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Can Agricultural Cultivation Methods Influence the Healthfulness of Crops for Foods?

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Supporting Information

ABSTRACT: The aim of the current study was to investigate if there are any health effects of long-term consumption of organically grown crops using a rat model. Crops were retrieved over two years from a long-term field trial at three different locations in Denmark, using three different cultivation systems (OA, organic based on livestock manure; OB, organic based on green manure; and C, conventional with mineral fertilizers and pesticides) with two field replicates. The cultivation system had an impact on the nutritional quality, affecting γ-tocopherol, some amino acids, and fatty acid composition. Additionally, the nutritional quality was affected by harvest year and location. However, harvest year and location rather than cultivation system affected the measured health biomarkers. In conclusion, the differences in dietary treatments composed of ingredients from different cultivation systems did not lead to significant differences in the measured health biomarkers, except for a significant difference in plasma IgG levels.

KEYWORDS: *organic food, rat model, fatty acids, antioxidants, vitamin E, immunity, IgG*

INTRODUCTION

There are several reasons consumers buy organic products, and two of the main ones are the belief that organic products have a better nutritional quality than conventional products, hence making organic products healthier.[1−3] Several systematic and nonsystematic reviews on the nutritional content and quality of organic produce have been published; some have concluded that organic produce has a higher nutrient content,[4,5] whereas others have reported that there are no consistent differences between conventionally and organically grown foods.[6−8] With the small impact of agricultural practice on major plant nutrients, research interest has shifted toward plant secondary metabolites and antioxidants.[6,9] Many of these plant compounds are still unknown, and only a very narrow range of compounds and their bioavailability have been studied in relation to the healthfulness of organic produce. As discussed by Brandt et al.,[10] it is necessary to have a systematic approach for identifying those plant compounds that might have health-promoting properties to evaluate the health-promoting effects of organic produce. Besides the nutrient content of the plants due to cultivation systems, other factors such as mycotoxin and endotoxin contamination of the plants could also have an impact on the nutritional quality of the products. Some studies have shown that cultivation systems, climatic variation, previous crops, etc., can influence the content of these in the crops.[11−14]

An additional problem for investigating if organically produced foodstuffs are healthier is how to measure health and to define health biomarkers.[1,15,16] Animal and human studies have been performed to evaluate the health effect;[17] however, as discussed in a systematic review by Dangour et al.,[18] evidence is lacking for nutrition-related health effects from the consumption of organic produce, in part because of a lack of solid experimental designs, that is, randomized controlled trials in humans or animals, with sufficient sample size, long and realistic dietary exposures, and, finally, accurate and objective measurements of dietary intake and relevant health outcomes. With a rapidly expanding organic market throughout Europe and the United States[19,20] and a changing modern organic production, the pivotal hypothesis that organic is more healthful than conventional has to be answered.

In a study by Lauridsen et al.[21] the objective was to define which measurable aspects of health would be affected by differences in cultivation methods, for more targeted future studies. A long-term feeding experiment with rats was performed, and several possible health outcomes were evaluated, that is, biochemical and physiological measurements (bioavailability of nutrients, metabolism, and nutritional status in blood and tissue), growth, physical activity, post-mortem evaluation, physiological functions of organs and intestine, and immunological analysis. Other studies have shown changes in some of the same or related indicators of health. A study by Huber et al.[14] evaluated health-related outcomes in a chicken model and concluded that both the specific and unspecific immune systems were affected by the cultivation system, as was the catch-up growth after an immunological challenge. A study by Finamore et al.[22] observed a difference in lymphocyte function in rats due to cultivation system.

In the aforementioned Lauridsen et al.[21] study complete diets rather than single food items were used,and the food ingredients came from strictly controlled and comparable fields. It is known that factors such as year, time of harvest, seasonal and climatic variations, genotype, and field location can affect the nutritional composition of the plants, which in turn could influence the healthfulness.[23−29] However, the Lauridsen et al.[21] experiment suffered, as most of the well-performed animal and human studies to date including the above-mentioned studies,[14,22] from

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Table 1. Chemical Composition of the Complete Diets[a]

	cultivation system				
	C	OA	OB	SEM	CO
gross energy (MJ/kg DM)	20.8	20.7	20.7	0.03	18.1
DM (g/kg feed)[b]	912	912	913	1.28	921
ash content (g/kg DM)	37.5	38.7	38.2	0.05	77.1
crude protein (N × 6.25) (g/kg DM)	155.6	147.5	149.1	2.92	217.5
starch (g/kg DM)	46.5	47.3	46.8	0.61	30.9
amino acids (g/kg DM)					
essential					
threonine	4.6	4.4	4.5	0.08	7.4
arginine	9.7	9.4	9.7	0.33	13.4
valine[c]	6.1	5.9	6.1	0.12	9.7
isoleucine	5.1	4.8	5.1	0.14	8.0
leucine	9.3	8.7	9.0	0.01	14.8
phenylalanine[d]	5.6	5.2	5.4	0.13	9.4
lysine	7.1	7.1	7.2	0.13	10.1
histidine	3.2	3.1	3.2	0.04	5.3
tryptophan	1.4	1.3	1.4	0.03	3.0
methionine[e]	6.6	6.4	6.6	0.12	4.1
nonessential					
asparagine	13.4	13.6	13.4	0.32	18.1
serine	6.1	5.7	5.8	0.12	9.7
glutamine	24.7a	21.9b	22.5ab	0.45	40.1
proline	7.7a	6.6b	7.0ab	0.20	12.7
glycine[c]	5.4	5.1	5.3	0.03	9.4
alanine	5.0	5.0	5.1	0.07	9.3
tyrosine	4.2	4.3	4.3	0.06	6.6
cystine	2.1	2.0	2.1	0.04	3.6
vitamins (mg/kg diet)					
α-tocopherol	41.2	39.9	39.9	1.00	124.0
γ-tocopherol	85.4a	81.3ab	74.4b	1.81	5.8
vitamin C[b]	77.5	80.0	66.9	4.57	19.0
rapeseed oil fatty acids (g FA/100 g FA)					
C16:0	4.4	4.5	4.5	0.04	
C18:0	1.7	1.8	1.8	0.02	
C18:1[b,c]	62.2a	63.2ab	63.6b	0.24	
C18:2[b,c]	18.4	18.0	18.1	0.11	
C18:3[b,c]	10.1a	9.6b	9.5b	0.05	
C20:1	1.2	1.1	1.1	0.03	
C20:2[c]	0.06a	0.05b	0.05b	0.00	
C22:0[c]	0.31a	0.29ab	0.28b	0.07	
SFA	7.2	7.2	7.2	0.06	
MUFA[b,c]	63.9	64.7	65.0	0.23	
PUFA[b,c]	28.5a	27.7b	27.6b	0.12	

[a]Fatty acids are only measured in the added rapeseed oil. C, conventional dietary group; OA, organic livestock manure based dietary group; OB, organic green manure based dietary group; CO, control diet; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. SEM is for the comparison of the diets within cultivation systems. Cultivation system LSMEAN values with different superscript letters within the same row are significantly different ($p < 0.05$). [b]Statistical difference due to year ($p < 0.05$). [c]Statistical difference due to location ($p < 0.05$). [d]Half of L-phenylalanine may be replaced by L-tyrosine. [e]Half of L-methionine may be replaced by L-cystine.

the fact that there were no field replicates per diet, which makes it impossible to estimate the variation due to factors such as location and year.

The overall objective of the present study was to investigate if there are any health effects of the long-term consumption of crops from different cultivation systems. We therefore performed a long-term feeding study with rats to study the influence of agricultural practice (conventional vs organic with either livestock manure or green manure based fertilization), year, and location on the nutritional composition of the crops and levels of some health-related biomarkers. We tested the hypothesis that the cultivation methods could influence a spectrum of health-related biomarkers that has proven interesting in previous studies: nutritional status, antioxidant status, immunological status, fatty acid profile, growth, post-mortem organ evaluation, and activity. A control diet (standard rat chow) was included in the study to establish the sensitivity of the rat model.

MATERIALS AND METHODS

Crop Production. Crops used for the diets were potatoes (*Solanum tuberosum* L. cv. Sava), winter wheat (*Triticum aestivum* L. cv. Tommi),

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spring barley (*Hordeum vulgare* L. mixture of cvs. Simba, Smilla, and Power), and fava beans (*Vicia faba* L. cv. Columbo) and were produced under controlled conditions in a long-term crop rotation field experiment with a randomized block design. [30] The current study included part of the treatments in the long-term experiment with 3 cultivation systems × 2 years × 3 locations × 2 field replicates = 36 diets. The cultivation systems had been maintained in the field experiments for about 10 years at the time the crops were grown for the current study. Winter rapeseed (*Brassica napus* L. cv. Elan (hybrid)) was not included in the long-term experiment, but was grown in a replicated field experiment with the same treatments and on the same sites as for the other crops. The agricultural treatments used were organic A (OA), organic cultivation with use of livestock manure without pesticides; organic B (OB), organic cultivation with green manure (legume-based cover crops) without pesticides; and conventional (C), inorganic fertilizer (NPK) plus pesticides. The legume-based cover crops were grown in autumn after the main crop and incorporated into the soil in spring before planting of the main crops to fix nitrogen from the atmosphere and prevent leaching of nutrients. Both organic systems were managed in full compliance with the Danish guidelines for organic farming outlined by the Danish Plant Directorate (http://www.pdir.fvm.dk/oekologi). The crops were grown in two successive years, 2007 and 2008 (years 1 and 2), at three different locations, Flakkebjerg (Fla), Jyndevad (Jyn), and Foulum (Fou), in Denmark. Further descriptions of field study design and of field and soil characteristics are given by Olesen et al.[30] and Laursen et al.[24]

There was a total crop loss of fava beans in year 2 at Flakkebjerg due to a severe attack of aphids (*Aphis fabae* L.) and Fla-year 2 was excluded from the study, because it was not possible to compose the diets. Hence, the final number of experimental diets was reduced to 30.

Preparation and Composition of Diets. The potatoes were washed, boiled, and frozen on arrival at the Department of Animal Science, Aarhus University, Denmark (ANIS). Afterward, they were chopped and freeze-dried (European Freeze-Dry, Kirke Hyllinge, Denmark) and packed into airtight bags. On arrival at ANIS the winter rapeseed was made into cold-pressed rapeseed oil. The freeze-dried potatoes, fava beans, winter wheat, and spring barley were ground through a 4 mm screen. Representative samples were taken of all crops, and the field replicate samples were pooled, before the chemical composition of the crops was analyzed (Supporting Information, Table S1). The crude protein content of the crops and literature data on vitamin, amino acid, and mineral content of the crops were used to calculate the proper diet composition, following nutrient requirement (NRC) recommendations for growing rats[31] (kg^{-1} complete diets; 130 g of rapeseed oil, 150 g of wheat, 270 g of barley, 270 g of fava beans, and 162 g of potatoes). All experimental diets were composed to contain the same percentage of each ingredient, to avoid elimination of any potential differences in nutrient content due to cultivation system, location, or year. However, it was not possible to design the diets to meet NRC completely using only these crops, so a minimum vitamin/mineral/amino acid mix (18.4 g/kg diet) was added (kg^{-1} complete diets; 11.6 g of $CaCO_3$, 1.1 g of NaCl, 0.7 mg of retinol, 10.0 mg of vitamin B_5, 6.0 mg of vitamin B_6, and 5.7 g of methionine) to avoid deficiency. The diets were packed in 1 kg plastic bags and stored at 4 °C for up to 1 week and at −18 °C for longer periods of the feeding experiment. Two additional control groups fed standard rat chow (CO) (Altromin, 1321) (Altromin Spezialfutter GmbH & Co. KG, Lage, Germany) were included.

Dietary Chemical Analyses. Dry matter (DM, g/kg feed) and ash content (g/kg DM) were determined according to AOAC methods. [32] Gross energy (MJ/kg DM) was determined with a Parr 6300 calorimeter (Parr Instrument Co., Moline, IL, USA) (ISO 9831:1998). Starch (g/kg DM) was analyzed enzymatically as described by Bach Knudsen.[33] Nitrogen was determined by thermal conductivity after complete combustion at 1300 °C in pure oxygen,[21] and crude protein (g/kg DM) was calculated as N × 6.25. The field replicates were pooled for analysis of amino acids (g/kg DM) and were analyzed by HPLC ((ISO 13903:2005), except tryptophan (trp) (ISO 13904:2005)). Lipids from the rapeseed oil were extracted according to the method of Bligh and Dyer.[34] The extracted lipids were transesterified into fatty acid methyl esters and separated by gas−liquid chromatography as described by Rotenberg and Andersen;[35] C17:0 was used as internal standard (expressed as g FA/100 g FA). α-Tocopherol, γ-tocopherol, and vitamin C (mg/kg diet) was determined by HPLC.[36] The chemical composition of OA, OB, C, and CO diets and the composition of selected fatty acids in the added rapeseed oils are shown in Table 1. Fatty acids presented in Table 1 include monounsaturated fatty acids (MUFA) (C16:1 + C17:1 + C18:1 + C20:1 + C22:1), polyunsaturated fatty acids (PUFA) (C18:2 + C18:3 + C20:2 + C20:3 + C20:4 + C20:5 + C22:5 + C22:6), and saturated fatty acids (SFA) (C14:0 + C15:0 + C16:0 + C18:0 + C20:0 + C22:0).

For endotoxin testing, the diets were pooled over cultivation systems and the samples were sent to Lonza, SPRL (Verviers, Belgium), where they were analyzed using the LAL kinetic chromogenic assay (European Pharmacopoeia section 2.6.14 methodology) (expressed as endotoxin units/mL).

The diet samples were extracted according to the method of Sulyok et al.[37] and screened for the two *Fusarium* mycotoxins deoxynivalenol (DON) and zearalenone (ZEA) by LC-MS/MS with the settings described in Josefsen et al.[38]

Animals and Housing. The experimental protocol was approved by The Danish Animal Experiments Inspectorate, Ministry of Justice, Denmark. Four-week-old female Wistar Hannover GALAS rats (N = 192) were obtained from Taconic Europe A/S (Laven, Denmark), weighing approximately 70−80 g on arrival. On arrival the rats were chipped and distributed randomly into 32 groups (30 experimental diet groups and 2 control diet groups = 32 groups) with 6 rats per group. After a week of acclimatization eating standard rat chow, the rats were provided their assigned experimental diets. Fresh water and feed were supplied ad libitum daily. They were housed in clear polycarbonate cages (59 × 39 × 20 cm) with stainless steel wire lids (Scanbur A/S, Lellinge, Denmark), shelter, biting sticks, and nesting material (Tapvei, Kortteinen, Finland). Room temperature and humidity were kept at 24−25 °C and 50−60%, respectively, with alternating dark/light cycles of 12 h.

Protocol and Analytical Procedures for in Vivo Measurements. Figure 1 outlines the study duration with the observations and measurements relative to the age of the rats. The duration of the complete feeding trial was approximately 6 months (range, weeks 29−30). Each week during the feeding trial a group-weighing was performed, feed intake was registered, and the rats were clinically evaluated. Every 4 weeks the rats were weighed individually. After week 12 (range, weeks 12−20), when fully matured, the rats were placed in individual cages for activity and heat production measurements. The rat's heat production and physical activity were measured with two open-air circuit respiration chambers in groups of six rats. Their energy metabolism and night/day activity were recorded over two days using passive infrared detectors.[21] The night (dark) period was from 6:00 p.m. to 6:00 a.m. the next morning and the day (light) period from 10:00 a.m. to 6:00 p.m., thus excluding the time when rats were fed and changed chamber. To ensure that there was no drift in the activity and heat production measurements over time, the CO group rats were measured at weeks 12, 16, and 20 as controls. The activity is expressed relative to activity at night of rats fed the conventional diet (C night = 100).

Protocol and Analytical Procedures for Post-Mortem Measurements. *Sampling.* Three randomly chosen rats from each group (N = (3 × 30 rats) + 3 CO rats = 93 rats) were weighed and euthanized by CO_2 asphyxiation for post-mortem measurements (weeks 29 and 30). Blood was collected by cardiac puncture and was centrifuged at 2000*g*, at

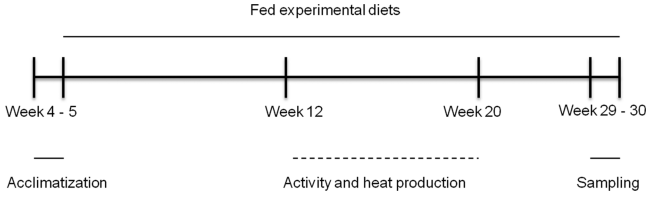

Figure 1. Timeline showing feeding of experimental diets, age of rats, and measurements and observations during the study.

4 °C for 15 min, and plasma and serum were obtained and stored at −20 °C. The weights (g/kg slaughter weight) of the heart, spleen, liver, lungs, pancreas, kidneys, adipose tissue, ovaries, and empty stomach were noted and the organs visually examined for any clinical signs of deviation from "normal health status"; the samples were stored at −20 °C for later analysis.

Nutritional Status and Fatty Acid Profile. Concentrations of blood glucose, triacylglycerol (TAG, mM), cholesterol (mM), and aspartate-aminotransferase (AST, EC.2.6.1.1, U/L) were determined according to standard procedures (Siemens Diagnostics Clinical Methods for ADVIA 1650). Concentrations of the nonesterified fatty acids (NEFA, μekv./L) were determined using the Wako NEFA C ACS-ACOD assay method. All analyses were performed using an autoanalyzer, ADVIA 1650 Chemistry System (Siemens Medical Solutions, Tarrytown, NY, USA). All intra-assay precision validations were within 3% (CV). Plasma insulin (μg/L) was measured using a rat insulin ELISA assay (EIA-2048), following the manufacturer's instruction (DRG Instruments GmbH, Marburg, Germany).

Fatty acid composition (g FA/100 g FA) of the liver, adipose tissue, and plasma was measured as described above for the diets.

Antioxidant and Immunological Status. Plasma and liver α-tocopherol, retinol, and β-carotene concentrations (μg/mL and μg/mg liver, respectively) were analyzed by HPLC.[39,40] Plasma IgG, IgM and IgA concentrations (μg/mL) were measured using commercially available rat ELISA kits (Bethyl Laboratories, Inc., Montgomery, TX, USA).

Calculations and Statistical Analysis. The data analyzed, Y_{yslb}, were the averages across the three rats that consumed the same diet. The responses were modeled with the linear mixed model

$$Y_{ylsb} = \mu + \beta_y + \delta_l + \gamma_s + \varepsilon_{ys} + \varepsilon_{yl} + \varepsilon_{ylb} + \varepsilon_{ylsb}$$

where μ is the generalized intercept, β_y (y = year 1, year 2) is the effect of year, δ_l is the effect of location (l = Foulum, Jyndevad, Flakkebjerg), and γ_s (s = C, OA, OB) is the effect of growth system. The errors (ε) are considered independently and normally distributed and represent corresponding variance components of interaction and ε_{ylb} (b = plot is the random effect of plot within year × location). The pairwise comparisons and their confidence intervals between the systems were adjusted to obtain a familywise error rate of 5%. The model was fitted using the Proc Mixed procedure of SAS/STAT software (http://support.sas.com/documentation/cdl/en/statugmixed/61807/PDF/default/statugmixed.pdf).[41]

All data are presented as LSMEANS and their standard deviation for the cultivation systems. When a statistical difference due to harvest year or location was observed, it is denoted in the table or the table footnote. The descriptive data for cultivation system × year × location is found in the Supporting Information (Tables S1−S8).

RESULTS

Diets. Table 1 shows the chemical composition of the different cultivation system diets. No difference was observed between cultivation systems in gross energy, DM, ash, and starch. However, a DM difference was observed between the two years (year 1 = 908 and year 2 = 917). Although there was no difference in crude protein between the cultivation systems, some significant differences in a few of the nonessential amino acids were observed, that is, glutamine and proline being higher in C than in OA and glycine being affected by location (Fla and Fou > Jyn; Supporting Information, Table S2). Vitamin E levels fulfilled NRC for growing rats. α-Tocopherol concentrations were similar between the diets, whereas γ-tocopherol was significantly lower in OB than in C. Year influenced vitamin C (year 1 = 5.81 and year 2 = 9.16) (Supporting Information, Table S2). Fatty acids C18:3, C20:2, and C22:0 were significantly higher in the conventional rapeseed oil than in the organic, whereas C18:1 was significantly lower in C than in OB. Several fatty acids in the rapeseed oil were affected by year (C18:1, C18:2, and C18:3) and location (C18:1, C18:2, C18:3, C20:2, and C22:0) (Table 1 and Supporting Information, Table S2). The CO diet was a commercial diet and had a completely different composition, and NRC requirements for growing rats were fulfilled.

None of the experimental diet samples contained DON and ZEA above the limits of detection, 400 and 100 ppb, respectively. Endotoxin levels (endotoxin units/mL) in the complete diets were year 1, Fou = 2.11, Jyn = 7.97, and Fla = 2.95; and year 2, Fou = 4.86 and Jyn = 2.87, which were similar to CO = 2.79.

Growth, Organ Weight, and Activity. The rats were clinically healthy throughout the study, and the feed was well accepted. One rat from the Jyn−year2 conventional diet died during the study, and the autopsy revealed no diagnostic reasons (N = 92). The rats performed well on both the experimental diets and the control diet. There was no statistical difference in final body weight, daily gain, or relative organ weight of the rats, between the cultivation systems, years, or locations (Table 2 and Supporting Information, Table S3). The relative activity of the rats was recorded during the measurement of the energy metabolism and is presented for day (light) and night (dark), respectively (Table 3). Activity was higher during the night as rats are nocturnal animals and, subsequently, heat production was higher. However, there was no difference between the cultivation systems.

Table 2. Growth and Relative Organ Weight[a]

	cultivation system				
	C	OA	OB	SEM	CO
initial wt (g)	79	79	75	2.20	77
final wt (g)[b]	249	250	249	4.42	247
daily gain (g)	1.05	1.06	1.07	0.04	1.03
organs (g kg^{-1} slaughter wt)					
liver	33.9	33.9	33.8	1.07	32.5
lungs	5.2	5.6	5.5	0.25	4.9
heart	3.1	3.1	3.1	0.09	3.4
spleen	2.2	2.2	2.2	0.07	2.0
pancreas	4.3	4.3	4.2	0.22	5.1
kidneys	6.9	7.0	7.0	0.19	6.6
adipose	67.6	66.0	62.9	4.32	62.0
ovaries	3.5	3.2	3.2	0.25	3.7

[a]C, conventional dietary group; OA, organic livestock manure based dietary group; OB, organic green manure based dietary group; CO, control diet. SEM is for the comparison of diets within cultivation systems. [b]Last weighing at same age (day 162).

Table 3. Heat Production and Relative Activity (C Night = 100)[a]

	cultivation system				
	C	OA	OB	SEM	CO
activity					
day	38	40	41	10	28
night	100	113	99	10	67
heat production (W, J/s)					
day	3.6	3.8	3.8	0.17	3.8
night	4.7	4.8	4.8	0.17	4.8

[a]C, conventional dietary group; OA, organic livestock manure based dietary group; OB, organic green manure based dietary group; CO, control diet. SEM is for the comparison of the diets within cultivation systems.

Nutritional Status and Fatty Acid Profile. No effect of cultivation system, year, or location was observed on the nutritional status of the rats (Table 4), except for an effect of location on plasma NEFA concentrations (Fou > Jyn; Supporting Information, Table S4). Likewise, there was no influence of cultivation system on the fatty acid profile of plasma, liver, and adipose tissue (Table 5). However, several fatty acids were affected by year: plasma, C18:3n-3; liver, C18:3n-3 and C20:4n-6; and adipose tissue, C16:0, C16:1, C18:1, C18:2n-6, and C18:3n-3. Adipose tissue C18:1 was also affected by location (Table 4 and Supporting Information, Tables S6 and S7). The CO diet led to a different nutritional status and fatty acid profile of the rats than the experimental diets.

Table 4. Nutritional Status[a]

	cultivation system				
	C	OA	OB	SEM	CO
glucose (mM)	10.4	10.9	11.1	0.66	12.3
NEFA[b] (μequiv/L)	663	643	652	75.0	573
TAG (mM)	1.76	1.81	1.59	0.20	1.42
cholesterol (mM)	1.84	1.89	1.94	0.08	1.66
insulin (μg/L)	0.49	0.59	0.56	0.07	0.32
phospholipid−choline (mM)	2.52	2.61	2.58	0.11	2.26
AST (U/L)	183	153	156	29.8	237.8
urea-N (mM)	5.36	5.20	5.16	0.28	8.29

[a]C, conventional dietary group; OA, organic livestock manure based dietary group; OB, organic green manure based dietary group; CO, control diet; TAG, triacylgycerol; NEFA, nonesterified fatty acids; AST, aspartate-aminotransferase. SEM is for the comparison of the diets within cultivation systems. [b]Statistical difference due to location ($p < 0.05$).

Antioxidant and Immunological Status. Plasma IgG was influenced by cultivation system, and rats eating diets from cultivation system C had a significantly higher plasma IgG concentration than rats eating OB. The dietary treatments had no effect on either IgA or IgM (Table 6). There was an effect of location on plasma retinol (Fla > Jyn > Fou; Supporting Information, Table S5), whereas vitamin E did not differ due to cultivation system, location, or year (Table 6).

Table 6. Immunological and Antioxidant Status[a]

	cultivation system				
	C	OA	OB	SEM	CO
plasma immunoglobulins (μg/mL)					
IgG	6988a	4878ab	4155b	888	3072
IgA	26.6	26.2	26.4	1.99	32.1
IgM	72.6	69	62.8	5.48	74.4
plasma antioxidants (μg/mL)					
α-tocopherol	7.41	7.90	7.91	0.84	9.70
retinol[b]	0.13	0.12	0.12	0.01	0.14
liver antioxidants (μg/mg liver)					
α-tocopherol	28.6	29.2	29.6	2.37	40.1
γ-tocopherol	0.65	0.69	0.54	0.08	0.15

[a]C, conventional dietary group; OA, organic livestock manure based dietary group; OB, organic green manure based dietary group; CO, control diet. SEM is for comparison of the diets within cultivation systems. Cultivation system LSMEAN values with different letters within the same row are significantly different ($p < 0.05$). [b]Statistical difference due to location ($p < 0.05$).

DISCUSSION

In previous studies only a very narrow range of nutrients and their bioavailability have been investigated in diets in relation to the effect of agricultural practices on nutrient quality and on the influence on health outcomes. In general, no major differences according to cultivation system have been reported in nutrient quality, except reasonable consistent trends of lower vitamin C and higher nitrate in conventional vegetables than in organic and lower protein content and higher phosphorus in organic cereal than in conventional.[4,5,7,8,29] Many plant components might still be unknown, and secondary metabolites and antioxidants are getting more attention in current research.[6,10] Few well-designed animal or human studies with clear health biomarkers looking at nutrition-related health effects have been performed.[1,18] No systematic nutrition-related health effects have been established

Table 5. Plasma, Liver, and Adipose Tissue Composition of Selected Fatty Acids[a]

	plasma (g FA/100 g FA)					liver (g FA/100 g FA)					adipose tissue (g FA/100 g FA)				
	cultivation system					cultivation system					cultivation system				
	C	OA	OB	SEM	CO	C	OA	OB	SEM	CO	C	OA	OB	SEM	CO
C16:0	12.6	12.5	12.4	0.50	20.4	12.2	12.1	12.1	0.52	18.0	10.4	10.0	10.1	0.38	25.3
C16:1	1.21	1.29	1.18	0.10	2.02	0.89	0.91	0.91	0.08	1.47	1.84	1.80	1.88	0.10	4.77
C18:0	12.0	11.8	12.4	0.43	13.2	18.9	18.8	18.6	0.31	21.7	2.05	2.05	2.00	0.06	3.21
C18:1	29.8	30.7	29.6	1.70	17.5	18.3	18.3	19.3	0.43	10.6	61.2	62.3	62.3	0.51	33.1
C18:2n-6	18.6	18.0	17.7	0.37	20.4	12.6	12.0	11.9	0.47	13.6	18.5	18.1	18.0	0.28	29.3
C18:3n-3	2.52	2.50	2.13	0.31	0.37	1.10	1.07	1.03	0.06	0.15	3.84	3.65	3.58	0.12	1.04
C20:4n-6	16.7	16.3	17.8	1.00	20.5	19.0	19.0	19.1	0.25	23.7	0.21	0.21	0.22	0.02	0.60
C20:5n-3	1.37	1.42	1.22	0.21	0.34	1.80	1.86	1.58	0.18	0.36	0.04	0.04	0.04	0.00	0.01
C22:5n-3	0.65	0.64	0.58	0.05	0.41	1.52	1.51	1.33	0.10	1.04	0.11	0.11	0.11	0.01	0.06
C22:6n-3	3.03	3.17	3.34	0.26	1.78	10.9	11.4	11.2	0.44	6.51	0.12	0.12	0.13	0.01	0.10
SFA	25.2	25.0	25.5	0.17	34.5	32.0	31.7	31.5	0.80	40.2	13.2	12.8	12.9	0.41	30.0
MUFA	31.4	32.5	31.2	1.62	20.0	19.8	20.0	20.9	0.52	12.3	63.8	64.8	64.9	0.54	38.4
PUFA	43.2	42.3	43.1	1.38	44.3	48.2	48.4	47.6	0.38	47.5	22.9	22.3	22.2	0.41	31.6

[a]C, conventional dietary group; OA, organic livestock manure based dietary group; OB, organic green manure based dietary group; CO, control diet; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. SEM is for the comparison of the diets within cultivation systems. Statistical difference due to year ($p < 0.05$): adipose tissue, C16:0, C16:1, C18:1, C18:2n-6, C18:3n-3, SFA, MUFA, PUFA; liver, C18:3n-3, C20:4n-6; plasma, C18:3n-3. Statistical difference due to location ($p < 0.05$): adipose tissue, C18:1, MUFA.

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either; however, some studies have shown that immunological parameters and growth might be sensitive to effects from cultivation systems.[14,21,22,42]

Cultivation Methods Effect on Nutritional Quality and Health Biomarkers. The rats used in the current study were all clinically healthy, with normal food intake and increase in body weight during the long-term feeding experiment without any remarkable deviations from a "normal physiological response". The essential amino acids (threonine, valine, leucine, lysine, and tryptophan) were just below NRC for growing rats in the experimental diets, but above NRC requirements for maintenance. However, the rats did not show any signs of amino acid deficiencies,[31] and the growth of the rats eating the experimental diets followed that of the CO group, whose diet had an abundance of amino acids. In addition, the inclusion of the CO diet in the experimental design showed that the rat model was sensitive and that it was possible to detect effects of intake of different nutrients in the measured biomarkers of health. In the present study, analysis of the diets showed that the diets made from conventional crops were higher in some nutritional components (glutamine, proline, γ-tocopherol) and some fatty acids (C18:3, C20:2, C22:0) than the organic diets, whereas the concentration of C18:1 was higher in the organic OB than in the conventional diet. However, the cultivation system differences in the measured nutritional quality parameters of the diets were not major differences and did not translate into any distinct differences in the measured health outcomes.

Interestingly, and in accordance with previous studies,[14,21] plasma IgG was affected by cultivation system. However, in the current study rats eating the conventional diet had a higher plasma IgG level than those eating the organic diet, which conflicts with the results of Lauridsen et al.[21] and Huber et al.[14] Lauridsen et al.[21] found a lower plasma IgG in rats eating a HIplusP (high fertilizer plus pesticide) diet, compared to rats eating LIminusP (low fertilizer input minus pesticide) and LIplusP (low fertilizer plus pesticide) diets. A higher level α-tocopherol was also detected in the LIminusP diet, and because it is known that vitamin E is important for the immune function,[43−45] it was suggested as a possible nutrient component that could have affected plasma IgG levels. However, in the current study there were no differences between the cultivation systems in dietary, plasma, or liver α-tocopherol. A difference in dietary γ-tocopherol was observed, but this difference could not be reproduced in liver γ-tocopherol. In the Huber et al.[14] study both the specific and unspecific immune systems were affected by cultivation system, and chickens eating organic diets had higher antibody titers than those eating the conventional diets. Several possible reasons for the change in the immune responsiveness were suggested, that is, immune-stimulating Gram-negative bacteria or LPS in the feed,[14,46] weight reduction/gain,[47] and alterations in metabolism. [48] However, in the current study there were no differences in the growth, activity levels, and heat production due to cultivation system. The endotoxin levels were measured only with regard to location and year, so it was not possible to say if there were any immune-stimulating effects of endotoxins due to cultivation system. Mycotoxins are also known to have immunomodulatory effects,[49] and in the Finamore et al.[22] study DON was suggested as having the effect on the altered lymphocyte function due to cultivation system. However, this was not the case because the level of mycotoxins was lower than that reported to affect the immune system. Fusarium mycotoxin (DON and ZEA) levels in the current study were below the detection limit, and hence no differences between cultivation systems were observed. However, the detection limits in the setup used in the current study were a little high, so there could have been cultivation system differences that we were not able to detect. Fatty acids are also known to influence immune responses and health;[50,51] however, the higher C18:3 level in the conventional diet than in the organic diets did not lead to any rise in plasma, liver, and adipose tissue C18:3n-3 levels in the C group rats. Therefore, in conclusion, it was not possible in the current study to explain the nutrient-related effect on plasma IgG levels or to conclude if a difference in plasma IgG should be considered an improvement of the immune status or not. Future studies should look at the impact of cultivation methods on immune function. Focus should be given to the immune system of young individuals in which the immune system is developing and therefore could be more sensitive. This could be done by a multigeneration study to investigate the long-term effect of the maternal diet during different sensitive stages such as pregnancy and lactation on the health of the offspring and the effect of early nutrition on health in adulthood.

The aforementioned studies indicate that consumption of feed from different cultivation systems can lead to differences in immunity health parameters, and trace elements are considered interesting in relation to nutritional quality and the ability to affect health. However, a study by Laursen et al.[24] performed on crops from the same field experiment concluded that there were no systematic differences between organic and conventional crop content of essential plant nutrients (C, N, K, Mg, P, S, Ca, Fe, Mn, B, Zn, Cu, Mo, Ba, Sr, and Na), and mineral content was also affected by year and location. Using the data from the individual crops in the aforementioned study, it was also possible to calculate the mineral content of the diets, and NRC requirements for growing rats was met.

Location and Year Effects on Nutritional Quality and Health Biomarkers. It must be stressed that the feed items used in the current study were produced from crops grown in well-designed and controlled field experiments with replication. The cropping systems had been maintained in the field experiments for about 10 years at the time the feed crops in our study were grown. The feed items produced therefore represented the long-term effects of different cultivation systems. Year and location were included in the experiment setup, because it is well documented that crop nutritive values are dependent on factors such as harvest year, specific field location, and time of harvest.[23−27] In accordance with the literature, results from the current study showed that year and location had as much of an effect as cultivation system on the measured nutritive values of the diets, and when health-related outcomes were evaluated, year and location influenced more variables than cultivation systems did. Diet samples were pooled across cultivation systems for endotoxin analysis, and it appeared that an interaction between the factors location and year could influence the levels, which in return could have an influence on health outcomes. The changes due to location and year in fatty acid composition of the rapeseed oils in the diets (C18:1, C18:2, and C18:3 and, hence, MUFA and PUFA) were also observed in the liver, plasma, and adipose tissue composition. In conclusion, year and location were the more important factors affecting the nutritive content and the related health implications, to a degree that might have overruled any potential effects of cultivation system. The effect of location and harvest year in the current study could possibly be due to unmeasured chemical components of the crops, such as secondary metabolites or phytochemicals, that can be affected

by factors such as climatic variation, soil, harvest time, year, and location.

Many studies have compared the nutritional values of organic and conventional foods, but few have investigated the health implications of eating organic and conventional foods. The strength of the present study lies in (1) the well-controlled long-term field experiment for producing feed, with three cultivation systems, three locations, and two years with two field replicates and (2) the long-term rat feeding study in which numerous health-related outcomes were investigated. From the study it could be concluded that besides plasma IgG levels, none of the measured health biomarkers were affected by cultivation system, although some differences in nutritional quality were observed. The small (or absent) differences between health outcomes of the different cropping systems may partly have been caused by the moderate rate of use of chemical fertilizers and pesticides in the conventional treatment in the experiment. This followed guidelines for farming in Denmark, where inputs of inorganic fertilizer and pesticides over the past 20 years have been lowered considerably to reduce the environmental pressure of farming practices. Finally, it was concluded that in the current study year and location affected nutritional quality and (maybe even more) the measured health biomarkers as much as cultivation system.

For future studies we find that it is important that the feed is produced in well-controlled field studies with year, location, and field replicates included, making it possible to correctly investigate the effect of cultivation system. More extreme cultivation systems could also be included to reveal possible thresholds in the composition of the crop products, for example, by further increasing fertilizer and pesticide inputs in the conventional treatment. In addition, we suggest that future studies designed to investigate the variability of cultivation systems should focus on immune parameters as health-related outcomes, maybe with a multigenerational setup and/or using an animal model in which health is studied in a more dynamic way by presenting challenges to the animals, for example, an immune challenge, to study the resilience of a physiological response.

■ ASSOCIATED CONTENT

Supporting Information

Mean ± SD of all combinations of cultivation system, location and year, for variables presented as LSMEANS and their standard error of mean for cultivation systems. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

ANIS, Department of Animal Science, AU, Denmark; C, conventional dietary group; DON, deoxynivalenol; Ig, immunoglobulin; LSMEANS, least square of means; MUFA, monounsaturated fatty acids; NPK, nitrogen−phosphorus−potassium fertilizer; NRC, National Research Council; OA, organic livestock manure based dietary group; OB, organic green manure based dietary group; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; ZEA, zearalenone.

■ REFERENCES

(1) Velimirov, A.; Huber, M.; Lauridsen, C.; Rembialkowska, E.; Seidel, K.; Bügel, S. Feeding trials in organic food quality and health research. *J. Sci. Food Agric.* **2010**, *90*, 175−182.

(2) Yiridoe, E. K.; Bonti-Ankomah, S.; Martin, R. C. Comparison of consumer perceptions and preference toward organic versus conventionally produced foods: a review and update of the literature. *Renewable Agric. Food Syst.* **2005**, *20*, 193−205.

(3) Nelson, L.; Giles, J.; MacIlwain, C.; Gewin, V. Organic FAQs. *Nature* **2004**, *428*, 796−798.

(4) Magkos, F.; Arvaniti, F.; Zampelas, A. Organic food: nutritious food or food for thought? A review of the evidence. *Int. J. Food Sci. Nutr.* **2003**, *54*, 357−371.

(5) Worthington, V. Nutritional quality of organic versus conventional fruits, vegetables, and grains. *J. Altern. Complement. Med.* **2001**, *7*, 161−173.

(6) Bourn, D.; Prescott, J. A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. *Crit. Rev. Food Sci. Nutr.* **2002**, *42*, 1−34.

(7) Woese, K.; Lange, D.; Boess, C.; Bögl, K. W. A comparison of organically and conventionally grown foods − results of a review of the relevant literature. *J. Sci. Food Agric.* **1997**, *74*, 281−293.

(8) Dangour, A. D.; Dodhia, S. K.; Hayter, A.; Allen, E.; Lock, K.; Uauy, R. Nutritional quality of organic foods: a systematic review. *Am. J. Clin. Nutr.* **2009**, *90*, 680−685.

(9) Rosen, J. D. A review of the nutrition claims made by proponents of organic food. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 270−277.

(10) Brandt, K.; Christensen, L. P.; Hansen-Moeller, J.; Hansen, S. L.; Haraldsdottir, J.; Jespersen, L.; Purup, S.; Kharazmi, A.; Barkholt, V.; Frøkiær, H.; Kobæk-Larsen, M. Health promoting compounds in vegetables and fruits. A systematic approach for identifying plant components with impact on human health. *Trends Food Sci. Technol.* **2004**, *15*, 384−393.

(11) Schneweis, I.; Meyer, K.; Ritzmann, M.; Hoffmann, P.; Dempfle, L.; Bauer, J. Influence of organically or conventionally produced wheat on health, performance and mycotoxin residues in tissues and bile of growing pigs. *Arch. Anim. Nutr.* **2005**, *59*, 155−163.

(12) Malmauret, L.; Parent-Massin, D.; Hardy, J. L.; Verger, P. Contaminants in organic and conventional foodstuffs in France. *Food Addit. Contam.* **2002**, *19*, 524−532.

(13) Godecke, R.; von Tiedemann, A. Effect of the previous crop on Fusarium spectrum and mycotoxin contamination in grains of winter wheat. *Zuckerindustrie* **2011**, *136*, 172−178.

(14) Huber, M.; de Vijver, L. P. L. V.; Parmentier, H.; Savelkoul, H.; Coulier, L.; Wopereis, S.; Verheij, E.; van der Greef, J.; Nierop, D.; Hoogenboom, R. A. P. Effects of organically and conventionally

produced feed on biomarkers of health in a chicken model. *Br. J. Nutr.* **2010**, *103*, 663−676.
(15) van Ommen, B.; Keijer, J.; Heil, S. G.; Kaput, J. Challenging homeostasis to define biomarkers for nutrition related health. *Mol.Nutr. Food Res.* **2009**, *53*, 795−804.
(16) Huber, M.; Bakker, M. H.; Dijk, W.; Prins, H. A.; Wiegant, F. A. The challenge of evaluating health effects of organic food; operationalisation of a dynamic concept of health. *J. Sci. Food Agric.* **2012**, DOI: 10.1002/jsfa.5563.
(17) Huber, M.; Rembialkowska, E.; Srednicka, D.; Bugel, S.; van de Vijver, L. P. L. Organic food and impact on human health: assessing the status quo and prospects of research. *Njas-Wageningen J. Life Sci.* **2011**, *58*, 103−109.
(18) Dangour, A. D.; Lock, K.; Hayter, A.; Aikenhead, A.; Allen, E.; Uauy, R. Nutrition-related health effects of organic foods: a systematic review. *Am. J. Clin. Nutr.* **2010**, *92*, 203−210.
(19) Wier, M.; Jensen, K. O.; Andersen, L. M.; Millock, K. The character of demand in mature organic food markets: Great Britain and Denmark compared. *Food Policy* **2008**, *33*, 406−421.
(20) Oberholtzer, L.; Dimitri, C.; Greene, C. *Price Premiums Hold on as U.S. Organic Produce Market Expands*; Economic Research Service, USDA: Washington, DC, 2005; pp 1−22, VGS-308-01,
(21) Lauridsen, C.; Yong, C.; Halekoh, U.; Bügel, S. H.; Brandt, K.; Christensen, L. P.; Jørgensen, H. Rats show differences in some biomarkers of health when eating diets based on ingredients produced with three different cultivation strategies. *J. Sci. Food Agric.* **2008**, *88*, 720−732.
(22) Finamore, A.; Britti, M. S.; Roselli, M.; Bellovino, D.; Gaetani, S.; Mengheri, E. Novel approach for food safety evaluation. Results of a pilot experiment to evaluate organic and conventional foods. *J. Agric. Food Chem.* **2004**, *52*, 7425−7431.
(23) Jørgensen, H.; Brandt, K.; Launidsen, C. Year rather than farming system influences protein utilization and energy value of vegetables when measured in a rat model. *Nutr. Res. (N.Y.)* **2008**, *28*, 866−878.
(24) Laursen, K. H.; Schjoerring, J. K.; Olesen, J. E.; Askegaard, M.; Halekoh, U.; Husted, S. Multielemental fingerprinting as a tool for authentication of organic wheat, barley, faba bean, and potato. *J. Agric. Food Chem.* **2011**, *59*, 4385−4396.
(25) Eppendorfer, W. H.; Eggum, B. O. Effects of sulfur, nitrogen, phosphorus, potassium, and water-stress on dietary fiber fractions, starch, amino-acids and on the biological value of potato protein. *Plant Foods Hum. Nutr.* **1994**, *45*, 299−313.
(26) Brunsgaard, G.; Kidmose, U.; Sorensen, L.; Kaack, K.; Eggum, B. O. The Influence of variety and growth-conditions on the nutritive-value of carrots. *J. Sci. Food Agric.* **1994**, *65*, 163−170.
(27) O'Brien, L. Genotype and environment effects on feed grain quality. *Aust. J. Agric. Res.* **1999**, *50*, 703−719.
(28) Harker, F. R. Organic food claims cannot be substantiated through testing of samples intercepted in the marketplace: a horticulturalist's opinion. *Food Qual. Pref.* **2004**, *15*, 91−95.
(29) Jensen, M. M.; Jørgensen, H.; Halkoh, U.; Watzl, B.; Thorup-Kristensen, K.; Lauridsen, C. Health biomarkers in a rat model after intake of organically grown carrots. *J. Sci. Food Agric.* **2012**, DOI: 10.1002/jsfa.5705.
(30) Olesen, J. E.; Askegaard, M.; Rasmussen, I. A. Design of an organic farming crop-rotation experiment. *Acta Agric. Scand., Sect. B* **2000**, *50*, 13−21.
(31) National Research Council. *Nutrient Requirements of the Laboratory Rat*; National Academy Press: Washington, DC, 1995.
(32) Association of Official Analytical Chemists. *Official Methods of Analysis*, 18th ed.; AOAC: Arlington, VA, 2005.
(33) Knudsen, K. E. B. Carbohydrate and lignin contents of plant materials used in animal feeding. *Anim. Feed Sci. Technol.* **1997**, *67*, 319−338.
(34) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Phys.* **1959**, *37*, 911−917.
(35) Rotenberg, S.; Andersen, J. O. Effect of dietary citrus pectin on fatty-acid balance and on the fatty-acid content of the liver and small-intestine in rats. *Acta Agric. Scand.* **1980**, *30*, 8−12.
(36) Kall, M. A.; Andersen, C. Improved method for simultaneous determination of ascorbic acid and dehydroascorbic acid, isoascorbic acid and dehydroisoascorbic acid in food and biological samples. *J. Chromatogr., B: Biomed. Appl.* **1999**, *730*, 101−111.
(37) Sulyok, M.; Berthiller, F.; Krska, R.; Schuhmacher, R. Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Commun. Mass Spectrom.* **2006**, *20*, 2649−2659.
(38) Josefsen, L.; Droce, A.; Sondergaard, T. E.; Sørensen, J. L.; Bormann, J.; Schäfer, W.; Giese, H.; Olsson, S. Autophagy provides nutrients for nonassimilating fungal structures and is necessary for plant colonization but not for infection in the necrotrophic plant pathogen *Fusarium graminearum*. *Autophagy* **2012**, *8*, 320−331.
(39) Jensen, S. K.; Jensen, C.; Jakobsen, K.; Engberg, R. M.; Andersen, J. O.; Lauridsen, C.; Sorensen, P.; Skibsted, L. H.; Bertelsen, G. Supplementation of broiler diets with retinol acetate, β-carotene or canthaxanthin: effect on vitamin status and oxidative status of broilers in vivo and on meat stability. *Acta Agric. Scand., Sect. A* **1998**, *48*, 28−37.
(40) Jensen, S. K.; Engberg, R. M.; Hedemann, M. S. All-rac-α-tocopherol acetate is a better vitamin E source than all-rac-α-tocopherol succinate for broilers. *J. Nutr.* **1999**, *129*, 1355−1360.
(41) The MIXED Procedure. In *SAS/STAT 92 User's Guide*; SAS Institute Inc.: Cary, NC, 2008.
(42) Baranska, A. M.; Rembialkowska, E.; Hallmann, E.; Lueck, L.; Cooper, J. M.; Leifert, C. The effects of organic v. conventional diets on immune variables in rats. *Proc. Nutr. Soc.* **2008**, *67*, E28, DOI: 10.1017/S002966510800637x2.
(43) Kaku, S.; Yunoki, S.; Mori, M.; Ohkura, K.; Nonaka, M.; Sugano, M.; Yamada, K. Effect of dietary antioxidants on serum lipid contents and immunoglobulin productivity of lymphocytes in Sprague-Dawley rats. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 575−576.
(44) Penn, N. D.; Purkins, L.; Kelleher, J.; Heatley, R. V.; Mascietaylor, B. H.; Belfield, P. W. The effect of dietary supplementation with vitamin-A, vitamin-C and vitamin-E on cell-mediated immune function in elderly long-stay patients − a randomized controlled trial. *Age Ageing* **1991**, *20*, 169−174.
(45) Meydani, S. N.; Barklund, M. P.; Liu, S.; Meydani, M.; Miller, R. A.; Cannon, J. G.; Morrow, F. D.; Rocklin, R.; Blumberg, J. B. Vitamin-E supplementation enhances cell-mediated-immunity in healthy elderly subjects. *Am. J. Clin. Nutr.* **1990**, *52*, 557−563.
(46) Parmentier, H. K.; Klompen, A. L.; De Vries, R. G.; Lammers, A. Effect of concurrent intratracheal lipopolysaccharide and human serum albumin challenge on primary and secondary antibody responses in poultry. *Vaccine* **2008**, *26*, 5510−5520.
(47) Parmentier, H. K.; Bronkhorst, S.; Nieuwland, M. G. B.; Reilingh, G. D.; van der Linden, J. M.; Heetkamp, M. J. W.; Kemp, B.; Schrama, J. W.; Verstegen, M. W. A.; van den Brand, H. Increased fat deposition after repeated immunization in growing chickens. *Poult. Sci.* **2002**, *81*, 1308−1316.
(48) Demas, G. E.; Chefer, V.; Talan, M. I.; Nelson, R. J. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *Am. J. Physiol.−Reg. I* **1997**, *273*, R1631−R1637.
(49) Bondy, G. S.; Pestka, J. J. Immunomodulation by fungal toxins. *J. Toxicol. Environ. Health B: Crit. Rev.* **2000**, *3*, 109−143.
(50) Fritsche, K. Fatty acids as modulators of the immune response. *Annu. Rev. Nutr.* **2006**, *26*, 45−73.
(51) Das, U. N. Beneficial effect(s) of n-3 fatty acids in cardiovascular diseases: but, why and how? *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2000**, *63*, 351−362.