



Final Report

For DARCOF II research projects financed by grants from
The Directorate for Food, Fisheries and Agro Business
under the Danish Ministry of Food, Agriculture and Fisheries

1. Research program

Research in organic farming 2000-2005 (DARCOF II)

Project title and number

Pig feeding under organic farming conditions with emphasis on nutrient utilisation,
product quality and health (II.7)

Head of project

Senior scientist Martin Tang Sørensen
Danish Institute of Agricultural Sciences
Department of Animal Nutrition and Physiology
P.O. Box 50
8830 Tjele
Phone +45 89 99 15 54
Fax +45 89 99 15 25
E-mail MartinT.Sorensen@agrsci.dk

Participating institutes

Danish Institute of Agricultural Sciences
Department of Animal Nutrition and Physiology
P.O. Box 50
8830 Tjele
Phone +45 89 99 13 76

Slutrapporten sendes elektronisk til Forskningscenter for Økologisk Jordbrug
foejo@agrsci.dk senest 3 måneder efter projektets afslutning.

Slutrapporten vedlægges et dansk resumé.

Fax +45 89 99 15 25
E-mail MartinT.Sorensen@agrsci.dk

Danish Meat Research Institute
P.O. Box 57
Maglegårdsvej 2
4000 Roskilde
Phone +45 46 30 32 51
Fax +45 46 30 31 32
E-mail ccm@danishmeat.dk

Other project staff

Jose A. Fernandez (JAF), Senior Scientist, Ph.D., Department of Animal Nutrition and Physiology, DIAS, Ph.: +45 8999 1374; Fax: +45 8999 1525; E-mail: José.Fernandez@agrsci.dk

Søren Krogh Jensen (SKJ), Senior Scientist, Ph.D., Department of Animal Nutrition and Physiology, DIAS, Ph.: +45 8999 1117; Fax: +45 8999 1166 E-mail: SorenK-rogh.Jensen@agrsci.dk

Hanne Damgaard Poulsen (HDP), Senior Scientist, Ph.D., Department of Animal Nutrition and Physiology, DIAS, Ph.: +45 8999 1386; Fax: +45 8999 1525; E-mail: HanneD.Poulsen@agrsci.dk

Knud Erik Bach Knudsen (KEBK), Professor, Ph.D., Department of Animal Nutrition and Physiology, DIAS, Ph.: +45 8999 1143; Fax: +45 8999 1378; E-mail: Knud-Erik.BachKnudsen@agrsci.dk

Ellen-Margrethe Vestergaard (EMV), Senior Scientist, Ph.D., Department of Animal Health and Welfare, DIAS, Ph.: +45 8999 1315; Fax: +45 8999 1500; E-mail: EllenM.Vestergard@agrsci.dk

Laurits Lydehøj Hansen (LLH), Senior Scientist, Ph.D., Department of Animal Product Quality, DIAS, Ph.: +45 8999 1255; Fax: +45 8999 1564; E-mail: LauritsLydehoj.Hansen@agrsci.dk

Karen Søegaard (KAS), Senior Scientist, Ph.D., Department of Crop Physiology and Soil Science, DIAS, Ph.: +45 8999 1834, Fax: +45 8999 1619; E-mail: Karen.Soegaard@agrsci.dk

Chris Claudi-Magnussen (CCM), Project leader, Ph.D., Danish Meat Research Institute, Ph.: +45 46 30 32 51; Fax: +45 46 30 31 32; E-mail: ccm@danishmeat.dk

Hanne Maribo (HMA), Consultant, Department of Nutrition and Reproduction, Danish Bacon and Meat Council, Ph.: +45 3373 2626; Fax: +45 3314 2517; E-mail: hma@danskeslagterier.dk

6. Project period (month, year)

Start of project: April 2001
End of project: June 2005

7. Final report

A. Project summary

A knowledge synthesis initiated by DARCOF revealed that some topics in relation to feeding of pigs under organic farming conditions needed to be studied. Thus the overall goal of this project was to provide new insight that can be applied in the formulation of feeding strategies and diets in organic pig production.

The demand for self-sufficiency in feed is increasing, and at the same time the feed sources must contain sufficient nutrients (amino acids, vitamins and minerals) to meet the demands of the pigs. Alternative feed crops and feed ingredients therefore have to be studied. With this background, a series of experiments was conducted with lupine as a substitute for more conventional protein sources. In this connection, a reduction in protein requirements would make it easier to formulate adequate diets.

Roughage can be a realistic alternative to pigs if feed uptake is not limiting for the production. This is the case for pregnant sows. Thus, the grass uptake of pregnant sows on pasture was investigated during the summer, and clover grass silage uptake was investigated during the winter. It was also investigated whether the requirements for essential nutrients are met (amino acids, vitamins and minerals).

In organic pig production the restrictive use of medicine increases the incitement of applying feed sources and feeding strategies that may prevent infections. This aspect was investigated with regard to reducing weaning diarrhoea and infection with intestinal worms.

Finally the effect of the feeds was investigated with regard to carcass and meat quality, e.g. the effect of lupine on the meat content of skatole.

The work was conducted in three work packages involving experiments with pregnant sows, weaned piglets and growing-finishing pigs. The expected achievements comprised:

Table A.1: Work package list (from application)

No.	Work package title	Participants*	Budget (1.000 DKK)	Start	End	Deliverable no(s):
-	Co-ordination	MTS	248.500	01.04. 2001	30.6 2005	
1	Adequate supply of dietary protein, vitamins and minerals to sows and growing-finishing pigs.	JAF	1.900.000	01.07. 2001	31.12. 2004	D1.2-D1.10
2	Feeding strategies for weaned and growing-finishing pigs with emphasis on gut health.	KEBK	1.900.000	01.07. 2001	30.06. 2005	D2.1-D2.5
3	Effects of nutrient supply for growing-finishing pigs on product quality.	CCM	951.500	01.01. 2002	31.12.2004	D3.1-D3.3
Total			5.000.000			

* Responsible participant

B. Objectives and expected achievements

The overall objective of the project was to obtain new knowledge, that can support the recommendation of suitable dietary composition and feeding strategies for organic pig production. The emphasis was placed on feedstuffs of organic farmed origin and the nutritional requirements for performance, carcass quality, meat quality and health of the pigs will be studied. Expected achievements comprise:

- Directions on how to increase self-sufficiency in the feed supply
- Increased knowledge with regard to the requirements for essential amino acids in organic pig production
- Increased knowledge with regard to the requirements for vitamins and minerals in organic pig production
- Development of feeding strategies to decrease weaning diarrhoea in organic pig production
- Development of feeding strategies to decrease the risk of infection with intestinal worms in organic pig production
- Description of the effects of selected feed sources and feeding strategies on carcass and meat quality

C. Progress and results

C.1 Description (summary) of main results and conclusions

[Her anføres resultater og konklusioner, samt resultaternes aktuelle anvendelighed og fremtidige perspektiver, herunder beskrives resultaternes eventuelle markedsmæssige potentiale og samfundsøkonomiske bidrag. Endvidere skal det anføres i hvilket omfang resultaterne har ført til nye kompetencer, fastholdelse af projektmedarbejdere, givet grundlag for nye projekter o.lign.]

WP 1. Adequate supply of dietary protein, vitamins and minerals to sows and growing-finishing pigs

Task 1.1. Production experiments with growing pigs

At the organic Research Station Rugballegård an experiment concerning protein supply for growing pigs has been carried out. Since suitable protein feeds of high quality for organic production are scarce, the aim was to study the effect of a reduced protein level in the feed and also to estimate the applicability of lupin as protein source.

Two hundred pigs were allocated to five diets: N=, N25, L12,5 and L25 where N and L mean Normal and Low protein supply, respectively while the numbers 0, 12,5 and 25 state percentage of lupin in the feed mixture. The pigs, which were housed in 20 pens with each five castrates and 20 pens with each five sows, were fed ad-libitum from feed dispensers in the weight interval 30-105 kg. Furthermore, they had free access to grass clover silage in an outdoor veranda.

The two levels of protein in the feed were according to the analyses at 137 and 127 g digestible crude protein per FEs for N and L, respectively while the content of digestible lysine was 7.5 and 6.5 g per FEs, respectively. The feed intake, daily growth and feed utilization were

not significantly influenced by the protein level. The low protein level caused that the meat content of the carcass was significantly reduced from 59.8 to 59.2%. However, the effect of a low protein level was found only at the castrates that were given feed without lupin (59.2 vs. 57.2) and this was caused by a significantly higher feed consumption. So, it is recommended to feed the castrates restrictively, at least in the terminal phase.

The pigs which were allocated protein according to the standards had in average an N-loss of 47 g while all the pigs that had reduced protein allocation, only excreted 40 g N per kg growth. This was a reduction of the excretion by 15%.

Lupin inclusion (up to 25%) had a negative effect (5-6%) on daily feed intake, growth and feed utilization but improved meat percentage by about 2% units. The excretion of N was increased from 35 to 52 g/kg gain and excretion of P from 8.6 to 9.3 g/kg gain.

The use of lupin in organic pig feeding causes that the dietary methionine deficit is exacerbated. Furthermore, the protein in lupin contains less lysine than the pigs require. Finally, the amount and the fibre type in lupin have the effect that the content of the gastrointestinal tract increases and causes larger slaughter loss (0.08% per % of lupin in the mixture). On the other hand, lupin contains more than 30% crude protein and may, however, be considered as a useful supplement to the other protein sources. Fibre content has, because of its high fermentability a favourable effect in the intestinal tract in the form of smaller strain from parasites and reduced skatole production which may give off-flavour to the meat (boar taint).

Lupin may very well be used for feeding organic pigs but for the abovementioned reasons, it is not recommended to include more than 10% in the mixture.

Protein supply of 100% organically fed pigs is actually a matter of providing enough methionine and lysine. Different combinations of feed and nutrient supply may contribute to this.

Task 1.2. Digestibility and balance experiments

Digestibility and balance studies were carried out with four of the diets used in the production experiment (i.e. diets with 0 or 25 % lupin, Task 1.1) and also with a batch of organic produced lupin respectively oats. Lupin and oats were diluted with wheat and potato starch in order to stimulate the pig's appetite.

The results showed that there were no major differences in the digestibility of phosphorus within the 4 diets used in the production experiment and the mean value was 48 %. The availability of zinc approximated 20 % in all four diets.

Increasing amounts of lupin (40, 55 and 70 %) showed that the digestibility of phosphorus and zinc was about 48 and 30 %, respectively.

The digestibility of phosphorus and zinc in oat (diluted with 10% starch) was determined to be 37 % and 44 % respectively.

Task 1.3. Production experiments with pregnant sows

A multiannual experiment comprising outdoor pregnant sows has been carried out at the organic experimental station Rugballegård. The aim of the experiment was to provide new knowledge about management of grazing fields and on utilisation of clover-grass in connection with outdoor sow units. Specifically, the grazing potential of clover-grass in summer and feeding clover-grass silage in winter were examined. This also included the possibilities for highest possible contribution to pregnant sow's requirement for energy, amino acids, minerals and vitamins from this crop. Three groups with each five pregnant sows were put in a clover-grass field divided into three identical grazing systems, which, again were divided into three rotation paddocks. Each group of sows in each grazing system was fed a standard complete diet for pregnant sows (F) or half F + half barley (FB) or only barley (B). In each system the sows grazed alternately one week in each of the paddocks.

The results of the chosen grazing strategy in this experiment, where a two week resting period

was included after one week of grazing, showed that the percentage of clover had a considerably higher level compared with continuous grazing in previous experiments. During the period the percentage of grass stems and dead material increased and the percentage of clover decreased. This development also influenced the grass quality in the form of decreasing protein- (EFN) and energy digestibility (EFOS) and consequently also the content of FE_{dr} during the grazing period.

The allocation of concentrates did not influence the intake of grass clover. Also, the intake of FEs was not different for the periods May-June and August-September.

The daily intake of energy from grass clover, calculated on the basis of the growth of the sows, was on average 1.63 FEs corresponding to 61% of their daily energy intake. Grazing contributed to the vitamin E and A supply of the sows with about 240 mg α -tokoferol and 35200 IE retinol per day. This has corresponded to 93 – 98% of the total vitamin A intake and 83 – 95 % of the total vitamin E intake.

Also the sows, which were fed silage, had their vitamin A and E requirement covered through the silage even though the vitamin A and E intake was lower compared with grazing sows. Mineral concentration in plasma and in milk was not reduced when the sows got reduced the amount of complete diet and thereby also the mineral supplement. However, there was a rise in the concentration of zinc in both plasma and milk of the sows having barley and clover-grass silage. This may thus indicate that sows are able to perform normally without supplementation of the minerals calcium, phosphorus and zinc during pregnancy. It ought to be mentioned though that selen supply has still to be monitored, since it was not included in this study.

The results show that pregnant sows are able to obtain a substantial nutrient supplement from grass or silage. Nutrient content of the supplemental concentrate (dietary protein, vitamins and minerals) can therefore be adjusted accordingly.

WP 2. Feeding strategies for weaned and growing-finishing pigs with emphasis on gut health

Task 2.1. Gut challenge and diet screening

A gut *E. coli* O 149 challenge model for piglets weaned at 4 weeks of age, which was established in another project, was modified and applied to piglets weaned at 7 weeks of age simulating organic conditions. All sows in the herd at Foulum Research Centre were tested for susceptibility to the attachment site of *E. coli* O 149 in the intestines (F4-fimbriae adhesion receptor). Only piglets from mono-zygotic susceptible sows were subsequently used in the challenge experiments. Initially a dose-response experiment was conducted, where three different doses of the *E. coli* O 149 (10^6 , 10^8 and 10^{10}) were applied to piglets by stomach tube daily on days 2-4 after weaning. Clinical recordings, assessment of diarrhoea and bacteriological recovery levels of *E. coli* O 149 showed that a dose of 10^8 bacteria was more suitable than the two other doses. Thus in the four subsequent experiments with four dietary factors, a dose of 10^8 was used.

Each of the four experiments consisted of 32 piglets from 4 sows. The design was 2 x 2 factorial with dietary factor and *E. coli* O 149 challenge as the two factors, each at two levels. The experimental period lasted for 10 days from weaning. The daily recordings included feed intake, growth, faces scoring (from 1=firm and solid to 6=yellow and watery) and clinical observations. Faces was sampled on days 2-5 after weaning and tested for *E. coli* strains. The following dietary factors were tested:

1. Ad libitum versus restricted feeding
2. Control versus low-protein diets at ad libitum feeding

3. Control versus lupine as a protein source at ad libitum feeding
4. Control versus extra E-vitamin at ad libitum feeding (approx. 60 versus 200 I.E. E-vitamin/kg feed)

As expected, feed restriction decreased the weight gain, while faeces characteristics were unaffected. *E. coli* challenge clearly compromised the faeces characteristics, and there was a tendency for an interaction between feeding level and *E. coli* challenge with regard to weight gain, i.e. the *E. coli* challenge may decrease weight gain for piglets on ad libitum feeding. Thus the data suggest that ad libitum feeding may be disadvantageous if piglets become *E. coli* infected, however the weight gain of these piglets were still higher than the weight gain of the restrictively fed piglets.

As expected, protein restriction decreased the weight gain. Faeces dry matter increased and faeces score decreased, i.e. protein restriction led to a trend towards less diarrhoea. There was an interaction between protein level and *E. coli* challenge with regard to faeces dry matter, i.e. the *E. coli* challenge decreased faeces dry matter for piglets on the control protein level. Thus the data suggest that control protein level may be disadvantageous if piglets become infected, however the weight gain of these piglets were still higher (albeit non-significantly) than the weight gain of piglets fed the low-protein diet.

Inclusion of lupine neither affected weight gain nor faeces characteristics. Regardless of an unexplainable interaction between lupine inclusion and *E. coli* challenge with regard to weight gain, it is concluded that lupine is an acceptable feed ingredient for piglets.

The faeces characteristics were affected by vitamin E, i.e. faeces dry matter decreased in parallel with an increase in faeces score and number of haemolytic bacteria in piglets fed supplementary vitamin E, while growth and feed intake were not affected. In the experiment with E-vitamin, additional samples (beyond the scope of the DARCOF project) were taken from the small intestine, gall bladder and liver for subsequent analysis of immunological responses including IgA and IgM, intestinal antibodies against *E. coli* O 149, activity of macrophages, and histological examination of the gut morphology. These data showed that Vitamin E supplementation improved the immune status of the piglets. There is no obvious explanation for the paradox that supplementary E vitamin affected the faeces characteristics towards diarrhoea while at the same time the immune status was improved.

Task 2.2. Effect of dietary factors on weaning diarrhoea

Based on results from task 2.1 as well as practical possibilities, E-vitamin supplementation was selected as the dietary factor to be tested in organic farmed weaned piglets. This experiment was conducted in three commercial organic farms. At weaning the piglets were split into two groups and one group (vit. E) received a supplement of 100 mg natural vitamin E pr. kg feed while the other group (Control, containing 55-150 mg alfa-tocopherol per kg feed) did not. Weight and faeces score were recorded at weaning, 5 days after weaning and 3-5 weeks after weaning. Although recordings are on individual pigs, the statistical analysis is based on group means (a total of 44 groups). In one of the three farms, there were frequent occurrence of weaning diarrhoea, while in the other two farms, weaning diarrhoea were rather infrequent. Evaluation of the data shows that vitamin E supplementation neither affected weaning diarrhoea nor weight gain. Thus, the data suggest that in one farm, the load of pathogens was of such a magnitude that extra vitamin E was insufficient to protect against weaning diarrhoea. In the two other farms the health status was apparently so good that extra vitamin E made no difference. Also the E-vitamin content in the control diet may have been at a level where an extra supplement would make no difference.

Conclusion with regard to weaning diarrhoea. It is obvious that the practise to decrease weaning diarrhoea by either restricted feeding or lowering the dietary protein level will be at the expense of weight gain. Further it can be concluded that lupine can be included as a feed ingredient for weaned piglets and that extra vitamin E in addition to the new norm (130 mg per FE, if given in the form of synthetic E-vitamin) has no effect. It must be stressed that the condition of weaning diarrhoea is multifactorial and that factors other than dietary such as infection load, hygiene and stress etc. must be taken into consideration.

Task 2.3. Effect of diets varying in digestible and non-digestible carbohydrates on *Trichuris suis* infection

Two diets with contrasting digestibility was formulated - the diet containing resistant carbohydrates (Diet 1) was based on triticale, barley, rapeseed cake and silage as the main ingredients while the diet with fermentable carbohydrates (Diet 2) was based on triticale, barley, blue lupines and chicory roots. Assuming that the pigs will consume approximately 7 % of the dry matter from silage and 15 % from chicory roots, the content of feed units for pigs (FEs) will be 0.96 and 1.09 FEs in Diets 1 and 2, respectively. The two types of diets were fed to four groups of 8 animals:

- Group 1, Diet 1, no infection
- Group 2, Diet 1, infected
- Group 3, Diet 2, no infection
- Group 4, Diet 2, infected.

The pigs were adapted for two weeks to the diets and then infected with 2000 infective eggs of *T. suis*. Faecal egg samples were taken twice weekly to determine the number of parasite eggs excreted. All the pigs were slaughtered 12 weeks post infection (pi). The large intestine was divided into caecum and 5 colon sections. pH was measured in each section and samples were taken for chemical examinations. The remaining intestinal contents were collected to determine the worm burden. The weight gain on the pigs receiving Diet 1 was lower than on Diet 2 presumably reflecting a lower energy intake, primarily because of less consumption of the silage as expected but there was difference between infected and non-infected pigs. The infected group receiving Diet 1 had higher faecal egg counts compared to the infected pigs receiving Diet 2 until 9 weeks pi, after which the two groups had similar mean egg counts until slaughter. However, the variation in faecal egg counts was substantial. No significant difference was found in the number of worms between the two groups.

The two diets introduced significantly differences in the gastrointestinal environment with pH generally lower and the concentration of short-chain fatty acids (SCFA) generally higher when feeding diet 2 (130-150 mmol/kg digesta) compared to diet 1 (105-125 mmol/kg digesta) but with no significant differences between either pH or SCFA of infected and non-infected pigs. The former diet also clearly stimulated butyrate formation in the large intestine.

Task 2.4. Effect of diets varying in carbohydrate composition on establishment of dual infections with *Trichuris suis* and *Brachyspira hyodysenteriae*

A. Influence of carbohydrates on experimental infection with *Brachyspira hyodysenteriae* – a pilot study

Two diets with contrasting fermentability were formulated. Diet 1 containing resistant carbohydrates was based on triticale, barley, rapeseed cake as the main ingredients supplemented with grass clover silage. Diet 2 containing fermentable carbohydrates was based on triticale, barley, blue lupines and dried grinded chicory roots. Furthermore, a standard pig diet in pel-

lets based on barley, soybean and wheat (Diet 3) was included, as it has been shown to induce high incidence of dysentery.

Thirty-eight pigs (female and castrates) tested negative for swine dysentery with an average weight of 26 kg were divided according to weight and sex into 4 groups as follows:

Group A: Diet 1 (10 pigs)

Group B: Diet 2 (10 pigs)

Group C: Diet 3 (10 pigs)

Group D: Diet 3 (8 pigs)

The pigs were fed once a day according to weight. After two weeks of adaptation to the diets pigs in groups A, B, and C were challenged with 5×10^6 colony-forming units of *B. hyodysenteriae* on three consecutive days. Group D remained as an uninfected control group. The pigs were weighed every other week and faecal samples were collected twice a week to monitor the infection. The faecal samples were cultured selectively for the presence of *B. hyodysenteriae* as well as scored according to consistency (normal, loose, watery/mucoid, and bloody diarrhoea), which was utilized as a measure of clinical disease. All the pigs were slaughtered 4 weeks post infection. At slaughter a macroscopic pathological examination was performed on each pig. Tissue samples were taken from caecum and the anterior part of the colon for histopathological examinations. The gastrointestinal tract was removed and the large intestine divided into caecum and colon, which were emptied and the tissue weighed. pH was measured in the contents from each compartment and content samples were taken for chemical analyses.

B. hyodysenteriae was not isolated from the control pigs (Group D) at any time during the study. The infected pigs fed Diet 3 (Group C) all developed clinical dysentery with bloody diarrhoea during the experiment. In Group A, four pigs had clinical disease, and in Group B, only 1 pig was observed with dysentery. *B. hyodysenteriae* was first isolated from faeces 6 days post challenge (p.c.) in Group C and 9 days p.c. in Group A. In both groups the bacteria were present until the end of the experiment (day 26 p.c.). In Group B, however, the bacteria was only isolated on day 12 and 15 post challenge. The isolation of bacteria was correlated with the clinical symptoms, *B. hyodysenteriae* was only isolated from pigs with clinical symptoms (watery/mucoid faeces and bloody diarrhoea). The weight gain of the control pigs fed the standard diet was higher compared with the infected groups.

Diet 2 and to a lesser extent Diet 1 seem to provide considerable protection against swine dysentery compared with Diet 3. Even though the disease is easily spread from pig to pig through ingestion of faecal material, the infection was not transmitted among pen mates.

B. Influence of carbohydrates on dual experimental infections with *Trichuris suis* and *Brachy-spira hyodysenteriae*

The objective of the study was: 1) to investigate the interaction of *T. suis* and *B. hyodysenteriae* and 2) to study the effect of carbohydrates with different fermentability on dual infections with *T. suis* and *B. hyodysenteriae*

Diet 1 and Diet 2 from the pilot study were used in this experiment.

Seventy-two pigs (female and castrates) tested negative for *T. suis* and swine dysentery with an average weight of 22 kg were divided into 8 groups (A-H) according to weight and sex as follows:

Group A: Diet 1, *T. suis* and *B. hyodysenteriae*

Group B: Diet 2, *T. suis* and *B. hyodysenteriae*

Group C: Diet 1, *B. hyodysenteriae*
 Group D: Diet 2, *B. hyodysenteriae*
 Group E: Diet 1, *T. suis*
 Group F: Diet 2, *T. suis*
 Group G: Diet 1, control
 Group H: Diet 2, control

The pigs were fed according to weight once a day. Two pigs (one from Group B and one from Group F) died before the experimental infections and were not replaced. After two weeks of diet adaptation, pigs in group A, B, E, and F were inoculated with 2000 infective *T. suis* eggs each. Pigs in group A, B, C, and D were challenged with 1×10^9 colony-forming units of *B. hyodysenteriae* each on three consecutive days. Group G and H were uninfected control groups. The pigs were weighed every other week and monitored daily for clinical symptoms. Faecal samples were collected twice a week to monitor the cause of dysentery. The faecal samples were cultured selectively for the presence of *B. hyodysenteriae* as well as scored according to consistency (normal, loose, watery/mucoid, and bloody diarrhoea), which was utilized as a measure of clinical disease. All pigs were slaughtered 6 weeks post infection (p.i.) over a course of 5 days. At slaughter a faecal sample was collected for examination of *T. suis* eggs and a macroscopic pathological examination was performed on each pig. Tissue samples were taken from the anterior part of the colon for histopathological examinations. The large intestine was divided into caecum and 5 colon sections. The sections were emptied and weighed and pH was measured in the contents of each section. Samples were taken from the contents for chemical analyses. The remaining contents were used for recovery of *T. suis*.

T. suis were not found at slaughter and no *B. hyodysenteriae* were isolated from any of the control pigs in group G and H. No clinical signs of dysentery were found in pigs experimentally challenged with *B. hyodysenteriae* that were receiving Diet 2 (Group B and D). Likewise, no bacteria were isolated from faeces in any of these pigs. In contrast, clinical symptoms of dysentery were observed in all experimentally *B. hyodysenteriae* pigs fed Diet 1 (Group A and C, except 1 pig in Group A) and similarly the bacteria were found in faeces of all these pigs at different times during the experiment starting from 6 days p.c.

There was no difference in worm counts between any of the *T. suis* infected groups (Group A, B, E, and F). The lowest worm burdens, however, were found in the pigs fed Diet 2 (Group B and F). The frequency of swine dysentery was lower in pigs with dual infections, but consistent throughout the course of the study. Pigs only infected with *B. hyodysenteriae*, on the other hand, had higher frequency of clinical symptoms, but only over a period of 2 weeks from 9 days to 23 days p.c.

The weight gain did not differ significantly between the groups, but was lowest in pigs with clinical swine dysentery (Group A and C).

Diet 2 prevented completely the occurrence of swine dysentery in both experimentally infected groups (A and C).

WP 3. Effects of nutrients for growing-finishing pigs on product quality

Task 3.1

In a 2x2 design, organic pigs are fed two levels of protein (norm and 85% of norm) and 0% and 25% lupine.

When the level of protein in the feed to organic pigs are lowered to 85% of norm, the lean meat percentage becomes up to 1 percent lower and the intramuscular fat (IMF) rises 0,5 percent. Drip loss, ultimate pH, temperature 24 hours after slaughter, content of pigment and texture for the loin muscle are not affected. The level of skatole in back fat becomes a little lower. The content of saturated fatty acids (SFA) in back fat rises; the content of monounsaturated fatty acids (MUFA) does not change while the content of polyunsaturated fatty acids (PUFA) falls. This means that the iodine value falls from 67 to 65. The eating quality expressed by the sensory profile of fried loin chops is only affected a little. Acidic and metal taste is a little less intensive and sweet taste is a little more intensive. In combination with 25% lupine though, the loins become more tender and juicy. The cooking loss when frying the loin chops becomes 1 percent higher but not if combined with feeding with 25% lupine.

When feeding with 25% lupine, the lean meat percentage rises by 1-1,5 percent. The temperature in the loin muscle 24 hours after slaughter is lower - perhaps because of a lower carcass weight. Drip loss ultimate pH, IMF, content of pigment and texture for the loin muscle are not affected. The content of SFA and MUFA in back fat falls while the content of PUFA rises resulting in a rise of iodine value from 63 to 69. The level of skatole in back fat is approx. halved for both gilts and castrates. Even though the levels of skatole are very low, it is recommended to test the effect of lupine feeding of entire male pigs with the purpose of reducing the occurrence of boar taint. Piggy odour and flavour of fried loin chops decreases, but the levels are in both cases very low. The intensity of fried meat odour and flavour rises a little.

The quality of the tested pork product (fried loin chops) is not affected in any decisive way by the tested feedstuffs. Especially the higher iodine value in the back fat by feeding with lupine may be of importance for the quality of other pork products. The effect of different fat sources in the feed for the fatty acid composition in the pig and the quality of different fresh meat and meat products is being investigated in an ongoing collaboration project ("Nye foderfædtkilder til svin") supported by the Danish Innovation Law. The effect of feeding with lupine on skatole in back fat of castrates and gilts should call upon an investigation of a similar effect for entire males where the level of skatole in certain cases may be considerably higher and thereby give rise to "boar taint" when heating the pork.

C.2 Fulfilment of deliverables and milestones

[Deliverables er forskellige former for offentligt tilgængelige produkter (artikler, rapporter, informationsmøder etc.) og det skal angives, hvilken form for produkt der er tale om. Milestones er væsentlige trin i forskningsprocessen. Der skal angives et tidspunkt for både deliverables og milestones (milestones ligger typisk tidligere end deliverables). Ændringer i deliverables og milestones forsyne med en notits om, at de er ændret i forhold til ansøgningen, og hvorfor de er ændret. Alle deliverables og milestones skal stå i rapporten, og de, der er passeret, mærkes af som udførte, eller der angives en ny dato. Større afvigelser kommenteres i D.]

(To be completed for each work package)

WP1: Adequate supply of dietary protein, vitamin and minerals to pregnant sows and growing-finishing pigs	Time schedule according to application	Deviations, if any*	Full filled
Deliverables			
D 1.1. Protocol for experiments with pregnant sows, 1 replicate	Quarter 3 – 2001		√
D 1.2. Protocol for digestibility experiments	Quarter 3 - 2001		√
D 1.3. Protocol for experiments with slaughter pigs, 1. replicate	Quarter 3 - 2001		√

D 1.4. Progress report	Quarter 4 - 2001		√
D 1.5. Progress report	Quarter 4 - 2002		√
D 1.6. Progress report including the results of digestibility and balance experiments	Quarter 4 - 2003		√
D 1.7. Progress report including the results of experiments with pregnant sows	Quarter 4 - 2004		√
D 1.8. Progress report including the results of experiments with slaughter pigs	Quarter 4 - 2004		√
D 1.9. Final report	Quarter 4 - 2004		√
D 1.10. Dissemination of results (DJF report)	Quarter 4 - 2004	*	√
Milestones			
M 1.1. Formulation of recommendations for organic pig production with respect to the usage of lupin under consideration of: Overall performance of pigs Impact on the environment of N and P output	Quarter 4 - 2004		√
M 1.2. Formulation of recommendations for organic pig production regarding the supply of vitamin and minerals to slaughter pigs	Quarter 4 - 2004		√
M 1.3. Formulation of recommendation for outdoor keeping of pregnant sows under consideration of summer and winter feeding methods	Quarter 4 - 2004		√

WP2: Feeding strategies for weaned and growing-finishing pigs with emphasis on gut health	Time schedule according to application	Deviations, if any*	Full filled
Deliverables			
D 2.1. Formulation of feeding strategies for weaned pigs in organic farming	Quarter 2 - 2005		√
D 2.2. Paper concerning challenge with <i>E. coli</i> O 149 and dietary factors	Quarter 3 - 2004	*	√
D 2.3. Paper concerning dietary effects on weaning diarrhoea in organic farming	Quarter 2 - 2005	*	√
D 2.4. Paper concerning the effect of diets varying in digestible and nondigestible carbohydrates on <i>T. suis</i> infection	Quarter 3 - 2004	*	√
D 2.5. Paper concerning the impact of carbohydrate composition on the establishment of dual infection with <i>T. suis</i> and <i>B. pilosicoli</i>	Quarter 2 - 2005	*	√
Milestones			
M 2.1. Preliminary evaluation of the procedure for gut challenge and diet screening	Quarter 3 - 2002		√
M 2.2. Final evaluation of the procedure for gut challenge and diet screening	Quarter 3 - 2002		√
M 2.3. Evaluation of dietary factors on weaning diarrhoea	Quarter 4 - 2003		√
M 2.4. Preliminary evaluation of dietary effects on <i>T. suis</i> infection	Quarter 4 - 2003		√
M 2.5. Final evaluation of dietary effects on <i>T. suis</i> infection	Quarter 1 - 2005		√
M 2.6. Evaluation of dietary effects on dual infection with <i>T. suis</i> and <i>B. pilosicoli</i>	Quarter 1 - 2005		√

WP3: Effects of nutrient supply for growing-finishing pigs on product quality	Time schedule according to application	Deviations, if any*	Full filled
Deliverables			
D 3.1. Protocol for collecting and analysing meat samples	Quarter 3 - 2002		√
D 3.2. Half way progress report	Quarter 3 - 2003		√
D 3.3. Final report	Quarter 4 - 2004		√

Milestones			
M3.1. Formulation of recommendations for organic pig production with respect to the usage of lupin and protein supply under consideration of: Product quality of organic pork Effect of lupin on reduction of skatole level in blood and backfat.	Quarter 4 - 2004		√

* *Deviations are to be further discussed in D*

D. Description of deviations and subsequent adjustments of plans

[Her skal der kun stå en kort forklaring på de afvigelser der er anført i C.2, og en beskrivelse af de ændringer i planerne det har givet anledning til. Der skal ikke gives resultater eller konklusioner]

*D1.10. Two manuscripts for DJF reports have been prepared regarding task 1.1 and task 1.3, respectively. Results from Task 1.2 are incorporated in the report concerning task 1.1.

*Not all papers in Organic Eprints are in the final version. Thus, the versions in the Organic Eprints will be upgraded as the publishing process proceeds.

E. Project publications and other products

[Produkter under 1 skal kopieres fra Organic Eprints. Dette gælder også for produkter, som kun delvist er finansieret af FØJO. Listen fra Organic Eprints kan findes på hjemmesiden <http://www.okoforsk.dk/projekt/index.html> under "Project publications" på de enkelte projekter.]

[Produkter under 2 er mundtlige præsentationer og andet, som ikke skal kunne findes i Organic Eprints]

1.

Products from Organic Eprints archive

English

Sørensen, Martin T.; Vestergaard, Ellen-Margrethe; Jensen, Søren K. and Højsgaard, Søren (2005) [Diarrhoea following weaning at seven weeks of age: Challenge with E. coli O 149 and effect dietary factors](#). Working Paper.

Thomsen, Lisbeth E.; Bach Knudsen, Knud Erik; Jensen, Tim K.; Christensen, Anja S.; Møller, Kristian and Roepstorff, Allan (2005) [The Effect of Fermentable Carbohydrates on Experimental Swine Dysentery and Whip worm Infections in Pigs](#). Working Paper.

Thomsen, Lisbeth E.; Bach Knudsen, Knud Erik and Roepstorff, Allan (2005) [The effect of organically grown diets with contrasting carbohydrate composition on the establishment of Trichuris suis in pigs](#). Working Paper.

Dansk - Danish

Claudi-Magnussen, Chris and Hansen, Laurits Lydehøj (2005) [Svinefodring - næringsstofudnyttelse, produktkvalitet og sundhed. Slutrapport for Work Package 3: Produktkvalitet. FØJO projekt II.7 \(OrganicPigFeed\)](#). Report no. 01793, Slagteriernes Forskningsinstitut.

Fernández, José A. and Danielsen, Viggo (2005) [Reduceret protein i foderet til økologiske slagtesvin fodret med forskellige mængder lupin](#). Report, Department of Animal Health, Welfare and Nutrition, Danish Institute of Agricultural Sciences.

Fernández, José; Danielsen, Viggo; Damgaard, Hanne; Krogh, Søren and Søgaard, Karen (2005) [Rationel anvendelse af afgræsning til drægtige søers ernæring og samspillet med kraft-](#)

[fodertildeling. Kløvergræs til drægtige søer, afgræsset og ensileret, og samspillet med kraftfodertildeling](#). Report, Department of Animal Health, Welfare and Nutrition, Danish Institute of Agricultural Sciences.

Sørensen, Martin T.; Kristensen, Jane; Vestergaard, Ellen-Margrethe and Jensen, Søren K. (2005) [E-vitamin til økologiske smågrise efter fravæning](#). Grøn Viden.

2. Other products (oral presentations, public meetings, field days, etc.)

At the workshop on "Organic Pig Production", April 24-25, 2003, Horsens, Denmark, three oral presentations were made. The titles were:

- The supply of protein, vitamins and minerals for sows and finishing pigs*
- An *E. coli* infection model to study weaning diarrhoea*
- Effects of carbohydrates on establishment of infection with *T suis**

At the EAAP Working Group on "Production and Utilisation of Meat from Entire Male Pigs", November 13-14, 2003, Dublin, Ireland, an oral presentation was made. The title was:

- Effect of Lupine and Protein level for Growing-Finishing Pigs on Skatole in Backfat (Pig off-odour) of Female and Castrated male pigs*

At the Organic Farming Congress, November 16, 2004, Odense, Denmark, an oral presentation was made. The title was:

- Improved feeding with home-grown protein crops*

At the NorFa Graduate course in Advanced Veterinary Parasitology, 18-23 April 2004 two oral presentation were given with the titles:

- Parasite-nutrition interactions: options for control of pig helminths*
- Dietary modulation of digestive physiology in pigs and implications for disease* susceptibility

At "Svinetemamøde", March 15 2005 an oral presentation with the title:

Fruktaner, dysenteri og parasitter - indflydelse af fermenterbare kulhydrater på tyktarmsinfektioner i slagtesvin.

At the Abildgaard Symposium, Copenhagen, Denmark, 26 August, 2005 an oral presentation:

- The influence of dietary carbohydrates on host susceptibility to helminth infections in pigs.

At the 12th Congress on Biotechnology - Bringing genomes to life, Lyngby, Denmark, 21-24 August, 2005 an oral presentation with the title:

- Chicory and lupine used as a prebioticum against swine dysentery changed the colonic microbiota of pigs.

Roepstorff, A., H. Mejer, L.E. Thomsen, S.M. Thamsborg, D.V. Byrne, L.L. Hansen, K.E. Bach Knudsen, K. Møller. 2005. Cikorierødder forbedrer smag og lugt i økologisk svinekød. FØJOenyt, Nyhedsbrev for Forskningscenter for Økologisk Jordbrug, nr. 3, juni 2005.

Thomsen, L.E. Kulhydrater kan være effektive mod svinedysenteri og indvoldsorm. Hyologisk, May, 2005.

* 25-75% financed by DARCOF

** 5-25% financed by DARCOF

F. Scientific education

By collaboration with the Danish Centre for Experimental Parasitology, KVL and the Danish Veterinary Laboratory, a joint PhD student funded by the Research School for Animal Production used data generated in tasks 2.3 and 2.4 for parts of her PhD thesis. The PhD thesis is expected to be finalised by the end of 2005.

The challenge model with *E. coli* O 149 applied in tasks 2.1 and 2.2. was partly developed in the Ph.D. project “The influence of feed intake during lactation on piglets susceptibility to post-weaning diarrhoea”, funded within the framework of the Research School for Animal Production.

A veterinary student has used data generated in task 2.2 for her final “speciale”.

G. National and international cooperation

This project has strong links to the EU project QLRT 30162 “Sustainability in the production of pork with improved nutritional and eating quality using strategic feeding in out-door production” (SUSPORKQUAL) through the project co-ordinator Anders Hans Karlsson, KVL. Furthermore, the co-ordinator of WP1 in this project (JAF) is also the co-ordinator of WP1 (Sustainable Pig Production) in the EU project. The two projects, although with different goals, will complement each other. The ultimate goal of SUSPORKQUAL is to improve the nutritional value, increase eating quality (tenderness), shelf life and technological quality of pork. This will be achieved by combining strategic feeding resulting in compensatory (accelerated) growth in the time up to slaughter, with known feeding strategies (enrichment with E-vitamin, PUFA etc.) that gives rise to improved nutritional value of the out-coming pork.

This project is also linked to the DARCOF II project I.5: “Grain legumes and cereals – new production methods for increased protein supply in organic farming systems”, which is co-ordinated by Erik Steen Jensen, professor at KVL and in which Knud Erik Bach Knudsen is partner. KEBK is further collaborating with the Danish Centre for Experimental Parasitology, KVL (Allan Roepstorff) and the Danish Veterinary Laboratory (Kristian Møller). Ellen-Margrethe Vestergaard is collaborating with professor Jens Peter Nielsen, KVL regarding the challenge model with *E. coli* O 149 used in tasks 2.1 and 2.2.

The project has links to two other DARCOF projects on organic pig production: II.9 “Resource use, environmental impact and economy in organic pig production systems”, co-ordinated by John E. Hermansen and II.8 “Management in relation to health and health safety in organic pig production co-ordinated by Jan Tind Sørensen. Both projects are parts of the same DARCOF research programme as this project. The three projects are complementary, but also synergetic, why the collaboration is relevant for the total output.

The project has also links to the project: “Organic production of steers and bioactive forages for grazing livestock”. The links are through the project co-ordinator Stig Milan Thamsborg and one of the tasks of the project “Influence of bioactive forages on animal health with emphasis on parasitic infections and effects on meat and eating quality” in steers and pigs. Furthermore, there are links to the organic project “Effect of organic pig production on meat and eating quality” co-ordinated by Henrik J. Andersen.

The project has links to lupine testing project “Lupinforsøg, Undersøgelser vedrørende kød- og spisekvalitet” by Danske Slagterier (Hanne Maribo).

The experiences gained in this project have provided a substantial background for input, comments and suggestions to the preliminary report “Possibilities and limitations of protein supply in organic poultry and pig production” by Sundrum, A., Schneider, K. and Richter, U. Project no. SSPE-CT_2004-502397

Finally, at the onset of the new regulative demanding 100% organic rations for livestock, the results obtained through the studies performed under this project are in high demand. The possibilities to carry out further research concerned with the protein supply of organic produced pigs are being surveyed in collaboration with the Danish organisation for Organic Production (Økologisk Landsforening).

H. Critical reflection on the project

[Her gives der en kritisk refleksion over projektets planer, forløb og resultater. Det kan rumme refleksioner over det videnskabelige håndværk med hensyn til fx metodevalg, prøvbarhed og udførelse; over eventuelle ændringer i relevans som følge af ændringer i omverdenen eller som følge af den læring der er sket i projektet; samt over aspekter af forskningsudvikling, især i relation til FØJOs mål om at udvikle tværgående og relevant forskning (og hvad der evt. kunne gøres bedre). Her diskuteres endvidere væsentlige justeringer af projektet som følge af afvigelser fra planen (fra C.2 og D) og andre væsentlige ændringer.]

As a whole, the project plans and designs were well suited to meet the purposes. Only two modifications of the experimental design have been implemented. (1) In task 1.1 the number of treatment groups was reduced from six to five (two levels of lupin at normal dietary protein and three levels of lupine at reduced dietary protein). Given the barn facilities at Rugballegård this modification was necessary to meet the time schedule. This modification had no consequences for task 3.1 concerning evaluation of product quality. (2) In task 2.4 it was decided to use *B. hyodysenteriae* rather than *B. pilosicoli*. This modification was implemented after thorough investigation and discussions with Danish Institute for Food and Veterinary Research.

8. Budget

A. Account for any change in budgets

B. Budget for the whole project (1.000 DKK)

Total consumption of funds from DARCOF and expected consumption this year and coming years

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Man-months					
Scientific personnel	49,8	31,7	14	2,5	48,2
Technical personnel	32,1	26,9	14		40,9

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Salaries					
Scientific personnel	2105,7	1260,1	604,6	108	1972,7
Technical personnel	901,9	722,2	342,7		1064,9
Other operational costs	1159,1	853,3	278,8	56,4	1188,5
Equipment					
Others (please specify)					
Direct costs	4166,7	2835,6	1226,1	164,4	4226,1
Indirect costs (20% of direct costs)	833,3	564,1	244,6	32,9	841,6
Total	5000	3399,7	1470,7	197,3	5067,7

Comments: Merforbrug kr. 67.700 i forhold til det oprindelige budget fordeler sig med et merforbrug i 2003 på kr. 43.500 samt et merforbrug i 2004 på kr. 24.200. Merforbruget er dækket af Danmarks JordbrugsForskning.

Due to reorganisation at DJF, i.e. closing Dept. of Analytical Chemistry where many chemical analyses previously were bought, some of "Other operational costs" is converted to "salaries".

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project Senior scientist Martin Tang Sørensen	Danish Institute of Agricultural Sciences	October 6, 2005	

 Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute: Danmarks JordbrugsForskning

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Man-months					
Scientific personnel	46,5	30	11,5	2,5	44
Technical personnel	28,5	22	12		34

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Salaries					
Scientific personnel	1906,5	1176	480,4	108	1764,4
Technical personnel	767	578	282		860
Other operational costs	1032	800,3	240,8	56,4	1097,5
Equipment					
Others (please specify)					
Direct costs	3705,5	2554,3	1003,2	164,4	3721,9
Indirect costs (20% of direct costs)	741	507,5	200,6	32,9	741
Total	4446,5	3061,8	1203,8	197,3	4462,9

Comments: Due to reorganisation at DJF, i.e. closing Dept. of Analytical Chemistry where many chemical analyses previously were bought, some of "Other operational costs" is converted to "salaries".

Budget for each participating institute (1.000 DKr)

Name of Institute: Slagteriernes Forskningsinstitut

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Man-months					
Scientific personnel	3,3	1,7	2,5		4,2
Technical personnel	3,6	4,9	2		6,9

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Salaries					
Scientific personnel	199,2	84,1	124,2		208,3
Technical personnel	134,9	144,2	60,7		204,9
Other operational costs	127,1	53	38		91
Equipment					
Others (please specify)					
Direct costs	461,2	281,3	222,9		504,2
Indirect costs (20% of direct costs)	92,3	56,6	44		100,6
Total	553,5	337,9	266,9		604,8

Comments:

Budget for each participating department (1.000 DKK)

Name of Institute and department: Danmarks JordbrugsForskning, HEF

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Man-months					
Scientific personnel	33	19,5	9	2,5	31
Technical personnel	22	15	11		26

Year:		Consumption before 2004	Consumption 2004	Consumption 2005	Total
Salaries					
Scientific personnel	1349,5	766	368,4	108	1242,4
Technical personnel	594	398	253		651
Other operational costs	777	625,7	145	56,4	827,1
Equipment					
Others (please specify)					
Direct costs	2720,5	1789,7	766,4	164,4	2720,5
Indirect costs (20% of direct costs)	544	357,9	153,2	32,9	544
Total	3264,5	2147,6	919,6	197,3	3264,5

Comments: Due to reorganisation at DJF, i.e. closing Dept. of Analytical Chemistry where many chemical analyses previously were bought, some of "Other operational costs" is converted to "salaries".

Budget for each participating department (1.000 DKK)

Name of Institute and department: Danmarks JordbrugsForskning, HSV

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Man-months					
Scientific personnel	8	5,5	2		7,5
Technical personnel	3	4			4

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Salaries					
Scientific personnel	331	218	85		303
Technical personnel	78	101			101
Other operational costs	136	115	36		151
Equipment					
Others (please specify)					
Direct costs	545	434	121		555
Indirect costs (20% of direct costs)	109	84,8	24,2		109
Total	654	518,8	145,2		664

Comments:

Budget for each participating department (1.000 DKK)

Name of Institute and department: Danmarks JordbrugsForskning, ARK

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Man-months					
Scientific personnel	3,5	3	0,5		3,5
Technical personnel	2,5	2	1		2,3

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Salaries					
Scientific personnel	145	115	27		142
Technical personnel	69	53	29		82
Other operational costs	119	55,6	59,8		115,4
Equipment					
Others (please specify)					
Direct costs	333	223,6	115,8		339,4
Indirect costs (20% of direct costs)	66,6	43,4	23,2		66,6
Total	399,6	267	139		406

Comments:

Budget for each participating department (1.000 DKK)

Name of Institute and department: Danmarks JordbrugsForskning, JPM

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Man-months					
Scientific personnel	2	2			2
Technical personnel	1	1			1

Year:	Original budget	Consumption before 2004	Consumption 2004	Consumption 2005	Total
Salaries					
Scientific personnel	81	77			77
Technical personnel	26	26			26
Other operational costs		4			4
Equipment					
Others (please specify)					
Direct costs	107	107			107
Indirect costs (20% of direct costs)	21,4	21,4			21,4
Total	128,4	128,4			128,4

Comments: