

Title: Organic food and health – a multigeneration animal experiment.

Acronym: Organic health

1. Introduction

Quality and safety of food are important issues, which during the last decade have received ever increasing attention by the public. One reason is that the efficient, industrialised methods of modern agriculture and food distribution has the effect that bad or unsafe food is sometimes distributed to many consumers before the problem is discovered and remedial action is taken, resulting in widely advertised “food scandals”. Another one is the decrease in the proportion of people with first-hand experience in primary food production, which allows for extensive myth-formation, particularly in city populations.

Together these and similar trends have created a very strong interest in organic food products, which many consumers in several European countries perceive as more beneficial for health than conventional ones (Beckman *et al.*, 2000). However, no widely recognised investigations have been published that clearly support or rule out such a difference. A large number of studies have addressed this question, primarily by analysing food compositions, but also a few experiments with animals or humans (Alföldi *et al.* 1998, Jensen *et al.*, Woese *et al.*, 1995, 1997). While none have had the design or extent necessary to definitively show differences of a type and magnitude, which is clearly associated with effects on health, they indicate that differences are either quite small or expressed mainly on a long term scale.

Previous initiatives of the Danish Research Centre for Organic Farming (DARCOF) have emphasised promoting the entire organic lifestyle, with emphasis on environment, sustainability and minimal consumption. While this is definitely the most important issue, it is becoming increasingly important to supplement it with a more specific study of possible physiological effects of eating certain food items. As organic products increase their market share and become more visible on the market, a polarisation of the debate occurs. Proponents of organic agriculture and those of conventional agriculture (with genetically modified organisms as the extreme example), mutually accuse each other of using unsubstantiated claims of risks or benefits to promote their own commercial interests. Consequently, it is in the interest of both consumers and policy makers to have access to independent scientific results, which can be used to secure the focus and improve the quality of the debate. All parties will have an interest in improved knowledge about which factors in the cultivation system promote health of the consumers, and the sincere proponents of either system will welcome subsequent efforts to introduce the best aspects of each into both types of systems.

In order to ensure that the results can be used in this positive way, it is important that the study is designed in a way that meets the requirements of both “parties”. That the choices and simplifications which are necessary in order to ensure comparability, are made in ways which comply with both the rules of good scientific practice, exemplified by the requirements for publications of major scientific journals, and (where relevant) with the rules of organic agriculture. That the types of measurements and analyses, which are considered important for different views of food quality are included and made on the same batches of materials. And also that all participants in the project and all users of the results are aware, that it is not possible in one study to cover the range found within each of the two systems. So the study must be made with specially designed treatments, which model relatively distinct versions of the organic and conventional agricultural systems, respectively. Preferably supplemented with factorial combinations of the major determinants of suspected effects on health: plant nutrient availability and pesticide use.

In order to keep the study at a manageable level of complexity, it will be restricted to only comprise plant foods, even though strict vegetarians are very small minority. However, if it is shown that the cultivation treatments affect fatty acid composition of the animals, subsequent studies should include meat products, since it has been shown recently, that changes in fatty acid composition of pig fat that are due to feed composition can have a potential to reduce blood cholesterol in human subjects (Lauridsen et al, 1999; Sandström et al., 2000).

2. State of the art

Previous comparisons of nutritional quality of organic and conventional food.

As mentioned above, numerous studies have compared one or more aspects of this. Most of these measure contents of well known vitamins or minerals in plant foods of more or less controlled origin, and conclude that differences are often significant, but generally relatively small. Normally they are less than differences found between different cultivars grown in the same system (Woese *et al.*, 1995, 1997, Alföldi et al. 1998, Justesen et al. 1998). While some general trends are clearly recognisable, e.g. higher average levels of vitamin C in organic than in conventional vegetables, these results do not by themselves prove any effects on health, since it is generally recognised that a normal, balanced, Western diet contains sufficient amounts of most vitamins and essential minerals. Conventional foods systematically contain more pesticide residues than organic ones, but normally at levels that are orders of magnitude lower than what is known to directly affect health.

Still, several feeding studies with animals have indicated differences in health and/or fertility between groups fed comparable organic and conventional diets. Two animal studies showed slight indications of improved immunological defence in animals fed an organic diet (Gottschewski, 1975; Plochberger, 1989), supplementing an epidemiological study showing reduced prevalence of allergies in families with an anthroposophic lifestyle (Alm et al. 1999). Reproductive health has been investigated in a number of animal feeding studies, and these have indicated that reproductive health including fertility may be improved in groups fed organic compared to conventional diets (Staiger, 1986; Velimerov *et al.*, 1992). Since exposure during fetal life may be of crucial importance for the reproductive function in adult life, single generation studies are not sufficient and two or three generation assessments are required (Giwerzman and Bonde, 1998). Some of the animal studies had shortcomings and most of them were older and may not be representative of today's organic and conventional farming systems. However, these studies still indicate that differences in reproductive health may be present, and that a more definitive study is justified. Plant foods contain other components with effects on health than the well-known nutrients, for example many biologically active secondary metabolites. And while there are good reasons to believe, that the contents of plant secondary metabolites will systematically differ among the agricultural systems (Brandt & Mølgaard), hardly any comparative studies have included measurements of any of these in the analyses.

Regarding which aspects of health that may be influenced by differences between organic and conventional diets, an initial filter is represented by the difference in health between the present and the situation before the use of artificial fertilisers and pesticides became widespread. Most of the agriculture before 1950 was organic, by necessity rather than by choice, so any health effects attributable to conventional agriculture must have appeared only after this time. Obesity, some cancers, diabetes and allergies, and illnesses associated with these conditions, are among the ones most strongly increased during this period. Also fertility problems in the first world may be related to changes in diet. Of course, the pre-1950 diet and entire lifestyle differed from the present one in many more aspects than what can be expected to be the case for an organic and a conventional one, but this reasoning provides an indication and upper limits to the types and magnitudes of the differences that can be expected.

Likely effects of secondary metabolites and fibres on human health.

None of them are known to be absolutely necessary for a long and healthy life (per definition - in the past, as soon as a specific physiological role was shown for a secondary metabolite, it was redefined as a vitamin). Secondary metabolites in plants have a variety of functions in the plant. However, the biologically active ones, which are most likely to influence human health, are primarily involved in defence against pests, herbivores and diseases, some of them as anti-nutrients, making the protein or other essential components less available to plant-eating animals or pests (Mawson et al. 1994, Waghorn 1990). Many commonly occurring secondary metabolites are known to be harmful if tested in high concentrations, and a few, such as linamarin from cassava and solanin from potato, are even harmful in regularly occurring concentrations in food plants. If the concepts for toxicity usually applied to pesticides and other synthetic chemicals are used for plant secondary metabolites, many of them would not pass a safety evaluation (Ames et al. 1990, Essers AJA, 1998). This is the evidence which leads some people to conclude that the intake of these compounds should generally be kept as low as possible.

On the other hand, a lot of plant products containing high concentrations of potentially toxic compounds are obviously harmless to humans, since many people have been using them for many years with no known harmful effects. Examples of this are coffee, pepper (both black and chili), parsley, radish and many others (Ames et al. 1990). So it is generally accepted that at normal levels of consumption, plant secondary compounds normally occurring in well-established food plants are not as harmful as would be expected from their LD₅₀ values. This is supported by epidemiological evidence firmly contradicting the concern that fruits and vegetables (the major sources of plant secondary metabolites) in general should pose a health hazard due to their potential toxicity. As it happens, many of the compounds presently under investigation for possible anti-cancerogenous effects (Lee et al. 1999, Sangwan et al. 1998), are better known as toxicants (Rosa et al. 1998). It is possible that those defence related plant secondary metabolites that retard growth and food utilisation, may simply promote health of over-fed animals (including humans) as a direct consequence of their "toxicity" (normally measured as retardation of growth of young animals) (Brandt et al. 2000). Thus the seemingly contradictory combination of harmful and beneficial effects makes sense if viewed from the angle of mimicking caloric restriction.

Also the dietary source and content of fibres may influence the nutritional value of food, and may affect physiological conditions in human subjects such as development of the gastrointestinal tract, digestibility and energy metabolism. For instance, the length of the intestine in broiler chickens increased with the dietary fibre level, and with increasing fibre intake the partitioning of retained energy between body protein and body fat changed in favour of protein (Jørgensen et al, 1996). Regarding rats, dietary pea fibre contributed with 5.4 kJ net energy per g fermented (Zhao et al., 1995).

Several ongoing studies at the institutes of the applicants address the questions of beneficial or toxic effects of plant non-nutrients, e.g. the project "Health promoting compounds in vegetables" financed by the Danish Food Technology and Development Programme (FØTEK), which comprises preliminary investigations of polyacetylenes from carrot, in terms of structure identification, bioavailability in humans and effects on cancer cells.

Effects of major and trace elements on human health

In human nutrition, minerals and trace elements can be classified as essential, neutral or toxic with respect to the individual effect of an element on the human health. This classification is changing with the development of new insight into the functions of the elements in the human body and evolution of analytical techniques with lower detection limits. Compared with the fact that the uptake of some elements from the human diets may be subject to competitive uptake of other elements, it is necessary not only to look at a few elements, but to as many as possible, when nutritional aspects are to be investigated. In the ongoing project: "Trace Elements in Danish Agricultural and Horticultural Products", financed by FØTEK, the elemental content of around 60 elements in different crops, milk, egg, pig meat, blood and organs are investigated in relation to conventional and organic

farming. Furthermore, a short-term human metabolic balance study with organic and conventional diets is performed as a part of the project. It has been shown that the elemental concentration profiles of some crops are very affected by the cultivation method, and the concentration of some elements may vary about 100 % in crops cultivated organically and conventionally (Gundersen et al. 2000). It will be of great importance for future work in the field to compare the results of the human balance study with an animal balance study performed with human diets. If the selected animal could be used as a model for human nutritional studies regarding uptake of for example minerals and trace elements, some normally very laborious, expensive and time consuming human studies could be replaced by cheaper, less expensive animal studies.

Picture-developing properties of organic and conventional food.

During the last decade there has been an ever increasing interest in more “holistic” approaches and methods to examine food and fodder quality that may complement traditional chemical analyses of primary compounds (Meier-Ploeger & Vogtmann, 1991). Among these methods we find the so-called picture-developing methods, which include crystallization and chromatographic techniques. It has been hypothesized that the methods visualize aspects of the organizational level / inner “order” of the samples in question, which are correlated to the nutritional value of the samples (Balzer-Graf, 1994). Based on a number of publications recent reviews have pointed to the potential of the picture-developing methods to differentiate conventional and organic samples, and to the fact that generally organic samples exhibit better picture-developing properties than comparable conventional samples (Woese *et al.* 1995; Alföldi *et al.* 1998). Concerning a possible correlation between nutritional and picture-developing properties only a single study has been reported. Here it was found that organic beet root samples exhibited better picture-developing properties than the corresponding conventional samples, and simultaneously in food preference test rats preferred organic over conventional samples (Mäder *et al.*, 1993).

Using animal models to study dietary effects on human health.

Animal models are widely used in nutrition science for various reasons. The limitations in extrapolating results of animal studies to human health is an ever ongoing debate. In general, results from animal studies are considered useful indicators, though, for the need of (much more expensive and difficult) human studies. Animal models are advantageous in cases such as long-term studies and other investigations, which for practical or ethical reasons cannot be studied in humans. The choice of species in animal models depends on a number of factors, including the resemblance to humans, the sensitivity to the investigated factors and of course also economy. In the connection of the present proposal we have considered carefully advantages and disadvantages of several animal species as the best model, and have chosen the rat. It is extensively used as a model for studies of factors affecting human health, and the cost per animal is relatively low. Another obvious choice would be minipigs, these animals are adapted to a diet more closely resembling the human one, and some investigations are much easier to perform on pigs than on rats, e.g. semen quality. However, the cost per animal is prohibitively high, under the limitations of the present proposal.

Long-term studies using realistic diets are rather few, and in this connection the most important ones are the studies of caloric restriction, which in many species have shown that diet can influence the ultimate health indicator, longevity, even when no potent chemicals are involved, nor any malnutrition ((McCarter 1995, McShane & Wise 1996). And that the diet of young animals can significantly influence their health as adults. It is also well established that differences in nutritional status of females will affect the growth and development of the offspring, and that this may further have consequences for their adult life, e.g. development of allergies (Leadbitter et al. 1999) or lung disease (Lopuhaa 2000). For these reasons and others, a study that extends for at least two generations is necessary to elucidate effects that are determined during the phases of rapid growth, but manifest themselves primarily after puberty.

Description of relevant secondary metabolites

Phenolic acids, flavonoids and coumarins:

Phenolic acids occur in most vegetable products and can be found either as the free acid, as glucosides and bound to polymeric materials (e.g. cellulose, lignin). In the free form, they exhibit broad spectrum antimicrobial properties. When bound, they assist in the stabilisation of texture, especially as dimeric phenolic acids, and as such affect the digestibility and fibre content and properties.

Coumarins are related defence chemicals found as phytoalexins in carrots and potatoes, and flavonoids are present in most plants as UV-defence. These compounds all have high antioxidant capacity and many also have interesting pharmacological properties, e.g effects related to prevention of cancer (Lee et al. 1999, Sangwan et al. 1998).

Polyacetylenes: Polyacetylenes are defence chemicals found in minor amounts in carrots and related crops. Polyacetylenes are highly potent compounds with known activity against cancer cells (Brandt and Christensen 2000).

Glycoalkaloids: Glycoalkaloids occur in potatoes and the content can be modulated by stress. The glycoalkaloids contribute to the characteristic flavour of potatoes and may promote resistance to infections (Gubarev et al. 1998), however, there is a narrow range between the glycoalkaloid intake from the potatoes consumed in a normal diet and the toxicity threshold. The major glycoalkaloid in potato is solanin.

Glucosinolates: Glucosinolates are present in cruciferous vegetables, and some have shown relevant biological properties, primarily prevention of cancer (Lee et al. 1999). Glucosinolates are easily degraded and the resulting products also have biological effects. Kale contains about 10 different glucosinolate analogs.

Carotenoids: Carotenoids are found in carrots and green vegetables and are precursors for vitamin A and some aroma compounds. They also act as antioxidants.

Phytins: Phytins can impede bioavailability of important minerals such as Fe and Zn (Reddy et al. 1996, Sandström et al. 1989) and are especially abundant in peas and cereals.

Volatiles: Differences in the aroma profile of vegetable products grown under different agricultural systems will affect taste and might thus influence the feeding preference. Some volatiles might also be important from a health perspective (Sangwan et al. 1998).

3. Objectives and expected achievements

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.
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 com-

pounds (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.

4. Description of workpackages including methods

Overall methodology:

The experiments must conform to the requirements of a blinded, placebo controlled trial. To ensure this, the batches are coded in each workpackage, when material is delivered from one task to another, and the codes are kept secret until the recordings of the analyses or experiments are completed. The feed grown conventionally with high fertiliser input is defined as the reference treatment, corresponding to placebo.

WP1 Cultivation of feed plants

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.

Note that the other factorial combination, high nutrient input without pesticides, is difficult or impossible to achieve, due to an increased incidence of diseases and pests under high nutrient conditions, and will not be attempted.

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 bisquits, oil produced from the rapeseed, and raw carrots and apples are shredded and freeze dried.

WP2 Characterisation of picture developing properties of plant materials and feed mixtures

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 computerized image analysis techniques (texture analysis of grey-levels) and / or by means of quantitative visual scoring techniques.

WP3 Characterisation of secondary metabolites in plant material

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, glucoalkaloids, phytin, coumarin and phenolic acids in potatoes, and phytins in wheat and peas.

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 are determined, and the biological value of major protein sources of feed plants will be assessed. Based on these results, 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.

WP5 Characterisation of mineral content and pesticide residues of feed mixtures

Concentrations of approx. 20 elements and 150 pesticides are estimated by analysis of the final diets produced in WP4 from the plant material harvested in WP1.

WP6 Management and recording of feeding and breeding

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.

WP7 Digestion and utilisation of nutrients and effect on health status

Subgroups of the second generation of rats are selected for an intensive study in which uptake and excretion of energy and protein 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.

WP8 Data management and analysis

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.

WP9 Coordination, dissemination and evaluation

Coordination and management of the project, primarily through periodic meetings and progress reports. Publications and other dissemination activities are planned and coordinated to maximise precision and extent of the impact of the results.

Table 1: Workpackage list

WP No	WP title	Responsible participant	Budget (1000 Dkr.)	Start	End	Deliverable, No
1	Cultivation of feed plants	JPM	431	1	10	1.1, 1.2
2	Characterisation of picture-developing properties of plant materials and feed mixtures	JOA	347	7	12/24	2.1, 2.2
3	Characterisation of secondary metabolites in plant material	EL	758	8	20/32	3.1
4	Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures	CL	335	13	18/30	4.1
5	Characterisation of mineral content and pesticide residues of feed mixtures	VG	186	17	24/36	5.1, 5.2
6	Management and recording of feeding and breeding	HNL	746	17	26/38	6.1, 6.2
7	Digestion and utilisation of nutrients and effect on health status	HNL	2290	19	36/48	7.1-7.3
8	Data management and analysis	JHB	566	4	40	8.1-8.3
9	Coordination, dissemination and evaluation	KB	466	1	48	9.1-9.7
Total			6125			

Note: Due to some errors detected too late in the editing process to allow for confirmation of changes, the budget is somewhat higher than the 6.0 million limit allowed. However, it is accurate, meaning that in order to reduce it, corresponding reductions in work content must be defined.

Note 2: Due to the significant uncertainties about the extent to which WPs 2-5 will be able to identify relevant differences between each of the 3 cultivation treatments, some flexibility in the budget must be allowed: To some extent it must be possible to change the distribution of resources among the WPs and participants, to ensure optimal use of the limited funds.

Note 3: The presently described project is close to the minimum number of generations and treatments to provide information about the subject. We have estimated that the optimal size of the central experiments (in terms of likelihood of determining a given difference, relative to the cost of the project) would be with 3 generations and 5 feed treatments, corresponding to using both the original and the protein- and energy-adjusted diets from the 3 cultivation treatments. This would cost approx. 8.7 mill. Dkr.

Exchanging rats with minipigs, with 2 generations and 3 treatments, would cost approx 8 mill. Dkr.

Table 2: Description of workpackages

WP1: Cultivation of feed plants

Workpackage number: 1
Start date or starting event: When the soil is ready to sow or plant the crops (month 1)
Responsible person: JPM
Contributing persons: Kristian Thorup-Kristensen, Hanne Lindhard Petersen
Person-months: 8

Objectives:

To produce plant material for the feed.

Description of work:

1. Production of plant material of spring wheat, rape, potato, carrot, kale, apples and dry peas, in 3 different cultivation treatments:
 - (1) Organic: No pesticides and low input of plant nutrients derived from organic matter.
 - (2) Conventional: With pesticides and high input of plant nutrients, provided as minerals.
 - (3) Intermediate: With pesticides and low input of plant nutrients, primarily derived from organic matter.
2. Processing the materials to become storable: Rape is pressed to produce oil, the residue is discarded. Potato, kale and split peas are cooked and freeze-dried. Carrot and apple are shredded, frozen and freeze-dried. Wheat is milled and the whole grain flour is made into biscuits.

Deliverables:

D1.1 (month 7): The harvested materials.
D1.2 (month 11): The processed materials.

Milestones:

M1.1 (month 7): The materials are harvested
M1.2 (month 11): The materials are processed.

WP2: Characterisation of picture-developing properties of plant materials and feed mixtures

Workpackage number: 2
Start date or starting event: When fresh plant material is available (month 7)
Responsible person: JOA
Contributing persons:
Person-months: 7

Objectives:

To characterise the picture-developing properties of plant materials produced in WP1 and the feed mixtures made in WP4.

Description of work:

Production of biocrystallization pictures on the basis of watery extracts of plant samples and feed mixtures. Development and optimization of experimental procedures in connection with extract production in the case of kale, apple and feed mixture samples. Examination of the field plot replicates of the treatments separately or pooled. Computerized image analysis and / or visual scoring of the crystallization textures. Organization of data with respect to the subsequent statistical analyses.

Deliverables:

D2.1 (month 24): Scientific manuscript on picture-developing properties of the examined samples.
D2.2 (month 48): Contribution to scientific paper(s) on the correlation between picture-forming characteristics and other data from the project.

Milestones:

M2.1 (month 16): The analyses are completed.

WP3: Characterisation of secondary metabolites in plant material

Workpackage number: 3
Start date or starting event: When some processed plant material is available (month 8)
Responsible person: EL
Contributing persons:
Person-months: 16

Objectives:

To characterise secondary metabolites in plant material.

To assist in determining if feed made from the 3 treatments in WP1 are sufficiently different to warrant the inclusion of all of them in the animal experiment.

Description of work:

Characteristic secondary metabolites are measured in selected feed material from each treatment:

1. Phenolic profile of all vegetable products used will be determined by HPLC and categorised as free and bound compounds, this comprises phenolic acids, coumarins and flavonoids where relevant. The 6 compounds for which the concentrations are most significantly different in the agricultural treatments will be isolated and characterised.
2. Volatile compounds are measured in carrot, kale, apple and rapeseed oil by dynamic headspace sampling followed by GC and GC-MS, and in the same materials carotenoids are measured by HPLC.
3. Phytins are measured in peas, wheat and potatoes by HPLC.
4. Glucosinolates in kale are measured by HPLC, glycoalkaloids in potatoes by LC-MS, and polyacetylenes in carrots by GC-MS.

Deliverables:

D3.1 (month 32): At least one scientific manuscript on the contents of secondary metabolites in the materials.

Milestones:

M3.1 (month 20): The analyses are completed.

WP4: Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures

Workpackage number:	4
Start date or starting event:	When all processed plant material is available (month 11)
Responsible person:	CL
Contributing persons:	Henry Jørgensen
Person-months:	8

Objectives:

To determine the biological value of protein and the energy content in the feed material from the 3 treatments in WP1.

To assist in determining if feeds made from the cultivation treatments are sufficiently different to warrant the inclusion of all 3 of them in the animal experiment.

To design and produce batches of feed from each cultivation treatment, if necessary adjusted in composition to obtain similar protein and energy content.

Description of work:

1. Characterisation of the plant ingredients produced in WP1 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).
2. 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 WP 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.

Deliverables:

D4.1 (month 32): Contribution to scientific manuscript with data on differences in macronutrient content and BV of protein in plant material produced in cultivation treatment, and the work involved in the design of the experimental diets.

Milestones:

M4,1 (month 18): Formulation and production of experimental diets for animal experiment.

WP5: Characterisation of mineral content and pesticide residues of feed mixtures

Workpackage number:	5
Start date or starting event:	When feed mixtures are available (month 18)
Responsible person:	VG
Contributing persons:	IEB and Fødevareregion København, Danish Food Control Agency or other subcontractor for pesticide analyses
Person-months:	2

Objectives:

To measure a large number of elements and pesticide residues in the 3 feed mixtures.

To determine which elements should be analysed in animal faeces and urine in WP7, based on the extent to which they differ among the mixtures.

Description of work:

1. Estimation of the concentrations of selected numbers of essential, toxic and potentially interesting elements in the final diets produced 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.
2. Estimation of the occurrence and concentration levels of 150 pesticides in the final diets.

Deliverables:

D5.1 (month 20): List of elements selected to be investigated in the balance study of the second generation in WP8.

D5.2 (month 20): Data on contents of pesticides in each of the diets.

Milestones:

M5.1 (Month 20): The analyses are completed.

WP6: Management and recording of feeding and breeding

Workpackage number:	6
Start date or starting event:	When feed mixtures are available (month 18)
Responsible person:	HNL
Contributing persons:	Subcontractor (Møllegården)
Person-months:	8

Objectives:

To manage the animals through 2 generations and record reproductive characteristics, weight gain and disease frequency (if relevant).

Description of work:

1. Separate sets of individually identifiable rats of a relevant genotype are fed with the 3 prescribed feed mixtures. For each of 2 generations, 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.

Deliverables:

- D6.1 (month 26): Weaned rats of second generation for WP7
D6.2 (month 36): Scientific manuscript on reproductive effects in rats fed organically versus conventionally produced feed over 2 generations.

Milestones:

- M6.1 (month 20): In the first generation, a sufficient number of young is produced in order to make the planned recordings and experiments.
M6.2 (month 24): In the second generation, a sufficient number of young is produced in order to make the planned recordings and experiments.

WP7: Digestion and utilisation of nutrients and effect on health status

Workpackage number:	7
Start date or starting event:	When the first generation of animals are weaned (month 21)
Responsible person:	HNL
Contributing persons:	CL, VG, IEB, SB, HF, LOD, EL
Person-months:	20 (HNL + SB) +13 (CL + HJ) + 6 (VG + IEB) + subcontractors (EL, LOD, HF)

Objectives:

To determine uptake of selected minerals, nutrients and secondary metabolites in each treatment
To characterise how selected biomarkers for health and well-being are affected by the treatments.

Description of work:

1. Preference tests is performed using animals (n=10/diet group) from the first generation. The animals are offered 2 test diets simultaneously over a period of 5 days. Intake is determined each day as the amount of food leftover subtracted from the amount of food given.
2. In the second generation a subgroup of rats (12 rats per feed treatment) are selected for a balance study. In this the uptake and excretion of energy and protein and of those minerals and secondary metabolites that are selected according to the results of WP5 and WP3, respectively, are measured to estimate bioavailability. In total four repetitions (one per week) of individual balance trials are performed. Simultaneously, a respiration study will be performed to assess differences in energy metabolism of the rats fed the different experimental diets. Two respiration chambers are available, and each chamber will be equipped with 6 balance cages (one per rat) and recorders to obtain the activity levels of the rats. Simultaneously the activity levels are recorded.
3. At the end of the balance trials rats will be sacrificed and blood and selected tissues will be obtained. A macroscopic analysis of the organs and a visual determination of the health status of the animals will be determined. Certain analyses (e.g. length and enzyme activity of intestine) will be included if relevant due to the treatments (e.g. variation in fibre content of the diets). Blood and tissue samples are used for studies of biomarkers related to health status, such as antioxidant status and blood lipids.
4. Selected immunological parameters are investigated in the second generation using 20 rats/diet group by *in vitro* analyses and by determining the level of antibodies in the animals after being fed the respective diets over a period of 6-10 weeks after weaning.
5. Life expectancy, frequency of disease and health status is investigated in rats from the second generation (n=10 rats/diet group). The rats are fed the respective diet during their entire life and weight and frequency of disease is recorded. The animals are autopsied after their death. After two years, the surviving animals are sacrificed and autopsied for abnormalities and signs of diseases, including signs of cancer and vascular diseases.

Deliverables:

- D7.1 (month 48): Contribution to scientific manuscript(s) on bioavailability of nutrients and secondary metabolites.
- D7.2 (month 48): Contribution to scientific manuscript on behaviour, activity levels and food preference.
- D7.3 (month 48): Contribution to scientific manuscript on immunological status, life expectancy and frequency of diseases.

Milestones:

M7.1(month 26): The tests on the first generation rats are completed.

M7.2 (month 30): The tests on the second generation rats are completed.

M7.3 (month 48): The life expectancy and occurrence of diseases of the second generation rats are characterised.

WP8: Data management and analysis

Workpackage number: 8
Start date or starting event: Month 4
Responsible person: JHB
Contributing persons:
Person-months: 9

Objectives:

To establish optimal experimental designs and methods, in order to ensure compatibility and utilisation of all results.

Description of work:

1. Detailed planning of interaction among experiments and exchange of materials and data.
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, and to provide inputs for the initial scientific papers (Dx-Dy).
3. Data from the animal experiments (WP 6-7) 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 (Dz-Då).

Deliverables:

D8.1 (month 10): Detailed plan for initial experiments and exchange of materials and data.
D8.2 (month 18): Optimised plan for the animal experiments.
D8.3 (month 40): Report or inputs to papers, with the relevant statistical analyses.

Milestones:

M8.1 (month 10): Detailed plan for experiments and exchange of materials and data is prepared.
M8.2 (month 18): Optimised plan for the animal experiments is prepared.

WP9: Coordination, dissemination and evaluation

Workpackage number:	9
Start date or starting event:	Month 1
Responsible person:	KB
Contributing persons:	All other participants
Person-months:	7

Objectives:

To coordinate the work and ensure that all subprojects support each other and the overall objective.
To evaluate the scientific progress in relation to the pre-set aims and to concurrent advances in other projects.
To disseminate the results of the project to relevant forums.

Description of work:

1. 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.
2. The data which are produced in WP's 2-8 and analysed in WP1 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. The target groups are nutrition scientists, agronomy scientists, farmers, consumers and politicians. Due to the high probability that the results will be somewhat controversial (almost irrespective of what they are), strict discipline will be required of the project members to avoid giving contradictory statements to the press, primarily in the pre-publication phases. 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.
3. When the results are determined, a report on implementation and further progress is prepared, providing recommendations for cultivation and marketing of organic products based on the results.

Deliverables:

D9.1 (month 1): Startup meeting M1
D9.2 (month 11): Progress meeting M2
D9.3 (month 18): 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 remainder of the project.
D9.4 (month 24): Progress meeting M4
D9.5 (month 34): Progress meeting M5
D9.6 (month 46): Progress meeting M6
D9.7 (month 48): Final report, including implementation and further progress.

Milestones:

M9.1 (month 18): Determination of detailed research plans for the animal studies.
M9.2 (month 24): Publication plan is prepared.
M9.3 (month 48): Final report on implementation and further progress is prepared.

7. Implementation and time schedule

Table 3: Deliverables list

Deliverable, No	Deliverable title	Delivery date	Meeting¹	Nature
9.1	Startup meeting	1	M1	Oral
1.1	The harvested materials.	7	M2	O
8.1	Detailed plan for initial experiments and exchange of materials and data.	10	M2	Re
1.2	The processed materials.	11	M2	O
9.2	Progress meeting M2	11	M2	Oral
8.2	Optimised plan for the animal experiments.	18	M3	Re
9.3	Progress meeting M3	18	M3	Oral
5.1	List of elements selected to be investigated in the balance study of the second generation in WP8.	20	M4	Re
5.2	Data on contents of pesticides in each of the diets.	20	M4	Re
2.1	Scientific manuscript on picture-developing properties of the examined samples.	24	M4	Pu
9.4	Progress meeting M4	24	M4	Oral
6.1	Weaned rats for WP7	26	M5	O
3.1, 4.1	At least one scientific manuscript on the contents of nutrients and secondary metabolites in the plant materials.	32	M5	Pu
9.5	Progress meeting M5	34	M5	Oral
6.2	Scientific manuscript on reproductive effects in rats fed organically versus conventionally produced feed over 2 generations.	36	M5	Pu
8.3	Report or inputs to papers, with the relevant statistical analyses.	40	M5	Re
9.6	Progress meeting M6	46	M6	Oral
2.2, 7.1, 7.2, 7.3	Several scientific manuscripts on the relationships between diet characterisation and animal health and well-being.	48	M6	Pu
9.7	Final report, including implementation and further progress.	48	M6	Re

¹ Number of the meeting where the deliverable will be evaluated. Normally the first meeting planned after the date of the deliverable.



8. Collaborative partners

The project is one among a large number of research projects in the Danish Research Centre for Organic Farming (DARCOF), which provide a framework for interaction with many relevant disciplines. In the context of the present project the close contact with colleagues working on the real agricultural systems is a particular advantage, to ensure that the connections between model treatments and the real systems are kept intact. In addition most of the involved research groups have extensive collaborative links, nationally and internationally, with other scientists and groups in their respective fields.

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