Mussel Meal in Poultry Diets – with Focus on Organic Production

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Abstract

The first limiting nutrients for poultry are the sulphur containing amino acids, particularly methionine. To fulfil the recommended requirement, conventional diets are supplemented with synthetic methionine. Since this is not allowed in organic production it becomes important to have access to alternative high quality protein feed ingredients. The aim of this thesis was to investigate whether blue mussels (*Mytilis edulis*) could be used as a protein source in diets for organic poultry and determine whether the amount of fish meal commonly used today in organic diets could be replaced with mussel meal.

Four experiments during both short time periods and during whole production cycles were conducted to evaluate production performance, egg quality and animal health when using mussel meal in the diets. Additionally, the effects of the mussel toxin okadaic acid ( ) were evaluated. is a toxin that unpredictably appears in mussels some years. The experiments were performed both in laying hens and in broiler chickens and for laying hens, both in furnished cages and floor systems. Inclusion levels of mussel meal in the diets of up to % for broiler chickens and % for laying hens were used.

Mussel meal in the diets did not affect production performance for laying hens or broiler chickens in any of the experiments. at moderate level used in diets for laying hens did not negatively affect the birds and no was detected in the egg yolk. When extremely toxic mussels were included in the diet, broiler chickens got diarrhoea, lower growth rate and feed intake than birds fed the control diet.

Egg yolk pigmentation differed significantly between diets in all experiments with laying hens. The egg yolk was more darkly coloured when the hens were fed a diet containing mussel meal. Off flavour and off odour did not differ in any eggs. In one experiment plumage conditions was evaluated and an improved total score was observed when mussel meal was included in the diet.

These results indicate that mussels may be a good and high quality protein source for poultry and may replace fish meal in organic diets for laying hens and broiler chickens.

Keywords: Mussel meal, fish meal, protein source, amino acids, organic production, laying hens, broiler chickens, okadaic acid, intestinal morphology.

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När du tittar på dina båda tassar, behöver du bara bestämma dig för vilken som är den högra, sedan kan du vara säker på att den andra är den vänstra.
Nalle Puh - A.A. Milne

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Jönsson, L. and Elwinger, K. ( ). Mussel meal as a replacement for fish meal in feeds for organic poultry – a pilot short term study. *Acta Agriculturae Scandinavica Section A. Animal Science* , – .

II Jönsson, L. and Holm, L. ( ). Effects of toxic and non-toxic blue mussel meal on health and product quality of laying hens. *Journal of Animal Physiology and Animal Nutrition*, In Press, DOI: . /j. – . . .x.

III Jönsson, L. and Waldenstedt, L. ( ). Blue mussel *(Mytilus edulis)* meal as a protein source in diets for broiler chickens (submitted).

IV Jönsson, L., Wall, H. and Tauson, R. ( ). Production and egg quality in layers fed organic diets with mussel meal (submitted).

Abbreviations

aa	Amino acids
CFU	Colony forming units
DHA	Docosahexaenoic acid (:)
DM	Dry matter
DPA	Docosapentaenoic acid (:)
DST	Diarrheic shellfish toxins
DTX-1	35-methylokadaic acid dinophysistoxin-1
E coli	*Escherichia coli*
EPA	Eicosapentaenoic acid (:)
FCR	Feed conversion ratio
GMO	Gene modified organism
HPLC	High performance liquid chromatography
HW	Hyline white, -
LSL	Lohmann selected leghorn
N	Nitrogen
OA	Okadaic acid
P	Phosphorous
PCB	Polychlorinated biphenyls
PST	Paralythic shellfish toxin
SD	Standard deviation
wk	Week

1 Introduction

1.1 Organic poultry production and regulations

In the past years there has been a rapid increase in organic production in Sweden as well as in many other countries. The increase has been a consequence of both a higher consumer demand for food produced in a certain way i.e. produced sustainably and with an expected high animal welfare (Blair,) and governmental goals. The goal for organic poultry production in Sweden is that % of the egg production and % of the broiler production should be produced organically (Report from The Swedish Government, / :). The overall aim of organic production is to produce safe food in a sustainable ecosystem with a low impact on the environment and with a high animal welfare.

Organic production is regulated and guided in general principles, recommendations and basic standards by the International Federation of Organic Agriculture Movements (,), an umbrella organisation which gathers certifiers from many countries in the world. The basic standards are the minimum requirements that a producer must meet to be certified as organic. The Swedish Animal Welfare Ordinance is the basis for all animal production, including organic production, in Sweden. In addition to this, organic animal production in Sweden is regulated by the European Council regulations (,) and by the organisation , both derived from the . The standards are a minimum and in some parts 's regulation are stricter. To ensure that the legislation is followed and that a high quality of the organic products is achieved, the production must be certified by a control organ accredited by the Swedish Board of Agriculture and National Food Administration. is the main accreditation organisation in Sweden but there are a few other certifiers on the market. For an overview of organic control organisations see *Figure* . The organic poultry production in Sweden today comprises approximately laying hens and broiler chickens per year corresponding to about % and < . % of the total production respectively.

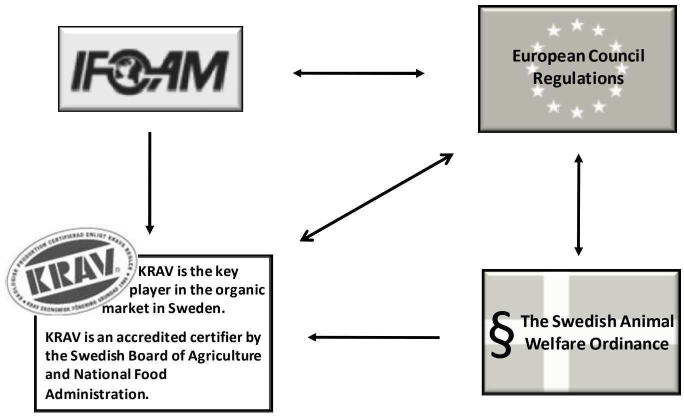

Figure 1. An overview of the organic control organisations.

1.2 Animal feeding

According to the regulations as well as the feed should be of high hygienic quality and the nutrient content should be adjusted to the animal's requirement. Restrictions on the use of feed ingredients in organic diets are as follows:

- No genetically modified grain, grain by-products or organisms, neither as feed ingredient, feed additives nor in the processing of feed ingredients.
- No antibiotics, hormones or drugs.
- No animal by-products, except milk products and fish meal.
- No grain by-products unless produced from certified organic crops.
- No chemically extracted feeds, such as solvent extracted soybean meal or other meals.
- No pure amino acids.
- Self-sufficiency on farms should be at least % on a year basis.
- The animals should have free access to roughage e.g. hay or vegetables and access to an outdoor run during the growing season.

1.3 Protein requirement and amino acid supply

Due to their plumage poultry have higher requirements of sulphur amino acids than any other food producing animals. Poultry feed are mainly based on cereals which are relatively low in protein content, especially methionine content, compared to the birds' needs. In conventional diets this problem is solved by adding pure (synthetic) amino acids but in organic production such additions are not allowed according to international (,) and Swedish standards, ( ). Fish meal is rich in sulphur amino acids and is frequently used in some organic diets today. However, overfishing of the seas, an intensive debate regarding the use of fish meal in feeds for animals and increasing costs will probably limit the availability of fish meal in the future.

The aim is to use % organically approved feed ingredients in diets for organic poultry. Today it is allowed to use % conventional ingredients because of the lack of available feed sources rich in protein and especially in the amino acid methionine. To fulfil the birds' requirement of methionine potato protein and corn gluten meal are used in the remaining % today. Successively this allowed conventional proportion will be phased out and by % of the ingredients must be organically approved. Today there is a limited number of organically approved protein sources available to solve the problem with fulfilling the birds' requirement of sulphur amino acids. Thus, if the organic poultry production is to survive in the future it is important to find new potential protein sources rich in methionine (Wilson, ; Elwinger *et al.*, ; Hammershøj & Steenfeldt, ; Elwinger & Wahlström,).

Shortage of good quality protein sources may jeopardize both animal health and production. Tiller ( ) showed that methionine deficiency in floor kept birds may cause severe welfare problems such as feather pecking and cannibalism, and Elwinger et al. ( ) found that a low methionine feed content impairs feather condition and reduces egg weight. Impaired feather cover results in body heat losses and in turn higher feed intake (Peguri & Coon, ; Tauson & Svensson,). Thus, the fulfilment of birds' methionine requirement is crucial both from a welfare and an economic point of view.

1.4 The Agro–Aqua cycle

For several reasons, meat from blue mussels (*Mytilus edulis*) may be an interesting alternative to fish meal. Mussels have a high content of protein with an amino acid pattern similar to fish meal (Jönsson & Elwinger, ; Berge & Austreng,), see *Table 1*, and have the possibilities to be approved according to the organic standards. Mussels are filter feeders and feed on phytoplankton and organic material. Under favourable conditions one mussel can filter - litres of water per hour (Lindahl *et al.*,). This means that mussels during the growth period have the capability to filter large volumes of coastal waters. In contrast to fish farms where feed is added the mussels utilize nutrients in the water through algae and plankton. In this process mussels contribute to the cleaning of these waters when an oversupply of nitrogen and phosphorous has leaked from the surrounding agricultural industry. See *Figure* for illustration of the Agro-Aqua cycle.

Modern mussel farming has been established in Sweden since the early ’s. The method most frequently used for mussel farming in Sweden is off-bottom cultivation with long-line systems where mussels attach to ropes hanging from a rope back-bone supported by large plastic floats. The production capacity of an average Swedish long-line unit is about - tons of mussels during a normal farming cycle of months. Each unit occupies a water surface area of about m^2 (Lindahl et al.,). Considering that approximately % and . % of the wet weight of mussels are nitrogen ( ) and phosphorous ( ), respectively (Lutz,), one unit of a mussel farm bring back about . tons and kg of from sea to land in an - month period.

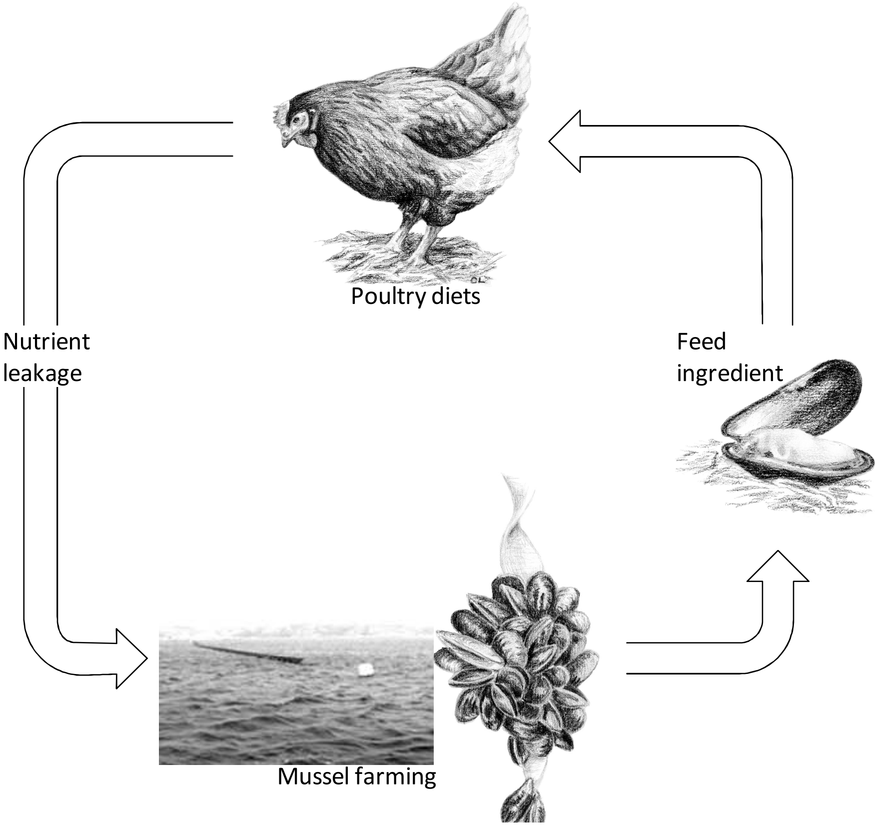

Figure 2. Recycling of nutrients in the Agro-Aqua system. (*Illustration by Cecilia Lindahl.)*

Eutrophication of coastal waters is a serious environmental problem. Hence, mussel filtration of the water creates a unique procedure where mussels recycle nutrients from sea to land. Thus, mussels could be a sustainable method for producing feed of high nutritional value and at the same time cleanse the coastal waters from and . Mussels from the Swedish West Coast, Limfjorden (Denmark) and Sognefjord (Norway), were used in the experiments included in this thesis, but in Sweden the future aim is to use locally produced mussels from the Swedish West Coast. Mussel farming may also be of environmental importance for the Baltic Sea and other regions where cultivation and harvesting are possible, for example in Lake Mälaren.[1]

[1] Personal communication, W. Goedkoop, .

Table 1. *Analysed protein, crude fat, ash and amino acid content of the mussel meal used in Paper I [A] and comparative data from Berge and Austreng ( ) using rainbow trout [B], Lutz ( ) [C] and Degussa*[1] *[D].*

	Mussel meal					Fish meal, (g aa/16 gN)	
		A		B	C	B	D
	g/kg DM	SD	g aa/16 gN				
Crude protein	711			645[2]	695[2]		705[2]
Crude fat	88			108[2]	159[2]		
Ash	99			72[2]	62[2]		
<u>*Amino acids:*</u>							
Alanine	35.93	2.09	5.23	4.3	4.37	6.6	5.9
Arginine	53.24	3.61	7.75	5.7	6.69	6.4	6.1
Aspartic acid	73.14	6.58	10.64	7.6	8.98	9.9	8.8
Cysteine	11.06	0.44	1.61	0.8	-	0.8	0.9
Phenylalanine	26.41	2.18	3.84	3.0	3.34	4.4	3.9
Glutamic acid	97.07	12.11	14.13	11.8	11.07	14.2	12.5
Glycine	40.43	3.39	5.89	5.8	5.71	7.8	6.0
Histidine	14.50	1.58	2.11	1.7	1.68	3.0	2.2
Isoleucine	32.76	1.92	4.77	3.3	3.68	4.3	4.0
Leucine	50.22	3.52	7.31	5.1	6.08	7.6	7.1
Lysine	53.45	2.57	7.78	6.0	6.86	8.5	7.4
Methionine	17.73	1.68	2.58	1.8	2.14	2.9	2.7
Proline	27.25	1.73	3.97	3.3	3.59	4.9	3.8
Serine	35.30	4.02	5.14	3.4	4.54	4.7	4.0
Threonine	33.13	2.74	4.82	3.9	4.18	4.8	4.1
Tyrosine	28.31	1.74	4.12	2.6	3.62	3.4	-
Valine	34.61	2.31	5.04	3.5	3.89	5.1	4.8

[1]Degussa AG, Feed Additives, Hanau-Wolfgang, Germany
[2]Values in g/kg DM

1.5 Hygienic aspects of mussels

By filtering large volumes of water mussels can accumulate and concentrate different pathogens such as bacteria and viruses and some different algae toxins. The potential risk for consumption of mussels is thus dependent on the occurrence and composition of these pathogens and toxins in the areas were the mussel farms are located (Rehnstam-Holm & Hernroth,). In Sweden there is a strict legislation regarding discharging of waste water, but despite the regulation there is a surprisingly high frequency of human pathogens in Swedish mussels. In a Scandinavian report both enteric viruses and

Escherichia coli (*E. coli*) were found in all evaluated mussel farms. The occurrence of *E. coli* has been low, in general, in the mussel farms further out from the shore (Hernroth et al.,). Mussels are consumed by humans either raw or slightly heated and thus the regulation regarding the pathogen content is strict.

During some periods harmful algal blooms can result in shellfish toxicity (Svensson,). Blue mussels farmed along the Swedish West Coast occasionally contain levels of okadaic acid ( ) above the tolerance limit for harvest of shellfish for human consumption (Rehnstam-Holm & Hernroth,). Diarrheic shellfish toxins ( ) seem to be the most predominant toxic threat to consumers of mussels in Europe, with diarrhoea and vomiting as a consequence. The major is , but there are a few other related s of the type dinophysistoxin ( ) (Vale & de M. Sampayo,). Other toxins related to mussels are paralytic shellfish toxins ( ) and substances with neurotoxic effects (Rehnstam-Holm & Hernroth,).

However, the content of pathogens and toxins in mussels vary considerably and is dependent on the location of the mussel farm, the year variation and environmental conditions, i.e. sea water temperature, salinity and land run-offs. In Sweden, toxin levels in harvested mussels are routinely analysed by the National Food Administration (Persson & Karlson,). During the preparation of mussels to produce mussel meal for poultry feed, mussels were first boiled to be able to separate the meat from shell. The meat was then dried at a temperature between – °C. Furthermore, in Sweden all poultry feeds from the manufacturer have to be heat-treated to a minimum of °C for two minutes to kill possible pathogens, e.g. salmonella. This further reduces the risk of the carry-over of some pathogens from the feedstuffs to poultry products.

Other contaminants are traces of heavy metals which occasionally may be found in mussels. The legal limits for heavy metals as well as dioxin and in mussels for human consumption are regulated by legislation, / . Data from blue mussels harvested on the Swedish West Coast show concentrations well below the legal limits for all substances analysed (Kollberg & Ljungqvist,).

Considering all these hygienic aspects, it is important to evaluate a wide range of environmental factors before deciding on the location of a mussel farm. Hence, all sites intended for mussel farms are subject to a thorough risk assessment concerning the hygienic quality of the water (Hernroth et al.,).

2 Aim

The overall aim of this thesis was to evaluate mussel meal as a protein source in poultry diets with focus on the use in organic production.

The objectives were to investigate:

- Effects on production parameters, egg quality, product quality and animal health when using mussel meal at different levels instead of fish meal in diets for both laying hens and broiler chickens.
- Effects of toxic mussel meal on animal health and digestive tract morphology and also to investigate whether production performance or egg quality were impaired.
- The possibilities to compose a diet with % organically approved feed ingredients using mussel meal as a major source of methionine.

The hypotheses were *i)* that mussel meal could be used as a high quality protein source in poultry diets and *ii*) that mussel meal could be a part of the solution needed to fulfil the protein requirements in organic diets by , i.e. when only feed ingredients approved according to the organic standards will be allowed.

3 Summary of investigations (Paper I-IV)

3.1 Materials and methods

This thesis includes four studies performed at the Funbo-Lövsta Research Station, at the Swedish University of Agricultural Sciences ( ) in Uppsala. The project was funded by Formas (Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning) and Eko Forsk ( ). The different housing systems used fulfilled the Swedish Animal Welfare Directives and the experiments were approved by the National Ethics Committee for animal research in Uppsala. The project was conducted in cooperation with Kristineberg Marine Research Station which supplied mussel meal for all experiments. Mussel meal prepared from dried and ground meat of mussels was used. Tristimus values of the , , standard colorimetric system with a -grade scale was used to measure egg yolk colour. All other methods and experimental procedures are described in detail in each paper. An overview of the experimental design is shown in *Table 2*.

Table 2. *Overview of the experimental designs.*

	Paper I	**Paper II**	**Paper III**	**Paper IV**
Laying hens	X	X		X
Broiler chickens			X	
Furnished cages	X	X		
Aviary system				X
Production performance	X	X	X	X
Egg quality	X	X		X
Fatty acid analyses	X			
Clostridium analyses			X	
Feed				
Mussel meal	X	X	X	X
Fish meal	X		X	X
Mussel toxin		X		
Experimental duration	11 wk	8 wk	36 days	20-72 wk of age

3.1.1 Mussel meal and diet preparation

Mussel meal was prepared from Blue mussels *(Mytilus edulis)*. A fresh live blue mussel roughly consist of three equal parts; shell, meat and water. The raw material used for the meal production must be fresh and the whole process must be carried out under the same hygienic conditions as for seafood production. In order to separate the meat from the shell, the mussels were steamed quickly and were thereafter spread on a shaking grid where the coagulated meat comes loose from the shell. It is then simple to separate meat and shell by using a density bath where the meat floats and the shell sinks. This is a standard technique used by the seafood industry to separate shell and meat. Meat and shells were crushed and separated in a swirl separator in Paper , and . The meat was dried at between and °C to about % water content and then ground[2].

The heating to – °C well fulfils the hygienic requirements for poultry feed (°C). Regardless of the heating procedure, each batch of meal used in Paper had to be tested for occurrence of *Salmonella* before it was allowed

[2] Personal communication, S. Kollberg and O. Lindahl, .

to be delivered to the feed factory and included in the complete feed. To control rancidity, ppm/kg feed of the antioxidant Vitalox (Helm, Hamburg, Germany) was added to the feed.

3.1.2 Pilot and toxin experiment (Paper I and II)

When the project started, two short-term studies were performed on a limited number of layers. These studies, referred to as the pilot and the toxin experiment respectively, resulted in Paper and .

Birds and management

In Paper and Lohmann Selected Leghorn ( ) hens were used. The pilot study in Paper included hens and the toxin study in Paper included hens. The birds were housed in furnished experimental cages designed by Victorsson and described in Wall and Tauson ( ). Eight hens and two hens were housed in each cage in Paper and respectively. The experiments were carried out when the hens were – weeks of age (Paper) and – weeks of age (Paper). The birds were given h light/ h at week (wk) and the light successively increased to . h light/ h at wk . The hens were vaccinated against coccidiosis, Marek´s disease, infectious bronchitis and avian encephalomyelitis during rearing.

Experimental diets

Four different experimental diets were used in Paper , with , , or % inclusion of mussel meal, respectively, replacing the same amounts of fish meal. In Paper three different experimental diets were used, one commercial control feed and two diets including % mussel meal either toxic or non-toxic (see *Table 3*). The calculated content in the mussel meal was µg/kg. This resulted in a level of . µg /kg in the diet with toxic mussel meal. Feed was pelleted and crumbled, and was available *ad libitum*.

Table 3. *Mussel and fish meal contents (%) in the experimental diets for Paper I and II.*

	Paper I				**Paper II**		
Diets	M0	M3	M6	M9	C	M	MTox[1]
Mussel meal (%)	0	3	6	9	0	15	15
Fish meal (%)	9	6	3	0	0	0	0

[1]The mussel meal contained

Registrations and analyses

Egg production, feed intake and mortality were recorded per cage daily and egg weight was recorded once every week. Eggs were collected and analysed for shell deformation, shell breaking strength, egg yolk pigmentation albumen height and albumen dry matter content (Paper and). Fatty acid composition was analysed (Paper) and each bird was weighed at the start and at the end of the trial (Paper).

In Paper eggs were collected to analyse the content of in the egg yolk according to the method of Quilliam ( ). Liver, intestine (cm from the proximal end of the duodenal loop), gizzard and proventriculus were collected and embedded for light microscopic evaluation. Intestinal villi height and crypth dept were measured and the number of goblet cells in the middle and in the top of villi and in the crypts of Liberkühn, and the number of epithelial cell divisions were calculated.

3.1.3 Broiler chicken experiment (Paper III)

This experiment was performed to evaluate mussel meal in diets for broiler chickens and to compare inclusion of mussel meal with similar inclusion of fish meal.

Birds and management

The experiment comprised as-hatched Ross broiler chickens, randomly distributed into pens (. x . m). The chicken house temperature started at °C and was gradually decreased to °C by the end of the experiment. The chickens had continuous artificial light the first three days, and thereafter the dark period was gradually increased to h at days of age.

Experimental diets and registrations

The chickens had free access to a control diet or a diet including either , , or % mussel meal, or equivalent inclusion levels of fish meal. In total nine different experimental diets were used.

Bird live weight gain and feed intake was recorded weekly until slaughter at days, and the feed conversion ratio ( ) was calculated. Quantitative determination of clostridial colony forming units ( ) was performed on the contents of one caecum/bird, on two birds per treatment, at , and days of age.

3.1.4 Long-term experiment with laying hens (Paper IV)

This experiment was performed to evaluate the effects of mussel meal during a whole production period on layers housed in a floor housing system. Experimental diets including mussel meal were compared with an organic diet available on the Swedish feed market.

Birds and management

The experiment comprised the time from wk to and included and Hyline White, W- , ( ) layers. The birds were transferred to the research station at wk and given . h of light per day. This was successively increased to h at wk . The birds were housed in pens (groups) in the Marielund aviary system described by Abrahamsson and Tauson ( ). This system consists of three welded wire tiers of which the two lower ones have feed troughs and the top resting tier has perches. Nipple drinkers were available on all tiers. The hens were vaccinated against coccidiosis, Marek´s disease, infectious bronchitis and avian encephalomyelitis during rearing.

Experimental diets and registrations

Three diets were used: a commercial organic diet as control and two experimental diets with either . or . % inclusion of mussel meal. In the % diet only feed ingredients that are organic available were used although ingredients in this diet were conventional. All birds had free access to chopped straw from alfalfa but the birds were prohibited to have access to outdoor runs due to directives about the bird flu at the time of the experiment.

All data were collected per group, i.e. hens housed in each aviary pen. A sample of birds was weighed at three occasions. Egg production, number of misplaced eggs and dead birds were recorded daily and feed consumption and were recorded on a four week basis. Egg weight was recorded once a week and proportion of cracked and dirty eggs was recorded at four occasions during the experiment. At three times eggs were collected and analysed for shell deformation, shell breaking strength, egg yolk pigmentation, albumen height and albumen dry matter content. Dry matter content in fresh excreta was analysed three times.

3.1.5 Statistical Analysis

Data were analysed by analysis of variance ( ) using the procedure (Paper , and) and the procedure (Paper) of the statistical system (,). Regression analysis were also used (Paper). Model assumptions, i.e. independence, homoscedasticity and normality of residual errors, were checked by tests and plots. In case of heteroscedasticity among treatments the Satterthwaite's adjustment for Student´s t-test was applied. Data in percentages were subjected to arcsine-root transformation (Snedecor & Cochran,) prior to analyses.

Student´s t-test (two-tailed) was used to compare live weights of the hens (Paper).

3.2 Main results

3.2.1 Production performance (Paper I, III and IV)

Production results were in general very good. In the experiments with layers inclusion levels from to % (Paper) and . or % (Paper) did not significantly affect production parameters i.e. , egg weight, egg mass production and laying percent. In the long-term laying hen experiment (Paper), feed intake, live weight at wk and , and proportion of misplaced, cracked and dirty eggs were also unaffected. There was a significant difference in feed consumption in the pilot experiment (Paper) where birds fed the and diet had a slightly increased feed intake.

In the broiler experiment (Paper) mussel meal inclusion up to % did not affect bird live weight, accumulated feed intake or .

Dry matter of the excreta was significantly lower for hens given the diet including % mussel meal than for hens fed the control diet or the diet containing . % mussel meal (Paper).

Mortality was close to zero (out of hens died) in the pilot experiment (Paper) and zero in the toxin experiment (Paper). The mortality in the broiler chicken experiment (Paper) and the long-term laying hen experiment (Paper) was about . % and between - % respectively.

Effects of genotype

In the long-term study (Paper) production performances differed significantly between the two laying hen genotypes studied. birds had lower feed intake, laying percent and egg mass but higher egg weight, resulting in a tendency for better than for birds. Eggs per hen housed and proportion of misplaced or dirty eggs were unaffected by hybrid but had higher proportion of cracked eggs than birds. birds were heavier than birds.

3.2.2 Egg quality

In all experiments with laying hens egg yolk pigmentation differed significantly between diets. The egg yolk was more coloured when feeding a diet containing mussel meal i.e. given a higher Roche score.

Inclusion level of mussel meal did not affect the egg quality parameters: shell deformation, shell breaking strength (Paper , ,), shell percentage (Paper), egg white dry matter content (Paper), albumen height (Paper ,) or proportion of blood and meat spots (Paper). Albumen dry matter content was significantly higher for the diets containing mussel meal in the long-term laying hen experiment (Paper).

Linolenic acid, docosahexaenoic acid ( ) and docosapentaenoic acid ( ) were unaffected by mussel meal inclusion, but eicosapentaenoic acid ( ) was negatively affected by a higher inclusion level of mussel meal (Paper).

Age and genotype effects

Age of the birds influenced all egg quality traits except proportion of meat and blood spots, and egg yolk pigmentation. Thus, with increasing age egg weight increased, albumen height and albumen dry matter content decreased, egg shell quality deteriorated, i.e. shell percentage decreased, shell deformation increased and shell breaking strength tended to decrease. The proportion of cracked eggs increased with hen age. Hybrid affected shell quality i.e. shell deformation, shell breaking strength, shell percentage and albumen height favouring birds, whereas eggs showed higher albumen dry matter content.

3.2.3 Production, histological evaluation and toxin analyses (Paper II)

Inclusion of toxic mussel meal in the diet for layers did not affect production performance or animal health negatively. Furthermore, no was detected in the egg yolk.

Intestinal villi height and crypth dept were unaffected by the diet containing mussel toxin ( ) at a daily intake of approximately . µg. In addition, the number of goblet cells in the middle of villi and in the crypts of Lieberkühn, and the number of epithelial cell divisions were unaffected.

The toxin diet, however, resulted in an increased number of goblet cells in the villi top.

3.3 Additional experiments and preliminary results

3.3.1 Plumage condition

The long-term laying hen experiment also included scoring of the birds' plumage condition at wk . Birds' feather cover was scored on six different parts of the body (neck, breast, cloaca, back, wings and tail) and a four grade scale was used. When the scores for the six different body parts are summarized a total score between and is generated where points is a full feathered bird and points is an almost naked bird (Tauson *et al.*, ; Tauson *et al.*,)

Both diet and genotype affected plumage condition significantly. Both hybrids showed an improved total score of the plumage condition with mussel meal included in the diet. birds generally had a better feather condition on all diets compared to (See *Figure*).

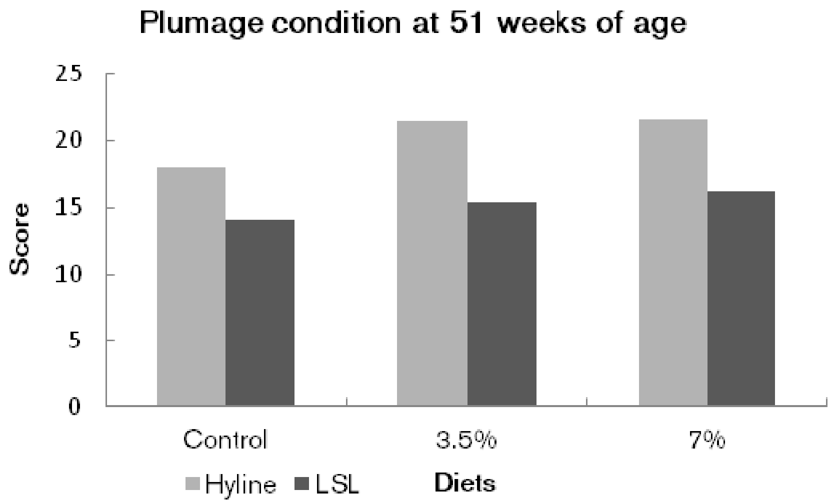

Figure . Plumage condition at weeks of age comparing three diets and two hybrids.

3.3.2 Broiler experiment with toxic mussel meal

In order to further study effects of toxic mussel meal an experiment with broiler chickens aged to days was performed. Two feeds were used: one diet similar to a commercial diet and one diet containing % toxic mussel meal. The toxin level in the mussel meal was μg – /kg and μg /kg, resulting in μg – and per kg feed. – is considered to be equivalent to regarding the toxic effects. With a daily feed consumption of approximately g at day this resulted in a total toxin intake of . μg/chicken/day and a daily feed intake of approximately g at day resulted in a toxin intake of μg/chicken/day. Housing and management were similar and registrations were performed in the same way as in the broiler chicken experiment (Paper). The chickens were randomly distributed into experimental pens with four birds in each pen resulting in seven replicates per treatment. Live weight and feed consumption differed significantly at and days of age. Birds given the toxic mussel meal feed had a lower live weight and less feed intake compared to the control birds. The numerical values show that the mussel toxin diet led to decreased live weight at day and feed intake being slightly lower already at day . was unaffected by treatment. The animals fed toxic mussel meal in their diet had a higher occurrence of diarrhoea than the control birds. There was an impaired litter condition in pens where the chickens received the toxic diet.

3.3.3 Sensory evaluation

Sensory evaluation was performed on eggs from hens fed the diets used in the long-term experiment with laying hens (Paper). The study comprised a total of hens of which half were and half Hyline Brown housed in furnished -hen cages. A descriptive test, conventional profiling (International Organisation for Standardisation,), was carried out by a trained six-member sensory panel, (International Organisation for Standardisation,). The sensory analysis was preceded by three training sessions where eggs were presented from the two laying hen genotypes fed five different diets of which three were the diets used in the long-term experiment with laying hens. The test was conducted on two consecutive days. Eggs from different treatments were randomly served to the panel members. Off flavour and off odour were found not to differ between the eggs. Egg flavour intensity was stronger in eggs from hens fed the two mussel meal diets and the standard organic diet compared to the conventional diet included in this experiment.

4 General discussion

Organic poultry production is facing the challenge that all ingredients in an organic diet should be organically approved by . The aim of this thesis was to evaluate whether blue mussels can be used as a protein source for poultry and, as such, present one possible way to solve the problem of protein supply in future organic poultry production. The thesis also aimed to investigate whether fish meal could be replaced by mussel meal and whether mussel meal containing certain level of toxins could be used.

The protein supply is important for the possibility to increase organic poultry production in the future (Wilson, ; Elwinger *et al.*, ; Hammershøj & Steenfeldt,). The experiments included in this thesis evaluated the use of mussels in poultry diets, both to laying hens (Paper , and and preliminary data) and to broiler chickens (Paper and preliminary data).

The experiments were conducted both in organic and conventional conditions. The first experiment was a pilot study (Paper) since mussel meal had not been given to laying hens in an experiment before. It was carried out on a small scale as a first step to evaluate mussel meal as a possible protein source. Hence, the results became important for further experiments. The next step was to use an available small amount of toxic mussel meal (Paper). The hens were exposed to for eight weeks, which can be regarded as long-term exposure when compared to previously published acute dose studies (Ito *et al.*, ; Berven *et al.*,). Paper took broiler chickens into consideration and in the long-term experiment with laying hens (Paper) a diet only containing feed ingredients expected to be organically approved in was evaluated.

4.1 Production performance

The results showed that mussel meal could be an excellent protein source in diets for poultry and that mussel meal can be used instead of fish meal in poultry diets. Production parameters referred to are laying percentage, egg weight, egg mass, feed consumption and (Paper , and) and growth performance, feed intake and (Paper).

No negative effects regarding production performance were observed in any of the experiments. Despite the long-term exposure to , production between the different treatments was unaffected (Paper). Bird performance was generally good for all treatments in the broiler chicken experiment (Paper) and even at high inclusion levels, up to %, no negative effects on performance were seen. Birds given mussel meal performed as well as those given fish meal. The long-term laying hen experiment (Paper) showed that with an inclusion of % mussel meal it is possible to compose a % organically approved diet and attain the same production level as with the inclusion of % conventional feed ingredients, being allowed at the time for the experiment. There was a small but significant difference in feed consumption in the pilot experiment (Paper) where birds fed the and diet had an increased feed intake. In the other experiments there has not been any difference in feed intake. This indicates that mussel meal is well accepted by the birds.

All these results show that mussel meal may become a substitute for the today commonly used fish meal in organic poultry diets. Mussel meal also has the potential to be approved as an organic feed component. Depending on the price for mussel meal it can also be considered to be used in conventional poultry diets.

In the experiments presented in this thesis, an inclusion level of mussel meal from to % was used. The conducted experiments showed no negative effects in any of the parameters measured regardless of the inclusion level of mussel meal. Based on these results it can be expected that the need for protein, especially methionine, and the price of mussel meal will decide the amount of mussel meal included in the diets in the future. A reasonable inclusion taken both the price and protein requirement in consideration would probably be between - %. This should be enough to fulfil the birds' methionine requirement and probably lead to reasonable feed costs for the producer.

The process used in the present studies when producing mussel meal is not yet fully developed and evaluated. Hence, variations in nutrient content in mussel meal depending on drying method are not described in this thesis. Such research is forthcoming within a project at Kristineberg Marine Research Station and will include a pilot manufacturer able to produce mussel meal that is commercially viable.

4.2 Egg quality

4.2.1 Yolk coloration

Today a layer diet is based mainly on feedstuffs that are low in carotenoid content, making it difficult to obtain an attractive colour of the egg yolk without adding other ingredients or substances. The colour of the egg yolk was affected in all experiments. Higher inclusion of mussel meal resulted in a deeper colour of the egg yolk. The intensity of the yolk colour depends on the accumulation of carotenoids. The effect of mussel meal on yolk pigmentation is mostly regarded as a positive effect from a consumer point of view. Consumers in general, want a coloured egg yolk. Laying hens are unable to synthesize carotenoids and are thus dependent on a dietary supply of these pigments (Nys,). Hence, synthetic carotenoids are widely used in laying hen diets in many countries, but they are not allowed in Sweden. The concern regarding synthetic additives and an increasing interest for natural alternatives have led to the use of e.g. pepper powder instead of synthetic additives (Karadas et al.,).

Mussels consume algae rich in carotenoids. Matsuno ( ) reported that mollusks contain carotenoids such as β-carotene, lutein A, zeaxanthin, astaxanthin, and also chlorophyll and other xanthophyll pigments originating from the algae consumed by the mussels. Carotenoid content in mussels varies due to season of the year and maturity state of the mussels. Total carotenoid content in fresh mussels is approximately mg/kg resulting in mg/kg dry matter (Campbell,). This is above the carotenoid content in for example corn gluten meal which contains on average – mg/kg (Belyavin & Marangos,). Hence, including mussels in the diet can replace the inclusion of other pigments and decrease the need for other expensive carotenoid-rich feed sources or synthetic substances.

Inclusion of vegetable sources such as corn gluten meal, alfalfa or access to grass in outdoor runs also affect the coloration of the egg yolk (Hammershøj & Steenfeldt, ; Leeson & Caston,). This factor probably contributed to the smaller difference in yolk pigmentation between the treatments in the long-term experiment with laying hens (Paper) compared to the pilot and toxin experiment (Paper and). In the long-term experiment the control diet and the . % diet included . % and . % corn gluten meal respectively and all hens had access to chopped alfalfa and outdoor runs.

4.2.2 Fatty acids

In the pilot experiment (Paper) type of diet had little effect on concentrations of the long chain fatty acids (:), (:) and (:), in the yolk. Dietary inclusion of mussel meal did not modify the fatty acid composition in the egg yolk to any considerable extent. The content of α-linolenic acid was lower in the and eggs compared to eggs from hens fed and , probably due to the lower content of rapeseed cake in those diets.

Mussel meal is known to be rich in the long-chain fatty acids , and while rapeseed cake contains more α-linolenic acid. The diets were not analysed for fatty acid content and composition but it was assumed that the amount of , and in the eggs from hens receiving the diet originated from the mussel meal. Since it has been shown that also housing system (Leskanich & Noble,), age (Van Elswyk,) and hybrid (Grobas *et al.*,) influence fatty acid composition in the egg yolk, comparison of results from this experiment with others is complicated. More reliable results regarding the effect of mussel meal on long-chain fatty acid content in the egg yolk could have been obtained if the experiment had included a control diet without both mussel and fish meal. One -g egg from hens fed mussel meal (diet) contained about mg and mg , which is about half as much as reported in omega three-enriched eggs on the Swedish market.

4.2.3 Dirty eggs and dry matter content

Excreta dry matter may be a factor affecting the incidence of dirty eggs. Smith et al. ( ) showed that a decrease in the dry matter content of the excreta was the major cause of the increase in proportion of dirty eggs. There were no significant differences regarding dirty eggs in the long-term experiment with laying hens (Paper) but small numerical differences were observed that implied that the % diet resulted in a slightly higher proportion of dirty eggs. However, since not only the mussel meal inclusion level differs between the diets used, it is not possible to conclude that the mussel meal is the only reason for the lower dry matter content in the excreta from hens fed the % diet.

4.2.4 Sensory evaluation

An important result of the sensory evaluation was the lack of differences found in eggs between diets in off-flavour and off-aroma. This indicates that no negative effects of mussel meal in the flavour and aroma of eggs were found at the levels used in this study. However egg flavour intensity differed. The egg flavour intensity was stronger in eggs from hens fed the two mussel meal diets and the standard organic diet compared to a conventional poultry diet. This reflects how intense the egg taste was perceived by the panel. The eggs were by no means considered unsavoury and the differences in flavour were not proof of whether an egg was perceived as more or less pleasant.

Previous studies have shown negative effects of diets containing fish meal on the flavour of broiler chicken meat. The fishy taint in poultry meat is associated with the intake of marine fat and related to an accumulation of long chain fatty acids, i.e. , and in the carcass (Krogdahl,). Kjos et al ( ) conclude that levels of . g or more of fish fat/kg feed caused off-flavour of chicken thigh meat. At levels lower than g fish fat/kg feed no problems with off-flavour were found. A likely inclusion level of mussel meal in poultry diets would be between - %, which will result in - g marine fat/kg feed, calculated on % fat content in the mussel meal (*Table*). No sensory evaluation was performed in the present broiler-chicken experiment (Paper). A professionally performed sensory evaluation of broiler chicken meat is needed before using mussel meal, especially in high inclusion.

4.3 Animal health

4.3.1 Methionine requirement

The amino acid methionine can not be synthesized by the bird and must be supplied by the diet. Methionine is important for feather formation and is generally the first limiting amino acid for poultry. Nutrient requirements in Europe are based on Nutrient Requirements of Poultry (,). One of the criticisms of the standards is that they are based on old research and publications. Hybrids used today have been intensively selected for even higher production which has resulted in a higher nutrient requirement, which do not account for (Novak *et al.*,). In Swedish practice the amount of methionine in poultry diets is in accordance with the breeder´s manual outline and is approximately % higher than the recom-menddations.

Deficiency of methionine can negatively affect the growth rate in broiler chickens (Bunchasak,). According to the Broiler Management Manual (Manual,) commercial recommendation of methionine intake for maximal production decrease from . % to . % of the diet with increasing age. Phase feeding was not used in the broiler chicken experiment (Paper). Thus, the same level of methionine was used during the whole growth period. The amount of methionine in the broiler chicken experiment diets was approximately . % and should fulfil the birds' requirement.

There is different information reported regarding the amount of methionine needed in diets for laying hens. A possible reason for this variation is differences in genetics, nutrition and management of hens in different experiments. Most studies investigate only production traits and not animal welfare e.g. feather condition. Shortage of methionine can result in both negative effects on animal health, such as impaired feather condition (Elwinger *et al.*, ; Van Krimpen *et al.*, ; Tiller,) and production traits, e.g. feed utilization (Novak *et al.*, ; Schutte *et al.*,). Elwinger et al ( ) reported impaired feather condition and decreased egg weight whereas number of eggs was the same when using very low levels of methionine. The ( ) recommendation of methionine is mg/hen/day. However, several experiments indicate a higher requirement of methionine for maximum production (Novak *et al.*, ; Schutte *et al.*, ; Schutte & Van Weerden,). Narvaéz-Solarte et al ( ) reported that increasing the intake of methionine over a certain level did not improve production further. These results indicate that there is a certain level of methionine intake that must be fulfilled in order to reach an acceptable production and avoid feather pecking behaviour. Further it appears that the birds may favour their reproduction before their welfare.

In the long-term experiment with laying hens the diets contained between . - . g methionine/kg feed. This implies an average daily intake of , and mg methionine for the control, . % and % diets, respectively. This is well above the recommendations but below the Lohmann breeder management guide during the peak production phase (Lohmann Tierzucht GmBH, Cuxhaven, Germany). In the pilot study the hens received , , and mg/day for the , , and diets respectively. Results from the long-term experiment and the pilot experiment show that despite a small lack of methionine during the pre-lay and peak production periods, the production may be considered acceptable, especially during organic conditions.

At present, use of pure amino acids are banned in organic diets because they are synthetic or derived from microbial fermentation by gene modified ( ) bacteria. These microbial organisms are gene modified and thus the produced amino acids are prohibited. The ban of pure amino acids can result in diets with low protein content and an unbalanced amino acid profile. To fulfil the birds' requirement for methionine the diets tend to oversupply the birds with crude protein resulting in secretion of unutilized protein leading to an oversupply of nitrogen to the environment (Blair,). This effect is probably in total contrast to the overall aim of organic production where the recycling of nutrients is an important factor.

It is questioned how animal health and welfare will be affected in if no new alternative protein sources will be available and the use of synthetic amino acids is still prohibited. It may be of importance to consider advantages and disadvantages with using synthetic methionine in diets for organic poultry. A disadvantage could be the consumer perception. Present directives make it difficult to have an efficient and sustainable organic production. Some countries actively seek a solution of the protein problem, including research on new potential protein sources, while other countries rely on a future change in the regulation, allowing synthetic amino acids (Blair,).

4.3.2 Plumage condition

Results from the long-term experiment with laying hens showed that hens fed mussel meal diets had a better plumage condition than hens fed the commercial organic diet used as control. The average total score was . , . and . for the commercial organic diet, . % and % diets respecttively. However, these diets differed regarding more ingredients than the mussel meal. For example the control diet included more than % of wheat while the mussel diets contained only between and % of wheat. Wahlström et al ( ) reported significantly impaired plumage condition of hens when the wheat fraction in the diet increased. Thus, other differences than the content of mussel meal may have contributed to differences in feather conditions.

4.3.3 Hygienic aspects of mussels

In the experiments included in this thesis no bacteria and virus analyses were conducted on diets or poultry products, but the effects of on laying hens were evaluated in the toxic experiment (Paper). Mussel meal used in Paper , and was produced from mussels approved for human consumption. No negative effects were registered when laying hens were given . µg of per kg diet, resulting in an average daily intake of µg per hen/day (~ µg/kg body weight). This is regarded as a low dose of compared to acute toxic studies done by Berven et al ( ) and Ito et al ( ) where levels of - µg/kg body weight were given to mice and rats intragastrically. Preliminary results from the second toxin study show that broiler chickens are affected at a level of µg - and /kg feed resulting in a daily - and intake of µg/day (~ µg/kg body weight) at days of age. The chickens ate and grew less and showed signs of diarrhoea when being exposed to mussel toxins during days. The mussels included in this experiment were considered extremely toxic and such a high level of toxin very seldom occurs in practice and, for sure, not in mussels checked for human consumption.

The reason why only two concentrations of the toxin (either . µg/kg or no detectable level) were tested in the first toxin study (Paper) was because this was the toxin level in the mussels harvested at that time. Thus, the level of toxin in the experiment is at a level that can occur in reality in the seas. The purpose of the study was to evaluate the effect of at a concentration that may commonly occur during the periods when mussels are toxic. Thus, the purpose was not to evaluate a dose-response effect of the mussel toxin. The level used in the second toxin experiment was extremely toxic, which is very rare in practice.

The limit for human consumption is according to The National Food Administration µg per kg mussels (,). The limit for animal consumption needs further evaluation. What we know so far is that laying hens seem to tolerate an content of up to µg per kg feed but broiler chickens react negatively on a - and content of µg per kg feed. On the other hand, it is practice, to delay the harvest of toxic mussels and give the mussels possibilities of cleaning themselves.

4.4 Experimental diets

It is difficult to compose an experimental diet formulated to evaluate a specific ingredient. Should the diet be optimized for as equal ingredient content or as equal nutrient composition as possible? In Paper , the diet was optimized for equal energy and protein content, focusing on the content of methionine that was considered the most important amino acids. With this method of optimization differences regarding the content of rapeseed cake and peas had to be accepted. The main reason for using rapeseed in the diets was to make the methionine content similar. Mussel meal was lower in lysine content and richer in methionine content than fish meal. Diets including fish meal had to be compensated for the lower methionine level in the fish meal, by using rape seed cake, which also resulted in an increased fat content. To compensate for the low levels of lysine, the diet got a slightly increased content of peas. It is therefore difficult to generalize differences between mussel meal and fish meal found in this experiment.

In the broiler chicken experiment (Paper) the inclusion of mussel meal in the diets varied between and %. This variation forced the amount of soya bean meal and peas to be altered and synthetic amino acids had to be used to achieve similar nutrient content levels. This was necessary to be able to evaluate possible effects of the inclusion of either mussel or fish meal.

Diets in the long-term experiment with laying hens (Paper) also required compromising regarding the feed composition. As a control a commercial organic diet was used and the experimental diet including . % mussel meal was made as a comparison to the commercial organic diet, allowing % conventional ingredients. The % mussel meal diet was optimized to comply with the regulations of , with only % organically approved ingredients. The diets were all composed in order to fulfil a practically applied situation which is why they differ both in composition and nutrient content.

4.5 Production systems and organic environments

Another difficulty when evaluating ingredients supposed for organic production is the differences regarding production system. In organic production many parameters i.e. outdoor run, stocking density and access to roughage can affect the results. In the broiler chicken experiment (Paper) all these factors which may influence the results were minimized. This is the reason why days was chosen as slaughter age instead of days according to the organic regulation and why the chickens were reared in pens indoors. In this way possible effects observed were assumed to arise from the mussel meal.

In the pilot experiment (Paper) furnished cages were used to be able to evaluate the effect of mussel meal without any influence of parameters originated from an organic environment. The possibilities with using cages are also that they provide more replicates. Thus in this case, when the access to mussel meal was limited, a pilot study in cages was assumed to give the most reliable results.

In the long-term study with laying hens (Paper) the housing environment was supposed to be similar to those at an organic farm, except from not having access to outdoor runs due to directives regarding the bird flu.

4.6 Practical implications

In Sweden the feed manufacturers use approximately to tons of fish meal every year, of which about tons are used in poultry diets, mainly in organic diets.[3] One ton of fresh mussels generates about kg mussel meat and after the drying and grinding process kg dried mussel meal remains. If all fish meal used in organic poultry diets today were to be replaced by mussel meal, tons of fresh mussels would be needed every year. Researchers at Kristineberg Marine Research Station at the Swedish West Coast have estimated that it is possible to grow and harvest about tons of mussels along the coastline, i.e. from the south archipelago around Gothenburg to the Norwegian border in the north.[4] Besides being a much more sustainable feed ingredient than fish meal, harvesting tons of mussels/year would result in a removal of tons of and tons of . Using meat from mussels in diets for poultry can thus contribute to a considerably better coastal water environment.

A realistic inclusion of mussel meal in poultry diets would be between and %. At these levels it will be possible to fulfil the birds' requirement and at the same time hopefully keep the feed cost at a reasonable level. If based on organic layers in Sweden today, consuming kg of feed during a production cycle (to wk) at an inclusion of mussel meal of either or

[3] Personal communication, Annsofie Wahlström, Lantmännen,
[4] Personal communication, Odd Lindahl,

% will require approximately or tons mussel meal respectively.

The price of mussel meal depends on the price for the harvested mussels. If the price of kg fresh mussels is and the management and processing cost per kg meal is calculated to be per kg, this will result in a price per kg meal of . The price for fish meal is today between – per kg, but could be expected to increase. The price for kg organic feed today is about . including roughly . % fish meal. If we replace the fish meal with mussel meal the feed cost will increase by . per kg, which is not possible for the producer to afford today. Feed conversion ratio in the long-term experiment with laying hens was about . kg feed per kg egg. Using . % mussel meal in the diet would result in an increased production cost of . per kg egg generating an increased cost of about per six eggs. Is it possible to make the consumer pay this extra cost for producing eggs with a feed that fulfils the birds' requirement and at the same time clean the coastal water? An important factor to consider is that it could be possible to get environmental subsidies for growing and harvesting mussels. The price for mussel meal could then reach the level of – per kg and would then probably be able to compete with fish meal in the near future.

5 Conclusions

- This thesis shows that mussel meal is an excellent source of protein and thus, from a nutritional point of view, a possible substitute for fish meal in diets for both laying hens and broiler chickens. Production performance, egg quality, product quality (eggs) and animal health were not impaired when mussel meal was used in the diets.
- Birds are sensitive to mussel toxins but a long-term daily exposure of laying hens to an environmentally relevant dose of mussel toxin did not negatively affect the hen intestine or animal health.
- Using mussel meal in the future would make it possible to compose feed containing % organic feed ingredients and at the same time fulfilling the birds' requirement of methionine.
- It is possible to include up to % and % mussel meal to laying hens and broiler chickens, respectively, without any negative effects on production, egg quality, animal health and quality. The cost of mussel meal will likely determine the use of mussel meal in the future.
- The use of mussel meal would also contribute to recovery of the environment as mussels effectively clean sea waters from agricultural wastes such as nitrogen and phosphorus.

6 Future research

As shown in this thesis, mussel meal so far has a good potential to become a new protein source for poultry. Despite the very promising results there are still some issues that need to be addressed and evaluated.

- In Europe a majority of the laying hens are brown hybrids. To be able to make the results in this thesis more applicable, additional research is needed with mussel meal to brown layers. Production parameters, egg quality and animal health need to be evaluated.

- A sensibility test of broiler meat is needed to assure that there is no off flavour in the meat from chickens fed a diet including mussel meal.

- Possible effects of mussel meal on the long chain fatty acid content in the egg yolk need further evaluation. This could increase the value of mussels used as a feed ingredient.

- More knowledge is needed about the metabolism of possible toxins in birds. A dose–response experiment would be able to evaluate the amount of toxin in the diet that poultry can tolerate without any negative effects. Further, if the use of mussels from the Baltic Sea will be a reality, evaluation of possible pathogens and other pollutants in those mussels need to be addressed. Since the Baltic is increasingly subjected to eutrophication of and , mussel production would play an important role in these waters.

- There is urgent need for a semi-commercial mussel meal plant in order to produce this feed ingredient. Such plans are in progress (Lindahl,).

- The carotenoid content in mussels may contribute to a decreased use of synthetic pigment sources. Further studies of factors affecting the carotenoid content and stability, such as processing and storage conditions, harvest season and location are therefore needed.

7 Populärvetenskaplig sammanfattning

Den ekologiska produktionen av särskilt ägg och delvis kyckling har ökat under de senaste åren. Idag finns nästan ekologiska värphöns i Sverige och produktionen av ekologisk slaktkyckling når upp till ca kycklingar per år. Det finns en ökande efterfrågan från konsumenter och ett mål från regeringen att % av äggen och % av kycklingköttet ska produceras ekologiskt . De ca ekologiska värphönsen utgör ungefär % medan produktionen av ekologisk slaktkyckling ännu bara har nått upp till < . % av den totala kycklingproduktionen i Sverige. Ett av hindren för att den ekologiska produktionen ska kunna öka och nå målen är problemet med att uppfylla djurens proteinbehov med ett ekologiskt godkänt foder.

7.1 Bakgrund

Värphöns och slaktkycklingar har särskilda behov av framförallt aminosyran metionin i sitt foder. För lite metionin i fodret kan t ex leda till att hönsen börjar plocka fjädrar och hacka på varandra. De flesta foderråvaror som ingår i ett fjäderfäfoder idag har ett lågt innehåll av metionin i förhållande till djurens behov. I konventionell produktion löser man detta genom att tillsätta syntetiska aminosyror. Dessa är inte tillåtna enligt de ekologiska reglerna och den ekologiska ägg- och slaktkycklingproduktionen är därför beroende av foderråvaror med ett högt innehåll av metionin. Försörjningen av fjäderfä med ekologiskt godkända proteinfodermedel kan bli ett stort problem då kravet på % ekologiskt godkända råvaror träder i kraft . Idag är det tillåtet att använda % konventionella råvaror i ett ekologiskt foder för att göra det möjligt att uppfylla djurens proteinbehov. Denna inblandning skall alltså fasas ut under – . Idag används även fiskmjöl som proteinfodermedel, men med tanke på utfiskning av våra hav är detta inte ett långsiktigt hållbart alternativ. Det finns också en mycket begränsad tillgång på -godkänt fiskmjöl och det är en allmän uppfattning att världshavens fiskbestånd bör förbehållas människan, så länge det inte rör sig om rena restprodukter.

En potentiell proteinkälla med högt metionininnehåll är blåmusslor. Proteininnehållet är jämförbart med fiskmjöl och musslor har goda förutsättningar att bli godkända enligt de ekologiska reglerna. Musselodlingar har dessutom en positiv inverkan på kustvattenmiljön genom deras unika förmåga att filtrera vatten och därmed rena våra hav från övergödning av näringsämnen, framförallt kväve och fosfor, som läcker ut från jordbruket. En enda mussla filtrera upp till – liter vatten per timme. Om allt fiskmjöl som används i ekologiskt fjäderfäfoder idag skulle bytas ut mot musselmjöl skulle det innebära odling av ton musslor per år, vilket skulle medföra att ton kväve och ton fosfor skulle återföras från havet till land.

7.2 Sammanfattning av studierna

Fyra försök är hittills utförda vid s forskningscentrum, Funbo-Lövsta, för att utvärdera musselmjöl som proteinråvara i foder till fjäderfä. Syftet med dessa studier har varit att studera hur ersättning av fiskmjöl med musselmjöl i foder till värphöns och slaktkyckling påverkar djurhälsa, produktion och äggkvalitet. I tre av fyra försök har de använda musslorna varit testade enligt Livsmedelsverkets normer för humankonsumtion och i ett försök användes giftiga musslor. Musslor kan ibland innehålla algtoxiner (bl.a. okadasyra) pga av algblomning. Musslorna skördas normalt i så fall inte förrän de åter är giftfria, men i ett försök ville vi testa giftiga musslor. I försöken användes vanligt förekommande djurmaterial i Sverige. Försök och var kortare försök med ett mindre antal värphöns och hönsen inhystes i inredda burar med tillgång till värprede, sittpinne och sandbad. I försök utvärderades effekter av att använda toxiskt musselmjöl i fodret. Försök utfördes på knappt slaktkycklingar under en hel produktionsomgång om dagar. De foder som användes innehöll upp till % inblandning av antingen musselmjöl eller fiskmjöl. I det sista försöket användes frigående värphöns i en miljö lik ekologisk produktion. Även detta försök pågick under en hel produktionsomgång, – veckor, och inblandningen av musselmjöl var upp till %. I detta försök bedömdes även hur väl befjädrade hönsen var.

7.3 Resultat

Resultaten visar att slaktkycklingar har lika bra tillväxt och hälsa med fiskmjöl som med musselmjöl i fodret. Äggproduktion, bra äggkvalitet och god djurhälsa upprätthålls när fiskmjöl i fodret byts ut mot musselmjöl till värphöns. I alla försök med värphöns har de höns som ätit musselfoder värpt ägg med gulare gula än de som har ätit kontrollfoder. Färgen på äggulan påverkas av mängden karotenoider (karotener och xantofyll) i fodret. Musslor innehåller dessa färgämnen naturligt, och bidrar därför till den gulare gulan. Hönsen som åt musselfodren hade en bättre befjädring än de som åt kontrollfodret. Detta kan också ha haft andra orsaker än musselinblandningen, t.ex. skillnader i fodrens innehåll av spannmål (vete). Dålig befjädring leder till ökade värmeförluster som i sin tur leder till att djuren behöver äta mer. Hönsen påverkades inte heller negativt av att utfodras med mjöl från giftiga musslor och okadasyra kunde inte påträffas i äggulan. Senare kompletterande studier visar även att musslor i fodret inte ger någon bismak till äggen.

7.4 Slutsatser

Avhandlingen visar att musselmjöl fungerar mycket bra som proteinfodermedel till både värphöns och slaktkyckling. God produktion, bra äggkvalitet och god djurhälsa bibehålls när fiskmjöl i fodret byts ut mot musselmjöl. Musslor är unika i det avseendet att de, utöver att vara en utmärkt proteinråvara, också hjälper till att rena våra övergödda hav. Kan vi öka musselodlingen innebär det inte bara att vi får ett bra proteinfodermedel till våra ekologiska värphöns och slaktkycklingar utan också att vi förbättrar havsmiljön genom att minska övergödningen. Till skillnad från fiskodlingar tillsätts ingen näring till musselodlingar då den redan finns i havet i form av plankton och alger. Eventuella effekter av musseltoxiner behöver dock utredas vidare. Hittills är det blåmusslor från västkusten som har använts i försöken, men målet är att samma utvärdering ska kunna göras även av de något mindre blåmusslorna i Östersjön. Om musselmjöl skall användas i framtida foderblandningar bör analyser på musselpartier utföras liknande de för humankonsumtion. Förhoppningsvis kan miljöstödspengar i kombination med storskalig produktion göra musselmjölet till en ekonomiskt konkurrenskraftig foderråvara så att vi på sikt kan äta ägg och kycklingkött och samtidigt rena våra kustvatten!

8 References

Abrahamsson, P. & Tauson, R. (1995). Aviary systems and conventional cages for laying hens. Effects on production, egg quality, health and birds location in three hybrids. *Acta Agriculturae Scandinavica* 45, 192-203.

Belyavin, C.G. & Marangos, A.G. (1989). Natural Products for Egg Yolk Pigmentation. In: Cole, D.J.A.*, et al.* (Eds.) *Recent Developments in Poultry Nutrition.* London: Butterworths. pp. 239-260.

Berge, G.M. & Austreng, E. (1989). Blue mussels in feed for rainbow trout. *Aquaculture* 81, 79-90.

Berven, G., Saetre, F., Halvorsen, K. & Seglen, P.O. (2001). Effects of the diarrhetic shellfish toxin, okadaic acid, on cytoskeletal elements, viability and functionality of rat liver and intestinal cells. *Toxicon* 39, 349-362.

Blair, R. (2008). *Nutrition and Feeding of Organic Poultry.* Trowbridge: CAB International.

Bunchasak, C. (2009). Role of Dietary Methionine in Poultry Production. *Journal of Poultry Science* 46, 169-179.

Campbell, S.A. (1969). Seasonal cycles in the carotenoid content in *Mytilus edulis*. *Marine Biology* 4, 227-232.

EC (2004). Council Regulation No 853/2004 on laying down specific hygiene rules for food of animal origin, *OJ L139 of 30.4.2004, p55.*

EC (2007). Council Regulation No 834/2007 on organic production and labeling of organic products and repealing Regulation (EEC) No 2092/91, *OJ L189 of 20.7.2007, p1.*

Elwinger, K., Tufvesson, M., Lagerkvist, G. & Tauson, R. (2008). Feeding layers of different genotypes in organic feed environments. *British Poultry Science* 49(6), 654-665.

Elwinger, K. & Wahlström, A. (2000). Effects of methionine deficiency on performance of laying hens in an aviary system. In: *Proceedings of Proc. 21th World's Poultry Congess*, Montreal, Canada.

Grobas, S., Méndez, J., Làzaro, R., De Blas, J.C. & Mateos, G.G. (2001). Influence of Source and Percentage of Fat Added to Diet on Performance and Fatty Acid Composition of Egg Yolks of Two Strains of Laying Hens. *Poultry Science* 80, 1171-1179.
Hammershøj, M. & Steenfeldt, S. (2005). Effects of blue lupin *(Lupinus angustifolius)* in organic layer diets and supplementation with foraging material on egg production and some egg quality parameters. *Poultry Science* 84, 723-733.
Hernroth, B.E., Conden-Hansson, A.C., Rehnstam-Holm, A.S., Girones, R. & Allard, A.K. (2002). Environmental factors influencing human viral pathogens and their potential indicator organisms in the blue mussel, Mytilus edulis: the first Scandinavian report. *Applied and Environmental Microbiology* 68(9), 4523-4533.
IFOAM (2005). The IFOAM basic standards for organic production and processing. *International Federation of Organic Movments*
International Organisation for Standardisation (1985). Sensory analysis - methodology - Flavour profile methods. Geneva, Switzerland.
International Organisation for Standardisation (1993). ISO 8586-1. General guidance for selection, training and monitoring of assessors. Geneva, Switzerland.
Ito, E., Yasumoto, T., Takai, A., Imanishi, S. & Harada, K. (2002). Investigation of the distribution and excretion of okadaic acid in mice using immunostaining method. *Toxicon* 40, 159-165.
Jönsson, L. & Elwinger, K. (2009). Mussel meal as a replacement for fish meal in feeds for organic poultry - a pilot short term study. *Acta Agriculturae Scandinavica Section A Animal Science* 59(1), 22-27.
Karadas, F., Grammenidis, E., Surai, P.F., Acamovic, T. & Sparks, N.H.C. (2006). Effects of carotenoids from lucerne, marigold and tomato on egg yolk pigmentation and carotenoid composition. *British Poultry Science* 47(5), 561-566.
Kjos, N.P., Herstad, O., Øverland, M. & Skrede, A. (2000). Effects of dietary fish silage and fish fat on growth performance and meat quality of broiler chicks. *Canadian Journal of Animal Science* 80, 625-632.
Kollberg, S. & Ljungqvist, L. (2005). Musslor som livsmedel och råvara inom lantbruket. http://www.miljomusslor.loven.gu.se (In Swedish).
Krogdahl, Å. (1985). Fish Viscera Silage as a Protein Source for Poultry. I. Experiments with Layer-type Chicks and Hens. *Acta Agriculturae Scandinavica* 35, 3-23.
Leeson, S. & Caston, L. (2004). Enrichment of eggs with lutein. *Poultry Science* 83, 1709-1712.
Leskanich, C.O. & Noble, R.C. (1997). Manipulation of the n-3 polyunsaturated fatty acid composition of avian eggs and meat. *World's Poultry Science Journal* 53(2), 155-183.

Lindahl, O., Hart, R., Hernroth, B., Kollberg, S., Loo, L.O., Olrog, L., Rehnstam-Holm, A.S., Svensson, J., Svensson, S. & Syversen, U. (2005). Improving Marine Water Quality by Mussel Farming: A Profitable Solution for Swedish Society. *Ambio* 34(2), 131-138.

Lutz, R.A. (Ed.) (1980). *Mussel Culture and Harvest: a North American Perspective*. Amsterdam: Elsevier Scientific publishing Company.

Matsuno, T. (1989). Animal carotenoids. In: Krinsky, N.I.*, et al.* (Eds.) *Carotenoids Chemistry and Biology*. New York: Plenum Press. pp. 59-74.

Novak, C., Yakout, H. & Scheideler, S. (2004). The combined effects of dietary lysine and total sulfur amino acid level on egg production parameters and egg components in Dekalb Delta laying hens. *Poultry Science* 83(6), 977-984.

NRC (1994). Nutrient Requirements of Poultry. 9th rev. ed. National Academy Press. Washington DC.

Nys, Y. (2000). Dietary carotenoids and egg yolk coloration - a review. *Archiv Für Geflügelkunde* 64(2), 45-54.

Peguri, A. & Coon, C. (1993). Effect of feather coverage and temperature on layer performance. *Poultry Science* 72(7), 1318-1329.

Persson, M. & Karlson, B. (2008). Kontrollprogrammet för tvåskaliga blötdjur (In Swedish). Sweden: National Food Administration.

Quilliam, M.A. (2003). Chemical methods for lipophilic shellfish toxins. In: Hallegraeff, G.M.*, et al.* (Eds.) *Manual on Harmful Marine Microalgae. Monographs on Oceanographic Methodology*. Paris: Unesco Publishing. pp. 211-246.

Rehnstam-Holm, A.S. & Hernroth, B. (2005). Shellfish and public health: A Swedish perspective. *Ambio* 34(2), 139-144.

Report from The Swedish Government (2005/06:88). Ekologisk produktion och konsumtion - Mål och inriktning 2010.

ROSS Manual (2002). *ROSS broiler management manual*. Aviagen Limited, Midlothian, Scotland.

SAS (2004). *SAS/STAT User's Guide, version 9.1*. Cary, N.C.: SAS Institute Inc.

Schutte, J.B., De Jong, J. & Bertram, H.L. (1994). Requirement of the laying hen for sulfur amino acids. *Poultry Science* 73, 274-280.

Schutte, J.B. & Van Weerden, E.J. (1978). Requirement of the hen for sulphurcontaining amino acids. *British Poultry Science* 19, 573-581.

Smith, A., Rose, S.P., Wells, R.G. & Pirgozliev, V. (2000). The effect of changing the excreta moisture of caged laying hens on the excreta and microbial contamination of their egg shells. *British Poultry Science* 41, 168-173.

Snedecor, G.W. & Cochran, W.G. (1989). *Statistical methods*. 8th edition. Ames, Iowa: Iowa State University Press.

Svensson, S. (2003). Depuration of okadaic acid (Diarrhetic shellfish toxin) in mussels, Mytilis edulis (Linnaeus), feeding on different quantities of nontoxic algae. *Aquaculture* 218, 277-291.

Tauson, R., Ambrosen, T. & Elwinger, K. (1984). Evaluation of procedures for scoring the integument of laying hens - independent scoring of plumage condition. *Acta Agriculturae Scandinavica Section A Animal Science* 34, 400-408.

Tauson, R., Kjaer, J., Maria, G.A., Cepero, R. & Holm, K.-E. (2005). Applied scoring of integument and health in laying hens. In: Siwiec, D. (Ed.) *Proceedings of 7th European Symposium on Poultry Welfare*, Lublin, Poland, 15-19 June.

Tauson, R. & Svensson, S.A. (1980). Influence of plumage condition on the hen's feed requirement. *Swedish Journal of Agriculture Research* 19, 35-39.

Tiller, H. (2001). Nutrition and animal welfare in egg production systems. In: *Proceedings of 13th European Symposium on Poultry Nutrition*, Blankenberge, Belgium pp. 226-232.

Vale, P. & de M. Sampayo, M.A. (2000). Dinophysistoxin-2: a rare diarrhoeic toxin associated with *Dinophysis acuta*. *Toxicon* 38, 1599-1606.

Wall, H. & Tauson, R. (2002). Egg quality in Furnished Cages for Laying Hens-Effects of crack Reduction Measures and Hybrid. *Poultry Science* 81, 340-348.

Van Elswyk, M.E. (1997). Comparison of *n*-3 fatty acid sources in laying hen rations for improvement of whole egg nutritional quality: a review. *British Journal of Nutrition* 78(Suppl. 1), S61-S69.

Wilson, S. (2009). Nutritional practice and environmental impact under different production systems. *17th European symposium on poultry nutrition*. Edinburgh, Scotland: Poultry Science The World´s Association

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