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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: Mussel meal (*Mytilus edulis*)

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Uusien luomuvalkuaisrehujen aminohappojen sulavuus sioilla: Sinisimpukkajauho (*Mytilus edulis*)

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Tiivistelmä

Tutkimuksen tavoitteena oli määrittää uuden, luonnonmukaisesti tuotetun eläinperäisen rehuaineen, sinisimpukkajauhon (*Mytilus edulis*), ravintoaineiden kokonaissulavuus ja aminohappojen standardoitu ohutsuolisulavuus porsailla. Simpukkajauhon käyttö sikojen ruokinnassa ei toistaiseksi ole sallittua, mutta käytön mahdollisuutta tullaan käsittelemään EU-lainsäädännössä.

Kokeessa oli 24 porsasta, 13 imisää ja 11 leikkoa, jotka painoivat kokeen alussa keskimäärin 19 kg. Porsaat olivat duroc-, maatiais- ja yorkshirerotujen risteytyksiä. Totutusjaksoilla siat saivat luomuporsasrehua vapaasti porsimiskarsinassa (koejakso 0) ja toista luomurehua siirryttyään lihasikalaan kahden porsaan karsinoihin (koejakso 1). Aminohappojen ohutsuolisulavuuden määritystä varten (koejakso 2) siat siirrettiin tärkkelyspohjaiselle rehulle. Koeruokintoja oli kaksi: 1) vähäproteiininen rehu aminohappojen endogeenisen perustason erityksen määritystä varten, 2) rehu, jossa simpukkajauho oli ainoa valkuaisen lähde, 30 % rehun kuiva-aineessa (KA). Ryhmässä 1 oli 10 porsasta ja ryhmässä 2 oli 14 porsasta pariruokinnalla. Koesuunnitelmaa oli muutettava, koska simpukkaryhmän porsaat sairastuivat ripuliin. Ravintoaineiden kokonaissulavuutta ei myöskään voitu määrittää ripulin vuoksi. Simpukkarehua laimennettiin heravalkuaisjauhoa sisältävällä rehulla. Muutosten jälkeen koekäsittelyt olivat: 1) vähäproteiininen rehu aminohappojen endogeenisen perustason erityksen määritystä varten, 2) simpukkajauho 12 % rehun KA:ssa ja 3) simpukkajauho 18 % rehun KA:ssa. Jokaisessa käsittelyssä oli 8 sikaa pariruokinnalla. Kokeen lopussa siat lopetettiin aminohappojen ohutsuolisulavuuden määrittämistä varten 3,5 h kuluttua ruokinnasta. Ohutsuolesta eristettiin noin 60 cm pituinen osa umpisuolen liittymäkohdasta alkaen, ja suolen sisältö kerättiin analyysejä varten. Myös mahahaavan esiintymistä arvioitiin ja tyhjän mahalaukun, maksan ja munuaisten painot mitattiin.

Simpukkajauho sisälsi 684 g raakavalkuaista, 105 g raakarasvaa ja 94 g tuhkaa/kg KA. Lysiiniä oli 47,8 g, metioniinia 17,0 g , kystiiniä 8,4 g ja valiinia 29,8 g/kg ka. Koerehujen aminohappojen näennäiset ohutsuolisulavuudet (AID) olivat 69,9–84,9 % alemmalla simpukkatasolla ja 77,9–87,2 % ylemmällä simpukkatasolla. Alemmalla tasolla aminohappojen AID:n hajonta oli huomattavasti suurempaa kuin ylemmällä simpukkatasolla.

Simpukkajauhon välttämättömien aminohappojen AID oli 66,3 – 88,5 % alemmalla simpukan lisäystasolla ja 71,8 – 87,6 % ylemmällä simpukkatasolla. Lysiinin ja metioniinin AID oli parempi ylemmällä simpukkatasolla.

Aminohappojen endogeeninen perustason eritys oli tavanomaista suurempi tässä kokeessa, joten simpukkajauhon aminohappojen standardoitujen ohutsuolisulavuuksien (SID) laskemiseen käytettiin kolmen muun ICOPP-projektin sulavuuskokeen endogeenisen erityksen keskiarvoja. Koska aminonohappojen näennäisen ohutsuolisulavuuden hajonta oli suurta alemmalla simpukkatasolla, SID -arvot laskettiin käyttämällä ainoastaan suuremman simpukkatason havaintoja. Simpukkajauhon välttämättömien aminohappojen SID -arvot olivat 80,9 – 92,5 %. Lysiinin standardoitu ohutsuolisulavuus oli 89,7 %, metioniinin 89,1 %, kystiinin 71,3 %, treoniinin 80,9 % ja valiinin 89,7 %.

Lähes kaikilla vähäproteiinista rehua saaneilla porsailla oli vakava mahahaava, joka todennäköisesti aiheuttaa kipua porsaalle. Simpukkaryhmissä 62,5 – 75 %:lla sioista ei ollut mahahaavaa, tai mahan limakalvomuutokset olivat vain vähäisiä. Noin kolmasosalla simpukkaryhmän porsaista oli vakavia mahahaavoja. Porsaiden munuaisten paino ja munuaisten paino suhteessa elopainoon kasvoivat, kun simpukkajauhoa lisättiin rehuun.

Tulokset osoittavat sinisimpukkajauhon sisältävän runsaasti hyvin sulavia aminohappoja, joten sen avulla voisi parantaa luomuporsasrehujen aminohappotasapainoa. Sinisimpukkajauhon vaikutuksia porsaiden terveyteen tulisi vielä tarkemmin selvittää ruokintakokeissa. Sinisimpukkajauho 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. Aminohappojen endogeeninen perustason erityksen määrästä porsailla tarvitaan myös lisätutkimuksia.

Avainsanat:

Sika

Porsas

Sulavuus

Ohutsuolisulavuus

Aminohapot

Sinisimpukka

Mytilus edulis

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Abstract

The objective of this study was to determine the apparent total tract digestibility (ATTD) of nutrients and the standardised ileal digestibility (SID) of amino acids in organically produced mussel (*Mytilus edulis*) meal in growing piglets. The use of mussel meal in pig feeding is not allowed for the time being, but feed legislation in the EU concerning the use of mussel meal for pigs is in progress.

The experiment was carried out with a total of 24 growing pigs, 13 gilts and 11 barrows, with the initial body weight of ca. 19 kg. The pigs 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 diet was changed to another organic diet when piglets 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 two dietary treatments: 1) low-protein diet to determine the basal endogenous losses of amino acids, 2) diet in which only protein source was mussel meal, 30% (of diet DM). There were 10 piglets in group 1 and 14 piglets in group 2. Experimental design needed to be changed due to diarrhoea in piglets of the mussel meal group, and the mussel meal diet had to be diluted with a diet containing whey protein concentrate (WPC). After modification of the experimental design there were 3 dietary treatments in pair feeding: 1) low-protein diet to determine the basal endogenous losses of amino acids, 2) mussel meal level 1 (12% mussel meal of diet DM) and 3) mussel meal level 2 (18% mussel meal of diet DM) and 8 pigs per treatment. At the end of the trial, 3.5 h after the morning feeding, the pigs were stunned by bolt pistol, bled and ileal digesta was collected for digestibility determination. Stomachs were visually estimated for gastric ulcers and the weight of empty stomach, liver and kidneys were measured.

Mussel meal contained 684 g crude protein, 105 g crude fat and 94 g ash per kg DM. There was 47.8 g of lysine, 17.0 g of methionine, 8.4 g of cystine and 29.8 g of valine per kg diet DM in mussel meal. The apparent ileal digestibility (AID) of essential amino acids was 69.9–84.9% in

diet with 12% mussel meal and 77.9–87.2% in diet with 18% mussel meal. The variation of the AID of amino acids was clearly higher in the lower inclusion level of mussel meal compared to the higher inclusion level. The AID of the essential amino acids in mussel meal varied between 66.3–88.5% (mussel meal level 1) and 71.8–87.6% (mussel meal level 2). The AID of lysine and methionine was higher in diet with 18% mussel meal than in diet with 12% mussel meal.

In the present trial the basal endogenous losses of amino acids were remarkably high, and therefore the mean values of the basal ileal endogenous losses of amino acids from three other digestibility trials in the ICOPP project were used for the calculation of the SID of amino acids in mussel meal. Due to high variation and several divergent values in the AID of amino acids in mussel in the lower inclusion level, the SID values for mussel meal were calculated only for the higher inclusion level. The SID of the essential amino acids in mussel meal varied between 80.9%–92.5%. The SID values for lysine, methionine, cystine, threonine and valine were 89.7%, 89.1%, 71.3%, 80.9% and 89.7%, respectively.

Most of the piglets fed low-protein diet had severe gastric lesions in the oesophageal area which are expected to cause pain and reduce the welfare of the piglets. When mussel meal was added to the diets, 62.5 to 75.0% of the piglets had no gastric lesions or the lesions were only minor. Severe gastric lesions were found in approximately one third of piglets fed with mussel meal but no grade 3 lesions were found. The weight of kidneys and the weight of kidneys in relation to live weight increased when mussel meal was added to the diets.

In conclusion, results indicate that mussel meal provides highly digestible amino acids, which can improve the amino acid balance in organic feeds for piglets. The effects of mussel meal on the health of piglets need to be further explored in feeding trials. Mussel meal could diversify the protein supply for organic pig production, but the economic aspects of the production of mussel 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. The basal endogenous losses of amino acids in piglets also need further research.

Keywords:

Pig

Piglet

Digestibility

Apparent ileal digestibility

Standardised ileal digestibility

Amino acids

Mussel

Mytilus edulis

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1 Introduction

1.1 Background

Mussel meal could be one option for novel protein source in pig feeding. Some promising results have already been received from poultry experiments. Jönsson and Elwinger (2009) conducted an experiment with laying hens. They included 0, 3, 6 and 9% of mussel meal in the diets at the expense of fishmeal. The 6% inclusion of mussel meal tended to improve laying percentage compared to control group, and yolk pigmentation increased significantly when mussel meal was added to diets. They concluded that if it is feasible to produce mussel meal at a reasonable price, mussels might be a valuable protein source for poultry. Also Jänsson and Holm (2009) concluded mussel meal to be functioning protein source for laying hens, and even the possible toxin in mussels, akadaic acid, will not be detrimental for laying hens or eggs at level of 198.6 µg/ kg feed. Feed legislation in the EU concerning the use of mussel meal for pigs is in progress. In addition to a possible protein source, mussel meal has also an interesting double purpose of being also an effective means to clean sea waters. Mussels remove nitrogen from the water while generating seafood, fodder and agricultural fertilizer, thus recycling nutrients from sea to land (Lindahl et al. 2005). Knowledge on the protein value of mussel meal for pigs is scarce and needs to be explored.

1.2 Objectives

The objective of this study was to determine the apparent total tract digestibility (ATTD) of nutrients and the standardised ileal digestibility (SID) of amino acids in mussel (*Mytilus edulis*) meal in growing piglets.

2 Materials and methods

2.1 Test feedstuffs

The mussel meal was shipped to MTT from SLU (Swedish University of Agricultural Sciences, Sweden). The mussel meal was finely ground. Clots in meal broke down easily when the meal was mixed with water (Appendix III). Salmonella test was done before the experiment and the result was negative. The chemical composition of mussel meal analysed for the ICOPP database of organic feedstuffs is presented in Tables 1 and 2 (Kyntäjä et al. 2014).

Table 1. Chemical composition of mussel meal.

		Mussel meal
Dry matter	%	95.6
Ash	g/kg DM	96.4
Crude protein	g/kg DM	681
Crude fat	g/kg DM	115
Sugars	g/kg DM	8.4
NDF	g/kg DM	31
ADF	g/kg DM	0
Lignin	g/kg DM	0
Amino acids		
Essential		
Arginine	g/16 g N	6.6
Histidine	g/16 g N	1.7
Isoleucine	g/16 g N	3.9
Leucine	g/16 g N	6.2
Lysine	g/16 g N	7.0
Methionine	g/16 g N	2.3
Phenylalanine	g/16 g N	3.4
Threonine	g/16 g N	4.3
Valine	g/16 g N	4.2
Non-essential		
Alanine	g/16 g N	4.5
Aspartic acid	g/16 g N	9.5
Cystine	g/16 g N	1.1
Glutamic acid	g/16 g N	11.4
Glycine	g/16 g N	5.6
Proline	g/16 g N	3.6
Serine	g/16 g N	4.5
Tyrosine	g/16 g N	3.5
In vitro ileal digestibility		
DM	%	89.2
N	%	90.3
In vitro total tract digestibility		
OM	%	91.1

Table 2. Mineral composition of mussel meal.

		Mussel meal
Minerals		
Calcium	g/kg DM	3.8
Magnesium	g/kg DM	1.8
Phosphorus	g/kg DM	11.3
Sulphur	g/kg DM	10.5
Potassium	g/kg DM	6.4
Sodium	g/kg DM	21.2
Fe	mg/kg DM	339.6
Cu	mg/kg DM	6.5
Zinc	mg/kg DM	138.8
Manganese	mg/kg DM	29.4
Selenium	mg/kg DM	2.7

2.2 Animals and housing

The experiment was carried out with a total of 24 weaned piglets, 13 gilts and 11 barrows, with the initial body weight of ca.19.4 kg (std. 1.27). The piglets were 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 three times per day and water was available *ad libitum* from drinking nipples. Wood shavings were used as bedding material. No bedding material was used for the piglets during faecal spot sample collection period and 3 preceding days to avoid contamination with the feeds. Wooden toys were put in pens for enrichment. Similar arrangements were done before slaughter of the animals to collect ileal digesta.

2.3 Experimental design and treatments

The experiment was carried out according to a completely randomised design. A total of 24 piglets were randomly distributed to pens (2 piglets/pen) and the pens were randomly allotted to two dietary treatments. There were two treatment groups: 1) low-protein diet group to determine the basal endogenous losses of amino acids, 2) mussel meal group with 30% mussel meal of starch-based diet DM (Table 3). Number of pens in groups 1 and 2 were 5 and 7.

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 period 0 was adaptation period after weaning (4 days) and piglets received the same organic diet which was fed during the suckling period. Period 1 (7 days) was for adaptation to the new environment and new pen

mate. The piglets were fed with another organic diet. The purpose of period 2 was to determine basal endogenous losses of amino acids, the ATTD of nutrients and the SID of amino acids in mussel meal.

Due to diarrhoea in the mussel meal group in the beginning of period 2, the original experimental plan needed to be changed and the ATTD was not possible to measure. The treatments were modified as follows: 1) low-protein diet to determine basal endogenous losses of amino acids, 2) mussel meal level 1 (12% mussel meal of diet DM) and 3) mussel meal level 2 (18% mussel meal of diet DM). One pen (2 animals) from original group 1 was moved to mussel meal feeding, so that the number of pens in all treatments was 4 (Table 4). In the actual trial period 2 lasted for 6 days for low-protein diet group and 7 days for mussel meal groups.

Table 3. The original experimental treatments for the determination of the ATTD of nutrients and the SID of amino acids (period 2) in organically produced mussel meal.

Group	Period 0	Period 1	Period 2	Pens	Piglets
1) Low-protein	Organic diet 620	Organic diet 631	Starch based low-protein diet 634	5	10
2) Mussel meal	Organic diet 620	Organic diet 631	Starch based mussel meal diet 635 (30% mussel meal of diet DM)	7	14

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

Group	Period 2	Pens	Piglets
1) Low-protein	Starch based low-protein diet 634	4	8
2) Mussel meal Level 1	40% starch based mussel meal diet 635 (contained 30% mussel meal) + 60% starch based WPC diet 640. Mussel meal level 12%.	4	8
3) Mussel meal Level 2	60% starch based mussel meal diet 635 (contained 30% mussel meal) + 40% starch based WPC diet 640. Mussel meal level 18%.	4	8

WPC = Whey protein concentrate

2.4 Diets and feeding

During the period 0 piglets received organically produced feed for piglets. Dietary ingredients were oats (10%), wheat (32.3%), barley (10%) rapeseed expeller (11.3%) peas (24%), commercial organic protein concentrate (12%, RehuX) and some minerals. During the period 1 piglets received an organic diet consisting of wheat (37%), barley (5%), vegetable oil (0.9%), peas (18.3%), commercial organic protein concentrate (36.7%, RehuX), soybean expeller (2%) and vitamin E product (Biofarm).

The starch based low-protein diet in group 1 in period 2 was similar to the low-protein diet that was used in an organic silage digestibility trial of ICOPP project to determine the basal endogenous losses of amino acids. The original starch based mussel meal diet contained 30% mussel meal and the same ingredients as the starch based low-protein diet, excluding WPC and rapeseed oil. In the actual experiment, starch based mussel meal diet (no. 635) in the original group 2 was diluted with a new, starch based diet (no. 640) containing 21% of whey protein concentrate

(WPC) (Table 5). Daily feed proportions for the mussel meal groups were thereafter either 40% or 60% of starch based mussel meal diet 635 and 60% or 40% of starch based WPC diet 640 (Table 4). Then the corrected amounts of mussel meal were 12% and 18% of diet DM in groups 2 and 3.

The targeted crude protein (CP) content in the mussel meal diets was at least 160 g/kg DM, so that the recommended threshold level was obtained (Fan et al. 1995). Titanium dioxide was used as an indigestible marker in all of the period 2 feeds (3 g/ kg DM) (Table 5).

Table 5. Dietary ingredients of the starch based experimental diets.

	Period 2		
Dietary ingredients, g/kg:	Low-protein diet 634	Mussel meal diet 635	WPC[1] diet 640
Mussel meal		300	
Barley starch	767.4	562.5	687.4
WPC75	50		210
Sugar	80	80	
Cellulose	30	30	30
Rapeseed oil	35		35
Monocalciumphosphate	16.1	2.5	16.1
Limestone	14.5	18	14.5
Mineral-vitamin mixture [2]	4	4	4
Titanium dioxide	3	3	3

[1]WPC = Whey protein concentrate

[2] 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. At the end of period 1 the piglets were gradually switched to starch based diets of period 2. This transition lasted for 3 days. The daily allowance was increased by 200 g DM at the beginning of period 2, but it was lowered in mussel meal group shortly after period 2 started as nearly all piglets in mussel meal group had diarrhoea. The feed was first diluted with low-protein feed (no. 634) at experimental day 18. On day 19 the new feed (no. 640) was made to dilute the mussel meal diet and piglets were fed according to the new feeding plan (Table 4).

The piglets were fed three times daily, at 11:00, 15:00 and the rest of the feed portion the next morning at 7:00. Feeds were hand-weighed on daily basis and mixed before feeding. Water was added on top of feed. Feeding was graded at the last morning of the trial before slaughtering so that all piglets were slaughtered 3.5 h after the last feeding. The experimental procedures during periods 0, 1 and 2 are summarised in Table 6.

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

Day		Period	Diets and procedures	Unit	piglets/ pen	Date	NB.
1	Thu	Period 0, adaptation to dry feeding	Organic diet 620, weighing of the piglets	farrowing pen	litter	24.1.2013	
2	Fri	Period 0, adaptation to dry feeding	Organic diet 620	farrowing pen	litter	25.1.2013	
3	Sat	Period 0, adaptation to dry feeding	Organic diet 620	farrowing pen	litter	26.1.2013	
4	Sun	Period 0, adaptation to dry feeding	Organic diet 620, weighing of the piglets	farrowing pen	litter	27.1.2013	
5	Mon	Period 1, adaptation to fattening unit and pen mate	Organic diet 631, weighing of the piglets, distribution to the experimental groups	fattening unit	2piglets/ pen	28.1.2013	
6	Tue	Period 1, adaptation to fattening unit and pen mate	Organic diet 631	fattening unit	2piglets/ pen	29.1.2013	
7	Wed	Period 1, adaptation to fattening unit and pen mate	Organic diet 631	fattening unit	2piglets/ pen	30.1.2013	
8	Thu	Period 1, adaptation to fattening unit and pen mate	Organic diet 631	fattening unit	2piglets/ pen	31.1.2013	
9	Fri	Period 1, adaptation to fattening unit and pen mate	Organic diet 631	fattening unit	2piglets/ pen	1.2.2013	
10	Sat	Period 1, adaptation to fattening unit and pen mate	Organic diet 631	fattening unit	2piglets/ pen	2.2.2013	
11	Sun	Period 1, adaptation to fattening unit and pen mate	Organic diet 631	fattening unit	2piglets/ pen	3.2.2013	

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

Day		Period	Diets and procedures	Unit	piglets/ pen	Date	NB.
12	Mon	Gradual change to Period 2 feeding	Increasing daily ratio, low-protein diet 634 for the	fattening unit	2piglets/ pen	4.2.2013	
13	Tue	Gradual change to Period 2 feeding	low-protein feed group and starch based diet 635 for	fattening unit	2piglets/ pen	5.2.2013	
14	Wed	Gradual change to Period 2 feeding	mussel meal group	fattening unit	2piglets/ pen	6.2.2013	
15	Thu	Period 2, adaptation to starch based diets	Diets 634 and 635	fattening unit	2piglets/ pen	7.2.2013	
16	Fri	Period 2, adaptation to starch based diets	Diets 634 and 635, removal of bedding material	fattening unit	2piglets/ pen	8.2.2013	diarrhoea
17	Sat	Period 2, adaptation to starch based diets	Diets 634 and 635	fattening unit	2piglets/ pen	9.2.2013	diarrhoea
18	Sun	Period 2, adaptation to starch based diets	Diets 634 and 635, attempt to take faecal spot samples	fattening unit	2piglets/ pen	10.2.2013	dilution of feed 635 with diet 634
19	Mon	Period 2, adaptation to starch based diets	Decreasing of the daily ratio due to diarrhoea. Diets 634, 635 and 640. Attempt to take faecal spot samples	fattening unit	2piglets/ pen	11.2.2013	dilution of feed 635 with diet 640
20	Tue	Period 2, adaptation to starch based diets	Diets 634, 635 and 640	fattening unit	2piglets/ pen	12.2.2013	dilution of feed 635 with diet 640
21	Wed	Period 2, adaptation to starch based. diets	Diets 634, 635 and 640. Low-protein feed group: Staggering of feeding according to the slaughter schedule, weighing of the piglets, slaughtering of the group 1 piglets 3.5h after morning feeding	fattening unit	2piglets/ pen	13.2.2013	dilution of feed 635 with diet 640
22	Thu	Period 2, adaptation to starch based diets	Diets 635 and 640	fattening unit	2piglets/ pen	14.2.2013	dilution of feed 635 with diet 640
23	Fri	Period 2, adaptation to starch based diets	Diets 635 and 640	fattening unit	2piglets/ pen	15.2.2013	dilution of feed 635 with diet 640
24	Sat	Period 2, adaptation to starch based diets	Diets 635 and 640	fattening unit	2piglets/ pen	16.2.2013	dilution of feed 635 with diet 640
25	Sun	Period 2, adaptation to starch based diets	Diets 635 and 640	fattening unit	2piglets/ pen	17.2.2013	dilution of feed 635 with diet 640
26	Mon	Period 2, adaptation to starch based diets	Diets 635 and 640. Mussel meal groups: Staggering of feeding according to the slaughter schedule, weighing of the piglets, slaughtering of the piglets in groups 2 and 3 3.5h after morning feeding	fattening unit	2piglets/ pen	18.2.2013	dilution of feed 635 with diet 640

2.5 Slaughter of piglets and collection of feed and ileal digesta samples

Sub-samples of experimental feeds were collected during the last three days of period 2, pooled per treatment for the analyses of proximate composition, amino acids, and markers. Faecal spot samples could not be taken as nearly all piglets in the original mussel meal group got diarrhoea.

At the end of the trial, on experimental day 21, all piglets in the low-protein feed group were slaughtered 3.5 h after the morning feeding. The piglets were stunned by bolt gun, bled and ileal digesta was collected for digestibility determination. 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. All the piglets in the mussel meal groups were slaughtered five days after the low-protein feed group piglets due to the change in the experimental design. The collected 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

The apparent ileal digestibility (AID) of amino acids in the diets was calculated by marker method as follows:

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

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

The AID of amino acids in the test feed (mussel meal) was calculated by difference method as follows:

$$B = (C \times (X + Y) - A \times X)/Y$$

Where
B = AID of AA in test feed
C = AID of AA in whole diet
X = AA intake from basal feed
Y = AA intake from test feed
A = AID of AA in basal feed

In this experiment A = AID of AA in Whey powder, low lactose, ash > 210 g/kg, 8009.626/2/0 in the CVB feed table from the Netherlands (CVB 2011).

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

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

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

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

The data was analysed with SAS® for Windows (Version 9.2) using the MIXED procedure and a linear model with fixed effects of sex and treatment and sex-by-treatment interaction. The SID of amino acids in mussel meal was obtained by GLM procedure of SAS® containing the effect of sex. General mean value of the SID in gilts and barrows was used as the SID value of mussel meal and the standard error of mean was calculated by dividing the root MSE by the square root of the total number of observations.

3 Results

3.1 Chemical composition of the experimental diets and feedstuffs

The diet in period 0 contained dry matter 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 diet in period 1 contained dry matter 878 g/kg, ash 59 g/kg DM, crude protein 216 g/kg DM and crude fibre 36 g/kg DM.

The analysed chemical composition of the final experimental diets in period 2 is presented in Table 7 and the analysed chemical composition of mussel meal is presented in Table 8.

Table 7. Analysed chemical composition of the experimental diets, g/kg DM.

	Low-protein diet 634	Mussel meal diet 635*	WPC diet 640*
Dry matter g/kg	923	924	926
Ash	40	56	39
Crude protein	30	217	109
Amino acids, g/kg DM			
Essential			
Arginine	0.9	13.5	2.7
Histidine	0.5	3.8	2.3
Isoleucine	1.9	8.8	7.2
Leucine	3.2	14.0	12.4
Lysine	2.7	15.3	10.5
Methionine	1.0	5.7	3.2
Phenylalanine	1.0	7.6	3.8
Threonine	2.1	9.7	8.0
Valine	1.8	9.5	14.4
Non-essential			
Alanine	1.5	10.0	5.7
Aspartic acid	3.5	20.8	12.8
Cystine	0.6	2.3	2.4
Glutamic acid	5.5	25.9	20.2
Glycine	0.8	13.0	2.4
Proline	1.9	8.2	6.9
Serine	1.6	9.9	6.0
Tyrosine	0.8	7.4	3.2

* Group 2: 400 g/kg DM mussel meal diet 635 + 600 g/kg DM WPC diet
Group 3: 600 g/kg DM mussel meal diet 635 + 400 g/kg DM WPC diet
Mussel meal diet 635 contained 30% mussel meal.

Table 8. Analysed chemical composition of mussel meal, g/kg DM.

	Mussel meal
Dry matter g/kg	956
Ash	94
Crude protein	684
Crude fat	105
NDF	59
ADF	17
ADF-N	1
Amino acids, g/kg DM	
Essential	
Arginine	45.0
Histidine	11.9
Isoleucine	27.0
Leucine	43.1
Lysine	47.8
Methionine	17.0
Phenylalanine	23.3
Threonine	30.6
Valine	29.8
Non-essential	
Alanine	31.7
Aspartic acid	65.1
Cystine	8.4
Glutamic acid	81.0
Glycine	39.4
Proline	25.7
Serine	31.3
Tyrosine	23.6

3.2 Apparent and standardised ileal digestibility of amino acids

The effect of mussel meal level on the AID of amino acids in experimental diets is presented in Table 9. The AID of essential amino acids was 69.9–84.9% in diet with 12% mussel meal and 77.9–87.2% in diet with 18% mussel meal. The variation of the AID values was much higher in diet containing 12% mussel meal (coefficient of variation, CV, for essential amino acids was 5.7−20.2% and for non-essential amino acids 16.0−76.7%) than in diet with 18% mussel meal (CV for essential amino acids was 3.9−7.5% and for non-essential amino acids 4.2−10.5%). The mussel meal level did not have a significant effect on the AID of most of the essential amino acids. Only the AID of lysine and arginine was higher in the diet with 18% mussel meal compared to the diet with 12% mussel meal (lysine 87% vs. 80% and arginine 87.2% vs. 77.3%)). The AID of all the non-essential amino acids, except tyrosine, was higher in piglets fed diet with 18% mussel meal compared to piglets fed diet with 12% mussel meal.

Table 9. The effect of mussel meal level on the AID of the amino acids in starch based experimental diets.

	Mussel meal inclusion			
	12%	18%	SEM	p diet
n	7	8		
Essential				
Arginine	77.3	87.2	3.22	**0.04**
Histidine	72.7	82.4	3.63	*0.08*
Isoleucine	81.7	83.7	2.15	0.52
Leucine	82.4	84.5	2.19	0.49
Lysine	80.0	87.0	2.40	**0.05**
Methionine	81.6	85.2	1.65	0.13
Phenylalanine	74.6	80.0	3.26	0.25
Threonine	69.9	77.9	3.21	*0.09*
Valine	84.9	85.9	1.88	0.70
Non-essential				
Alanine	69.6	79.6	3.24	**0.05**
Aspartic acid	70.3	81.0	3.61	**0.05**
Cystine	56.8	72.5	7.89	**0.04**
Glutamic acid	67.0	81.6	4.14	**0.03**
Glycine	46.0	76.0	8.67	**0.03**
Proline	69.7	82.1	3.78	**0.04**
Serine	70.1	78.8	2.96	**0.05**
Tyrosine	67.0	76.6	5.63	0.24

The effect of sex on the AID of the amino acids in experimental diets is presented in Table 10. There were no differences in the AID between gilts and barrows. The AID of cystine tended to be higher in gilts than in barrows (p=0.07).

Table 10. The effect of sex on the AID values of the amino acids in mussel meal diets.

	Gilts	Barrows	SEM	p sex
n	8	7		
Essential				
Arginine	84.8	79.7	3.22	0.27
Histidine	81.1	74.0	3.63	0.18
Isoleucine	83.4	82.0	2.15	0.65
Leucine	84.5	82.4	2.19	0.50
Lysine	85.8	81.1	2.40	0.17
Methionine	84.0	82.9	1.65	0.62
Phenylalanine	79.0	75.6	3.26	0.45
Threonine	76.6	71.2	3.21	0.24
Valine	86.3	84.4	1.88	0.47
Non-essential				
Alanine	77.5	71.8	3.24	0.22
Aspartic acid	79.2	72.0	3.61	0.17
Cystine	71.3	58.0	4.89	*0.07*
Glutamic acid	78.7	69.8	4.14	0.14
Glycine	70.1	51.9	8.67	0.15
Proline	78.8	72.9	3.78	0.28
Serine	77.0	71.9	2.96	0.23
Tyrosine	75.4	68.2	5.63	0.37

The interaction of mussel meal inclusion level and sex is presented in Table 11. The AID of lysine was higher in gilts than in barrows when inclusion level was lower. When mussel meal inclusion level was higher, the AID of lysine was higher in barrows than in gilts. Similar effect was found in the AID of aspartic acid, cystine and glutamic acid.

Table 11. The interaction effect of level and sex on the AID of the amino acids in mussel meal diets.

	Mussel meal inclusion					
	12%		18%			
	Gilts	Barrows	Gilts	Barrows	SEM	p diet x sex
n	4	3	4	4		
Essential						
Arginine	83.6	70.9	86.0	88.4	4.87	0.11
Histidine	81.0	64.5	81.2	83.6	5.49	*0.08*
Isoleucine	84.5	78.9	82.2	85.1	3.25	0.18
Leucine	85.5	79.2	83.4	85.6	3.30	0.18
Lysine	86.5	73.4	85.2	88.8	3.63	**0.03**
Methionine	83.5	79.7	84.5	86.0	2.50	0.26
Phenylalanine	79.5	69.7	78.5	81.4	4.93	0.18
Threonine	76.7	63.0	76.4	79.4	4.85	*0.08*
Valine	87.9	81.9	84.8	86.9	2.85	0.14
Non-essential						
Alanine	77.1	62.2	77.9	81.3	4.89	*0.06*
Aspartic acid	79.3	61.2	79.1	82.8	5.46	**0.05**
Cystine	72.9	40.7	69.8	75.3	7.39	**0.02**
Glutamic acid	78.0	55.9	79.4	83.7	6.26	**0.04**
Glycine	67.2	27.9	73.0	79.0	13.11	*0.07*
Proline	76.5	62.8	81.1	83.0	5.72	0.16
Serine	76.6	63.6	77.5	80.1	4.48	*0.08*
Tyrosine	74.1	59.9	76.6	76.6	8.50	0.38

The AID of amino acids in mussel meal was calculated by difference method using table values of AID (whey powder, low lactose, CVB 2011, Table 12) for basal feed in which whey protein powder (WPC) was the only protein source. The original mussel meal diet did not contain other protein sources but due to diarrhoea problems the amount of mussel meal needed to be reduced and WPC was added to basal feed to maintain sufficient protein level in the two new diets. Due to high variation of the AID of amino acids in diet with 12% mussel meal, calculation of the AID by regression method was not reasonable either.

The AID and the SID of amino acids in mussel meal was calculated with two different data; using observations from both mussel meal inclusion levels and using observations only from the higher level. However, due to variation of the AID values in the lower inclusion level, several divergent observations needed to be rejected. Table 12 shows that the AID of the essential amino acids varied between 66.3–88.5% in mussel meal level 1 and 71.8–87.6% in mussel meal level 2. The AID of lysine and methionine was higher ($p<0.05$) and the AID of arginine and cystine tended to be higher in diet with 18% mussel meal than in diet with 12% mussel meal.

Sex of the piglet had no effect on the AID values of mussel meal. Interaction with mussel meal level and sex of the piglet was detected with the AID of lysine. Gilts had higher AID values of lysine in lower inclusion level compared to barrows and barrows had higher AID values in higher mussel meal inclusion level compared to gilts (p=0.07).

Table 12. The effect of mussel meal level on the AID of amino acids in mussel meal (observations from groups 2 and 3). The AID values of whey powder was used as basal feed.

	Mussel meal inclusion						Whey powder
Amino Acid	12%	n	18%	n	SEM[1]	p treatment	(CVB 2011)
Essential							
Arginine	81.4	6	87.6	8	2.61	*0.09*	84
Histidine	78.9	5	80.5	8	3.56	0.71	87
Isoleucine	78.6	6	80.8	8	3.04	0.58	89
Leucine	79.2	6	81.3	8	2.80	0.58	90
Lysine	74.9	6	85.2	8	3.66	**0.05**	91
Methionine	74.3	7	83.5	8	2.73	**0.03**	90
Phenylalanine	73.4	6	77.7	8	3.21	0.33	87
Threonine	66.3	5	71.8	8	3.64	0.15	88
Valine	88.5	6	84.8	8	3.16	0.39	87
Non-essential							
Alanine	72.7	5	76.9	8	3.95	0.39	87
Aspartic acid	75.3	5	78.1	8	4.70	0.62	88
Cystine	43.5	5	61.3	8	7.43	*0.07*	89
Glutamic acid	71.9	4	78.3	8	5.24	0.33	88
Glycine	78.6	4	76.6	8	4.49	0.71	71
Proline	77.0	5	81.5	8	7.63	0.63	83
Serine	71.2	5	76.4	8	3.43	0.23	85
Tyrosine	69.4	6	73.9	8	3.90	0.39	86

n = number of observations
[1]SEM is presented for the lowest number of observations.

The basal endogenous losses of amino acids (g/kg DM intake) are presented in Table 13. In the present trial the endogenous losses were clearly higher than in the other three digestibility trials done in the ICOPP project. Therefore, the mean values of the basal ileal endogenous losses of amino acids from three other digestibility trials in ICOPP project were used for the calculation of the SID values of mussel meal. 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 13.

Table 13. Mean flow of basal ileal endogenous amino acids (g/kg DM intake).

	Mussel meal experiment		Other ICOPP experiments in MTT[1]		Jansman et al. (2002)[2]
	Mean	Std.	Mean	Std.	Mean
Essential					
Arginine	0.65	0.24	0.45	0.01	0.36
Histidine	0.33	0.11	0.23	0.06	0.21
Isoleucine	0.63	0.20	0.44	0.06	0.51
Leucine	1.03	0.30	0.70	0.10	0.54
Lysine	0.86	0.27	0.60	0.10	0.44
Methionine	0.51	0.17	0.26	0.08	0.12
Phenylalanine	0.59	0.17	0.39	0.06	0.36
Threonine	1.15	0.37	0.77	0.11	0.72
Valine	0.81	0.26	0.56	0.07	0.74
Non-essential					
Alanine	0.95	0.37	0.60	0.04	0.56
Aspartic acid	1.57	0.48	1.05	0.13	0.95
Cystine	0.33	0.07	0.24	0.07	0.28
Glutamic acid	1.93	0.60	1.39	0.27	1.75
Glycine	1.84	0.83	0.98	0.15	0.70
Proline	1.86	1.44	1.33	0.88	0.76
Serine	1.06	0.30	0.73	0.10	0.91
Tyrosine	0.60	0.20	0.41	0.04	0.30

[1]Mean values of the basal ileal endogenous losses of amino acids from three other digestibility trials in ICOPP project were used for the calculation of the SID values of mussel meal.
[2] Basal ileal endogenous losses of amino acids from diets with casein/wheat gluten.

The SID values for mussel meal are presented in Table 14. Due to high variation and several divergent values in the AID of amino acids in mussel in the lower inclusion level, the SID values of mussel meal were calculated only for the higher inclusion level. The SID of the essential amino acids in mussel meal varied between 80.9%–92.5%.

Table 14. The SID values of the amino acids in mussel meal (observations from group 3).

Amino Acid	Mussel meal	n.	SEM
Essential			
Arginine	92.5	8	1.80
Histidine	87.7	8	2.23
Isoleucine	86.2	8	2.74
Leucine	86.5	8	2.49
Lysine	89.7	8	1.56
Methionine	89.1	8	1.99
Phenylalanine	84.1	8	2.94
Threonine	80.9	8	2.36
Valine	89.7	8	2.77
Non-essential			
Alanine	84.1	8	2.18
Aspartic acid	84.1	8	2.31
Cystine	71.3	8	4.47
Glutamic acid	84.2	8	3.14
Glycine	87.8	8	2.50
Proline	95.8	5	2.06
Serine	85.1	8	2.08
Tyrosine	81.1	8	3.72

n = number of the observations

3.3 Gastric health and organ weight

Effect of feeding with starch based low-protein diet and two starch based diets with mussel meal on incidence and severity of gastric lesions and the results of visual evaluation of stomach contents are presented in Table 15. Most of the piglets 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 II). Their stomach contents were in liquid form without visible particles. When mussel meal was added to the diets, 62.5 to 75.0% 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). Severe gastric lesions were found in piglets fed with mussel meal but grade 3 lesions were not found. The stomach contents were in liquid form but feed particles were also present.

Table 15. The incidence and severity of gastric lesions and consistency of digesta in stomach of piglets fed starch based low-protein diet and diets containing mussel meal.

	Starch based diets					
	Low-protein		Mussel meal 12%		Mussel meal 18%	
	n[1]	%[2]	n	%	n	%
Severity of gastric lesions						
Grade 0	0		0		1	12.5
Grade 1	1	12.5	5	62.5	5	62.5
Grade 2	2	25.0	3	37.5	2	25.0
Grade 3	5	62.5	0		0	
Consistency of digesta in stomach[3]						
Grade 1	8	100.0	0		0	
Grade 2	0		8	100.0	8	100.0
Grade 3	0		0		0	

[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 16. The live weight and the weight of empty stomach was the lowest in the piglets fed low-protein diet before slaughter. Compared to piglets fed low-protein diet, the weight of empty stomach and live weight of the piglet were higher in piglets fed mussel meal diets but values were similar between mussel meal groups. There were no differences in percentage of stomach weight of the live weight. The weight of liver and 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 mussel meal was added to the diets.

Table 16. The effect of diet on the live weight and organ weights of piglets.

	Starch based diets				
	Low protein	Mussel meal 12%	Mussel meal 18%	SEM	p diet
n piglets	8	8	8		
Live weight, kg	25.0[a]	29.2[b]	31.5[b]	1.07	**0.001**
Empty stomach, kg[1]	0.167[a]	0.191[b]	0.202[b]	0.007	**0.003**
Empty stomach, % of live weight	0.67	0.65	0.64	0.02	0.65
Liver, kg[1]	0.797	0.844	0.886	0.004	0.26
Liver, % of live weight	3.21	2.90	2.81	0.14	0.13
Kidneys, kg[1]	0.102[a]	0.163[b]	0.213[c]	0.007	**0.001**
Kidneys, % of live weight	0.41[a]	0.56[b]	0.68[c]	0.02	**0.001**

[1]Stomach, liver and kidneys were weighed immediately after slaughter, 3.5 h after feeding.
[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 17. Gilts had less severe gastric lesions than barrows (30.8% vs. 72.8%). The live weight, the weight of empty stomach, liver and kidneys and their proportion of live weight did not differ between gilts and barrows but the weight of kidneys in relation to live weight tended to be higher in barrows than in gilts (Table 18).

Table 17. The effect of sex on incidence and severity of gastric lesions and consistency of digesta in stomach.

	Gilts		Barrows	
	n[1]	%[2]	n	%
Severity of gastric lesions				
Grade 0	0		1	9.1
Grade 1	9	69.2	2	18.2
Grade 2	1	7.7	6	54.6
Grade 3	3	23.1	2	18.2
Consistency of digesta in stomach[3]				
Grade 1	4	30.8	4	36.4
Grade 2	9	69.2	7	63.6
Grade 3	0		0	

[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 18. The effect of sex on the live weight and organ weights of piglets.

	Gilts	Barrows	SEM	p sex
n piglets	13[2]	11		
Live weight, kg	28.5	28.6	0.89	0.98
Empty stomach, kg[1]	0.186	0.187	0.005	0.86
Empty stomach, % of live weight	0.65	0.66	0.02	0.80
Liver, kg[1]	0.829	0.856	0.003	0.53
Liver, % of live weight	2.92	3.03	0.12	0.51
Kidneys, kg[1]	0.153	0.166	0.006	0.13
Kidneys, % of live weight	0.53	0.57	0.02	*0.09*

[1]Stomach, liver and kidneys were weighed immediately after slaughter, 3.5 h after feeding.

4 Discussion and conclusions

The content of crude protein, crude fat and ash in mussel meal was comparable to the results of Jönsson and Elwinger (2009) and Berge and Austreng (1989). Okumuş and Stirling (1998) studied seasonal variation in the composition of mussels and found that the organic matter of mussel meat contained 518−824 g/kg DM crude protein, 26−127 g/kg DM crude fat and 86−358 g/kg DM carbohydrates. The ash content of mussels was 42−142 g/kg DM. The mussel meal in the present study also contained small amounts of NDF and ADF but in other studies fibre content of mussels is seldom analysed. The content of ADF-N which represents nitrogen not available for the animal was negligible. The carbohydrates in mussel meal are mainly glycogen which the mussels utilize when the supply of food is low. The amino acid content in mussel meal was comparable to earlier studies. Jönsson and Elwinger (2009) reported slightly higher content of amino acids and Berge and Austreng (1989) slightly lower values in case of some amino acids than in the present study.

The content of crude protein and crude fat in mussel meal in the present trial was comparable to feed table values for fish meal. The content of ash was slightly lower in mussel meal compared to fishmeal. The content of lysine, methionine and threonine in mussel meal was comparable to the table values presented for fishmeal. The content of cystine in mussel meal was slightly higher and the content of valine was slightly lower than in fishmeal (CVB 2011, EvaPig 2008).

The original aim was to feed mussel meal as the only source of protein in the diet (300 g/kg DM). However, the piglets got diarrhoea soon after offering the mussel meal diet. There were also many other changes for the piglets during a short period of time: weaning, change from the company of littermates to pair feeding with unfamiliar pen mate, change of diet, environment and temperature. However, the mussel meal feeding could be one reason for diarrhoea as the piglets fed low-protein diet did not suffer from diarrhoea. The lower inclusion level of mussel meal in the final experimental diet (12% of DM) remained rather low which can be one reason for the high variation in the AID of amino acids in that diet. Due to this variation, the SID values were calculated only for the higher inclusion level (18% of DM).

The flow of basal ileal endogenous amino acids is needed for the calculation of the SID of amino acids from the AID values. In the present trial the basal endogenous losses of amino acids in piglets were high compared to the earlier results determined with older pigs fed diets containing highly digestible protein sources (e.g. Jansman et al. 2002). Therefore, the mean values from three other digestibility trials in the ICOPP project were used for the calculation of the SID values of mussel meal. There is limited data available on the flow of basal ileal endogenous amino acids in young pigs (<30 kg live weight). Feeding level and length of the pre-test period may also affect the basal endogenous losses of amino acids.

The AID and the SID of essential amino acids were rather high in mussel meal (AID 66.3–88.5% and SID 80.9%−92.5%). The SID of cystine was the lowest (71.3%). The SID of lysine, methionine, valine and histidine was comparable to the SID values of fish meal in feed tables but

the SID of other essential amino acids was lower than in fish meal (CVB 2011, EvaPig 2008). Compared to plant based protein sources, the SID of lysine, methionine and valine was comparable to that of soybean meal but the SID of other essential amino acids was lower. However, mussel meal had clearly higher SID of amino acids than peas (CVB 2011, EvaPig 2008).

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 mussel 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 and piglets fed mussel meal had also visible particles in their stomach contents. 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 mussel meal to piglets increased the size of kidneys and the proportion of kidneys in relation to live weight. It remains unclear whether there were harmful substances in mussel 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, results indicate that mussel meal provides highly digestible amino acids, which can improve the amino acid balance in organic feeds for piglets. The effects of mussel meal on the health of piglets need to be further explored in feeding trials. Mussel meal could diversify the protein supply for organic pig production, but the economic aspects of the production of mussel 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. The basal endogenous losses of amino acids in piglets also need further research.

5 References

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6 Appendix

I References for analytical methods

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 vitro (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.

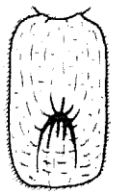
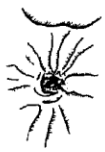
Sugars

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 mussel meal (Photo: Tapio Helenius)