, adaptation to starch based diets		fattening unit	2piglets/ pen	29.5.2013
14	Thu	Period 2, adaptation to starch based diets		fattening unit	2piglets/ pen	30.5.2013
15	Fri	Period 2, adaptation to starch based diets	Removal of bedding material	fattening unit	2piglets/ pen	31.5.2013
16	Sat	Period 2, adaptation to starch based diets		fattening unit	2piglets/ pen	1.6.2013
17	Sun	Period 2, adaptation to starch based diets	Staggering of feedings according to the slaughter schedule	fattening unit	2piglets/ pen	2.6.2013
18	Mon	Period 2, adaptation to starch based diets	Staggering of feedings according to the slaughter schedule, weighing of the piglets, slaughtering of the piglets 3.5h after morning feeding	fattening unit	2piglets/ pen	3.6.2013

2.5 Slaughter of pigs and collection of ileal digesta

At the end of the trial, the piglets were stunned by bolt gun, bled and ileal digesta was collected for digestibility determination. After slaughter, the abdominal cavity was opened, and a 0.5–0.6m piece of ileum, backwards from ileo-caecal junction, was isolated. Digesta was collected from the isolated intestine and if that part of ileum was empty additional 0.5–0.6m piece was isolated and digesta was collected. The digesta was frozen immediately. Digesta samples were freeze-dried and analysed for DM, ash, amino acids and markers. All the analytical methods used are presented in Appendix I.

Liver and kidneys of the piglets were removed, weighed and examined visually for abnormalities. The stomach of the piglets was opened, emptied and the weight of empty stomach was measured. The oesophageal area of the stomach was examined for gastric ulceration according to Hautala and Rautiainen (1991) (Appendix II). The consistency of digesta in stomach was evaluated as follows: Grade 1 = liquid, Grade 2 = liquid with visible particles, Grade 3 = mushy.

2.6 Calculation of digestibility and statistical analyses

AID of amino acids were calculated by marker method as follows:

$$AID, \% = [1 - (AA_{digesta}/AA_{diet}) \times (M_{diet}/M_{digesta})]$$

AA = amino acid, and M = marker concentration, g/kg DM

Basal ileal endogenous losses of amino acids (IAA_{end}), g/ kg DM intake, were calculated from ileal samples of low-protein diet according to Stein et al. (2007) as follows:

$$\text{Basal } IAA_{end} = AA_{digesta} \times (M_{diet}/M_{digesta})$$

The standardised ileal digestibility of amino acids was calculated according to Stein et al. (2007) as follows:

$$SID, \% = AID + [(\text{basal } IAA_{end} / AA_{diet}) \times 100].$$

The AID and the SID of amino acids in the two different *Hermetia* meals and in the basal feed was calculated by regression method (Fan and Sauer 1995) using the GLM procedure of SAS® for Windows (Version 9.2).The AID of diets was analysed with SAS® for Windows (Version 9.2) using the MIXED procedure with fixed effects of *Hermetia* batch, inclusion level and sex of the piglets and interactions of *Hermetia* batch and level, *Hermetia* batch and sex, and inclusion level and sex. Organ weight data was analysed with SAS® for Windows (Version 9.2) with fixed effects of treatment, sex of the piglets and interaction of treatment and sex.

3 Results

3.1 Chemical composition of the experimental diets and feedstuffs

The diet in periods 0 and 1 contained DM 877 g/kg, ash 58 g/kg DM, crude protein 177 g/kg DM, crude fibre 53 g/kg DM, lysine 8.5 g/kg DM, threonine 7.3 g/kg DM, and methionine + cystine 6.8 g/kg DM. The analysed chemical composition of the experimental diets in period 2 is presented in Table 7.

Table 7. Analysed chemical composition of the experimental diets.

	Starch based low-protein diet	Starch based diet [1]
Analysed chemical composition, g/kg DM		
Dry matter g/kg	913	927
Ash	40	28
Crude protein	32	107
Amino acids, g/kg DM		
Essential		
Arginine	0.8	2.6
Histidine	0.9	2.4
Isoleucine	1.9	7.1
Leucine	3.4	12.4
Lysine	2.8	10.3
Methionine	1.3	3.2
Phenylalanine	1.2	3.9
Threonine	2.1	7.9
Valine	1.7	6.8
Non-essential		
Alanine	1.5	5.6
Aspartic acid	3.5	12.7
Cystine	0.6	2.1
Glutamic acid	5.4	20.0
Glycine	0.7	2.3
Proline	1.9	6.8
Serine	1.7	6.0
Tyrosine	0.9	3.2

[1] Used with *Hermetia* meal according to experimental group, see Table 3.

In group 2: 898 g/kg DM starch based diet + 102 g/kg DM *Hermetia* meal batch 1

In group 3: 796 g/kg DM starch based diet + 204 g/kg DM *Hermetia* meal batch 1

In group 4: 907g/kg DM starch based diet + 93 g/kg DM *Hermetia* meal batch 2

In group 5: 814 g/kg DM starch based diet + 186 g/kg DM *Hermetia* meal batch 2

The analysed chemical composition of the *Hermetia* meal is presented in Table 8. Mechanically extracted *Hermetia* meal in batch 1 contained more fat than hexane extracted *Hermetia* meal in batch 2. Mechanically extracted *Hermetia* meal contained a little less crude protein than hexane extracted *Hermetia* meal (629 and 705 g/kg DM, respectively) and therefore also less amino acids. The content of glutamic acid in mechanically extracted *Hermetia* meal was much lower compared to hexane extracted *Hermetia* meal (64.7 and 73.0 g/kg DM, respectively). Mechanical extraction of fat leaves more fat to the *Hermetia* than hexane extraction (crude fat content 185 and 90 g/kg DM, respectively). Both batches contained 12 g ADF-N /kg DM. ADF-N was interpreted to represent the part on nitrogen that is unavailable to the animal.

Table 8. Analysed chemical composition of *Hermetia* meal.

	Hermetia meal mechanical fat extraction	*Hermetia* meal hexane fat extraction
Analysed chemical composition, g/kg DM		
Dry matter g/kg	925	906
Ash	51	53
Crude protein	629	705
Crude fat	185	90
NDF	318	287
ADF	136	143
ADF-N[1]	12	12
Amino acids, g/kg DM		
Essential		
Arginine	30.5	32.0
Histidine	18.4	20.8
Isoleucine	26.9	30.8
Leucine	43.5	49.5
Lysine	31.7	37.8
Methionine	12.0	14.1
Phenylalanine	24.1	28.0
Threonine	24.3	27.4
Valine	39.6	44.2
Non-essential		
Alanine	39.6	45.6
Aspartic acid	57.4	63.5
Cystine	3.5	3.7
Glutamic acid	64.7	73.0
Glycine	34.3	40.4
Proline	36.3	41.2
Serine	27.1	30.1
Tyrosine	39.7	43.8

[1]ADF-N = The nitrogen in this form may be unavailable to the animal.

3.2 Apparent and standardised ileal digestibility of amino acids

The effect of *Hermetia* meal inclusion level and batch and sex of the piglets on the AID of amino acids in experimental diets is presented in Table 9.There were no differences in the AID of amino acids in the experimental diets between the inclusion levels or batches. The AID of cystine tended to be slightly higher at the lower inclusion level of *Hermetia* meal compared to higher inclusion level (p=0.09). The sex of the piglets did not affect the AID of amino acids in the experimental diets.

Table 9. The effect of *Hermetia* meal inclusion level and *Hermetia* meal batch and sex of the piglets on the AID of amino acids in experimental diets (%).

	Hermetia level 1 and 2				*Hermetia* batch Mechanical fat extraction	Hexane fat extraction			Sex Gilts	Barrows		
			SEM	p level			SEM	p batch			SEM	p sex
n	16	16			16	16			17	23		
Amino Acid												
Essential												
Arginine	80.3	81.5	2.49	0.72	79.8	82.1	2.56	0.50	81.3	80.6	2.66	0.84
Histidine	80.7	78.7	2.61	0.58	77.9	81.5	2.69	0.33	79.9	79.5	2.80	0.91
Isoleucine	86.0	84.8	1.64	0.62	84.2	86.5	1.69	0.32	85.6	85.1	1.76	0.83
Leucine	86.6	85.5	1.73	0.65	84.8	87.4	1.78	0.29	86.5	85.7	1.85	0.74
Lysine	84.3	81.7	2.01	0.35	81.5	84.6	2.07	0.27	82.3	83.8	2.15	0.61
Methionine	87.2	85.8	1.74	0.56	85.2	87.9	1.79	0.28	86.3	86.7	1.86	0.89
Phenylalanine	83.5	83.8	2.14	0.92	82.1	85.3	2.20	0.29	84.7	82.6	2.28	0.48
Threonine	77.9	75.4	2.30	0.43	74.7	78.5	2.37	0.25	76.9	76.3	2.46	0.85
Valine	80.6	80.5	2.11	0.97	79.0	82.1	2.17	0.30	81.1	80.0	2.26	0.73
Non-essential												
Alanine	79.1	79.3	2.43	0.96	77.5	80.9	2.50	0.33	79.0	79.4	2.60	0.93
Aspartic acid	81.6	78.7	2.48	0.41	78.2	82.1	2.55	0.28	79.9	80.4	2.65	0.89
Cystine	73.0	62.1	4.60	*0.09*	63.4	71.7	4.74	0.21	68.0	67.1	4.92	0.89
Glutamic acid	81.3	76.7	3.14	0.29	76.8	81.3	3.23	0.31	78.0	80.1	3.35	0.63
Glycine	58.6	60.2	5.19	0.82	54.8	64.1	5.34	0.21	59.5	59.4	5.55	0.99
Proline	78.8	77.3	2.15	0.62	76.1	80.0	2.21	0.22	78.8	77.3	2.30	0.61
Serine	76.1	75.1	2.56	0.78	73.5	77.8	2.64	0.24	75.7	75.5	2.74	0.95
Tyrosine	77.8	79.4	3.31	0.72	76.0	81.1	3.41	0.28	81.7	75.5	3.54	0.20

n = number of animals

SEM is the highest standard error

The interaction of the *Hermetia* meal inclusion level and *Hermetia* meal batch in the experimental diets is presented in Table 10. The AID of tyrosine was higher in diet with mechanically extracted *Hermetia* meal when the inclusion level was higher. In diet with hexane extracted *Hermetia* meal the AID of tyrosine was higher when the inclusion level was lower (p=0.01). Tendency to similar interaction was detected with arginine, isoleucine and valine.

Table 10. The interaction of the two different *Hermetia* meal batches and inclusion levels and the effect on the apparent ileal digestibility of amino acids in the experimental diets (%).

	Hermetia, mechanical fat separation		*Hermetia*, hexane fat extraction			p
	Level 1	Level 2	Level 1	Level 2	SEM	Batch x Level
n	8	8	8	8		
Essential						
Arginine	75.9	83.6	84.8	79.5	3.70	*0.07*
Histidine	76.3	79.5	85.1	77.9	3.89	0.17
Isoleucine	82.8	85.7	89.1	84.0	2.44	*0.09*
Leucine	83.2	86.3	90.0	84.7	2.58	0.10
Lysine	81.5	81.4	87.2	82.0	2.99	0.37
Methionine	84.5	85.9	90.0	85.8	2.58	0.26
Phenylalanine	79.4	84.7	87.6	82.9	3.17	0.11
Threonine	73.6	75.8	82.1	74.9	3.42	0.16
Valine	76.1	81.9	85.1	79.1	3.14	*0.06*
Non-essential						
Alanine	74.7	80.3	83.5	78.3	3.61	0.12
Aspartic acid	77.2	79.2	85.9	78.2	3.68	0.17
Cystine	64.5	62.3	81.5	61.9	6.84	0.19
Glutamic acid	77.0	76.6	85.7	76.9	4.66	0.34
Glycine	48.3	61.3	69.0	59.2	7.72	0.13
Proline	74.4	77.9	83.2	76.7	3.20	0.11
Serine	71.2	75.7	81.0	74.5	3.81	0.14
Tyrosine	68.9	83.1	86.6	75.7	4.93	**0.01**

n. = number of observations
SEM is the highest standard error in *Hermetia* batch x *Hermetia level*

The AID of amino acids in two *Hermetia* meal batches is presented in Table 11. The AID of amino acids in *Hermetia* meal and basal feed was calculated by regression method. The AID values were higher in mechanically extracted *Hermetia* meal compared to hexane extracted *Hermetia* meal. The AID of essential amino acids in mechanically extracted *Hermetia* meal was 79.3–93.2% and 61.2–79.9% in hexane extracted *Hermetia* meal. In mechanically extracted *Hermetia* meal the AID of cystine was very low (42.9%) and the results showed no reliable value for the AID of cystine in hexane extracted *Hermetia* meal as the value was negative.

Table 11. The apparent ileal digestibility of amino acids in two *Hermetia* meal batches.

	Hermetia meal mechanical fat extraction		*Hermetia* meal hexane fat exraction	
	Mean	SEM	Mean	SEM
n	16		16	
Essential				
Arginine	92.9	10.71	77.2	7.96
Histidine	83.1	13.41	71.9	8.53
Isoleucine	91.5	11.26	75.2	8.42
Leucine	93.2	12.48	74.7	9.15
Lysine	79.4	16.81	75.6	10.22
Methionine	88.5	12.65	79.9	7.75
Phenylalanine	92.6	12.41	79.0	7.52
Threonine	79.3	19.38	61.2	11.73
Valine	91.5	11.73	72.4	8.88
Non-essential				
Alanine	88.8	12.55	74.7	8.65
Aspartic acid	81.9	16.50	67.9	10.28
Cystine	42.9	62.51	-17.3	28.44
Glutamic acid	73.1	25.43	63.0	15.00
Glycine	75.9	19.56	54.5	15.67
Proline	82.6	13.34	69.7	8.70
Serine	83.8	17.60	67.4	9.77
Tyrosine	99.7	13.51	64.2	11.4

n = number of animals
SEM=standard error

The basal endogenous losses of amino acids (IAA_{end}) of this experiment are presented in Table 12. For comparison, the mean values of basal ileal endogenous losses of amino acids from diets with casein/wheat gluten from the research of Jansman et al. (2002) are presented in Table 12.

Table 12. Mean flow of basal ileal endogenous amino acids (g/kg DM intake).

	Hermetia meal experiment		Jansman et al. (2002)[1]
	Mean	Std.	Mean
n	8		
Essential			
Arginine	0.46	0.106	0.36
Histidine	0.18	0.049	0.21
Isoleucine	0.38	0.107	0.51
Leucine	0.59	0.172	0.54
Lysine	0.51	0.165	0.44
Methionine	0.18	0.038	0.12
Phenylalanine	0.32	0.103	0.36
Threonine	0.65	0.152	0.72
Valine	0.50	0.133	0.74
Non-essential			
Alanine	0.55	0.127	0.56
Aspartic acid	0.90	0.236	0.95
Cystine	0.16	0.050	0.28
Glutamic acid	1.13	0.307	1.75
Glycine	1.10	0.468	0.70
Proline	2.34	1.506	0.76
Serine	0.61	0.127	0.91
Tyrosine	0.45	0.169	0.30

n = number of animals
Std. = standard error
[1]Basal ileal endogenous losses of amino acids from diets with casein/wheat gluten.

The SID of amino acids is presented in Table 13. The SID of amino acids in *Hermetia* meal and basal feed was calculated by regression method. The SID of amino acids was higher in mechanically extracted *Hermetia* meal compared to hexane extracted *Hermetia* meal. The SID of essential amino acids in mechanically extracted *Hermetia* meal was 81.3–94.8% and 64.0–81.8% in hexane extracted *Hermetia* meal. In mechanically extracted *Hermetia* meal the SID of cystine was very low (49.8%) and the results showed no reliable value for the SID of cystine in hexane extracted *Hermetia* meal as the value was negative.

Table 13. The standardised ileal digestibility of amino acids in two *Hermetia* meal batches.

	Hermetia meal mechanical fat extraction		*Hermetia* meal hexane fat exraction	
	Mean	SEM	Mean	SEM
n	16		16	
Essential				
Arginine	94.3	10.71	78.6	7.96
Histidine	84.4	13.41	73.0	8.53
Isoleucine	93.2	11.26	76.7	8.42
Leucine	94.8	12.48	76.1	9.15
Lysine	81.3	16.81	77.2	10.22
Methionine	90.7	12.65	81.8	7.75
Phenylalanine	94.2	12.41	80.4	7.52
Threonine	82.5	19.38	64.0	11.73
Valine	92.9	11.73	73.6	8.88
Non-essential				
Alanine	90.4	12.55	76.1	8.65
Aspartic acid	83.7	16.50	59.6	10.28
Cystine	49.8	62.51	-10.8	28.44
Glutamic acid	75.3	25.43	64.9	15.00
Glycine	78.8	19.56	56.9	15.67
Proline	86.3	13.34	72.9	8.70
Serine	86.4	17.60	69.8	9.77
Tyrosine	100.7	13.51	65.1	11.40

n = number of animals
SEM=standard error

3.3 Gastric health and organ weight

Effect of feeding with starch based low-protein diet and four starch based diets with *Hermetia* meal on incidence and severity of gastric lesions and the results of visual evaluation of stomach contents are presented in Table 14. Most of the piglets (87.5%) fed low-protein diet had severe gastric lesions in the oesophageal area (grades 2 and 3) which are expected to cause pain and reduce the welfare of the piglets (Hautala and Rautiainen 1991) (Appendix III). Their stomach contents were in liquid form with and without visible particles. In diets with *Hermetia* meal in which fat was extracted mechanically, 75–87.5% of the piglets had no gastric lesions or the lesions were so small that they should not affect the welfare of the piglets (grades 0 and 1). Corresponding values for *Hermetia* meal in which hexane fat extraction was used were 75–100%. Severe gastric lesions were found in piglets fed with *Hermetia* meal but grade 3 lesions were not found. The stomach contents of piglets fed *Hermetia* meal were in liquid form but feed particles were also present. Two pigs fed diet with *Hermetia* meal, in which hexane fat extraction was used, had stomach contents in mushy form.

Table 14. The incidence and severity of gastric lesions and consistency of digesta in stomach of piglets fed starch based low-protein diet and diets containing *Hermetia* meal.

	Starch based diets									
			Hermetia meal mechanical fat extraction				*Hermetia* meal hexane fat extraction			
	Low-protein		Level 1		Level 2		Level 1		Level 2	
	n[1]	%[2]	n[1]	%[2]	n[1]	%[2]	n[1]	%[2]	n[1]	%[2]
Severity of gastric lesions										
Grade 0	0		1	12.50	0		1	12.50	1	12.50
Grade 1	1	12.50	6	75.00	6	75.00	7	87.50	5	62.50
Grade 2	3	37.50	1	12.50	2	25.00	0		2	25.00
Grade 3	4	50.00	0		0		0		0	
Consistency of digesta in stomach[3]										
Grade 1	5	62.50	0		0		0		0	
Grade 2	3	37.50	8	100.00	8	100.00	7	87.50	7	87.50
Grade 3	0		0		0		1	12.50	1	12.50

[1]n=number of piglets within diet and grade.
[2]%=percentage distribution within diet and grade.
[3] Consistency of digesta in stomach: Grade 1 = liquid, Grade 2 = liquid with visible particles, Grade 3 = mushy.

The effect of diet on organ weights is shown in Table 15. The live weight was the lowest in the piglets fed low-protein diet before slaughter. Compared to piglets fed low-protein diet, the live weight was higher in piglets fed *Hermetia* meal but similar with each other. There were no differences in the weight of empty stomach between the experimental groups. The weight of empty stomach in relation to live weight was the lowest in piglets fed the diet with *Hermetia* meal in which fat was extracted mechanically, and it was significantly lower than that of piglets fed the low-protein diet. The weight of liver was the lowest in piglets fed low-protein diet. The weight of liver in relation to live weight did not differ between the experimental groups. The weight of kidneys and the weight of kidneys in relation to live weight increased when *Hermetia* meal was added to the diets.

Table 15. The effect of diet on the live weight and organ weight of piglets.

	Starch based diets						
		Hermetia meal mechanical fat extraction		*Hermetia* meal hexane fat extraction			
	Low-protein	Level 1	Level 2	Level 1	Level 2	SEM	p diet
n piglets	8	8	8	8[2]	8		
Live weight, kg	19.4[a]	24.9[b]	25.8[b]	24.2[b]	25.6[b]	1.259	**0.0015**
Empty stomach, kg[1,2]	0.147	0.157	0.168	0.173	0.172	0.01	0.227
Empty stomach,% of live weight[2]	0.77[a]	0.64[bc]	0.65[bc]	0.71[ac]	0.67[ac]	0.028	**0.0085**
Liver, kg[1]	0.559[a]	0.788[b]	0.819[b]	0.735[b]	0.812[b]	0.051	**0.001**
Liver, % of live weight	2.90	3.17	3.18	3.05	3.16	0.15	0.53
Kidneys, kg[1]	0.078[a]	0.142[b]	0.150[b]	0.135[b]	0.146[b]	0.009	**<0.001**
Kidneys, % of live weight	0.40[a]	0.57[b]	0.58[b]	0.56[b]	0.59[b]	0.041	**0.004**

[1]Stomach, liver and kidneys were weighed immediately after slaughter, 3.5 h after feeding.
[2]One observation was rejected due to divergent value in empty stomach weight and empty stomach weight in relation to live weight in group 4 (*Hermetia* meal, hexane fat extraction, level 1).
[a,b,c] Means with different superscript differ significantly ($p<0.05$).

The effect of sex on the incidence of gastric lesions is shown in Table 16. Gilts had less severe gastric lesions than barrows (23.52% vs. 34.79%). The live weight, the weight of empty stomach, liver and kidneys and their proportion of live weight did not differ between gilts and barrows (Table 17).

Table 16. The effect of sex on the incidence and severity of gastric lesions and consistency of digesta in stomach.

	Gilts		Barrows	
	n[1]	%[2]	n	%
Severity of gastric lesions				
Grade 0	2	11.76	1	4.35
Grade 1	11	64.71	14	60.87
Grade 2	2	11.76	6	26.09
Grade 3	2	11.76	2	8.70
Consistency of digesta in stomach[3]				
Grade 1	3	17.65	2	8.70
Grade 2	13	76.47	20	86.96
Grade 3	1	5.88	1	4.35

[1]n=number of piglets within sex and grade.
[2]%=percentage distribution within sex and grade.
[3] Consistency of digesta in stomach: Grade 1 = liquid, Grade 2 = liquid with visible particles, Grade 3 = mushy.

Table 17. The effect of sex on the live weight and organ weight of piglets.

	Gilts	Barrows	SEM	p sex
n piglets	17	23[1]		
Live weight, kg	24.4	23.5	0.78	0.39
Empty stomach, kg[1,2]	0.165	0.162	0.006	0.73
Empty stomach, % of live weight[1]	0.68	0.69	0.02	0.72
Liver, kg[2]	0.753	0.732	0.031	0.62
Liver, % of live weight	3.10	3.08	0.10	0.92
Kidneys, kg[2]	0.127	0.134	0.005	0.32
Kidneys, % of live weight	0.52	0.56	0.03	0.16

[12]One observation was rejected due to divergent value in empty stomach weight and empty stomach weight in relation to live weight in group 4 (*Hermetia* meal, hexane fat extraction, level 1).
[2]Stomach, liver and kidneys were weighed immediately after slaughter, 3.5 h after feeding.

4 Discussion and conclusion

The content of crude protein in *Hermetia* meal in the present trial was comparable to feed table values for fish meal. The content of crude fat in hexane extracted *Hermetia* meal was comparable to feed table values for fish meal. In mechanically extracted *Hermetia* meal the content of crude fat was higher than in fish meal. The content of ash was lower in *Hermetia* meal compared to fish meal. The content of lysine and methionine in *Hermetia* meal was lower than the feed table values presented for fish meal. The content of threonine in *Hermetia* meal was close to that of fish meal. The content of cystine in *Hermetia* meal was clearly lower and the content of valine was clearly higher than in fishmeal (CVB 2011, EvaPig 2008). Estimated from the content of NDF, one third of the *Hermetia* meal is chitin.

The AID and SID of amino acids were higher in mechanically extracted *Hermetia* meal (batch 1) compared to hexane extracted *Hermetia* meal (batch 2). The SID of essential amino acids in *Hermetia* meal in batch 1was 81.3–94.8% and 64.0–81.8% in batch 2. Hexane extraction may have affected the digestibility of amino acids in *Hermetia* meal.

The SID of lysine and threonine in mechanically extracted *Hermetia* meal was somewhat smaller than presented in feed tables for fish meal, but the SID of arginine, methionine, isoleucine, valine, leucine, phenylalanine and histidine was similar or even higher to that of fish meal (CVB 2011, EvaPig 2008). Compared to plant based protein sources, in mechanically extracted *Hermetia* meal the SID of nearly all essential amino acids, exept for threonine and histidine, was higher than in soybean meal. The SID of arginine was similar to soy bean meal. The SID of amino acids is generally higher in mechanically extracted *Hermetia* mea compared to peas (CVB 2011, EvaPig 2008). However, the SID of cystine is very low in *Hermetia* meal.

Feeding starch based low-protein diet to determine basal endogenous losses of amino acids had very negative effect on gastric health of the piglets. After the seven day feeding period, seven of eight piglets fed low-protein diet had severe gastric ulcers which are expected to cause pain for the animals. Gastric ulcers in pigs can develop quickly, even in 12 hours, and healing can occur relatively quickly as well (Friendship 2004). Severe gastric ulcers can develop even in young pigs (Fossi et al. 2010). The piglets fed starch based diets with *Hermetia* meal had less gastric lesions and the most severe lesions were not found. The stomach contents of piglets fed low-protein diet were in liquid form with or without visible particles and the stomach contents of piglets fed *Hermetia* meal were mostly in liquid form with visible particles. Two piglets fed diet of mechanically extracted *Hermetia* meal had stomach contents in mushy form. According to Nielsen and Ingvartsen (2000), gastric ulcers can be prevented by feeding factors which increase the firmness of stomach contents. The starch in the experimental diets was very fine-grained. The fine feed structure and small particle size have been shown to increase the prevalence of gastric lesions in pigs (Mahan et al. 1966).

In this experiment, feeding *Hermetia* meal to piglets increased the size of liver and kidneys and the proportion of kidneys in relation to live weight. It remains unclear whether there were harmful substances in *Hermetia* meal which could result to the increased size of kidneys and whether the rate of increase on the size of kidneys is detrimental for the animal.

In conclusion, in this experiment two fat extraction methods were compared and the extraction method affects the AID and the SID of amino acids, as the digestibility values were lower in hexane extracted *Hermetia* meal compared to mechanically extracted *Hermetia* meal. The results indicate that mechanically extracted *Hermetia* meal provides highly digestible amino acids, which can improve the amino acid balance in organic feeds for piglets. *Hermetia* meal could diversify the protein supply for organic pig production, but the economic aspects of the production of *Hermetia* meal for pig feeding need to be explored. The fine-grained starch based feeds, especially the low-protein feed used for the determination of the basal endogenous losses of amino acids, caused gastric ulcers for the piglets. The research methods should be developed to minimize the disadvantages to animal welfare.

5 References

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6 Appendix

I References for analytical methods used

Dry matter

DM content was determined by drying at 105°C for 16 h.

Ash

Ash was determined by 600°C for 2 h or alternatively 510°C 16 h. (AOAC, 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Inc., Arlington, VA. 1298 p. ISBN 0-935584-42-0).

Ether extract after hydrolysis with 3M HCl

Accredited In-house methods No. 4.21 and 4.22: Determination by Soxcap-Soxtec-Analyzer. (AOAC Official Method 920.39 Fat (Crude) or Ether Extract in Animal Feed and Foss Tecator Application Note AN 390).

Nitrogen (Crude protein) by Kjeldahl method

Accredited methods 1120, 1122 and 1125 Kjeldahl; Standard methods (AOAC, 1990. Official Methods of Analysis. Association of Official Analytical Chemists, Inc., Arlington, VA. 1298 p. ISBN 0-935584-42-0) using Cu as a digestion catalyst and using Foss Kjeltec 2400 Analyzer Unit (Foss Tecator AB, Höganäs, Sweden).

Neutral detergent fibre (NDF) with filtering apparatus

by Van Soest, P.J., Robertson, J.B. and Lewis, B.A. 1991. Methods for dietary fibre, neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74: 3583-3597.
Sodium sulfite was used in NDF-detergent solution and α-amylase in case of samples containing starch. NDF is expressed without containing residual ash.

Acid Detergent fibre (ADF) and Lignin (Permanganate-lignin)

by Robertson, J.B. and Van Soest, P.J. 1981. The detergent system of analysis and its application to human foods. In: James, W.D.T. and Theander, O. (eds.). The Analyses of dietary Fibre in Foods. New York, NY, Marcell Dekker. p. 123-158.

Amino acids

In-house method No. 5000: Determination of amino acids (UPLC).
European Commission (1998). Commission Directive 98/64/EC. Community Methods of Analysis for the determination of amino acids, crude oils and fats, and olaquindox in feeding stuffs and amending Directive 71/393/EEC. Official Journal L 257, 19/09/1998 p. 14-28.
Total (peptide bound and free) amino acid analysis was performed with Biochrom 20 amino acid analyser (Biochrom Ltd, Cambridge, England) using Sodium Buffer –system.
Since 1.1.2009 the equipment used was Waters Finland MassTrak UPLC (Waters Corporation, Milford, U.S.A) and the application was UPLC Amino Acid Analysis Solution®.

In virto (pigs), apparent ileal digestibility of N and dry matter

by Boisen, S. and Fernàndez, J.A. 1995. Prediction of the apparent ileal digestibility of protein and amino acids in feedstuffs and feed mixtures for pigs by in vitro analyses. Animal Feed Science and Technology, 51: 29-34.

In vitro (pigs), total tract digestibility of organic matter

by Boisen, S. and Fernàndez, J.A. 1997. Prediction of the total tract digestibility of energy in feedstuffs and in pig diets by in vitro analyses. Animal Feed Science and Technology, 68: 277-286

Minerals and trace elements (Ca, P, K, Na, Mg, Mn, Fe, Cu, Zn, S)

by Luh Huang, C.-Y. and E.E. Schulte. 1985. Digestion of plant tissue for analysis by ICP emission spectrometry. Communications in soil science and plant analysis 16: 943-958.

Measurement was performed with ICP-OES (inductively coupled plasma optical emission spectrometry) (Thermo Jarrel Ash Iris Advantage, Franklin, USA).

Titanium

Digestion of samples for Titan analysis was made according to van Bussel, W., Kerkhof, F., van Kessel, T., Lamers, H., Nous, D., Verdonk, H., and Verhoeven, B. 2010. Accurate determination of Titanium as Titanium Dioxide for limited sample size digestibility studies of feed and food matrices by inductively coupled plasma optical emission spectrometry with real-time simultaneus internal standardization. Atomic Spectroscopy 31 (3): 81-88.

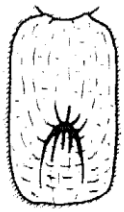
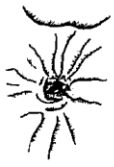
Sugars

Somogyi, M. 1945. A new reagent for the determination of sugars. Journal of Biological Chemistry 160: 61-68.

II The scale for the evaluation of the gastric lesions in pigs (Hautala and Rautiainen 1991)

The scale for the evaluation of the gastric lesions of the pig

OR=the oesophageal region
GR=the glandular region

	GRADES	A SCHEME OF THE OESOPHAGEAL PART	CHARACTERIZATION	NOTE
NO EFFECT ON THE WELFARE OF THE PIG	0		OR: A well demarcated skinlike area. Colours seen: white, yellow, grey as well as a mixture of two or even three of these colours. The surface undulates slightly.	GR: Lesions only limited to the mucosa are not included in the evalution. OR: The mild very minute preliminary changes can be differentiated only histologically from the grade 0 changes.
	I		OR: Roughness and a flaky scale in part of the area or in the whole area AND/OR the area is pitted (seen as streaky grooves or dark spotty holes) or minutely eroded AND/OR mature scarring without stenosis of the oesophageal opening.	GR: Lesions only limited to the mucosa are not included in the evaluation.
HAVE AN EFFECT ON THE WELFARE OF THE PIG	II		OR: Ulceration (= erosion down to the muscular part of the mucosa or even deeper) < 50% of the area. GR: Ulceration without haemorrhage.	
	III		OR: Ulceration ≥ 50% of the area AND/OR haemorrhagic ulceration AND/OR perforated ulcer AND/OR the wall of the stomach has clearly thickened and there is an inflammation of the serosa around the lesion. GR: Haemorrhagic or perforated ulcer.	
	Stenosis of the oesophageal opening		OR: The scarring has narrowed the opening of the oesophagus. At the same time there can be seen first, second or third grade lesions.	The stenosis feels like a rigid ring, when one or two fingers are pushed into the opening of the oesophagus.

III Photograph of dry *Hermetia* meal (Photo: Tapio Helenius)