Danish Research Centre for Organic Farming

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)

2. Project title and number

Organic food and health – a multigeneration animal experiment. No. III.4.

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

Slutrapporten vedlægges et dansk resumé.

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6. Project period (month, year)

Start of project: 01.05.01
End of project: 31.12.04

7. Final report

A. Project summary

Introduction

The project aimed to determine if differences between organic and conventional cultivation systems can result in differences in the health of those who eat the products.

So the principle was to grow crops in models of organic and conventional cultivation systems, process them to food, and feed them to rats in a multigeneration experiment. Then the products and the rats were thoroughly investigated to determine if and what differences occur.

The project was funded in two rounds, the first grant of 6 mill. Dkr. was given in 2001 to establish a multigeneration experiment using plant material produced in one growth season. However, with only one growth season the results would only be applicable to this particular year, since the differences in climate between years may be more important for the effect on health than the difference between cultivation methods. So an extension of the project was applied for, with one more growth season and one more animal generation, using the offspring of the same rats, and an additional grant of 1.83 mill. Dkr. was received in 2002. This resulted in a combined project to provide independent data on the fertility of rats raised on feed from 2001 as well as on feed produced in 2002, and thus provide the data needed for a statistical evaluation of the results.

In 2003 the central part of the project took place, the animal feeding experiments over 3 generations of rats, and during 2004 the material and data from the animals were analysed, and most of remaining analyses on the plant material were carried out. The first papers have been submitted for review in scientific journals and more are on the way.

Project overview:

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

No.	Work package title	Participants*	Budget (1.000 DKr)	Start	End[$]	Deliverable No(s):
1	Cultivation of feed plants	JPM, KTK, HLP	753	April 01	Oct. 02	1.1, 1.2
2	Characterisation of picture-developing properties of plant materials and feed mixtures	JOA	485	Nov. 01	Dec. 02/ Dec. 03	2.1, 2.2
3	Characterisation of secondary metabolites in plant material	RN, UK	1124	Dec. 01	Aug. 03/ Aug. 04	3.1
4	Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures	CL, HJ	620	May 01	June 03/ June 04	4.1
5	Characterisation of mineral content and pesticide residues of feed mixtures	SHB, LF, KB + subcontractors	382	Sept. 01	Dec. 03/ Aug. 04	5.1, 5.2
6	Management and recording of feeding and breeding	MR	1113	Sept. 01	Feb. 04/ Oct. 04	6.1, 6.2
7	Digestion and utilisation of nutrients and effect on health status	CL, SHB, LF + sub-	2206	Nov. 01	Aug. 04/	7.1-7.3

		contrac-tors			Dec. 04	
8	Data management and analysis	UH	721	Jan. 02	Dec. 04	8.1-8.3
9	Coordination, dissemination and evaluation	KB + all	450	April 01	Dec. 04	9.1-9.7
Total			7.860			
x	Screening of phytochemical changes in plant material	HR	(400)	Aug. 01	April 02/ April 03	x.1

* Responsible participants are underlined

$ Duplicate end dates for work relating to both growing years or more than one generation

Methods used in each workpackage (WP)

WP1 Cultivation of feed plants
The agricultural treatments used were the following:
1. A model of a distinct organic cultivation system, with low input of nutrients through animal manure and use of catch crops, and no pesticides (LIminusP).
2. A model of a distinct conventional cultivation system, with high input of nutrients through mineral fertiliser and use of as much pesticides as is allowed (HIplusP).
3. A combination of 1 and 2, with low input of nutrients, primarily through animal manure, and use of as much pesticides as is allowed (LIplusP).
This design provided 3 separate cultivation system models, 2 of which are similar to those actually used for food production. And it also allowed an evaluation of the relative importance of nutrient supply compared with plant protection, for any effects on product composition or animal health.

The treatments were established on 2 sites, wheat, potatoes and oilseed rape were produced in Foulum, and carrots, kale and mature peas in Årslev.
Potato, peas and kale were cooked and freeze dried, oil produced from the rapeseed, and raw carrots and apples were shredded and freeze dried. The plan was to grind wheat and bake it to bisquits, but a fire destroyed the material, so the plans were changed to make instead a feed without wheat, with mature peas and potatoes as the major components.
The processing was intended to ensure that the feed mixture would contain only components that are also parts of human diets, this was the reason for avoiding eg. raw peas or raw wheat.

The cultivation took place in 2001 and 2002. Using two years provided material for a repetition of the analyses of the materials, and in the feeding trial.

WP2 Characterisation of picture developing properties of plant materials and feed mixtures
The picture-developing properties of fresh plant material were examined for carrot, potato, kale, apple and pea samples, as well as for processed feed mixtures by means of biocrystallization. The pictures were quantified by means of computerized image analysis techniques (texture analysis of grey-levels) and / or by means of quantitative visual scoring techniques.
Combined with the other WPs this work would allow a quantification of the reproducibility and precision of the biocrystallization method for detecting effects of cultivation conditions.

WP3 Characterisation of secondary metabolites in plant material
Characteristic secondary metabolites were measured in selected feed material from each treatment: Polyacetylenes, isocoumarins, β-carotene and volatile compounds in carrot, volatiles, β-carotene and phenolic compounds in apples, glucosinolates, volatiles, β-carotene and phenolics (including flavonoids) in kale, glucoalkaloids, coumarin, β-carotene and phenolic acids in potatoes, and β-carotene in peas.
This WP would provide data on bioactive components suspected, but not known, to be important for the effects of food on health. It would also provide fingerprint-type data, which are known to be sensitive detectors of differences in the physiological status of plants (metabolomics).

WP4 Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures
The major nutrients in the feed plants of the cultivated treatments of WP1 were determined, and the biological value of major protein sources of feed plants were assessed. Based on these results, feed mixtures were prepared based on defined weight percentages of each material from each treatment.
The proportions of each crop were chosen as far as possible to provide at least adequate amounts of each known nutrient in any of the diets. For a few nutrients, including methionine and vitamin E, any mixture of these crops would be inadequate to meet the tabulated demands of the reproducing animals. So adequate amounts of each of those nutrients was added to the diets, the same amount to each treatment. Otherwise the health of the rats would be strongly influenced by nutrient deficiencies, which would be irrelevant for the purpose of the project of providing a model for human health, since no deficiencies of any of the nutrients in question are likely to occur in a European population.
Based on this, the composition of the diet became: 30% potatoes, 13% carrots, 47.2% pea, 1% kale, 1% apple and 13% rapeseed oil. To this was added the amino acid methionine as well as selected vitamins and minerals, for which it was assessed that none of the treatments would be able to provide the recommended daily intake for rats. The content of rapeseed oil was higher than recommended, corresponding to the average level in Danish human diets. The intention with this choice was to be able to assess health aspects related to a superoptimal energy intake, which are particularly relevant for human health in developed countries.

WP5 Characterisation of mineral content and pesticide residues of feed mixtures
Concentrations of 16 elements were measured by ICP-OES (Ca, Cu, Fe, K, P, Mg, Mn, Na and Zn) or ICP-MS (Cd, Co, Cr, Mo, Pb, Te og V) on the plant material harvested in WP1, and a standard multi-analysis of 140 pesticides was carried out on each of the final diets.
The data on minerals was used to determine which minerals would be further studied for bioavailability in WP7, and were also used to determine what extent of supplementation of single essential minerals was necessary to avoid deficiencies.

WP6 Management and recording of feeding and breeding
Young rats were fed with the 3 feed mixtures, and bred for 3 generations. It was originally planned that the first two generations would be raised on plant materials grown in 2001, while the third generation would receive feed from 2002-material. The plan was revised so now half of the rats (and their offspring) were raised on feed grown in the 2001 season throughout the experiment, while the other half received feed grown in 2002 during the second and the third generation. The numbers and genotypes of rats

was determined in pilot experiments with 5 different strains, and it was determined that the experiment was done with 2 strains for the first generation (Brown Norway and GK/mol), while one strain (GK/mol) continued for the two subsequent generations. Reproductive characteristics and weight gains were recorded in each generation.

WP7 Digestion and utilisation of nutrients and effect on health status
Subgroups of the second generation of rats from WP6 were selected for an intensive study in which uptake and excretion of energy and protein and selected micronutrients were determined. Respiration trials were performed to assess the energy metabolism, and simultaneous measurements of the activity levels of the rats were performed. A non-invasive method using analysis of isotope composition of exhaled air after ingestion of specific substrates was used to evaluate the liver function and the gastric motility. In addition, blood and tissue samples of the rats were obtained to study the effect of the dietary treatments on the immunological, antioxidant, and health status of the rats.
A preference-test was performed with rats of the same strain (GK/mol), using male rats of the third generation raised under WP6, and the test took place immediately after weaning.

WP8 Data management and analysis
Data from the experiments were assembled and analysed using relevant models, and combined with the data on feed to determine which associations were significant, and to provide inputs for the final scientific papers. A model for a discrimination analysis of the biocrystallization pictures was developed, as well as the methods for the data analysis for WP6.

WP9 Coordination, dissemination and evaluation
Coordination and management of the project, primarily through periodic meetings and progress reports. Publications and other dissemination activities were planned and coordinated with the intention to maximise precision and extent of the impact of the results. In particular it was attempted (although not with complete success) to ensure that the more far-reaching results would be subjected to a peer review process before they were released to the public, even though this would mean that most of the results would only be published after the project was finished. Since due to the substantial public interest in the issue, it would be a particularly serious problem if preliminary data were released, and later had to be corrected.

WPx Screening of phytochemical changes in plant material
TLC screening of fresh plant material from each crop and cultivation treatment for phytochemical changes was carried out in an attempt to provide an estimate of similarity and facilitate the selection of analysis and isolation of secondary compounds. This WP was financed from external funding (co-financing). NERI’s work was based on support from a company that expected to develop a test to reveal whether a plant has been sprayed with pesticides. The patterns provided a quick screening to show which types of compound differ among treatments, and could therefore provide a valuable lead for optimising the work in WP3.

B. Objectives and expected achievements

The overall objective was to determine if a well controlled animal feeding experiment comparing food products produced by conventional and organic methods shows differences in animal physiology, of a type and magnitude that indicates that such products can affect humans differently.

Partial objectives were the following:

1. To produce food products from strictly controlled, comparable fields, representing the versions of agricultural treatments described in the methods section below, to ensure that the field treatments were the only important variables related to the food, and that the diet made from the food products was both nutritionally adequate for the experimental animals and a relevant model for human diets.

2. To determine if the foods showed differences in contents of macronutrients (protein amount and composition, energy content and distribution) of such a magnitude that this was likely to affect food consumption and/or growth of animals under nutrient-limited conditions.

3. To determine if the foods showed significant differences in picture-developing characteristics of the fresh sample materials or processed feed mixtures, or differences in contents of any other compounds (secondary metabolites, vitamins or minerals) of such a magnitude that this was likely to affect consumption and/or growth of animals.

4. To determine if the 3 diets described above affected growth and/or fertility of experimental animals for 3 generations.

5. To analyse biomarkers for relevant disease indicators, adaptations to biological value of the feed, bioavailability of selected minerals and secondary metabolites, level of activity and other health related responses for differences among the 3 groups during the 2nd generation.

C. Progress and results

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

WP 1: It was possible to obtain adequate yields of all the crops in all treatments. The materials from the 2001 and 2002 harvests were processed.

WP2: For the crops potato, pea, kale and carrot, as well as for the feed mixtures, biocrystallization pictures of sufficient quality (in terms of percentage of single-centered pictures) for comparisons within and among years were produced from material from the 2001 and 2002 harvests.

For the crop samples, each of five image analysis parameters and each of the three/four resolution scales a linear model were analysed, containing the main factors 'crop', 'treatment', 'year' and the interaction factor 'year x treatment', after omitting initially as non-significant the interaction factor 'crop x treatment'. The correlation structure of the data due to the repeated measurements of images from the same extract was incorporated into the analysis by a generalised equation (GEE) approach. Also two multivariate models (MANOVA) were analysed, containing 'year', 'crop' and 'treatment' and additionally excluding respectively including 'year x treatment', based on the five image analysis parameters.

It was seen that the crops were all discriminated from each other by the parameter “Diagonal Moment” (figure 1). LIminusP (1) and LIplusP (2) were discriminated significantly, whereas LIplusP (2) was not discriminated from HIplusP (3) ($p > 0.30$ and 0.70 at scale 1 and 2, respectively). The interaction 'year x treat' was highly significant ($p < 0.01$), treatment 1 and 2 being higher than treatment 3 for year 1, and smaller for year two. The parameter Cluster Shade showed a very similar pattern of discrimination.

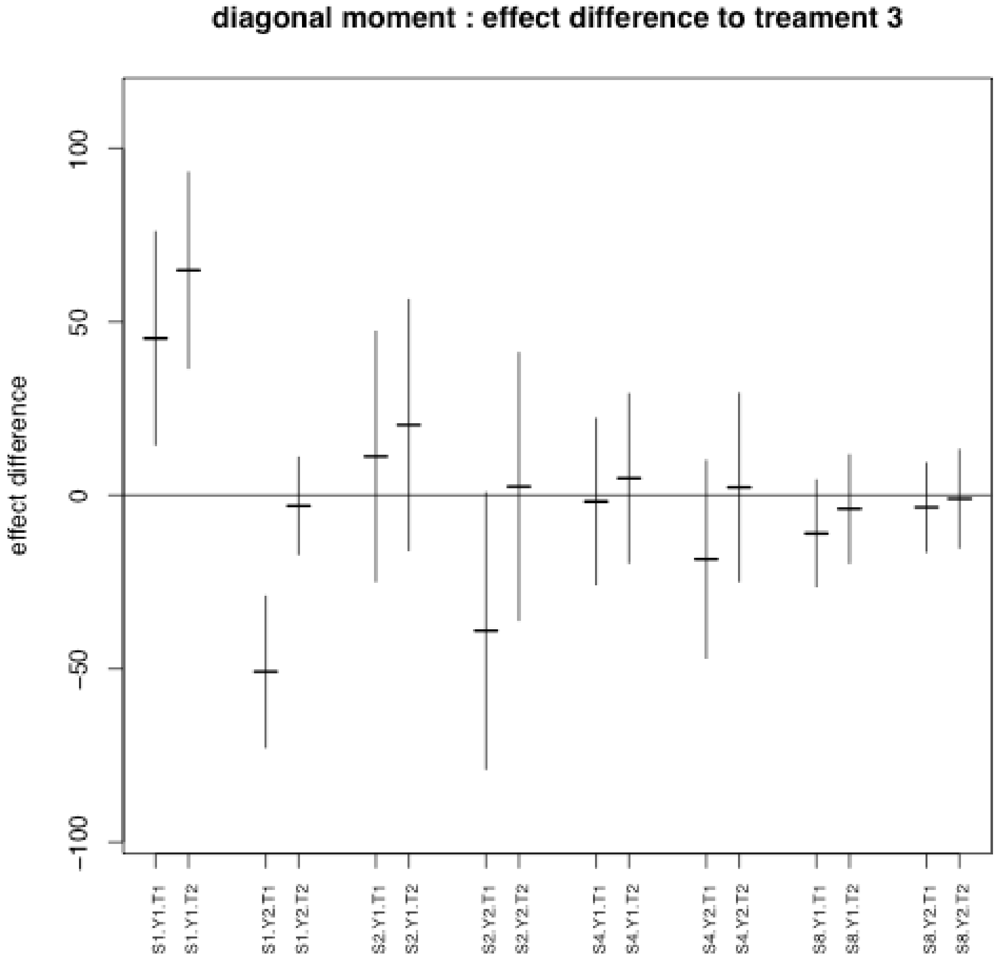

Figure 1 gives a graphical overview of the effects of treatment 1 LIminusP and 2 LIplusP relative to treatment 3 HIplusP, for all years and scales for Diagonal Moment. On the x-axis 'Sa.Yb.Tc', refers to scale a, year b and treatment c (1=LIminusP, 2=LIplusP, 3=HIplusP). The vertical line depicts a 95% confidence interval.

For the feed samples, no parameters discriminated for Treatment, whereas four of the five parameters did discriminate for Year for one or more of the scales 1, 2 and 4.

So while both crop type and cultivation year could be distinguished by several parameters, none of the tested parameters showed any effects of treatments that represented consistent trends between years.

WP3: All crops except rapeseed oil were analysed for phenolics, including flavonoids and phenolic acids. Relevant materials were analysed for β-carotene, volatile compounds, polyacetylenes, glycoalkaloids and coumarins. Unfortunately a few key analyses were delayed so the data are still not available (identification of the volatile compounds, analyses of glucosinolates, a repetition of polyacetylene analyses with an improved method), so the overall picture is still not complete.

Still, in can be seen that the analyses of composition of the various foods that have been evaluated have shown differences of the expected magnitude (10-25 %). Some, but all, differences were significant, which will be defined when the complete dataset has been obtained and analysed statistically. For some types of compounds (e.g. content of volatile secondary metabolites) it appears that the difference between years of cultivation tended to be greater than the effect of cultivation treatment, as it was also seen for the biocrystallization analyses (Figure 1). However, for most crops the effect of the cultivation treatment was systematic across years for the volatile compounds, in other words, the ranking of the three treatments was the same in both years.

For example, a "crude summary" of the data for volatile compounds in apples:

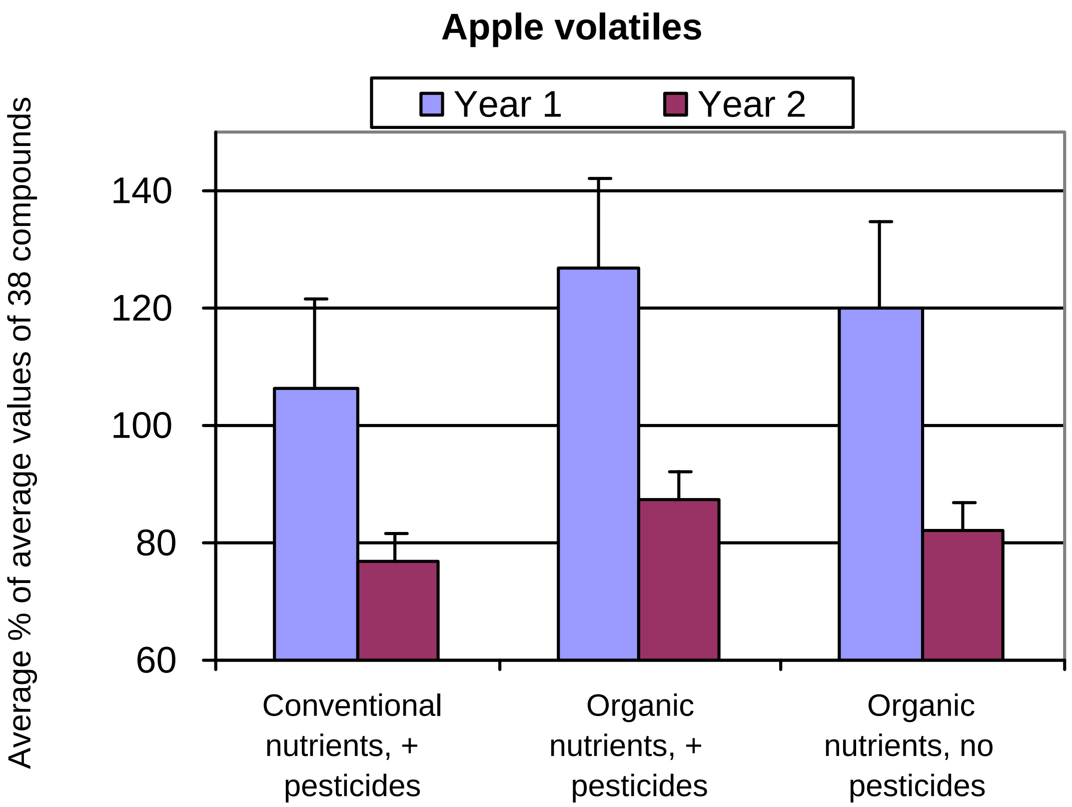

Figure 2: Effect of cultivation treatments on 38 volatile compounds in freeze-dried apples. For each compound the average content of that compound is set at 100%, and the value of each treatment is expressed as the percentage of the average value. In the figure is shown the average percentages obtained for a large number of compounds. This method for visualisation highlights trends that are common for many compounds and is not affected by the absolute amount of each compound (which varies by several factors of magnitude).

WP4: Rapeseed oil was analysed for total fat and fatty acids. All other crops were analysed for ash, protein and amino acids including tryptophane, dietary fat and fatty acids, crude fibre and gross energy. The biological availability of the nutrients of the feed plans was assessed by digestibility and balance experiments with growing male Wistar rats.
The main results: A strong statistical effects of cultivation year with regard to the nutri-

ents composition of the feed ingredients (protein, fat fibre, amino acids, fatty acids, as well as total and digestible energy). Generally a negative correlation was found between the content of crude fibre and the digestibility of energy. Furthermore, there was an indication of improved biological value of the organically grown products in terms of total energy and protein.

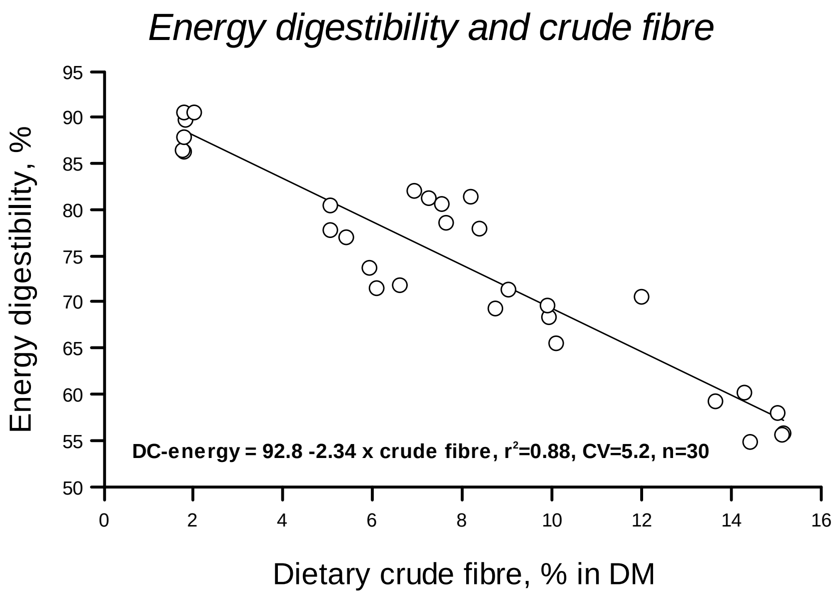

Figure 3. Relation between the digestibility of energy and the dietary content of crude fiber.

The protein was evaluated by comparing the requirement of preschool children by the protein digestible corrected amino acid score (PDCAAS). Interestingly, in spite of low protein content in apples and a low digestibility the amino acid composition was well balanced. Lysine turned out to be the limiting amino acid in the carrot and green kale. The conclusion was that the PDCASS could be used to rank protein quality of the ingredients giving the following ranking: apples, carrots, potatoes, green kale and peas. The amino acid pattern in peas and potatoes complement each other well as dietary sources.

Based on these analyses the diet was composed as follows (in g per kg):

Table 1. Ingredients composition of the experimental diets

	Organic	Minimally fertilized	Conventional
Potatoes	300,0	300,0	300,0
Carrots	50,0	50,0	50,0
Peas	472,4	472,4	472,4
Green kale	10,0	10,0	10,0
Apples	10,0	10,0	10,0
Rapeseed oil	130,0	130,0	130,0
DL-methionine	6,4	6,4	6,4
CaCO3	12,5	12,5	12,5
Salt	0,7	0,7	0,7
Vitamin/mineral mixture	8,0	8,0	8,0

WP5: The crops except rapeseed oil were analysed for selected macrominerals (Ca, Cu, Fe, K, P, Mg, Mn, Na Zn) and micro elements. The results showed that the trace element

and mineral contents of the individual foodstuffs carrot, kale, pea, potato, and apple, grown using one of three cultivation systems in year 2001 and 2002 showed no tendency towards a difference in the mineral content (Ca, Mg, P, K, Na, Cu, Fe, Zn) between the two harvest years (2001, 2002), nor was there an identifiable significant difference with regard to cultivation system used (LIminusP, LIplusP, HIplusP). Overall, there was a larger variation in the content of trace elements (Mo, Cd, Co, and V) with different harvest year, and also with different cultivation systems. However, no consistency was seen with regard to which harvest year or cultivation system produced the largest mineral or trace element content.

There were no overall tendency towards a difference in the mineral and trace element content of the diets produced from foodstuffs grown in year 2001 and 2002. However, there was a higher Mo (P=0.0205) and a lower K (P=0.0154) content in the diet samples from 2001 than from 2002. The trace element content showed a few significant differences according to cultivation system used, in that the Mo content was higher in samples from the two organic cultivation systems (LIminusP and LIplusP) than from the conventional cultivation system (HIplusP) (P=0.0122), and a higher V content was found in the diet sample from the LIminusP cultivation system (P=0.0439).

Mean mineral content of the two diets based on ingredients from three different cultivation systems harvested in 2001 and 2002, respectively, presented as means (SEM) (n=3).

	Harvest year 2001 ***Cultivation System***			***Harvest year 2002*** ***Cultivation System***		
	LIminusP	***LIplusP***	***HIplusP***	***LIminusP***	***LIplusP***	***HIplusP***
Ca, g/kg	7.32 (0.11)	7.17 (0.06)	7.37 (0.22)	7.49 (0.14)	7.69 (0.16)	7.46 (0.35)
Mg, g/kg	1.08 (0.01)	1.02 (0.02)	1.08 (0.01)	1.21 (0.02)	1.19 (0.01)	1.12 (0.02)
P, g/kg	3.51 (0.08)	3.56 (0.15)	3.65 (0.08)	4.05 (0.13)	3.73 (0.02)	3.90 (0.11)
K, g/kg	9.87 (0.22)	9.57 (0.23)	10.31 (0.49) *	11.40 (0.27)	11.81 (0.16)	11.92 (0.43)
Na, mg/kg	623 (172)	628 (153)	632 (114)	869 (183)	802 (113)	722 (151)
Cu, mg/kg	6.19 (0.33)	5.68 (0.15)	6.12 (0.17)	6.36 (0.58)	5.58 (0.46)	5.65 (0.17)
Fe, mg/kg	93.4 (9.8)	86.0 (11.8)	88.2 (1.85)	123 (23.1)	92.2 (14.5)	89.8 (3.0)
Mn, mg/kg	14.78 (0.44)	13.45 (0.57)	13.55 (0.01)	15.14 (0.43)	14.63 (0.31)	6.31 (0.14)
Zn, mg/kg	22.33 (0.73)	23.04 (1.11)	26.54 (3.50)	28.23 (3.32)	24.16 (1.92)	27.06 (1.92)
Mo, µg/kg	1650 (26) [a]	1578 (18) [a]	1087 (11) [b]*	1494 (29)	1379 (12)	796 (17)
Cd, µg/kg	58 (3)	57 (4)	68 (5)	54 (1)	54 (1)	72 (5)
Co, µg/kg	100 (12)	77 (3)	69 (6)	88 (2)	82 (3)	75 (3)
V, µg/kg	51 (8) [a]	36 (0) [b]	39 (2) [b]	58 (4)	41 (4)	39 (6)

* Difference between year 2001 and 2002 independently of cultivation method, $P<0.05$.
[ab] Different letters in same row indicate difference between cultivation methods independently of year, $P<0.05$

WP6: The breeding experiment was successfully carried out as planned through the 3 generations.

Fertility
In the first generation, breeding pairs of the GK-mol fed on LIminusP diets achieved a significantly larger weight per litter compared to HIplusP and LIplusP diets, Fig. 4. This was due to more pups were born alive per litter and in addition they also had larger average individual weight.

In the second and third generation, no consistent effects were seen on the breeding performance, the difference between years of cultivation for some breeding components seemed greater than the effect of cultivation treatment for the last two generations.

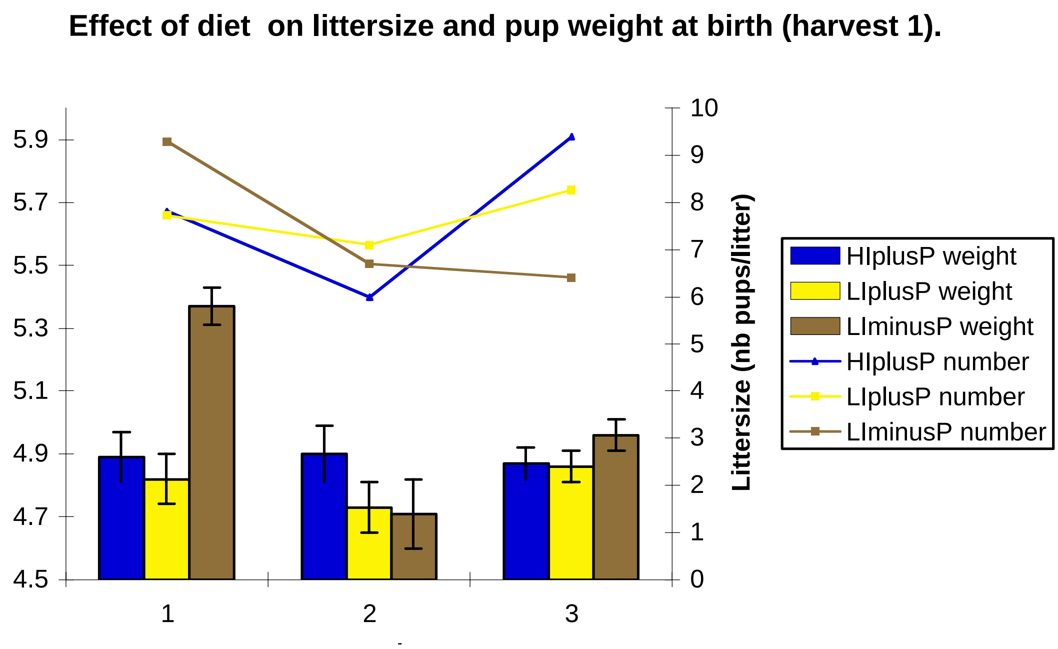

Figure 4
Weight per pub and number of pups per litter during 3 generations, for GK-mol strain, data for 2001 harvest.

Food intake and growth
No consistent effects on growth and food intake were found between diets nor cultivation years or generations was seen in the three generations.

Semen quality
A master student (see section F) additionally examined various aspects of semen quality of the male rats after breeding, but no significant differences were found.

WP7: Using rats of the second generation after breeding, the long term effects of the diets prepared from crops grown in 2001 were assessed by testing a wide range of health related markers and properties of the rats, both non-invasive such as activity level and gastric emptying and post-mortem evaluations such as composition of blood, organs and tissues. The following results were obtained:

Measurement of health

The health of the rats was examined through physiological response measurements that comprised nutrient utilisation, function of organs and physical activity. Post mortem (44 weeks of age) blood and tissue samples were collected for analysis of biomarkers of health such as immune function, antioxidant status and nutritional status

In addition to the presently reported results, the main project comprised a range of other issues related to reproduction of the rats, bioavailability of the nutrients in the ingredients of diets, and annual variation in the content of nutrients and secondary metabolites in the ingredients.

Improved immune status

Immune status of the rats was measured as the total content of immunoglobulins in the blood serum. The results showed that rats fed on organic and minimally fertilised diets had a higher content of immunoglobulin G (IgG) than rats fed on the conventionally grown diet (Figure 5). There were no differences in the serum contents of immunoglobulins A and M.

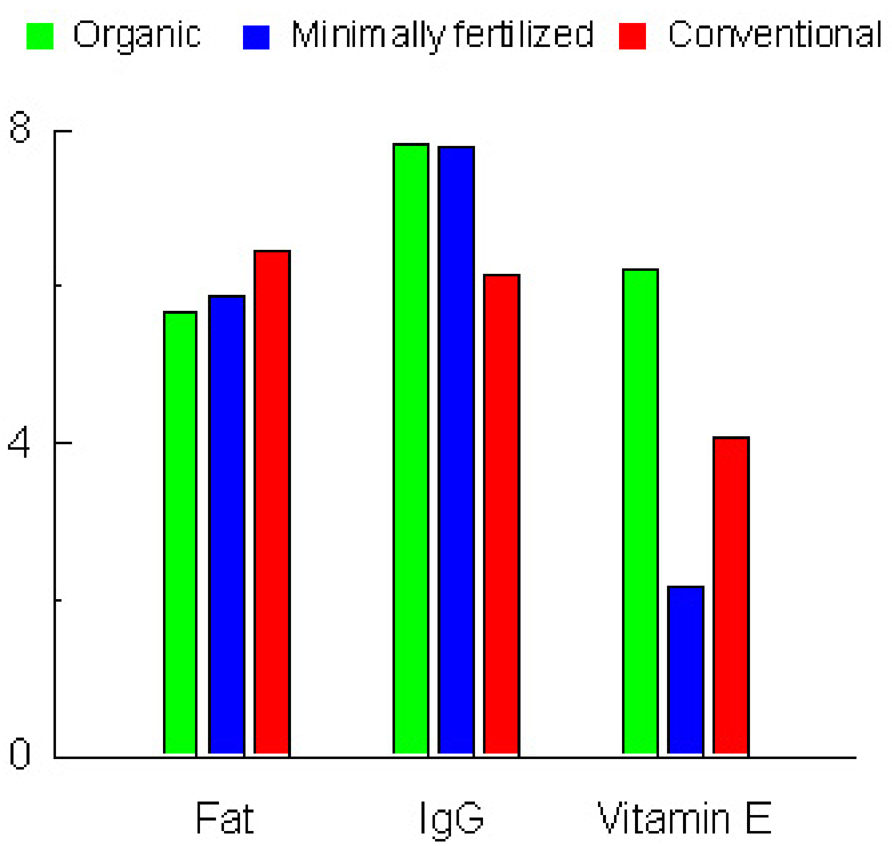

Figure 5. Content of adipose fat in the body (%), and plasma content og immunoglobulin G (IgG, mg/ml) and vitamin E (mg/l) in rats fed organic, minimally fertilized or conventional diets.

At present, we have no explanation of the lower content of IgG in the rats that were offered the conventionally grown diet. Yet, it is noteworthy that the conventional diet had a higher content of the secondary metabolite falcarindiol than the other diets. It cannot be excluded that falcarindiol may have an inhibitory effect on initiation of the immune response.

Tendencies towards less adipose tissue and better rest

The rats thrived on all three diets and only showed minor differences with respect to utilisation of energy and nutrients. Even though the rats were genetically disposed for diabetes, there was no visual sign of this disease among the rats.

The rats had only a slight increase in weight after eating the diets for 25 weeks. However, the data showed a tendency towards a lower weight and a lower content of adipose tissue in the rats that were fed on the organic diet as compared to the other diets (Figure 5).

Concurrently with the measurements of energy utilisation, we measured the physical activity of the rats using infrared sensors. Rats are active at night, and there were no differences between the dietary groups with respect to activity at night. However, during daytime, when the rats are supposed to rest, our data indicated that rats fed on the organic diet was more relaxed than rats feed on the other diets (Figure 6).

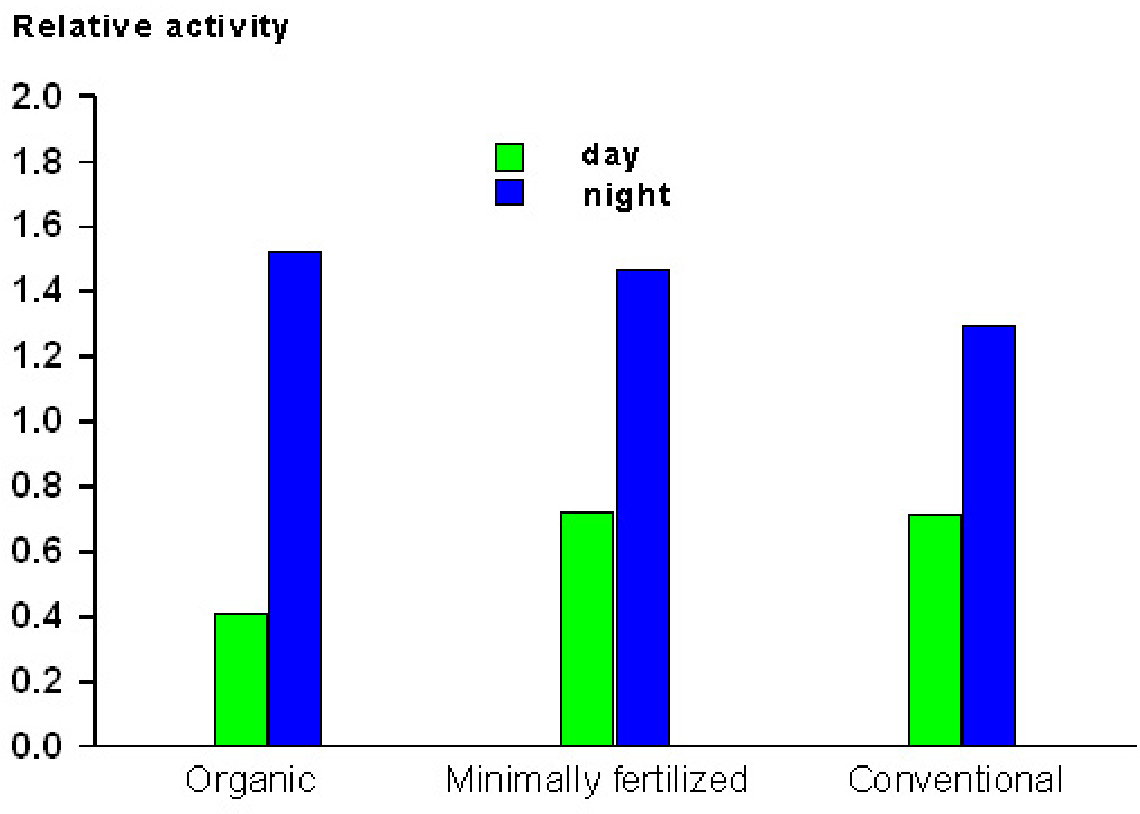

Figure 6. Relative activity of rats at day and night. The rats were fed organic, minimally fertilized or conventional diets.

Differences in vitamin E

Rapeseed oil comprised 25 pct. of the energy content of the diets. Due to experimental problems there was a different composition of fatty acids and a lower vitamin E content in the rapeseed oil from the minimally fertilised treatment (Table 2). This caused some differences in the fatty acid composition of the serum and the tissue of the three dietary groups of rats. Also, the vitamin E content was lower in blood plasma from rats that received the minimally fertilised diet.

Table 2. Analysed chemical composition of selected nutrients in the experimental diets

	Organic	Minimally fertilized	Conventional
Dry matter (DM), g/kg	966	972	962
Energy, MJ/kg DM	21,0	21,2	21,3
Protein, g/kg DM	160,5	160,6	160,9
HCl-fat, g/kg DM	156,8	154,7	157,9
Vitamin E, mg/kg DM	32,1	19,2	31,7
Saturated fatty acids, %	8	7	8
Monounsaturated fatty acids, %	62	72	63
Polyunsaturated fatty acids, %	30	21	29

The content of vitamin E in the organic and the conventional diet was similar (Table 2). Yet, there was a higher content of vitamin E in the blood of the rats that were fed with the organically grown diet (Figure5).
There were no differences in the vitamin E content of liver and adipose fat tissue between rats from the three dietary groups.

WPx: TLC biomarker patterns were produced from potato, pea, kale, apple and carrot from the 2001 harvest, showing an average of 4 compounds differing by at least 50 % in spot size between the two most different treatments. However, the commercial support was withdrawn before the work was completed, and the data obtained until then were not sufficiently systematic nor well defined to allow their use in relation to the chemical analyses, so this part did not fulfil the expectations (note that it did not use any DARCOF funds, it only consumed only a few kg of surplus plant material from the cultivation experiment).

Other additional work based on the materials produced in the project:
Sensory evaluation of carrots harvested in 2001, performed by students at the Royal Veterinary and Agricultural University, showed significant differences in sweetness and bitter aftertaste between blanched carrots grown with the fully organic or the fully conventional treatments, while the third treatment was not different from any of the others. A test of the same carrots served as raw showed smaller differences, only significant for sweetness, but the treatment averages were ranked in the same sequence. From the 2002 harvest a similar test was made for green, blanched peas, and also here small, but significant differences were detected.
Based on these pilot experiments, the “Foundation for Organic Agriculture” (Fonden for økologisk landbrug), a section of the Danish agricultural levy foundations, allocated 129000 Dkr. for a separate project in 2004, where a trained panel at DIAS carried out a full scale sensory evaluation of the frozen material of carrots and kale from project III.4 for both the 2001 and 2002 harvests. The results showed a significant variation in the sensory attributes among kale and carrot. A large part of the sensory variation was attributed to the cultivation years, and only a minor part of the variation in the sensory quality was determined by the cultivation methods. For both crops this included bitterness being generally highest in the organic samples.

C.2 Fulfilment of deliverables and milestones

WP 1 Cultivation of feed plants	Time schedule according to application	Deviations, if any*
Deliverables		
D1.1 The harvested materials.	Nov. 01 and Nov. 02	Achieved in 2001 and 2002.
D1.2 The processed materials.	March 02 and March 03	Most crops OK, the wheat was lost (burned!). *
Milestones		
M1.1 The materials are harvested	Nov. 01 and Nov. 02	Achieved in 2001 and 2002 (no wheat was grown in 2002). *
M1.2 The materials are processed.	March 02 and March 03	Achieved for all crops except wheat*

** Deviations are to be further discussed in D1*

WP 2 Characterisation of picture-developing properties of plant materials and feed mixtures	Time schedule according to application	Deviations, if any*
Deliverables		
D2.1 Scientific manuscript on picture-developing properties of the examined samples.	Aug. 03 *	Achieved (partially) with a delay of 10 month
D2.2 Contribution to scientific paper(s) on the correlation between picture-forming characteristics and other data from the project.	Dec. 04	Achieved on time
Milestones		
M2.1 The analyses are completed.	April 03	Achieved with a delay of 4 months

** Deviations are to be further discussed in D2*

WP 3 Characterisation of secondary metabolites in plant material	Time schedule according to application	Deviations, if any
Deliverables		
D3.1 At least one scientific manuscript on the contents of secondary metabolites in the materials.	Aug. 04 *	Most work completed on time, a few parts delayed substantially, will be completed Summer 2005.
Milestones		
M3.1 The analyses are completed.	Aug. 03 *	A few analyses are still missing

** Deviations are to be further discussed in D3*

WP 4 Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures	Time schedule according to application	Deviations, if any
Deliverables		
D4.1 Contribution to scientific manuscript	Dec. 03	Achieved on time*
Milestones		
M4,1 Formulation and production of experimental diets for animal experiment.	Oct. 02 and June 03	Achieved with a delay of 1 month

** Deviations are to be further discussed in D4*

WP 5 Characterisation of mineral content and pesticide residues of feed mixtures	Time schedule according to application	Deviations, if any*

Deliverables		
D5.1 (month 20): List of elements selected for the balance study of the second generation in WP7.	Dec. 02	Achieved with a delay of 5 months*
D5.2 (month 20): Data on contents of pesticides in each of the diets.	Dec. 02 and Aug. 03	Achieved with a delay of 2 months*
Milestones		
M5.1 (Month 20): The analyses are completed.	Dec. 02 and Aug. 03	Achieved with a delay of 7 months*

** Deviations are to be further discussed in D5*

WP 6 Management and recording of feeding and breeding	Time schedule according to application	Deviations, if any*
Deliverables		
D6.1 Weaned rats of second generation for WP7	June 03	Achieved with a delay of 1 month
D6.2 Scientific manuscript on reproductive effects.	April 04	On time
Milestones		
M6.1 In the first generation, a sufficient number of young is produced in order to make the planned recordings and experiments.	Dec. 02	Achieved with a delay of 1 month
M6.2 In the second generation, a sufficient number of young is produced in order to make the planned recordings and experiments.	April 03	Achieved with a delay of 1 month
M6.3 In the third generation, a sufficient number of young is produced in order to make the planned recordings and experiments.	Aug. 03	Achieved with a delay of 1 month

** Deviations are to be further discussed in D6*

WP 7 Digestion and utilisation of nutrients and effect on health status	Time schedule according to application	Deviations, if any*
Deliverables		
D7.1 Scientific manuscript(s) on bioavailability of nutrients (and secondary metabolites).	Dec. 04 *	Achieved with a delay of 4 months (not for secondary metabolites)
D7.2 Scientific manuscript(s) on behaviour, activity levels and food preference.	Dec. 04	Achieved on time
D7.3 Scientific manuscript(s) on immunological status, frequency of diseases and other aspects of health status in aged animals.	Dec. 04	Achieved on time
Milestones		
M7.1 The tests on the first (now third) generation rats are completed.	June 03	Achieved with a delay of 8 months
M7.2 The tests on the second generation rats are completed.	Oct. 03	Achieved with a delay of 4 months
M7.3 The occurrence of diseases and deficiencies in aged animals of the second generation rats are characterised.	Dec. 04	On time

** Deviations are to be further discussed in D7*

WP 8 Data management and analysis	Time schedule according to application	Deviations, if any*
Deliverables		
D8.1 Detailed plan for initial experiments and exchange of materials and data.	Feb. 02	On time (changed to WP 9)
D8.2 Optimised plan for the animal experiments.	Oct. 02	Achieved with a delay of 6

		months
D8.3 Report or inputs to papers, with the relevant statistical analyses.	Aug. 04 *	Achieved on time for data that were available on time
Milestones		
M8.1 Detailed plan for experiments and exchange of materials and data is prepared.	Feb. 02	On time (changed to WP 9)
M8.2 Optimised plan for the animal experiments is prepared.	Oct. 02	Achieved with a delay of 6 months

** Deviations are to be further discussed at in D8*

WP 9 Coordination, dissemination and evaluation	Time schedule according to application	Deviations, if any*
Deliverables		
D9.1 Startup meeting M1	April 01	Achieved
D9.2 Progress meeting M2	March 02	Achieved
D9.3 Progress meeting M3. At this meeting those details of the research plans for WPs 6 and 7, which depend on WPs 2-5, and, if necessary, relevant redistributions of resources, are determined for the remains of the project	Oct. 02	Achieved with a delay of 1 month
D9.4 Progress meeting M4	April 03*	Achieved with a delay of 2 months
D9.5 Progress meeting M5	Feb. 04	On time
D9.6 Progress meeting M6	Oct. 04	On time (June)
D9.7 Final report, including implementation and further progress.	Dec. 04	Achieved with a delay of 3 months
Milestones		
M9.1 Determination of detailed research plans for the animal studies.	Oct. 02	Achieved with a delay of 6 months
M9.2 Publication plan is prepared.	April 03*	Not achieved
M9.3 Final report on implementation and further progress is prepared.	Dec. 04	Achieved with a delay of 3 months

** Deviations are to be further discussed in D9*

WP x Screening of phytochemical changes in plant material	Time schedule according to application	Deviations, if any*
Deliverables		
Dx.1 Participation in publications where the results are used to identify secondary compounds.	Dec. 03	On time/reduced
Milestones		
Mx.1 The analyses are completed.	Dec. 02	On time/reduced

** Deviations are to be further discussed in Dx*

D. Description of deviations and subsequent adjustments of plans

D1, deviations in WP1.

For processing, the wheat from all 3 treatments was milled, and the flour was taken to a biscuit factory for the preparation and baking of biscuits from it. However, a technical error occurred, which caused the oven to overheat and catch fire, and all material from one of the treatments was lost. We considered possible actions and decided to continue the project plans based on the use of only the crops that were successfully processed (potatoes, cabbage, carrots, apples, peas).

In October – November 2001 problems appeared for Hanne Nygaard Larsens position at The Royal Veterinary and Agricultural University, Research Department of Human Nutrition. Due to a new strategy the Department requested that this part of the project be moved to another institute, and during this process Hanne Nygaard Larsen decided to

leave the project for another job.
In relation to WP1 this delayed the calculation of the amount of material that was due to be processed, and therefore most of the processings in 2001-2002 were delayed by approx. 2 months.
Another problem concerned the second year of crop production, where it turned out to be impossible to arrange the relevant cultivation conditions for oilseed rape, so we could not grow a second crop of rape. However, after the recalculation mentioned above, it turned out that the amount of rapeseed oil produced in the first year was adequate to meet the needs of the entire project. So part of this oil was stored and used for the second batch of material. Since for rape only part of the crop (the oil) was used, this was assessed as manageable in relation to interpretation of the results.

D2, deviations in WP2.
While most of the pictures made from carrots, kale, potatoes and peas were single-centered, the sample preparation procedure for the 2001 apple samples resulted in multi-centered pictures, which are known not to be suitable for visual nor instrumental evaluation. Thus it would not be possible to obtain useful comparisons, neither within the 2001 data nor with data from the 2002 harvest, so in 2002 apples were deleted from the analysis programme, as well as the wheat analyses originally planned. Instead additional analyses of the other crops were carried out in 2002, including a study of the repeatability of the extraction procedure used.
The work was further delayed during 2003 due to other tasks which could not be postponed, both for the picture preparation, which was completed in August 03, and for the statistical analysis of the results. The extent and content of the experimental work was not changed and the final completion was within the originally expected timeframe. However, due to the nature of the results it was decided not to write an article exclusively on this topic, but instead write the results as contributions to incorporate into the more general papers in preparation, about the crop composition and the breeding performance.

D3, deviations in WP3.
Since the measurements were made on the processed material, the start of the analyses was delayed due to the delayed processing. And just when the material was ready for analysis, the responsible scientist, Erik Larsen, resigned from his job. The tasks were taken over by two other scientists in the same research group, Rikke Nørbæk and Ulla Kidmose, and several minor tasks delegated to other scientists (Jens Hansen-Møller, Lars P. Christensen), so the work could be done, although with some delays. A few analyses (glucosinolates, glucoalkaloids, identification of volatiles) were further delayed due to other commitments and changes in responsibilities, however, it is still expected that the remaining analyses will be completed within a few months.

D4, deviations in WP4.
Despite the delays in processing, the materials were delivered to this WP almost at the planned time. However, then the supplier of the rat strain used for this experiment experienced disease problems, and was therefore unable to deliver as frequent as usual. Combined with a structural reorganisation of the department (Department for Analytical Chemistry) that analyses the samples for assessment of biological value and nutritional content of the cultivated crops, a delay of approximately 1 month had accumulated when the animal work was finished in November 2002.
A few analyses of amino acids in the feed material were performed late in 2004 due to technical problems, and this has delayed the work with a scientific manuscript for a journal accordingly. However, all other tasks were done approximately on time, and some of the results of cultivation year 1 were presented as a poster in 2004.
Due to the delayed delivery of crops from the second harvest, diet preparation was de-

layed by approximately one month.

D5, deviations in WP5.
The scientist originally responsible for this WP, Vagn Gundersen, had to leave his working area and thus the project, due to a changed strategy at Risoe. His scientific responsibilities were taken over by Susanne Højbjerg Bügel. The laboratory work was still made by Vagn's assistant Lis Vinther Kristensen, as originally planned, together with post doc. Lars Frøsig. This has caused some changes in the distribution of funds between Risø and The Royal Veterinary and Agricultural University, see the section on budget changes.
After the analyses of feed material were completed, the evaluation of the data was delayed due to paternity leave for a researcher. This means that the work on bioavailability was postponed to 2004/2005 (with corresponding revisions of the timing of the budget for KVL).
The pesticide analyses of the feed were delayed due to minor logistic issues, they were finished in November 2003.

D6, deviations in WP6.
This WP was supposed to have been the central part of Hanne Nygaard Larsens work. When she left the project, and the department did not want to provide another scientist for the work, several other possibilities were considered, and the choice fell on the Biomedical Laboratory (BL) of University of Southern Denmark. BL tends laboratory animals for all parts of the University, and carries out research in relation to the use of animals as experimental models for human physiology and diseases, so it has all relevant facilities and expertise for this task. This was also confirmed by external reviewers in relation to the application for supplementary funding, where BL was named as responsible.
The start of the experiment experienced a minor delay due to delays in provision of the feed. The progress since then has been according to schedule.

D7, deviations in WP7.
The parts of this WP that Hanne Nygaard Larsen should have taken care of was transferred to DIAS, where it was carried out by Charlotte Lauridsen and Henry Jørgensen, as described in the section on budget changes.
The plans were changed slightly to make them fit better with other activities and provide better data than in the original plan. The investigations planned for animals from the first generation was changed to the third generation, and the health assessment and life expectancy studies were combined to a study using the parents from the second generation rather than young rats, as it was also recommended by reviewers. The original plan was to feed relatively few rats (10) for a maximum of 2 years on each of the respective diets. The recordings consisted of registration after death of abnormalities and signs of diseases, including signs of cancer and vascular diseases. In order to fulfil the purpose of this work optimally, we made use of the rats that will be raised for the other studies. We chose to keep a selected group of rats for a longer period than originally planned for the other (non-invasive) experiments, by using the mother rats in generation 2 after they had been used for breeding. By using non-invasive methods, such as ^{13}C-infrared isotope analyser for monitoring function of vital organs and intestinal flora, as well as analysis of nutritional status and biomarkers indicative of health status in blood samples, the plan was to provide measures of the onset of physiological disorders without killing the animals. This revised study design would answer the question regarding the influence of the respective diets on the listed aspects of health status and general well-being of the animals at different age using the same animal material, and thus provide more useful data in a shorter time and without added costs.
This made it necessary to transfer some funds from 2003 to 2004 and 2005, as reflected

in the budgets for DIAS and KVL.
The results of the bioavailability estimation of energy and protein are presented in the scientific manuscript as planned according to D3 of WP7, which is achieved on time. The 4 months delay regarding the scientific manuscript on the bioavailability of selected minerals is commented under WP5. A scientific manuscript of the bioavailability of secondary metabolites (selected under WP3) was originally planned, but will not be conducted due to explanations as presented and commented under WP3 and WP9.

D8, deviations in WP8.
Due to other obligations Jens Henrik Badsberg, who was originally responsible for this WP, first postponed his engagement and later completely withdrew from the project. His colleague Ulrich Halekoh has taken over the work. However, this is also affected by the delays in other parts of the project, the person-months that were originally planned for 2001, and then transferred to 2002, were transferred further to 2004, which is when they were most needed. This ensured the placing of the most intensive effort during the period when the data on the results were being processed and published.

D9, deviations in WP9.
The plans were delayed as described above, and a lot of effort has been invested in keeping the project on track despite all those changes. The publication plan was postponed several times, and is not yet completely finalised, a major problem being that the last remaining analyses (on crop bioactive components) logically should be reported in the first paper to be published, with the details of the cultivation systems and crop characteristics, which the other papers should then refer to, so the absence of these data makes the whole plan more difficult. In January 2004 the project coordinator Kirsten Brandt also changed job, although an agreement was made with her new employer, University of Newcastle, that she could continue in this role until the project was finished while in the new job. However, serious delays have occurred in the management, in particular in relation to planning and writing of publications as well as follow-up on remaining analyses, primarily due to unexpected problems appearing in summer and autumn of 2004 in another project, which are fortunately now subsiding.

Dx, deviations in WPx.
The participation of NERI was planned to be financed by a company Biotech Line AS, according to an agreement between this company and NERI. However, the budget was reduced, so the work presented in the workplan was reduced to consist of a screening fresh plant material of apples, potatoes, kale, peas and carrots from the 2001 harvest, while screening of fresh plant material obtained in 2002 could not be performed. Still, NERI has completed the work that was in progress, to a large extent based on the work of two students.

E. Project publications and other products

1. Products from Organic Eprints archive

* 25-75% financed by DARCOF
** 5-25% financed by DARCOF

Kidmose U, Thybo A, Christensen LP & Brandt K. 2005. **Sensory evaluation of organic, processed vegetables.** Abstracts. Danish Food Science 2005. From Molecule to man, Food Congress 2005, DTU March 9-10 2005 p. 82.

Jensen, MN 2004. **Organic diet and fertility: Possible effects of diet on rat male reproduction parameters. MSc thesis, University of Southern Denmark.

Lauridsen, C., Jørgensen, H., Halekoh, U., Christensen, L.P., and Brandt K. 2005. **Økologisk kost gavnede rotters sundhed.** Nyhedsbrev fra Forskningscenter for Økologisk Jordbrug, Februar 2005, No. 1, http://www.foejo.dk/enyt2/enyt/feb05/sundhed.html

Lauridsen, C., Jørgensen, H., Halekoh, U., Christensen, L.P., and Brandt K. 2005. **Organic diet enhanced the health of rats** (same content as the previous one in Danish)**.** Article on the website for Danish Research Centre for Organic Farming, February 2005, http://www.darcof.dk/research/health.html

Chen, Y., Jørgensen, H., Halekoh, U. & Lauridsen, C., 2004. **Rats show indiviual preference for short-term choice of three human diets**. In: Challenges to food science and technology. Abstract collection, Food Congress, DTU, March 17.-18., 145

Jørgensen, H. & Lauridsen, C, 2004. **Nutrient composition and bioavailability of protein and energy in common fruits and vegetables prepared for human consumption**. In: Challenges to food science and technology. Abstract collection, Food Congress, DTU, March 17.-18., 135.

Ravn H. W., Christensen T. F., Diedrichsen B., Kristensen C.V., Jensen A. K., Husted C. L., Lindhard Pedersen H., Brandt K., & Andersen J.B. 2004. **Plant Biomarker Pattern, Apples grown with various availability of organic nitrogen and with or without the use of pesticides**. Poster at ECO-FRU-VIT. 11th International Conference on Cultivation technique and Phytopathological problems in Organic Fruit-Growing.

Brandt, K., Bügel, S.H., Ritskes-Hoitinga, M., Frøsig, L. et al. 2003. **Organic food and health – a multigeneration animal experiment.** Poster presented at the EFFoST conference "New Functional Ingredients and Foods - Safety, Health and Convenience" April 9-11 2003 in Copenhagen.

Brandt, K., Ejlersen, A., Nørbæk, R. & Lindhard Petersen, H. 2003. ***Effects of Cultivation Conditions for Apples on Growth Rates of Fruit Fly Larvae and Contents of Phenolics.** Poster presented at the EFFoST conference "New Functional Ingredients and Foods - Safety, Health and Convenience" April 9-11 2003 in Copenhagen.

Brandt, K. and Kristensen, E.S. 2003. **Investigations of organic food and health.** Innovations in Food Technology 20, 68-69.

Jensen, M.N, Halekoh, U., Brandt, K., Jegstrup, I.M., Ritskes-Hoitinga, M. 2003. **The effects of organically and conventionally cultivated plant feed on fertility and health in two inbred rat strains**. Oral presentation at Scandlas Meetings, Lahti in Finland 2003.

Brandt K. 2002. **Organic food and human health**. Oral presentation at the LMC "Food Congress", 17-18 Jan. 2002. Abstract and presentation.

Brandt, K. & Kidmose, U. 2002, ***Nutritional Consequences of Using Organic Agricultural Methods in Developing Countries.** In: Impacts of Agriculture on Human Health and Nutrition, edited by Cakmak I., Graham R.D., and Welch R.M., in Encyclopedia of

Life Support Systems (EOLSS), Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford, UK, [http://www.eolss.net].

Christensen, T.F., Diedrichsen, B., Adsersen, A., Ravn, H.W., & Andersen, J.B. 2002. **Plant Biomarker Pattern, Screening Programme for Phytochemical Differences in Plants Exposed to Stress**. Poster presented at Natur og Miljøforskningskonferencen (The Conference on Research on Nature and the Environment), H.C. Ørsted Instittutet, Copenhagen, 22-23- August 2002.

Ravn, H.W., Christensen, T.F., Diedrichsen, B. 2002. **A new Phytochemical Screening Programme used for Organic and Conventional Crops**. M.Sc. thesis report from NERI and The Royal Danish School of Pharmacy, Dept. of Medicinal Chemistry, Copenhagen. Published on the Organic Eprints website as pdf-file.

Brandt, K. & Mølgaard, J.P., 2001. ****Organic agriculture: does it enhance or reduce the nutritional value of plant foods**? J. Sci. Food Agric. 81, 924-931.

Brandt K., Nygaard Larsen H., Andersen J.-O., Mølgaard J.P., Lauridsen C., Jørgensen H., Gundersen V., Larsen E., Badsberg J.H. and Thorup-Kristensen K.. 2001. **Organic Food and Health: A new project to study the effects of plant cultivation methods (organic and conventional) on nutritional value, health and reproduction in an animal experiment.** Poster presented at “FOOD and NUTRITION for BETTER HEALTH” (HEALFO Conference), 13-15 June 2001, Lanciano, Italy.

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

The project was presented at the “FØJO open house” event in Årslev on Aug. 7, 2003.

The part of the project that comprises cultivation of vegetables in 2002 was presented at the “FØJO open house” event in Årslev on Aug. 8, 2002.

The part of the project that comprises biocrystallisation analyses were presented at a visit of the Special Committee for Organic Agriculture and local organic committees, 65 persons in total, at Biodynamic Research Association, Herskind, on May 30, 2002

The part of the project that comprises cultivation of vegetables in 2001 was presented to representatives of the Danish Association of Vegetable Growers, including organic growers and consultants, during a field excursion on Aug. 16, 2001.

Slot, J., Andersen, T.S. and Kristensen, H.T. 2003. ***Sensorisk bedømmelse af ærter** (Sensory evaluation of peas). Report from a 3-week course in sensory evaluation at The Royal Veterinary and Agricultural University, taught by prof. Magni Martens, using material provided from the project. This report is not available in electronic form.

Nøddekær, T., Sandberg, C, Kreutzmann, S., and Albrechtsen, H.O. 2002. ***Sensorisk bedømmelse af gulerødder** (Sensory evaluation of carrots). Report from a 3-week course in sensory evaluation at The Royal Veterinary and Agricultural University, taught by prof. Magni Martens, using material provided from the project. This report is not available in electronic form.

Several interviews to the press regarding the DARCOF newsletter (Lauridsen et al.

2005) in February 2005 (the results of WP7):
Nationally: Interviews with C. Lauridsen in the news of both major Danish TV-stations, all major Danish newspapers wrote about the findings as well as many commentaries and discussions of the topic.
Internationally: In the UK the story was used by the Guardian (front page story), The Times, The Independent, The Journal, (Newcastle), The Herald (Glasgow), Northern Echo (Durham), and by BBC News.
At present (early April 2005) at least 200 pages in English refer to the story according to Google.

Plan for remaining publications:

Manuscripts submitted:
Lauridsen C, Yong C, Halekoh U Bügel S, Brandt K, Christensen LP and Jørgensen H: **Rats show differences in health when eating diets based on ingredients produced with three different cultivation strategies**. Submitted to British Journal of Nutrition in January 2005.

Yong C, Halekoh U, Jørgensen H and Lauridsen C, **Rats show individual preference for short-term choice of three human diets.** Submitted to Journal of The Science of Food and Agriculture in January 2005.

Manuscripts in advanced state of preparation (draft present):
Jørgensen, H., Brandt K & Lauridsen, C: **Organic food and health: Growth conditions, chemical composition and digestibility in common fruits and vegetables prepared for human consumption** (additionally, it may be decided to incorporate the crop part of the data on bio-crystallisation in this manuscript).

Bügel S, Kristensen M, Jørgensen H, Halekoh U, Østergaard LF, Brandt K, and Lauridsen C: **The effect of plant cultivation methods on mineral content of foodstuffs and mineral balance in rats.**

The bio-crystallisation data have been prepared by Andersen J-O as contributions ready to incorporate into two manuscripts.

Manuscripts under initial stages of preparation (experiments finished, data processed, but first draft not yet circulated):
Jegstrup, IM, Jensen MN, Brandt K, Halekoh U and Ritskes-Hoitinga, J: **Fertility of rats raised on diets produced with three different cultivation strategies** (additionally, it may be decided to incorporate the feed part of the data on bio-crystallisation in this manuscript).

Manuscripts planned (experiments and data processing not fully finished):
Brandt K, Nørbæk, R, Kidmose, U, Thybo A, Hansen-Møller J, Lindhard Petersen H, Thorup-Kristensen K, Mølgaard J-P, Jørgensen H & Christensen LP: **Secondary metabolites and sensory properties of food plants grown with three different cultivation strategies** (may be divided into several papers, each covering specific crops).

F. Scientific education

The only Ph.D.-student involved in the project was Ulla Kidmose, who worked on the project for approximately one month, analysing β-carotene, which was also the subject of her thesis work. Her thesis “Kidmose U. (2005). Effects of storage, processing and household preparation on carotenoids in commonly consumed vegetables” was successfully defended on 29th October 2004, but is still under publication and therefore not yet available, also not in electronic form.

The project group comprises 4 post doctoral scientists from Denmark (RN, IMJ, LF and JOA). In addition a Chinese post doc (Chen Yong) worked on the project during the period October 2003 to August 2004.

The M. Sc. student Marianne Nygaard Jensen, University of Southern Denmark, was affiliated with the project. Her thesis work regarded male rat fertility evaluated from quantitative and qualitative measures of semen along with testicular histology. She made use of the male rats after breeding was completed, and successfully defended her thesis “Organic diet and fertility: Possible effects of diet on rat male reproduction parameters” on 8th November 2004.

M. Sc. students Trine F. Christensen and Brigitte Diedrichsen, The Royal Danish School of Pharmacy, Dept. of Medicinal Chemistry and NERI, were affiliated with the project for their thesis on biomarkers visualised by TLC (see section E1).

Apple material was used by B.Sc. student Astrid Ejlersen in experiments with the growth rate of fruit flies reared on material made using different cultivation strategies. And her results were published together with some of the analyses of secondary metabolites in apples (see section E1).

Material from the project (raw and processed carrots) was used in the experimental course “Sensory evaluation”, the Royal Veterinary and Agricultural University, June 2002, and peas from the project were used in the same course in June 2003 (see section E1).

G. National and international cooperation

Together with project no. III.8, the project forms part of the basis for an EU-project for support for a concerted action on “Recommendations for improved procedures for securing consumer oriented food safety and quality of certified organic foods from plough to plate (Organic HACCP)”. This project, which is coordinated by Kirsten Brandt, started in February 2003 and was finish in January 2005. The project website is http://www.organichaccp.org.

Another related EU-project, the Integrated Project “Improving quality and safety and reduction of cost in the European organic and “low input” food supply chains” (QualityLowInputFood) is coordinated by the University of Newcastle upon Tyne in UK. In this project one of 7 subprojects is on investigations of impact of production systems on nutrient content, safety and health, which is coordinated by Kirsten Brandt, and part of this work incorporates concepts from the presently reported DARCOF project, as well as several other DARCOF activities.

The biocrystallisation investigations were performed in cooperation with Biodynamic Research Association, Denmark (BRAD), DK-8464 Galten. This cooperation constituted an important element in initiating the ongoing validation of the biocrystallisation method, which takes place in cooperation with University of Kassel (D) and Louis Bolk Institute (NL).

DARCOF has become member of the International Organic FQH Research Association (http://www.organicfqhresearch.org/) with Martin Tang Sørensen as contact person. In the Netherlands, the Dutch government has just (March 2005) expressed its willingness to support a somewhat similar project on chickens with 1 million Euros, which builds on and extends many of the concepts and experiences of the present project.

H. Critical reflection on the project

Regarding the work and collaboration within the project:
This project was extremely multidisciplinary, comprising participants from 9 departments in 5 different institutes, with very different traditions and modes of work, and an interest in organic agriculture and health as the only common feature. In addition, it attempted to address a question that is notoriously difficult to answer, and where there is a high risk that results will be used by different interest groups to support their different views, no matter what the results actually show. Still, and despite several significant problems in the first years of the project, we have managed to use the need for changes to develop the planning further in a positive way, so the project eventually became substantially better designed than when it started.

Regarding the perspectives of the work for society and science:
Even though the work is difficult, it is clearly very important that the question of the project is addressed, in a good scientific manner, because it is a field where well controlled data are very seriously needed. Without a project like this, the press and various interest groups will be completely unrestrained in their statements. Keeping in mind that not all the results have yet been completely assessed, the project has already provided a very significant contribution to the development of interdisciplinary research relevant for organic (and conventional) agriculture, which will increase as the results progress through peer review and become integrated in the general scientific basis of this area.

Particularly important is the message that diet differences can have significant effects on well-being, immunity and obesity, even though the tested diets all had adequate and almost identical contents of all macro- and micro-nutrients, and showed no differential effect on fertility (nor on bio-crystallisation). This challenges one of the most central dogmas in present day nutritional science, and at the same time shows how it is feasible to experimentally test the diet/behaviour interactions that are quite often reported among e.g. school children, but for ethical and practical reasons cannot be tested in humans, and therefore often are not taken serious. If the results of the present project are followed up in other projects using a similar design, and confirmed in comparisons of other relevant diet types, e.g. with or without artificial colourings, the possible impact can be massive.

During the course of the project, the discussions at the meetings, and in particular the processes of preparing and receiving evaluations of the two applications that provided its funding, we have also become very aware of the limitations of the project, and how important it is not to extrapolate its data too much. No matter what the presently known and remaining results show, they will primarily be valuable as basis for further research, which should then be designed to dissect and quantify the correlations or differences we find in the project. We can provide an upper limit for an estimate of how much the differences among model cultivation systems could affect the health of rats. It cannot tell us how much real cultivation systems affect the health of humans, only define which types and magnitudes of effects should be concentrated on in further research. But due to its inclusion of both tropic levels, the results of this project is still much better for this purpose than many other studies, which attempt to address this question by working only on part of the production chain, analysing only the outcome of primary production or that of market surveys.

8. Budget

A. Account for any change in budgets

The following budget changes have occurred after 2003:

1. Some funds at KVL, Institute of Human Nutrition, moved from 2004 to 2005 and between the different personnel categories due to the further postponement of 3 person-months of work, and the conversion of some technical assistance to scientific personnel.

Budget changes that occurred between 2002 and 2003:

2. Some funds at KVL, Institute of Human Nutrition, were moved from 2003 to 2004 due to the postponement of 5 person-months of work. See details in D7.

3. Some funds at DIAS, Department of Animal Nutrition and Physiology were moved from 2003 to 2004 due to the postponement of 3.7 person-months of work. See details in D7

4. Some funds at Risø were moved from 2003 to 2004 due to the postponement of 2.2 person-months of work. See details in D5

Budget changes that occurred before 2002:

5. Vagn Gundersens departure from the project caused a transfer of funds to SHB (for bioavailability work) and KB (for subcontracting of pesticide analyses). Details in section D5.

6. Hanne Nygaard Larsens departure from the project caused a transfer of funds to MR (for WP6) and CL+HJ (for WP7). See details in sections D1, D6 and D7.

7. Delays in data inputs caused a postponement of 2 months of work in WP8. Details in section D8

8. NERI’s contribution was reduced (affecting only self-funded activities). Details in section Dx.

Since most changes are due to redistributions of responsibilities for work or allocations for delays, rather than actual changes in plans, and all revisions of plans have been within the WPs affected, these changes have not affected the amount of funding for each workpackage, nor on the load or content of the work.

B. Budget for the whole project (1.000 DKK)

Year:	Original budget	Consumption before 2003	Consumption 2003	Con-sump-tion 2004	Con-sump-tion 2005	Total
Man-months						
Scientific personnel	90.1	41	23.1	20.9	3.0	88.0
Technical personnel	42.2	28.3	32.0	6.7	0.0	67.0

Year:	Original budget	Consumption before 2003	Consumption 2003	Con-sump-tion 2004	Con-sump-tion 2005	Total
Salaries						
Scientific personnel	3604	1550	948	854	92	3444
Technical personnel	1109	653	730	166	0	1549
Other operational costs	1785	689	432	300	12	1433
Equipment	125	10	115	0	0	125
Others (please specify)	0	0	0	0	0	0
Direct costs	6623	2903	2225	1320	104	6552
Indirect costs (20% of direct costs)	1310	580	444	263	22	1310
Total	7860	3483	2669	1583	126	7862

Comments: The only change since the 2003 report is for The Royal Veterinary and Agricultural University, which has transferred some funds to 2005.

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project			

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute: Danish Institute of Agricultural Sciences

Year:	**Original budget**	**Consumption before 2003**	**Consump-tion 2003**	**Consump-tion 2004**	**Total**
Man-months					
Scientific personnel	50.1	25.3	14.8	18.1	58.2
Technical personnel	31.8	21.3	18.5	4	43.8

Year:	**Original budget**	**Consumption before 2003**	**Consump-tion 2003**	**Consump-tion 2004**	**Total**
Salaries					
Scientific personnel	2084	988	621	742	2351
Technical personnel	727	494	388	79	961
Other operational costs	855	545	357	271	1173
Equipment	20	0	20	0	20
Others (please specify)	0	0	0	0	0
Direct costs	3686	2028	1386	1092	4506
Indirect costs (20% of direct costs)	737	405	277	219	901
Total	4423	2433	1663	1311	5407

Comments:

Name of Institute: The Royal Veterinary and Agricultural University

Year:	**Original budget**	**Consumption before 2003**	**Consumption 2003**	**Con-sump-tion 2004**	**Con-sump tion 2005**	**Total**
Man-months						
Scientific personnel	32.0	8.7	1	1,40	3	14.1
Technical personnel	6.6	0	4,05	1,04	0	5.1

Year:	**Original budget**	**Consumption before 2003**	**Consumption 2003**	**Con-sump-tion 2004**	**Con-sump tion 2005**	**Total**
Salaries						
Scientific personnel	1168	322	1	1,40	3	14.1
Technical personnel	173	0	4,05	1,04	0	5.1
Other operational costs	682	38	1	1,40	3	14.1
Equipment	0	0	4,05	1,04	0	5.1
Others (please specify)	0	0	1	1,40	3	14.1
Direct costs	2022	360	4,05	1,04	0	5.1
Indirect costs (20% of direct costs)	404	72	1	1,40	3	14.1
Total	2427	432	4,05	1,04	0	5.1

Comments: The funds used in 2005 were transferred from the 2004 budget. This only affected the Department of Human Nutrition (see the Department's budget on page 37).

Name of Institute: Risø National Laboratory

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months					
Scientific personnel	4.3	1	1	0.5	2.5
Technical personnel	4.8	1	2	1.7	4.7

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries					
Scientific personnel	192	42	44	22	108
Technical personnel	155	33	65	55	153
Other operational costs	89	0	14	0	14
Equipment	105	10	95	0	105
Others (please specify)	0	0	0	0	0
Direct costs	542	85	218	77	380
Indirect costs (20% of direct costs)	108	17	44	15	76
Total	650	102	262	92	456

Comments:

Name of Institute: University of Southern Denmark

Year:	**Original budget**	**Consumption before 2003**	**Consumption 2003**	**Consumption 2004**	**Total**
Man-months					
Scientific personnel	0	6	6.3	0.9	13.2
Technical personnel	0	6	7.4	0	13.4

Year:	**Original budget**	**Consumption before 2003**	**Consumption 2003**	**Consumption 2004**	**Total**
Salaries					
Scientific personnel	0	198	244	37	479
Technical personnel	0	126	167	0	293
Other operational costs	0	106	46	5	157
Equipment	0	0	0	0	0
Others (please specify)	0	0	0	0	0
Direct costs	0	430	457	42	929
Indirect costs (20% of direct costs)	0	86	91	8	185
Total	0	516	548	50	1114

Comments:

B. Budget for each participating department (1.000 DKK)

Name of Institute and department: Danish Institute of Agricultural Sciences, Department of Food Science

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months					
Scientific personnel	21.3	15.4	4.4	1.6	21.4
Technical personnel	12.9	10.6	2.3	0	12.9

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries					
Scientific personnel	880	597	196	86	879
Technical personnel	309	250	59	0	309
Other operational costs	414	360	117	7	484
Equipment	0	0	0	0	0
Others (please specify)	0	0	0	0	0
Direct costs	1602	1208	372	93	1673
Indirect costs (20% of direct costs)	320	242	74	19	335
Total	1923	1449	446	112	2007

Comments:

Name of Institute and department: Danish Institute of Agricultural Sciences, Department of Animal Nutrition and Physiology

Year:	**Original budget**	**Consumption before 2003**	**Consumption 2003**	**Consumption 2004**	**Total**
Man-months					
Scientific personnel	14.1	4	7	11.1	22.1
Technical personnel	12.2	4	16.2	4	24.2

Year:	**Original budget**	**Consumption before 2003**	**Consumption 2003**	**Consumption 2004**	**Total**
Salaries					
Scientific personnel	521	142	262	384	788
Technical personnel	260	85	329	79	493
Other operational costs	297	60	230	255	545
Equipment	20	0	20		20
Others (please specify)	0	0	0	0	0
Direct costs	1097	287	841	718	1846
Indirect costs (20% of direct costs)	219	57	168	144	369
Total	1316	344	1009	862	2215

Comments:

Name of Institute and department: Danish Institute of Agricultural Sciences, Department of Agricultural Systems

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months					
Scientific personnel	2.9	2.9	0	0	2.9
Technical personnel	6.7	6.7	0	0	6.7

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries					
Scientific personnel	110	110	0	0	110
Technical personnel	159	159	0	0	159
Other operational costs	117	117	0	0	117
Equipment	0	0	0	0	0
Others (please specify)	0	0	0	0	0
Direct costs	386	386	0	0	386
Indirect costs (20% of direct costs)	77	77	0	0	77
Total	463	463	0	0	463

Comments:

Name of Institute and department: Danish Institute of Agricultural Sciences, Department of Animal Breeding and Genetics

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months					
Scientific personnel	11.8	3	3.4	5.4	11.8
Technical personnel	0	0	0	0	0

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries					
Scientific personnel	574	139	163	272	574
Technical personnel	0	0	0	0	0
Other operational costs	27	8	10	9	27
Equipment	0	0	0	0	0
Others (please specify)	0	0	0	0	0
Direct costs	601	147	173	281	601
Indirect costs (20% of direct costs)	120	29	35	56	120
Total	722	177	208	337	722

Comments:

Name of Institute and department: The Royal Veterinary and Agricultural University, Research Department of Human Nutrition

Year:	Original budget	Consumption before 2003	Consumption 2003	Con-sump-tion 2004	Con-sump-tion 2005	Total
Man-months						
Scientific personnel	22.2	0	0	1.40	3	4.4
Technical personnel	6.6	0	4.05	1.04	0	5.1

Year:	Original budget	Consumption before 2003	Consumption 2003	Con-sump-tion 2004	Con-sump-tion 2005	Total
Salaries						
Scientific personnel	927	0	0	53	92	145
Technical personnel	197	0	110	32	0	142
Other operational costs	799	0	10	24	12	46
Equipment	0	0	0	0	0	0
Others (please specify)	0	0	0	0	0	0
Direct costs	1924	0	120	109	104	333
Indirect costs (20% of direct costs)	385	0	24	21	22	67
Total	2308	0	144	130	126	400

Comments: By 31st December 2004 the project's accounts show a remaining amount of approx. 126 t. kr., compared with the budget. This is requested transferred to the 2005 period for 3 months salary to scientific personnel and travel (mainly for travels to Foulum as well as presentation of results).

Name of Institute and department: The Royal Veterinary and Agricultural University, Organic Farming Unit

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Man-months					
Scientific personnel	9.7	8.7	1	0	9.7
Technical personnel	0	0	0	0	0

Year:	Original budget	Consumption before 2003	Consumption 2003	Consumption 2004	Total
Salaries					
Scientific personnel	361	322	39	0	361
Technical personnel	0	0	0	0	0
Other operational costs	43	38	5	0	43
Equipment	0	0	0	0	0
Others (please specify)	0	0	0	0	0
Direct costs	404	360	44	0	404
Indirect costs (20% of direct costs)	81	72	9	0	81
Total	485	432	53	0	485

Comments:

C. Budget for co-financing from each participating institute (1.000 DKK)

Name of Institute: National Environmental Research Institute

Year:	**Original budget**	**Consumption before 2003**	**Consumption 2003**	**Consumption 2004**	**Total**
Man-months					
Scientific personnel	3	2	0	0	2
Technical personnel	6	3	0	0	3

Year:	**Original budget**	**Consumption before 2003**	**Consumption 2003**	**Consumption 2004**	**Total**
Salaries					
Scientific personnel	150	90	0	0	90
Technical personnel	150	75	0	0	75
Other operational costs	50	50	0	0	50
Equipment	50	0	0	0	0
Others (please specify)	0	0	0	0	0
Direct costs	400	215	0	0	215
Indirect costs (20% of direct costs)	0	43	0	0	43
Total	400	258	0	0	258

Comments: This was partly financed from NERI basal funds, since an anticipated external funding source was reduced, after the work was started, and the first set of results produced. The value of the work of 2 students is not included.