



Annual Status Report 2001 and Application for Continuation in 2002

For 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

III.4 Organic food and health – a multigeneration animal experiment

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7. **Start of project: April 2001**
End of project: December 2004
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8. Annual report/Application for continuation in 2001

A. Objectives and expected achievements (from application)

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

Partial objectives are 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 are the only important variables related to the food, and that the diet made from the food products is both nutritionally adequate for the experimental animals and a relevant model for human diets.
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2. To determine if the foods show differences in contents of macronutrients (protein amount and composition, energy content and distribution) of such a magnitude that this is likely to affect food consumption and/or growth of animals. If this is the case for protein and/or energy, an additional objective is to produce protein- and energy-adjusted diets where the proportion of peas and wheat starch, respectively, is increased in the organic treatments to compensate for deficiencies in protein content or quality and in energy density.
3. To determine if the foods show 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 is likely to affect consumption and/or growth of animals.
4. To record the growth, fertility and level of activity of experimental animals on the 3 diets described above, throughout 2 generations.
5. To analyse biomarkers for relevant disease indicators, such as blood lipids, antioxidant and immunological status, adaptations to biological value of the feed and bioavailability of selected minerals and secondary metabolites for differences among the 3 groups during the 2nd generation.
6. To determine other relevant differences and stress responses, e.g. longevity and cause of death, on selected animals in the 2nd generation.

B. Project summary (from application)

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

The agricultural treatments used are 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.
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.
3. A factorial combination of 1 and 2, with low input of nutrients, primarily through animal manure, and use of as much pesticides as is allowed.

The treatments will be established on 2 sites, wheat, potatoes and oilseed rape will be produced in Foulum, and carrots, kale and mature peas in Årslev.

Potato, peas and kale will be cooked and freeze-dried, wheat is ground and baked to biscuits, oil produced from the rapeseed, and raw carrots and apples are shredded and freeze dried.

The picture-developing properties of fresh plant material are examined for carrot, potato, kale, apple, pea and spring wheat samples, as well as for processed feed mixtures by means of biocrystallization. The pictures are quantified by means of computerised image analysis techniques (texture analysis of grey-levels) and / or by means of quantitative visual scoring techniques.

Characteristic secondary metabolites are measured in selected feed material from each treatment: Polyacetylenes, isocoumarins and volatile compounds in carrot, volatiles and phenolic compounds in apples, glucosinolates, volatiles and phenolics (including flavonoids) in kale, glycoalkaloids, phytin, coumarin and phenolic acids in potatoes, and phytins in wheat and peas.

The major nutrients in the feed plants of the cultivated treatments of WP1 are determined, and the biological value of major protein sources of feed plants will be assessed. Based on these results, 3 feed mixtures are prepared, either based on defined weight percentages of each material from each treatment, or, if substantial differences are found between material from the cultivation treatments, one feed mixture of each cultivation treatment is designed in such way that that the feed mixtures are adjusted to provide the same availability of protein and energy. Concentrations of approx. 20 elements and 150 pesticides are estimated by analysis of the final diets.

Rats are fed with the 3 prescribed feed mixtures for 2 generations, with 25 females per treatment and generation. Reproductive characteristics and weight gain are recorded. Subgroups of the second generation of rats are selected for an intensive study in which uptake and excretion of energy and pro-

tein and selected micronutrients are determined. Respiration trials are performed to assess the energy metabolism, and simultaneous measurements of the activity levels of the rats are performed. In addition, blood and tissue samples of the rats will be obtained to study the effect of the dietary treatments on the immunological, antioxidant, and health status of the rats.

Data from the experiments are assembled and analysed using relevant models, and combined with the data on feed to determine which associations are significant, and to provide inputs for the final scientific papers. Publications and other dissemination activities are planned and coordinated to maximise precision and extent of the impact of the results.

Table 1: Work package list (from application)

No.	Work package title	Participants*	Budget (1.000 DKr)	Start	End	Deliverable No:
1	Cultivation of feed plants	<u>JPM</u> , KTK, HLP	431	1	10	1.1, 1.2
2	Characterisation of picture-developing properties of plant materials and feed mixtures	<u>JOA</u>	347	7	12/24	2.1, 2.2
3	Characterisation of secondary metabolites in plant material	<u>EL</u>	758	8	20/32	3.1
4	Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures	<u>CL</u> , HJ	345	13	18/30	4.1
5	Characterisation of mineral content and pesticide residues of feed mixtures	<u>VG</u> + subcontractors	186	17	24/36	5.1, 5.2
6	Management and recording of feeding and breeding	<u>HNL</u> + subcontractor	746	17	26/38	6.1, 6.2
7	Digestion and utilisation of nutrients and effect on health status	<u>HNL</u> , CL, VG, SHB, EL + subcontractors	2193	19	36/44	7.1-7.3
8	Data management and analysis	<u>JHB</u>	548	4	40	8.1-8.3
9	Coordination, dissemination and evaluation	<u>KB</u> + all	456	1	44	9.1-9.7
Total			6.000			

* Responsible participants are underlined

C. Progress

C.1 Annual description (resume) of main results and conclusions

The project has now been in progress for 7 months and proceeds on schedule. The cultivation of the 7 different feed crops is completed, harvest will be complete by the end of October. The processing has started for some of the crops, and is expected to be completed by February 2002, as planned. The yields were sufficient to produce the feed calculated as necessary to carry out the planned experiments, although the planned safety margin is somewhat smaller than expected.

C.2 Fulfilment of tasks and deadlines in individual work packages

(To be completed for each work package)

WP 1 Cultivation of feed plants	Time schedule according to application	Deviations, if any*
Task		
1 Production of plant material	Month 1-7	Achieved
2 Processing the materials to become storable	Month 6-11	On time
Deliverables		
D1.1 The harvested materials.	Month 7	Achieved
D1.2 The processed materials.	Month 11	On time
Milestones		
M1.1 The materials are harvested	Month 7	Achieved
M1.2 The materials are processed.	Month 11	On time

* *Deviations are to be further discussed at C3*

WP 2 Characterisation of picture-developing properties of plant materials and feed mixtures	Time schedule according to application	Deviations, if any*
Task		
1 Characterise picture-developing properties	Month 7-16	On time
2 Relate picture-developing properties to other results of the project	Month 16-44	-
Deliverables		
D2.1 Scientific manuscript on picture-developing properties of the examined samples.	Month 24	-
D2.2 Contribution to scientific paper(s) on the correlation between picture-forming characteristics and other data from the project.	Month 44	-
Milestones		
M2.1 The analyses are completed.	Month 16	On time

* *Deviations are to be further discussed at C3*

- *Too early to assess if deviations are going to occur*

WP 3 Characterisation of secondary metabolites in plant material	Time schedule according to application	Deviations, if any*
Task		
1 Analyses selected compounds in plant products:	Month 8-20	On time
Phenolic profiles	Month 12-20	-
Volatile compounds	Month 8-12	On time
Phytins	Month 15-18	-
Glucosinolates, glycoalkaloids and polyacetylenes	Month 8-15	On time
2 Publish results of the project	Month 16-32	-
Deliverables		
D3.1 At least one scientific manuscript on the contents of secondary metabolites in the materials.	Month 32	-
Milestones		
M3.1 The analyses are completed.	Month 20	-

* *Deviations are to be further discussed at C3*

- *Too early to assess if deviations are going to occur*

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*
Task		
1 Characterisation of plant ingredients	Month 11-16	On time
2 Design and produce batches of feed from each cultivation treatment	Month 16-18	-
Deliverables		
D4.1 Contribution to scientific manuscript	Month 32	-
Milestones		
M4,1 Formulation and production of experimental diets for animal experiment.	Month 18	-

* *Deviations are to be further discussed at C3*

- *Too early to assess if deviations are going to occur*

WP 5 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*
Task		
1 Concentrations of selected elements	Month 18-20	-
2 Occurrence and concentration of pesticides	Month 18-20	-
Deliverables		
D5.1 (month 20): List of elements selected for the balance study of the second generation in WP8.	Month 20	-
D5.2 (month 20): Data on contents of pesticides in each of the diets.	Month 20	
Milestones		
M5.1 (Month 20): The analyses are completed.	Month 20	-

- *Too early to assess if deviations are going to occur*

WP 6 Management and recording of feeding and breeding	Time schedule according to application	Deviations, if any*
Task		
1 Management and recordings of the animals for 2 and a half generations	Month 18-26	-
Deliverables		
D6.1 Weaned rats of second generation for WP7	Month 26	-
D6.2 Scientific manuscript on reproductive effects.	Month 36	-
Milestones		
M6.1 In the first generation, a sufficient number of young is produced in order to make the planned recordings and experiments.	Month 20	-
M6.2 In the second generation, a sufficient number of young is produced in order to make the planned recordings and experiments.	Month 24	-

- *Too early to assess if deviations are going to occur*

WP 7 Digestion and utilisation of nutrients and effect on health status	Time schedule according to application	Deviations, if any*
Task		
1 Preference tests with animals from the first generation.	Month 20-26	-
2 Balance study with animals from the second generation.	Month 26-32	-
3 Determination of the health status of animals from balance study	Month 32-36	-
4 Investigation of selected immunological parameters in the second generation.	Month 26-32	-
5 Life expectancy study with animals from the second generation.	Month 24-44	-
Deliverables		
D7.1 Scientific manuscript(s) on bioavailability of nutrients and secondary metabolites.	Month 44	-
D7.2 Scientific manuscript(s) on behaviour, activity levels and food preference..	Month 44	-
D7.3 Scientific manuscript(s) on immunological status, life expectancy and frequency of diseases.	Month 44	-
Milestones		
M7.1 The tests on the first generation rats are completed.	Month 26	-
M7.2 The tests on the second generation rats are completed.	Month 30	-
M7.3 The life expectancy and occurrence of diseases of the second generation rats are characterised.	Month 44	-

- Too early to assess if deviations are going to occur

WP 8 Data management and analysis	Time schedule according to application	Deviations, if any*
Task		
1 Detailed planning of interaction among experiments and exchange of materials and data.	Month 7-10	On time (changed to WP 9)
2 Data from the preliminary experiments (WP 1-5) are assembled and analysed using relevant models, and used to optimise the design of the animal experiments in WPs 6 and 7	Month 10-18	-
2 Data from the animal experiments (WP 6-7) are assembled and analysed using relevant models	Month 18-40	-
Deliverables		
D8.1 Detailed plan for initial experiments and exchange of materials and data.	Month 10	On time (changed to WP 9)
D8.2 Optimised plan for the animal experiments.	Month 18	-
D8.3 Report or inputs to papers, with the relevant statistical analyses.	Month 40	-
Milestones		
M8.1 Detailed plan for experiments and exchange of materials and data is prepared.	Month 10	On time (changed to WP 9)
M8.2 Optimised plan for the animal experiments is prepared.	Month 18	-

* Deviations are to be further discussed at C3

- Too early to assess if deviations are going to occur

WP 4 Coordination, dissemination and evaluation	Time schedule according to application	Deviations, if any*
Task		
1 Coordination and management of the experimental work	Month 1-44	On time
2 Coordination and management of the scientific publication activities	Month 10-44	-
2 Coordination and planning of recommendations and follow-up activities	Month 20-44	-
Deliverables		
D9.1 Startup meeting M1	Month 1	Achieved
D9.2 Progress meeting M2	Month 11	On time
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	Month 18	-
D9.4 Progress meeting M4	Month 24	-
D9.5 Progress meeting M5	Month 34	-
D9.6 Progress meeting M6	Month 42	-
D9.7 Final report, including implementation and further progress.	Month 44	-
Milestones		
M9.1 Determination of detailed research plans for the animal studies.	Month 18	-
M9.2 Publication plan is prepared.	Month 24	-
M9.3 Final report on implementation and further progress is prepared.	Month 44	-

* *Deviations are to be further discussed at C3*

- *Too early to assess if deviations are going to occur*

C.3 Discussion on the progress, incl. deviations and achievements in the project as a whole and in the individual work packages

Until now, only WP's 1, 2, 3 and 9 have had any substantial activity, and no delays have occurred. For WP 3 and to some extent WP 2 this has been mainly in the form of method optimisation (as planned), so no results have yet been produced (as expected at this time). The only change, and a very minor one, is that some of the work described in the application to be placed in WP 8, task 1, of planning and managing the exchange of material and data among participants, has been taken over by the coordinator and thus will be moved to WP 9. Since it was a misunderstanding in the first place, the scientist responsible for WP 8 never intended to do it, but this detail was overlooked in the process of making the application.

In WP1 the yields were recorded and sufficient amounts were produced that are needed to carry out the planned experiments, although for one of the crops, peas, the planned safety margin has turned out to be somewhat smaller than expected. The harvest only provided 15% more than necessary for the calculated feed requirement in the treatment with the lowest yield. While this does not give any problems for carrying out the project exactly as planned, it reduces the possible options for later changes to the workplan.

In WP2 biocrystallization pictures have been produced from the carrot samples from all treatments. Procedures for producing extracts of kale, apples and potatoes have been elaborated, and work is in progress to produce biocrystallization pictures from these crops.

D. Description of plans and future work in the project as a whole and in the work package (Including plans for publication and communication)

The project is expected to progress as planned.

As described in the application, the work that will take place in 2002 is the following:

WP 1: Processing of all remaining material, will be completed by February 2002. The material that it is almost certain that will be used will be freeze dried to be ready for preparation of feed pellets, while some additional material will be stored frozen, but not dried, as a reserve, that can be freeze dried later if needed. This WP will be completed in 2002.

WP 2: The picture-developing properties will be investigated in the remaining crops, that were not tested in 2001, and in the final feed mixtures when these are prepared, later in 2002. The data are published and/or prepared for later use in joint publications with the other participants in the project, when the results of the animal experiments are known. This WP will be completed in 2002.

WP 3: The concentrations of the selected secondary metabolites are measured in the remaining crops, in addition to those tested in 2001. The data are published and/or prepared for later use in joint publications with the other participants in the project, when the results of the animal experiments are known. This WP will be completed in winter 2002/2003.

WP 4: The plant ingredients produced in WP1 are characterised with respect to their nutrient content and value. The nutrient analysis will include dry matter, crude fat, crude protein, ash, crude fibre and, in selected ingredients, the composition of amino acids and fibres. Biological value (BV) of protein in the major protein contributing plant ingredients is assessed in a balance experiment with rats (5 per treatment). The data are published and/or prepared for later use in joint publications with the other participants in the project, when the results of the animal experiments are known.

It is determined if the plant ingredients differ in their energy content and BV of protein to such an extent, that according to previous experiences it is likely to influence growth and health of the rats. If this is the case, the feed mixtures of the plant material of the cultivation treatments will be designed in such way that the energy and protein contents become similar. This will be done by adjusting the content of energy and protein contributing ingredients (e.g. wheat flour and pea meal). Depending on the results of this and of WP's 2, 3 and 5, such adjusted feed(s) will either be used to replace treatment 3 with an adjusted version of treatment 1, or both treatments 1 and 3 will be used only in adjusted form. This WP will be completed in 2002.

WP 5: The concentrations of selected numbers of essential, toxic and potentially interesting elements are estimated in the final diets produced in WP 4 from the plant material harvested in WP1. The elements may be Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Se, Zn, Ba, Cd, Pb, Sr, Ce, Cs, Rb, and V. The final selection of elements to be measured in feed will be executed when a running human metabolic balance study is finished. Of these, elements that differ significantly among treatments, or where there is an indication that bioavailability can differ depending on other food components, will be selected for measurement in WP7. The occurrence and concentration levels of 150 pesticides are estimated in the final diets.

The data are published and/or prepared for later use in joint publications with the other participants in the project, when the results of the animal experiments are known. This WP will be completed in 2002.

WP 6: Separate sets of individually identifiable, newly weaned rats of a relevant genotype are fed with the 3 prescribed feed mixtures. For each of 2 generations (only the first one of these will be in 2002), 25 pairs of rats per diet group are allowed to breed, one litter per female. For each female, reproductive characteristics are recorded, including numbers of attempts before pregnancy, litter size, female/male ratio, stillborn/live born pups, litter size at weaning and possibly age of sexual maturity and anugenital distance. After weaning, a female and a male pup from each female rat are selected to form the next generation. Subgroups of rats from the 2 generations are enrolled in the experiments described in WP7. For all rats, weight gain is recorded weekly (for pups starting at the age of 4 weeks) and any signs of the presence of disease are recorded. This WP will continue in 2003, where it will be completed.

The project group is presently considering several options for which genotype of rats will be used in the experiment. Since the overall objective of the project is to gain information that relates to humans,

not only to rats, it is very important that the selected strain of rats has responses to feed composition that parallels those of humans as closely as possible. Also it can be an advantage if the rats are not too fertile, since this will enable both positive and negative deviations from the values expected from other experiments with standard feed, and thus may give better chance to determine if significant differences occur.

WP 7: Further investigations of the health and longevity of the animals from WP 6. Determination of uptake of selected minerals, nutrients and secondary metabolites in each treatment. Characterisation of how selected biomarkers for health and well-being are affected by the treatments. No activity in 2002, except some method optimisation. The main activity is in 2003, continuing in 2004.

WP 8: Data from the preliminary experiments (WP 1-5) are assembled and analysed using relevant models, and used to optimise the design of the multigeneration animal experiments in WPs 6 and 7, and to provide inputs for the initial scientific papers. Later in 2002 work is initiated to assemble and analyse data from the animal experiments (WP 6) using relevant models, combine with the data on feed to determine which associations are significant, and to provide inputs for the final scientific papers. The main activity will be in 2002, continuing in 2003 and 2004.

WP 9: Coordination and management of the project, primarily through periodic meetings and progress reports. At each meeting, progress is evaluated and reported in relation to the pre-set aims and to concurrent advances in other projects, and relevant action is decided and implemented if deviations occur. One more meeting (the second) will be held later in 2001, and at this meeting the detailed plans for the remaining work of the initial workpackages (2-5) will be finalised. At least one progress meeting is planned for 2002, to take place in summer/autumn, when WP 4 is completed. No later than at this meeting the detailed plans for the multigeneration experiments (WP 6-7) will be finalised.

The data which are produced in WP's 2-7 and analysed in WP8 are assembled into scientific and popular papers and conference presentations, which are submitted to the most influential journals or events to reach the target audiences in the relevant fields, nationally and internationally. A detailed plan will be prepared, describing which information will be disseminated where and under which conditions. E.g. some popular or conference publications must await acceptance of a particular paper by a journal before they can be released. This will start up in 2002, with the main activity in 2003 and continuing in 2004.

After mutual agreement between the involved departments, Hanne Nygaard Larsen will move from the Research Department of Human Nutrition to Department of Animal Science and Animal Health, both at The Royal Veterinary and Agricultural University, since this Department is better able to provide the scientific support for this type of work. As soon as possible an associate professor at this department will be appointed as supervisor for HNL. Since Susanne Højbjerg Bügel is still at Research Department of Human Nutrition, the budget for this department is split in two parts, where the part relating to HNL's work is transferred to Department of Animal Science and Animal Health, while SHB's part remains at Research Department of Human Nutrition.

The project group has applied for funding for extending the project with an additional year of cultivation and an additional generation of rats, that would be fed with material from the second year. The proposal is presently under consideration by DARCOF after a call for new proposals in September, as well as two other proposals for follow-up projects (see section G) using the material already cultivated.

E. Project publications

1. Articles in international, scientific journals with review procedures

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.

2. Presentations at congresses, symposiums etc.

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.

3. Articles in agricultural journals etc.

None yet

4. Other presentations at meetings, field days etc.

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

- **Scientific education (ph.d. and post doc.), including visiting scientists and visits abroad**

National: No Ph.D.-students are presently involved in the project.

The project group comprises 3 post doctoral scientists (CL, HNL and JOA).

The M.Sc. student Sabine Jensen, University of Southern Denmark, is affiliated with the project. Her thesis work regards the measurement of degradation products of glucosinolates in cruciferous vegetables, and she will analyse the kale material produced in the project for those compounds.

International:

No operative collaboration (working visits of a week or longer duration) has yet been established, but several contacts have been made, primarily regarding various holistic methods.

A research group at the Ludwig Boltzmann Institute in Vienna, Austria, represented by Dr. Alberta Velimirov, have included collaboration with the project as part of a national Austrian application "Suitability of methods to determine specific product characteristics in regard to biological cultivation", which is presently under consideration for funding. If successful, this project will start early in 2002. The anticipated collaboration will be in the form of a visit by an Austrian post-doctoral scientist, Dr. Bettina Brandtner, who will stay for a training period at DIAS in Årslev to learn some analysis techniques for secondary metabolites in carrots, and to discuss and compare the results of the two projects, since the planned Austrian project also comprises the comparison of organic and conventional cultivation treatments.

G. National and international co-operation

The project forms part of the basis for an EU-proposal currently under preparation, for support for a concerted action on "Safety, quality and health effects of organic food". A START-grant (support for project preparation) has just been awarded for the preparation of the application, which will be submitted to the European Commission ultimo January 2002.

A Nordic workshop on quality in organic agriculture was arranged to take place in Stange, Norway 22.-24.10.2001, with additional support from NKJ. However, due to an insufficient number of participants it was cancelled, although there is still a possibility that it will be held at a later date.

For some or all of the harvested crops, some surplus plant material will be saved (without freeze drying), for the possible use in other, related projects, that may be established in parallel with the present one. Specifically some of the participating departments have applied for two such projects in the DARCOF call for proposals presently under consideration. One of these proposed follow-up projects is on investigation of sensory properties of the plant products, the other is on a thorough investigation of intestinal health in two genotypes of rats, one of which is diabetic, fed on the three diets of the present project.

Whether or not these proposals are successful, the material will be saved for a while, since some of these possibilities may attract other collaborators, including students, that are interested to carry out additional tests in a project with well-defined material.

