Organic feed makes the immune system more alert

Summary

The aim of the project 'Organic, More Healthy?' was to search for 'biomarkers' that show potential health effects of organic food. An important reason for many consumers to buy organic is the assumption that organic products are healthier than conventional produced products. If beneficial effects on health are confirmed this motivates the consumer to buy organic products and therefore could stimulate the growth of the organic market. Until now, however, very little research has been performed to study the effect of organic food on health.
More than 100 studies have made the comparison of nutrient content in organically and conventionally grown products. Some of these studies provide findings that organically grown products can be different from conventionally grown products, e.g. higher levels of dry matter, more anti-oxidants, higher or equal amounts of vitamin C and minerals, and a comparable or better protein quality in the organic compared to the conventional products. Furthermore, less contaminants such as pesticide residues, in most cases lower or equal amounts of nitrate and often lower or equal amounts of mycotoxins have been described.
Differences in nutritional content may underlie the differences in health effects, found in the few recent studies performed in animals (Staiger 1988, Finamore 2004, Lauridsen 2007).
To study the effect of organic products on health, thus far representative parameters to study health effects are lacking. Especially when health effects on human subjects are to be studied, it is important to have adequate 'biomarkers'. Biomarkers are indicators of biological processes, and should be adequate for the question investigated. Much research has been performed in finding biomarkers for specific diseases, much less is been developed as yet on biomarkers for health or fitness in healthy individuals. In this study an attempt is made to search biomarkers to study the health effects of different feeding regimes in healthy subjects. For this study a chicken model is used. But the final goal is to find biomarkers suitable for studies in humans, in the future.

The present study was designed by an international group of research institutes, members of the international association of organic Food Quality & Health research, FQH. First of all the study searched for differences in feed ingredients from different production systems, organic or conventional. Second a feeding experiment was performed to study biomarkers which may be indicative from the perspective of health.
The study was performed by a Dutch consortium of institutes, Louis Bolk Institute, TNO Quality of Life, RIKILT Institute of Food Safety and Wageningen University-Department of Animals Sciences, and several other institutes in the Netherlands and abroad, several of them being members of FQH.
The project design was reviewed and approved by the Scientific Advisory Committee of FQH, as is the report.
The study was financed by the Dutch Government (Ministry of Agriculture, Nature and Food Quality and Ministry of Economic Affairs), Rabobank and Triodos Bank.

Working hypothesis

Organically grown products have a more beneficial effect on health.

Research questions

1. Can differences be found between ingredients for chicken feed, obtained from organic and conventional production systems?
2. Can biomarkers be identified for health effects, related to the consumption of organic compared to conventional feed?
   a. Is there a difference in the developing immune system of chicken fed with the two different feeds?
   b. Do differences occur in the functioning of other organ systems, related to positive or negative health effects, connected to the consumption of the different feeds?

Research design

The study comprised a blinded animal feeding experiment with identically composed feeds from either organic or conventional products. The animals were two generations chicken from the Wageningen Selection Lines, laying hens that during 25 generations were selected for their either high or low antibody reaction to SRBC (sheep red blood cells) 5 days after injection at the age of 37 days. Next to these lines a random bred control group (C-line) of chicken was available, from the same original parental stock, representing the 'original paternal wild type' of the animals. The animals are described as high line (H-line) for the group with a high reaction to SRBC and low line (L-line) for the group with a low reaction to SRBC. The control line (C-line) concerns the group of animals, which represents the whole 'normal' genetic variation in reactions to SRBC. Parallel to the hens of the first generation, a group of roosters from the same three lines was raised on both feeds.

A feed was composed, according to existing norms for organic chicken feed, with six ingredients that were produced organically as well as conventionally. Wheat, barley, triticale, peas, maize and soy were sought, preferably from controlled production. If this was not possible, products were chosen from neighbouring farms - so called 'farm pairs' -, preferably known as 'best practice farms', with the same basic soil and climatic conditions. The use of the same variety of produce is considered ideal, but only if this is realistic within the specific farming system. Wheat, barley and triticale were obtained from the Netherlands, peas from Denmark, maize and soy from Austria. Only soy was of the same variety. Of barley and wheat a choice could be made out of two farm pairs. Here the products from the DOK-trials in Switzerland, a 25 year running comparison trial between (bio)Dynamic, Organic and conventional production systems, served as an indication of a 'golden standard' for the choice of the preferred farm pair.

The ingredients were screened for residues of pesticides or mycotoxins, to prevent adverse health effects due to these compounds, which might disturb the interpretation of the results. Products were accepted if they were clean, or if residues were below the maximum residue limit (MRL). In one maize sample a mycotoxin was found, below MRL, which was considered not dangerous for the young chicken. Later in the study more checks were performed for a selected number of other possible anti-nutritional, inhibiting factors. The ingredients that were accepted for feed production, as well as the feeds, were extensively analysed for macronutrients, micronutrients, trace elements, micro-organisms and bioactive ingredients.

The feeds were produced from these ingredients, by a well known manufacturer of organic animal feeds. To prevent shortages in the nutritional needs of the chicken, some additions were made. Added were potato protein (from conventional origin as this was guaranteed without solanin), the amino acid methionine, a small dosage of a mix of vitamins and minerals (Fx Layers Premix), chalk, grid, salt and $NaCO_3$. After production, the feeds were checked for amino acid content and if a shortage existed, essential amino acids were added up to the minimum level of the Norm (recommended level). Then the feeds were blinded by the manufacturer, named either A or B, and transported to Wageningen University, where the chicken were housed.

The first generation was housed in individual cages. This was necessary to be able to identify each individual egg, and so to secure the identity of the animals of the next generation. The second generation was housed in indoor runs, in groups of 6 animals, 2 hens from each line. The runs were spacey and enriched, to ensure optimal natural behaviour of the animals, and thus to facilitate the expression of possible health effects.

The animals of the first generation started their life on the usual conventional chicken feed. From week 11 of age the animals were fed the experimental feeds. The animals of the second generation received the experimental feeds from the first day of their life. Both generations could eat ad libitum. The second generation lived till 13 weeks of age and was then sacrificed.

In this study the aim was to obtain biomarkers for possible health effects. The health effects could be either positive or negative. Physiological markers were sought in general health features, immunological parameters, metabolite measurements in plasma and liver through metabolomics, gut functioning through genomics, and in a post mortem evaluation through pathological anatomy. As both groups of animals received balanced and sufficient feed, no large differences were expected. A disturbance was considered necessary, to evaluate the animals potential to react to and recover from that. A non-pathogenic, often used, immunological trigger was chosen as disturbing challenge, being an injection with a protein (KLH) at the age of 9 weeks in the second generation.
General health effects were evaluated by measuring weekly feed intake, weighing the animals and documenting egg production, health disturbances, illnesses and deaths.
The immune system was expected to show most obvious effects of the different feeds, as it is known that in young, developing organisms, the contact of the gut with the consumed food stimulates the development of the immune system. Therefore, a broad range of immunological measurements were performed, in both generations.
In the first generation the effects of the change from original feed towards the experimental feeds, in week 11 on immune parameters, were examined. In the second generation the strongest influence was expected from the immunological challenge by KLH, in week 9. The period before and after the challenge, was monitored by immunological measurements. In the second generation even more analyses were added. Blood, drawn before and after the challenge, was analysed by metabolomics.
In week 13 the animals' life ended and section was performed. Tissue samples were taken for metabolomics of the liver, for genomics of the gut, for pathological anatomy of the organs, for sensory analysis of the meat and for biobanking of material. The immunological measurements were performed on the whole group of animals. The measurements of metabolomics, genomics and pathological anatomy were performed in a subgroup of animals. In this reduced group all C-line animals were analysed, but – from each feed group – only 6 randomly selected H-line animals and 6 L-line animals.

The study was performed blinded, till the majority of the examinations of feeds and animals and their interpretation were finished, to prevent interpretations to be influenced. To allow the interpretation with respect to the feed parameters that could underlie the differential effects in the chicken, the results of the feed analyses, were connected to the results of the chicken analyses, however still coded A or B. Only just before the printing of this report the codes of the origin of the feeds were broken. Feed A turned out to be organic, feed B was conventional.

Results of the feeds

- Most consistent were differences observed in the amount of proteins, which was about 10% higher in the conventional feed (B). In wheat, soy and barley the amino acids were 10-40% higher in the ingredients used for the preparation of the conventional feed. The digestibility of the amino acids appeared better in the conventional feed.
- The level of phytosterols was higher in conventional soy and barley (feed B).
- Most organic ingredients (feed A) were higher in vitamin K, organic soy was higher in isoflavones and vitamin E, especially alpha tocopherol, and organic peas were higher in folate.
- The vitamins B5 and C were higher in respectively the conventional wheat and the conventional maize and peas (feed B).
- In the period of the KLH challenge slight differences in fatty acids in the feed applied, with higher levels of unsaturated C18 in feed A.
- With respect to microbiology, no large differences were observed between the organic and conventional feeds.
- Moulds were more common in the feeds from organic origin, and in general the organic feeds (A) had a higher aerobic colony count, as well as a higher amount of Enterobacteriaceae.
- Higher contents of LPS endotoxins were measured in the conventional feeds (B), especially those provided in the first generation.
- Complementary analyses by biophoton measurements, protein ratio and biocrystallizations showed that ingredients from the two agricultural systems could be differentiated. Where experience with the ingredients was available, researchers were able to identify the organic samples blindly.

Results of the chicken

Both generations and all three chicken lines were evaluated. However, in the evaluation of the effects, the results of the control (C-) line animals of the second generation are considered to be most informative, as these reflect the natural genetic variety of the population. If not indicated otherwise, the results of these chicken are presented.

- All animals of the second generation were diagnosed as being perfectly healthy. However, the groups on the two different feeds showed clear differences in several aspects of their physiology.
- Weight gain. Animals on the conventional feed (B) were significantly heavier throughout the experimental period. Relative growth was significantly higher in the animals on the conventional feed in the first 5 weeks of life, but then the animals on the organic feed (A) started to grow slightly more. After the KLH challenge a 20-30% decline in growth was observed in both groups for about two weeks. After the decline the animals on the organic feed showed a larger growth (catch-up growth) than the animals on conventional feed.
- Several immunological parameters showed differences between the animals on the two different feeds. This was true for both the humoral and cellular components of the immune system, both innate and adaptive, by higher LPS-antibody titers in blood (C-line, H-line reverse), KLH-induced classical complement activation (reflecting an activated innate immune system), higher vaccination antibody titers e.g. directed to Gumboro indicating activation of the adaptive system and the in vitro response to feed extracts in the presence of Con A of peripheral blood leukocytes, which were higher in the organically fed animals. The immunological results

were not fully consistent, but were overall interpreted as indicating a higher potential for immunological reactivity of the animals fed the organic feed.

- The metabolomics results of the blood showed a clear distinction between the animals on the two feeds, especially after the challenge. In this period an increase of several so called free fatty acids and unsaturated lipo poly saccharides were observed, more in the A- than in the B-animals. This led to the interpretation that the animals on the organic feed showed, after the challenge, a stronger reaction and connected metabolism, indicating a stronger acute phase reaction, than the animals on the conventional feed.
- The metabolomics results of the liver showed an increased pentose phosphate pathway activity in the animals on the organic feed (feed A), as well as more markers of liver metabolism and food intake (vitamin E).
- The genomics showed, in the animals on the conventional feed, a down-regulation of genes connected with cholesterol biosynthesis. These findings were confirmed by a follow-up analysis. The expectation of higher plasma cholesterol levels could however not be confirmed by metabolomics measurements.
- Evaluation by pathological anatomy showed some differences in the weight of specific organs between the feed groups, in the different lines. This finding is not yet understood.

Conclusions

Concerning the feeds it can be concluded that the analytical differences in ingredients and feeds were most consistent in the amount of proteins and amino acids. Further differences in some micronutrients were found. Although differences were observed, the feeds were sufficiently nutritious for the growing chicken, and with the exception of the proteins, no large effects from these differences on the chosen parameters of health were expected. Though *all the chicken were healthy*, a clear difference in the measured parameters was observed. The animals on the conventional feed gained more weight, whereas the animals on the organic feed showed a stronger immune reactivity, a stronger reaction to the challenge, as well as a slightly stronger recovery from the challenge in terms of regained growth.

Results are based on findings in the control line animals, as these represent the natural genetic variety. However results in the special high and low responding chicken in this animal model supported the conclusions of increased potential for immune reactivity in the organically fed animals.

Concerning the factors in the feed that could explain these differences, the higher protein content in the conventional feed is considered to be the factor, causing the stronger weight gain in the animals on this feed. The factor(s) in the feeds that might cause the physiological differences in relation to the challenge, are not yet clear. There are indications in literature that an enhanced status of immune reactivity in the animals on the organic feed, may be related with lower body weight gain.

The implications of these different physiological reactions in the context of short term and long term ‘health’ of these animals, is still unclear. A follow up study should clarify that.

Overall the study provided an enormous amount of information and caused effects which were not foreseen. An important outcome of this study is that feed ingredients from different origins can have small but clear immunological and metabolic effects in healthy animals. Further it became clear that the concept of ‘health’, and the physiology and immunology of health, are a still quite unexplored field of research.

Evaluation of the working hypothesis and research questions

The working hypothesis was that 'Organically grown products have a more beneficial effect on health'.
Regarding a potential 'larger' beneficial effect on health of one of the two feeds, no clear conclusions can be drawn. Both feeds were 'healthy' as such. The concept of 'health' and the connected physiology and immunology, need to be worked out further, before clear conclusions can be drawn.
With respect to the first research question: 'Can differences be found between ingredients for chicken feed, obtained from organic and conventional production systems?', it can be concluded that the ingredients for the chicken feed, obtained from organic and conventional production systems, differed most clearly with respect to protein and amino acid content. However, at the same time it is clear that many but not all nutritional and anti-nutritional factors s in the feeds were analyzed.
With respect to the second research question: 'Can biomarkers be identified, for health effects, related to the consumption of organic compared to conventional feed?', and the two sub questions about effects on the immune system and other organ systems, these can be answered confirmatively, although the implications of the observed differences with respect to health, are not clear yet and need further investigation. It must be noted that only one harvest and selection of products has been investigated in this project and that it cannot be excluded that different selections might have produced different results.

Biomarkers identified

Biomarkers which in this study clearly presented the different effects of the two feeding regimes are growth and, especially after exposure to a challenge, immune responsiveness, metabolic reactions, gene regulation in the gut system and observations by pathological anatomy.

Recommendations

The results of the present study are most promising. This study showed that small differences in feeds, because of differences in agricultural background, have implications for immune reactivity, metabolites and gene activity in healthy animals. Before these results can be used in studies in humans (the final goal), the results need to be confirmed. Confirmation should preferably first be sought, again, in chicken, which need to be followed longer (e.g. till natural death) and should be studied during a stronger challenge with an infection model or other disease model. Feed ingredients for such follow-up research should be obtained from 'best practice' farms in the same area. The feeds need to be extensively analysed, to give the possibility to relate observed effects in the animals to nutritional factors in the feeds. Next to this the ingredients should be analysed thoroughly, also in relation to products as purchased by the consumer, to get more insight in the representativity of the products.
Towards the future a confirmation in mammals is recommended, preferably in pigs, as these animals are most comparable to human beings. Research in humans is the ultimate goal.

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