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Effect of Crop Protection and Fertilization Regimes Used in Organic and Conventional Production Systems on Feed Composition and Physiological Parameters in Rats

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Supporting Information

ABSTRACT: Very little is known about the effects of an organic or conventional diet on animal physiology and health. Here, we report the effect of contrasting crop protection (with or without chemosynthetic pesticides) and fertilization (manure or mineral fertilizers) regimes on feed composition and growth and the physiological parameters of rats. The use of manure instead of mineral fertilizers in feed production resulted in lower concentrations of protein (18.8 vs 20.6%) and cadmium (3.33 vs 4.92 μg/100g) but higher concentrations of polyphenols (1.46 vs 0.89 g/100g) in feeds and higher body protein (22.0 vs 21.5%), body ash (3.59 vs 3.51%), white blood cell count (10.86 vs 8.19 × 10^3/mm^3), plasma glucose (7.23 vs 6.22 mmol/L), leptin (3.56 vs 2.78 ng/mL), insulin-like growth factor 1 (1.87 vs 1.28 μg/mL), corticosterone (247 vs 209 ng/mL), and spontaneous lymphocyte proliferation (11.14 vs 5.03 × 10^3 cpm) but lower plasma testosterone (1.07 vs 1.97 ng/mL) and mitogen stimulated proliferation of lymphocytes (182 vs 278 × 10^3 cpm) in rats. There were no main effects of crop protection, but a range of significant interactions between fertilization and crop protection occurred.

KEYWORDS: *organic food, conventional food, rat, feeding study, health, fertilization, crop protection, physiological parameters*

INTRODUCTION

The intensification of agricultural production over the last 40 years is estimated to have doubled global crop yields but also has resulted in a 2- to 4-fold increase in mineral fertilizer, pesticide, and plant growth regulator input.[1] However, concerns are increasing about negative environmental, food quality, and health impacts, as well as food security risks associated with the use and dependence on agrochemicals.[2−4] As a result, demand for foods produced to organic farming standards, which prohibit the use of chemosynthetic mineral fertilizers, pesticides, and growth regulators, has expanded rapidly over the last 20 years. Also, consumer concerns (especially about negative impacts on health) have resulted in the introduction of government legislation and supermarket quality assurance schemes that restrict agrochemical inputs in conventional crop production.[5,6] However, while environmental impacts associated with mineral fertilizer and pesticide use are well documented,[2−4] there is still controversy on whether agrochemical use results in significant differences in crop composition and whether the consumption of organic foods should be recommended from a health point of view.[7−10] Most studies into the potential effects of mineral fertilizers and pesticides have focused on comparing the chemical composition of fruits, vegetables, and cereals produced to organic and conventional farming standards.[7,8] Recent reviews of such

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Table 1. Previous Crops, Planting and Harvesting Dates, Crop Protection, and Fertilization Regimes Used for Wheat, Potato, Carrot, and Onion Cultivation

	wheat	potato	carrot	onion
previous crops	winter wheat	winter wheat	2 years grass/clover; winter wheat	winter wheat
planting date	14/10/2004	02/05/2005	31/05/2005	05/05/2005
harvesting date	23/08/2005	13/09/2005	07/11/2005	06/10/2005
Fertility Management (F)				
organic	no input	composted cattle manure to equivalent to 170 kg N/ha	composted cattle manure to equivalent to 85 kg N/ha	composted cattle manure to equivalent to 170 kg N/ha
conventional	ammonium-nitrate (Nitram): 50 kg N/ha in mid March and 130 kg N/ha mid April to equivalent to 180 kg N/ha and Superphosphate and KCl as a compound fertilizer (0:20:30) to equivalent to 64 kg P/ha and 96 kg K/ha	ammonium-nitrate (Nitram) to equivalent to 180 kg N/ha and Superphosphate and KCl as a compound fertilizer (0:20:30) to equivalent to 134 kg P/ha and 200 kg K/ha	ammonium-nitrate (Nitram) to equivalent to 20 kg N/ha and Superphosphate and KCl as a compound fertilizer (0:20:30) equivalent to 100 kg P_2O_5/ha and 150 kg K_2O/ha	ammonium-nitrate (Nitram) to equivalent to 75 kg N/ha and Superphosphate and KCl as a compound fertilizer (0:20:30) equivalent to 100 kg P_2O_5/ha and 150 kg K_2O/ha
Crop Protection (P)				
organic				
weed control	2 × mechanical weeding	2 × ridging	2−3 × hand-hoeing	5−6 × mechanical weeding
pest control			Capatex protective netting (1.3 mm mesh size)	
disease control		copper oxychloride; 5 applications per year		
conventional				
weed control	Isoproturon (3 L/ha), mecoprop (1 L/ha), pendimethalin (1.5 L/ha) (applied in November)	Linuron (1 application per year)	Linuron (1.4 L/ha, 1 application per year)	Propachlor (9 L/ha)
pest control		Aldicarb (1 application per year)	Temik 10G (aldicarb) (33.5 kg/ha)	
disease control	1st tank mix (applied in April): epoxiconazole (0.5 L/ha), chlorothalonil (1 L/ha), fenpropimorph (0.2 L/ha), chlormequat (2.3 L/ha) 2nd Tank mix (applied in May): epoxiconazole (0.75 L/ha), chlorothalonil (0.75 L/ha), fenpropidin (0.15 L/ha), trifloxystrobin (0.25 L/ha)	fluazinam (4 applications per year), mancozeb and metalaxyl-M (3 applications per year)	Corbel (Fenpropimorph) (1 L/ha, 1 application per year); Amistar (chlorothalonil) (3 applications per year)	Rovral (Iprodione) (2 L/ha, 3 applications per year); Amistar (chlorothalonil) (1 L/ha, 4 applications per year); Rovral (Iprodione) (2 L/ha, 3 applications per year)

comparative studies have reported overall trends toward lower levels of pesticide residues and nitrates in crops, similar or lower levels of protein and mycotoxins in cereals, and similar or higher concentrations of certain secondary metabolites in vegetables and fruit from organic production compared to conventional production.[7,8] However, these type of comparisons cannot identify the effect of and interactions between individual agronomic practices (rotation, fertilization, and crop protection regimes) used in organic or conventional crop production. The importance of using factorial experiments was demonstrated by a recent study into the relative effects of rotational position, fertilization (organic vs mineral fertilizers), and crop protection (with or without pesticides) regimes on heavy metal concentrations in wheat.[11] The study reported that mineral fertilizers and pesticides have contrasting (and sometimes opposing) effects on heavy metal concentrations; the use of mineral fertilizers increased Cd, Ni, Cu, and Zn concentrations compared to crops fertilized with compost, whereas the use of pesticides resulted in decreased Cu and Zn but similar Cd and Ni concentrations compared to those of nonpesticide treated crops.[11]

Some of the secondary metabolites (phenolic compounds and other antioxidants) occurring in higher concentrations in organic crops[8] have been linked to the reduced risk of degenerative and cardiovascular diseases and certain cancers, a result that is thought to be at least partially due to their antioxidant properties.[12]

However, there have been few studies into the impact of organic food consumption on human or animal health. *In vitro* studies reported that extracts made from organic crops had a higher antimutagenic and antioxidative activity and resulted in a more efficient inhibition of cancer cell proliferation than extracts from conventional crops.[13,14] Controlled studies on animal models have reported that diets based on organic crops have significant effects on the reproductive ability,[15,16] immune system,[17−19] and development of rats, rabbits, and chickens. For example, chickens on an organic crop-based diet had higher immune system reactivity and improved growth rates following an antigenic challenge than chickens on a conventional crop based diet.[19] Also, higher lymphocyte proliferation, blood immunoglobulin concentrations, and immune system reactivity was recorded in rats fed organic rather than rats fed a conventional diet.[17,18] In contrast, a recent study by Jensen et al.[20] reported no significant differences in rat growth and a range of health biomarkers (including blood glucose, nonesterified fatty acids, triacylglycerol, cholesterol, plasma insulin, phospholipid-choline, aspartate-aminotransferase, urea-N, immunoglobulins, and plasma and liver antioxidants) between rats raised on feeds produced from conventional or organic crops, except for IgG. However, there are no published factorial studies in which the effects of individual production system components (e.g., fertility management and crop protection regimes) on animal physiology were compared.

Environmental exposure to pesticides has been linked to an increased risk of a range of diseases (e.g., certain cancers and neurological and reproductive disorders),[21] but controversy exists on whether dietary exposure to concentrations below the maximum permitted residue level poses a health risk.[8] However, the finding that children consuming organic foods had lower concentrations of organophosphorus pesticides in their urine indicated that organic food consumption can lower food-based pesticide exposure.[22]

The main aim of the study reported here was therefore to quantify the effects of contrasting crop fertilization (mineral vs organic fertilizers) and protection (with or without pesticides) regimes used in commercial organic or conventional crop production on the (a) composition of rat feeds and (b) body composition, growth, and hormonal and immune status parameters of rats. This screening study was designed to obtain data that allows the formulation of hypotheses for more targeted dietary intervention studies into the effect of feeds from contrasting production systems on animal health.

■ MATERIALS AND METHODS

Crop Production Methods. Wheat, potatoes, carrots, and onions for the production of rat feeds were grown in experimental plots with contrasting crop protection (P) and fertilization (F) regimes within the Nafferton Factorial Systems Comparison (NFSC) trial at the University of Newcastle's Nafferton Experimental Farm, Northumberland, United Kingdom (54:59:09 N; 1:43:56 W). Each system was represented by four replicate blocks. The NFSC is a long-term trial (consisting of 4 individual experiments) that was set up in 2001.[11] It was established to quantify the effects of, and interactions between, crop protection (pesticide-based protocols used in conventional farming [CP] or crop protection according to organic farming standards without chemosynthetic pesticide use [OP]) and fertility management (mineral fertilizer-based protocols used in conventional farming [CF] or composted manure inputs according to organic farming standards [OF]). Experiments are based on a factorial split-plot design with 4 blocks in which two crop protection main plots (12 m × 48 m) are divided into two fertility management subplots (12 m × 24 m).[23,24] The location of crop protection plots and fertility management subplots within the experiment was randomized, and 10-m unplanted separation strips were established between crop protection main plots, and 5-m unplanted separation strips were placed between fertilization subplots. This design allows four agricultural systems to be compared: (a) organic (organic crop protection with organic fertilization (OPOF)), (b) low input crop protection (organic crop protection with conventional fertilization (OPCF)), (c) low input fertilization (conventional crop protection with organic fertilization (CPOF)), (d) conventional (conventional crop protection with conventional fertilization (CPCF)). For a more detailed description of the field trial design and crop management practices used for wheat, see ref 11. The crop protection and fertilization regimes that were used for potato, onion, and carrot are described in Table 1.

Experimental Diets. Crops from replicate plots of the 4 production systems were harvested separately and transported refrigerated to the Warsaw University of Life Sciences in Poland. All crops were dried to 11 to12% water content in the Institute of Agricultural and Food Biotechnology (Warsaw, Poland) and further processed into pelleted rat feeds by the Feeds Production Plant A. Morawski (Kcynia, Poland) to produce 16 experimental pellet feeds (corresponding to 4 agricultural systems × 4 replicate blocks). The amounts of individual crops and supplements (lactoalbumin, casein, rape seed oil, vitamins, and minerals) included in the experimental feed (Table 2) were designed to satisfy the nutritional requirements of rats.[25] The nutrient composition of crops was calculated based on published mean composition data.[26] Feeds were stored in dry and dark conditions at room temperature and used before the expiry date set by the manufacturer.

Analysis of Rat Feeds. Moisture, protein (nitrogen × 6.25), lipid, fiber, and ash in the experimental feeds were determined in the Laboratory of Chemistry, Kielanowski Institute of Animal Physiology and Nutrition, by AOAC methods.[27] Experimental feeds also were analyzed for total polyphenols as described by Singleton and Rossi,[28] for flavonols using the Christ-Müller method described by Strzelecka,[29] and for β-carotene and lutein using the method described by Saniawski and Czapski[30] (see Supporting Information for details of the analytical protocols). Total antioxidant activity of feeds was

Table 2. Feed Composition (% Weight from Different Crops and Supplements)

crops[ab]	wheat	54.50
	potato	10.20
	carrot	3.92
	onion	0.95
feed supplements[c]	lactoalbumin[d]	6.80
	casein[e]	11.03
	rape seed oil[f]	5.79
	minerals + vitamins[g]	6.81

[a]Weight after drying to a uniform moisture content of 11% ±1%. [b]From different experimental treatments. [c]The same in all experimental diets. [d]Whey powder (Pozmlecz Sp. z o.o., Znin, Poland). [e]Sodium caseinate (Hortimex Sp. z o.o., Konin, Poland). [f]First pressing Kujawski oil (ZT Kruszwica SA, Poland). [g]Sixty-four percent calcium carbonate (Labtar, Sp. z o.o., Tarnów Opolski, Poland), 8% calcium phosphate monohydrate (PPH Standard, Sp. z o.o., Lublin, Poland), 8% NaCl (Solino SA, Inowroclaw, Poland); 20% vitamin and mineral supplement (Cargill Sp. z o.o., Kiszkowo, Poland): Ca (30.7%), vit. A (1,500,000 unit/kg), vit. D_3 (100,000 unit/kg), vit. E (8,000 mg/kg), vit. K_3 (300 mg/kg), vit. B_1 (400 mg/kg), vit. B_2 (400 mg/kg), vit. B_3 (1,500 mg/kg), vit. B_6 (600 mg/kg), vit. B_{12} (5000 μg/kg), biotin (20 mg/kg), folic acid (100 mg/kg), choline (75,000 mg/kg), Fe (7,500 mg/kg), Mn (1,000 mg/kg), Cu (800 mg/kg), Zn (2,500 mg/kg), I (15 mg/kg), Co (15 mg/kg), Se (40 mg/kg), antioxidant (3,000 mg/kg), and calcium pantothenate (1,000 mg/kg).

assayed with a spectrophotometric method using ABTS synthetic cation radicals, as described by Re et al.,[31] and results were presented as TEAC (Trolox equivalent antioxidant capacity), i.e., as μmol of Trolox per 100 g of feed. The calculations were carried out for the solutions showing the ability to scavenge ABTS radicals within the range of 20−80% based on the curve calibration equation. The analyses were carried out in the specialist analytical laboratory of the Faculty of Human Nutrition and Consumer Science, Warsaw University of Life Sciences, using validated published protocols and methods.

The individual feed components also were analyzed for heavy metal content and pesticide residues, and concentrations in feeds were calculated based on the proportion of different crops in the rat feed. Concentrations of the heavy metals, cadmium (Cd), copper (Cu), lead (Pb), and nickel (Ni), were determined in the analytical laboratories at Sabanci University (Istanbul, Turkey) as described previously.[11] The analysis for residues of pesticides, herbicides, fungicides, and the growth regulator chlormequat (CCC) were performed by the Institute of Chemical Technology (Prague, Czech Republic) by LC-MS/MS and gas chromatography (see Supporting Information for details of the methods used). The pesticides tested for included herbicides, fungicides, and pesticides applied to crops in the field experiments (see Table 1).

Experimental Animals and the Rearing/Housing Systems Used. The animal feeding experiment was carried out with male Wistar rats (strain: Wistar Cmd:WI(WU)). All procedures complied with the Polish regulations for animal experimentation and were approved by the first Local Ethical Commission in Warsaw, Poland. Adult female (n = 32) and male (n = 16) rats obtained from the rat breeding house of the Polish Academy of Sciences' Medical Research Centre (Warsaw, Poland) were randomly assigned to 16 dietary groups (2 females and 1 male per group) each receiving one of the 16 experimental feeds ad libitum (4 production systems treatments × 4 replicate blocks) for 3 weeks. The same groups of animals were then transferred to breeding cages (2 females and 1 male per cage). All females included became pregnant and gave birth. Pregnant females were separated from males and continued to be fed with the same experimental feeds throughout the pregnancy and lactation period. The litter size (mean of two females) was similar in each of the 16 dietary groups. At the age of 3 weeks, 6 randomly selected male pups from each group were placed in individual cages and maintained on the same feed as their parents for another 9 weeks. Throughout the experiment, all the animals were kept under conditions of controlled light (L/D 12:12) and temperature (22−23 °C), and had ad libitum access to the experimental feeds and fresh water. The evaluation of the dietary effects on animal growth and animal physiology was carried out on young male rats.

Rat Growth Assessment. The body weight of young rats was recorded weekly, and total weight gain was calculated as a difference between the initial (first after weaning) and the final (on the last day of the experiment) body weight. Feed intake was recorded daily, and the feed conversion ratio (FCR) was calculated as a total intake of feed in the experimental period divided by total weight gain. Normal housing cages without litter were used to allow feed pellets dropping onto the floor to be collected and taken into account in the calculation of daily feed intakes. On the basis of the daily records of feed intake and the results of feed composition analyses, daily intakes of specific feed chemical compounds were calculated.

Sampling for Body Composition, Blood, and Spleen Analysis. Sampling was performed on 12 week old rats, after rats were subjected to a 12-h fasting period. Rats were anesthetized by peritoneal thiopental injection (120 mg/kg of body weight), and blood was collected directly from the heart into polypropylene tubes with heparin. The spleens were dissected aseptically from 3 randomly selected rats per dietary group; only 8 of the 16 dietary groups (2 random field replicates per treatment) were included in the spleen analyses due to resource limitations. Spleens from the 3 rats per dietary group were pooled and immediately used for the preparation of in vitro cell cultures. The carcasses were collected and stored at −20 °C for body composition analyses. Fresh whole blood was used for the evaluation of basic hematological parameters, and plasma was obtained by centrifugation (15 min, 600g, 4 °C) of blood and stored at −20 °C until analyses of hormonal and immune system parameters.

Body Composition and Basic Physiological Parameters. Rat carcasses were defrosted, autoclaved (7.5 h, 121 °C, 1 MPa), and homogenized with a blender (Bamix, Mettlen, Switzerland) with the addition of water (50 mL or more if necessary). Percentage content of dry mass, ash, protein, and fat in the homogenate was determined by standard analytical methods.[27]

The blood hematological parameters and white blood cell numbers were estimated by standard laboratory methods immediately after the collection of blood. Red and white blood cell numbers (RBC and WBC, respectively) were counted using a hemocytometer. Packed cell volume (PCV) was determined in heparinized capillary tubes after centrifuging in a microcentrifuge at 18 700g for 3 min. Hemoglobin content (Hb) was measured by Drabkin's colorimetric method.[32] Results were expressed in cells/mm^3 (RBC and WBC), % (PCV), and g/100 mL (Hb). Plasma glucose concentration was determined using a commercial enzymatic kit (Hydrex, Poland) and was expressed in mmol/L.

Body chemical composition and plasma glucose concentration were analyzed for all rats in each dietary group (4 agricultural production systems × 4 field plot replicates × 6 rats = 96 rats). Hematological parameters (Hb, WBC, RBC, and PCV) were analyzed for rats raised on feeds made from crops from 2 field replicates only (4 agricultural production systems × 2 field plot replicates × 6 rats = 48 rats). All body composition and hematological analyses were done in triplicate per sample. All analyses were carried out in the specialist analytical laboratory of the Faculty of Human Nutrition and Consumer Science, Warsaw University of Life Sciences using validated published protocols or in accordance with the manufacturers' instructions for the use of test kits.

Endocrine and Immune System Parameters. Insulin and leptin plasma concentrations were measured using the Linco Research, Inc. radioimmunoassay (RIA) kits (St. Charles, Missouri).[33,34] Insulin-like growth factor 1 (IGF-1), corticosterone, and testosterone plasma concentrations were assayed using the Mouse/Rat RIA kits (Diagnostic Systems Laboratories, Inc., Webster, Texas). Growth hormone (GH) was analyzed using a Mouse/Rat Growth Hormone ELISA kit (Diagnostic Systems Laboratories, Inc.). The concentration

Table 3. Effects of Crop Protection and Fertilization Regime on the Chemical Composition of the Experimental Feeds (per 100 g FW)[a]

	protein (g)	polyphenols (g)	β-carotene (mg)	lutein (mg)	TEAC[b]	Cd (μg)	CCC[c] (μg)
Crop Protection (P)							
organic (n = 8)	19.7 ± 0.4	1.26 ± 0.16	1.16 ± 0.10	0.709 ± 0.057	21.9 ± 1.1	4.26 ± 0.38	0.00 ± 0.00
conventional (n = 8)	19.7 ± 0.3	1.09 ± 0.09	1.19 ± 0.08	0.597 ± 0.036	22.2 ± 0.9	3.99 ± 0.34	17.35 ± 4.13
Fertility Management (F)							
organic (n = 8)	18.8 ± 0.1	1.46 ± 0.11	1.21 ± 0.08	0.710 ± 0.056	21.6 ± 1.1	3.33 ± 0.13	13.91 ± 5.37
conventional (n = 8)	20.6 ± 0.2	0.89 ± 0.04	1.13 ± 0.09	0.596 ± 0.037	22.6 ± 0.9	4.92 ± 0.25	3.44 ± 1.36
ANOVA *P*-Values							
P	NS	NS	NS	*0.0965*	NS	NS	0.0015
F	<0.0001	0.0004	NS	*0.0515*	NS	<0.0001	<0.0001
P × F	NS	0.0177[d]	0.0002[d]	0.0287[d]	0.0114[d]	NS	<0.0001[d]

[a]Data are presented as the mean ± SE with ANOVA *P*-values. Only the results for feed compounds where there was a significant effect of crop protection or fertilization, or a significant interaction, are included in the table; see Supporting Information for dry matter, ash, lipids, fiber, flavonols, Cu, Ni, and Pb concentrations. [b]Trolox equivalent antioxidant capacity (μmol Trolox equiv). [c]Chlormequat. [d]See Figure 1 for details of the interaction; *n*, number of samples (field replicates).

of IgA in blood plasma was measured using a commercially available rat ELISA kit (Bethyl Laboratories, Montgomery, Texas),[35] and plasma concentration of C-reactive protein (CRP) was determined using an ELISA kit designed for the detection and quantification of rat CRP (BD Biosciences, Franklin Lakes, New Jersey).[36] The same or similar kits were widely used in physiological studies with rats.[18,37,38]

Plasma hormone concentrations were analyzed for all rats in each dietary group (= 4 agricultural production systems × 4 field plot replicates × 6 rats = 96 rats). All of the hormone analyses were repeated 2 times per rat plasma sample, and results were expressed in ng/mL (insulin, leptin, GH, corticosterone, and testosterone) or μg/mL (IGF-1) of plasma. Plasma immune parameters were analyzed for rats on feeds made from crops from 1 field replicate only (4 agricultural production systems × 1 field plot replicate × 6 rats = 24 rats). Analyses were repeated 3 times per rat plasma sample, and results were expressed in μg/mL (IgA) and ng/mL (CRP) of plasma.

Cells isolated from rat spleens were cultured for analysis of their proliferation capacity, both spontaneous and mitogen stimulated. In vitro assays were based on the method described by Bik et al.[39] but included a different growth medium (Roswell Park Memorial Institute medium, RPMI, Invitrogen, Grand Island, New York, containing L-glutamine and sodium bicarbonate) and two mitogens, concanavalin A (ConA, a T-cell specific mitogen) and lipopolysaccharide (LPS, a B-cell specific mitogen) (Sigma-Aldrich, St. Louis, Missouri) in concentrations of 0.125 μg/well and 2 μg/well, respectively. Data for mitogen stimulated proliferation were used for the analysis and presentation, after the subtraction of values obtained in control cultures consisting of cells incubated with RPMI alone (spontaneous proliferation).[40] Results were expressed in counts per minute (cpm). Analyses were repeated 6 times (mitogen stimulated proliferation) or 12 times (spontaneous proliferation), respectively. All analyses were carried out in the specialist analytical laboratory of the Faculty of Biology, University of Warsaw, and the Faculty of Human Nutrition and Consumer Science, Warsaw University of Life Sciences.

Statistical Analyses. The main effects and interactions of crop protection (P) and fertilization management (F) were assessed using analyses of variance derived from linear mixed-effects models.[41] Crop protection and fertility management were fixed factors in the analyses, with block and crop protection random factors given the split−split−split experimental design,[42] and the analyses were carried out using the nlme package in the R statistical environment.[41] Variance was estimated by maximum likelihood estimation (MLE). Differences between the four crop management combinations (OPOF, OPCF, CPOF, and CPCF) were tested using Tukey's honestly significant difference (HSD) tests, based on a mixed-effect model, using the multcomp package in R, as in ref 11. Residual normality was tested using the qqnorm function and D'Agostino-Pearson tests in R. No data transformations were required. In addition, the relationship between measured feed composition parameters and rat physiological parameters were investigated using the standard Pearson's product−moment correlation analyses, carried out in R.

The relationships between concentrations of feed components (ash, lipids, protein, fiber, antioxidant activity, β-carotene, flavonols, lutein, polyphenols, Cd, Cu, Pb, Ni, and chlormequat) and (a) plasma concentration of hormones (corticosterone, growth hormone, insulin-like growth factor 1, leptin, and testosterone), (b) rat body composition (body ash, dry mass, fat, and protein), and (c) growth parameters (total weight gain and feed conversion ratio) were investigated using redundancy analyses (RDA). Relationships with immune system parameters could not be investigated since immune parameters were determined only for rats fed feeds based on crops grown in 1 of the 4 replicate blocks. RDAs were carried out using the CANOCO package,[43] with the importance of intake parameters assessed with automatic forward selection within RDAs, no interaction terms being tested in each of the RDAs, using the constrained ordination in combination with Monte Carlo permutation tests: 9999 permutations for each randomization test.

■ RESULTS

Effects of Crop Management Practices on Feed Composition. There were no detectable pesticide residues, except for the residues of the growth regulator CCC that were detected in wheat grain from plots under the conventional crop protection regime (Table 3). Compared to mineral fertilizer-based conventional fertilization, organic fertilization regimes based on cattle manure compost resulted in lower concentrations of protein and Cd but higher concentrations of polyphenols and CCC residues in rat feeds. At the same time, fertilization regimes had no effect on the concentrations of lipids, ash, fiber, flavonols, β-carotene, lutein, total antioxidant capacity (TEAC), and the heavy metals Cu, Pb, and Ni in rat feed (Table 3; Supporting Information, Table 5). For polyphenols, β-carotene, lutein, CCC, and TEAC in feed, there were significant interactions between crop protection and fertility management, but there were differences in the interactions (Figure 1). When conventional fertilization regimes were used, polyphenol concentrations were not affected by crop protection, whereas under organic fertilization regimes, organic crop protection resulted in higher polyphenol concentrations than conventional crop protection. If organic crop protection was used, β-carotene concentrations were higher with organic fertilization, but under conventional crop protection, conventional fertilization resulted in higher concentrations. The fertilization regime had no effect on lutein concentrations or TEAC in feeds, if crops from plots under

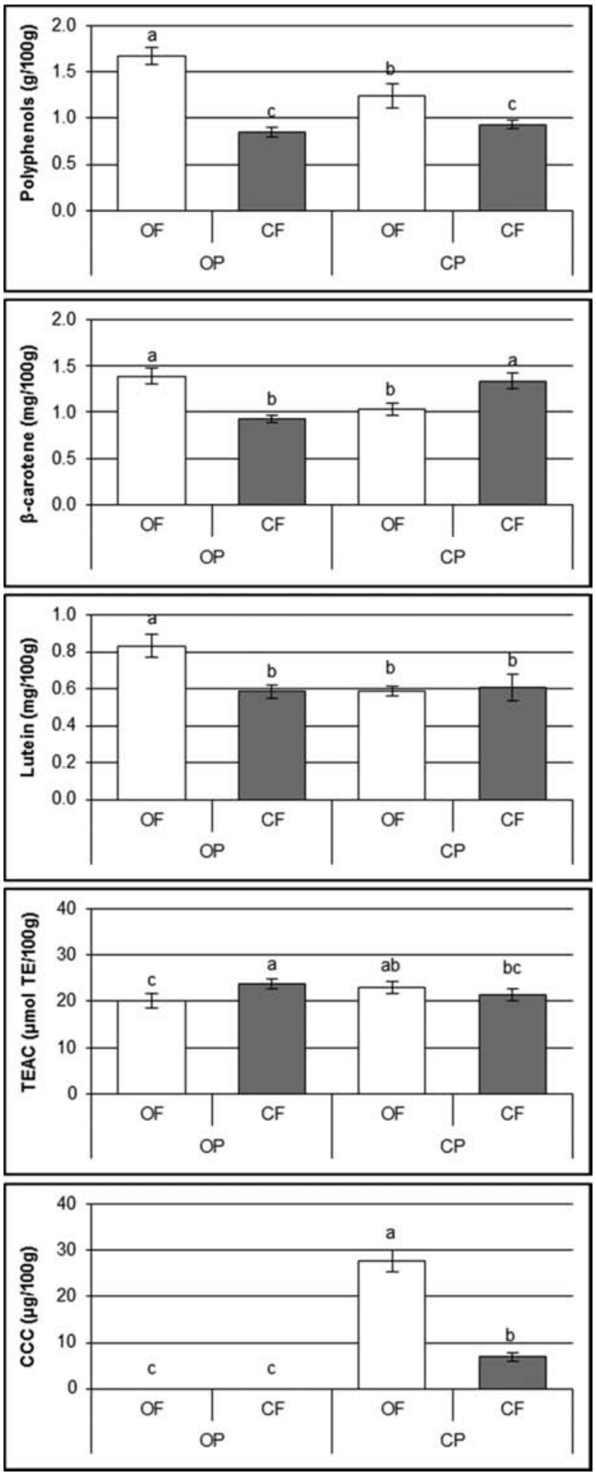

Figure 1. Effects of organic crop protection (OP) or conventional crop protection (CP) and organic fertility management (OF) or conventional fertility management (CF) on the concentration of polyphenols, β-carotene, lutein, chlormequat (CCC), and on antioxidant activity (TEAC) in experimental feeds. Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P = 0.05$).

conventional crop protection were used. In contrast, if crops from organic crop protection plots were used, lutein concentrations were higher, and TEAC was lower in feeds made from organically fertilized crops. There were no CCC residues in feeds made from crops under organic crop protection regimes, but under conventional crop protection, CCC concentrations were more than 3 times higher in feeds made from organically fertilized crops than from conventionally fertilized crops (Table 3). See Supporting Information (Tables 1−4) for results on heavy metal (Cd, Cu, Ni, and Pb) concentrations in the individual feed components (wheat, potato, carrot, and onion).

Intake of Feeds and Selected Nutrients. There was a significant effect of fertilization but not crop protection on daily feed intake. Rats consuming feeds made from organically fertilized crops had a slightly, but significantly lower, feed intake than rats on feeds made from conventionally fertilized crops (Table 4). There was a significant negative correlation between feed intake and polyphenol ($r = -0.23$, $P = 0.025$) concentrations in feeds. Also, there were trends toward or significant positive correlations between feed intake and (a) protein ($r = 0.18$, $P = 0.075$), (b) Cu ($r = 0.17$, $P = 0.095$), and (c) Cd ($r = 0.21$, $P = 0.044$) contents in feeds.

Although there was a significant effect of fertilization on daily feed intake, this was very small (<3%). Differences in feed composition were therefore the main driver for the daily intake of specific compounds (see Supporting Information, Table 6 and 7 and Figure 2 and 3).

Rat Growth, Body Composition, Plasma Glucose, and Hematological Parameters. The growth rates of rats were similar with all four types of feeds (Figure 2), and final body weights were not significantly affected by treatment (see Supporting Information, Table 8). Organic fertilization resulted in a significantly higher body weight at weaning, but lower total weight gain and higher FCR (Table 4). However, there were also significant interactions for body weight at weaning and FCR. A significantly lower body weight at weaning was detected only when rats were raised on feeds produced from conventionally fertilized crops under organic crop protection. A significantly higher feed conversion ratio was detected only when organic fertilization was used in combination with conventional crop protection (Figure 3).

Significant main effects of fertilization were detected for total body protein, body ash, plasma glucose, and white blood cell count (WBC), with feeds made from organically fertilized crops resulting in higher body protein (2.3%) and ash (2.3%) (Table 4), and plasma glucose (33%) and WBC (16%) (Table 5). No significant effect of contrasting feeds on total body dry mass and fat content, red blood cell count (RBC), packed cell volume (PCV) (Supporting Information, Table 8), and blood hemoglobin concentration (Hb) (Table 5) could be detected. However, there was a significant crop protection × fertilization interaction for blood Hb. Crop protection had no effect on blood Hb if organic fertilization was used to produce feed crops, but if feeds were produced from conventionally fertilized crops, conventional crop protection resulted in higher Hb concentrations than organic crop protection (Figure 3).

Endocrine Parameters. The use of rat feeds made from organically fertilized crops resulted in higher concentrations of leptin, corticosterone, and IGF-1 (29%, 18%, and 46%, respectively) but 45% lower concentration of testosterone (Table 5). In contrast, no significant main effects of crop protection could be detected for the other endocrine parameters assessed (insulin and growth hormone;, see Supporting Information, Table 8). However, significant crop protection × fertilization interactions were found for leptin and IGF-1. Fertilization had no effect on IGF-1 and leptin

Table 4. Effects of Crop Protection and Fertilization Regime on the Intake of Feed, Body Growth, Feed Conversion Ratio (FRC) and Body Composition[a]

	feed intake (g/d)	body weight at weaning (g)	total weight gain (g)	feed conversion ratio	body protein (%)	body ash (%)
Crop Protection (P)						
organic ($n = 8 \times 6$)	20.8 ± 0.2	122 ± 4	251 ± 5	5.87 ± 0.07	21.7 ± 0.2	3.56 ± 0.03
conventional ($n = 8 \times 6$)	20.5 ± 0.3	128 ± 3	232 ± 4	6.12 ± 0.08	21.9 ± 0.1	3.54 ± 0.03
Fertility Management (F)						
organic ($n = 8 \times 6$)	20.3 ± 0.3	129 ± 4	231 ± 4	6.20 ± 0.08	22.0 ± 0.1	3.59 ± 0.03
conventional ($n = 8 \times 6$)	21.0 ± 0.2	121 ± 3	251 ± 5	5.79 ± 0.07	21.5 ± 0.1	3.51 ± 0.03
ANOVA *P*-Values						
P	NS	NS	NS	*0.0768*	NS	NS
F	0.0238	0.0315	0.0012	<0.0001	0.0071	0.0209
P × F	NS	0.0080[b]	NS	0.0430[b]	NS	NS

[a]Data are presented as the mean ± SE with ANOVA *P*-values. Only the results for the body composition parameters where there was a significant effect of crop protection or fertilization, or a significant interaction, are included in the table; see Supporting Information for final body weight, body dry mass, and body fat content; *n*, number of samples (number of field replicates × number of experimental rats). [b]See Figure 3 for details of the interaction.

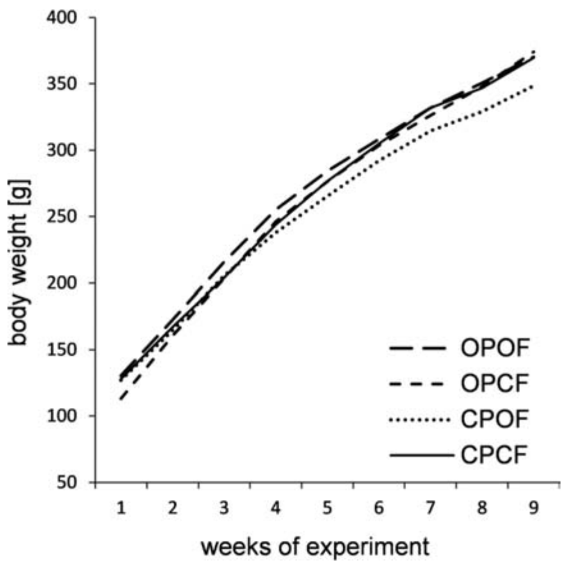

Figure 2. Body weight gain of rats fed feeds based on crops cultivated according to four crop management strategies based on organic crop protection (OP) or conventional crop protection (CP) and organic fertility management (OF) or conventional fertility management (CF).

concentration when feeds were made from crops grown under conventional crop protection regimes. In contrast, organic fertilization resulted in higher leptin and IGF-1 concentrations, when feeds were made from crops produced under organic crop protection regimes (Figure 3). Significant correlations between plasma IGF-1 (but not leptin, corticosterone, testosterone, growth hormone, and insulin) and glucose concentrations ($r = 0.27$, $P = 0.013$) also could be detected.

For insulin, there were positive correlations with total weight gain ($r = 0.32$; $P = 0.009$), body dry matter ($r = 0.34$; $P = 0.006$), body fat ($r = 0.35$; $P = 0.005$), negative correlation with body ash ($r = -0.43$; $P < 0.001$), and body protein ($r = -0.44$; $P < 0.001$) and no correlation with feed conversion ratio. For growth hormone, there were negative correlations with total weight gain ($r = -0.22$; $P = 0.049$) and body dry matter ($r = -0.25$; $P = 0.021$), but none of the other growth and body composition parameters were recorded. For testosterone, there was only a significant negative correlation with feed conversion ratio ($r = -0.26$; $P = 0.019$).

Immune Parameters. As expected for nonstimulated cells, spontaneous lymphocyte proliferation (sp-LP) *in vitro* was low when compared to concanavalin A stimulated lymphocytes (ConA-LP). However, LPS stimulated lymphocyte proliferation (LPS-LP) was low, with no stimulation of cells from rats on feeds from organically fertilized crops (Table 6).

Significant main effects of fertility management were detected for sp-LP, ConA-LP, and LPS-LP. The use of feeds made from organically fertilized crops resulted in a 121% increase in sp-LP, a 35% lower ConA-LP, and a more than 4-fold reduction in LPS-LP compared to those of conventionally fertilized crops (Table 6). In contrast, no significant main effects of crop protection could be detected for the immune parameters assessed (Table 6). However, significant crop protection × fertilization interactions occurred for sp-LP, ConA-LP, and LPS-LP. Fertilization had no effect on sp-LP if crops were grown under conventional crop protection, but if organic crop protection regimes were used, organic fertilization resulted in 3 times higher sp-LP. For ConA-LP, the opposite was found; there was no effect of fertilization regimes, if feeds were made from crops under organic crop protection regimes, while organic fertilization resulted in 47% lower ConA-LP, if feeds were made from crops under conventional crop protection regimes (Figure 4). No significant effect of any of the experimental factors on plasma CRP and IgA could be detected (Supporting Information, Table 8).

Relationship between Feed Composition, Plasma Hormone Concentrations, and Rat Growth/Body Composition Parameters. The biplot derived from RDA in Figure 5 shows the relationships between feed composition parameters (those found to be significantly affected by crop protection or fertilization regimes) and (a) body composition, (b) growth parameters, and (c) plasma hormone concentration. Axis 1 explained 73.1% of the variation, and axis 2 explains a further 1.2%. Most additional variance was explained by concentrations of polyphenols ($F = 51.15$, $P = 0.002$), TEAC ($F = 12.95$, $P = 0.002$), lutein ($F = 6.74$, $P = 0.004$), β-carotene ($F = 6.25$, $P = 0.016$), protein ($F = 4.79$, $P = 0.026$), flavonols ($F = 4.26$, $P = 0.032$), fiber ($F = 4.08$, $P = 0.058$), Ni ($F = 1.90$, $P = 0.170$), Pb ($F = 1.46$, $P = 0.230$), and lipids ($F = 1.44$, $P = 0.194$) in rat feeds. Concentrations of other metals (Cd and Cu), CCC, and ash in feeds explained less ($F < 1.20$; $P \geq 0.300$) of the additional variance than the above factors. There were positive associations along the negative axis 1 between plasma IGF-1

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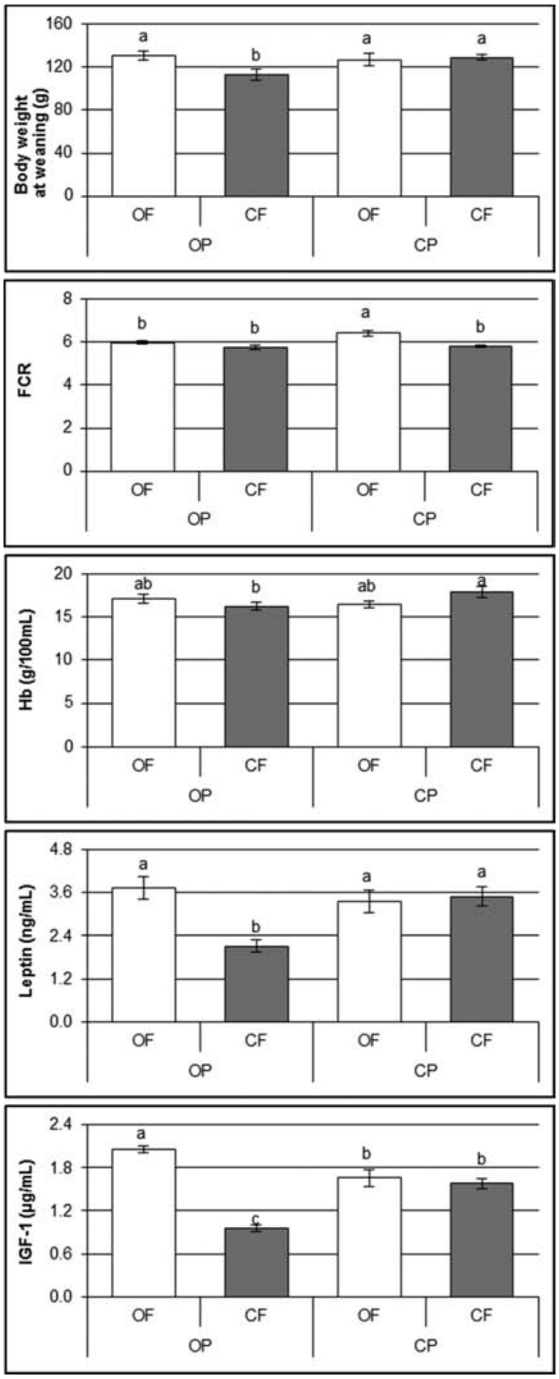

Figure 3. Effects of organic crop protection (OP) or conventional crop protection (CP) and organic fertility management (OF) or conventional fertility management (CF) on body weight at weaning, feed conversion ratio (FCR), blood hemoglobin content (Hb), and plasma leptin and IGF-1 concentration in experimental rats. Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P = 0.05$).

and leptin concentrations and polyphenol, β-carotene, flavonols, and lutein content in rat feeds. There were also positive associations along the positive axis 1 between plasma testosterone concentration and weight gain of rats, and the total antioxidant activity and protein, fiber, and Cd in rat feeds.

■ DISCUSSION

Although there is now a significant body of evidence for composition differences (especially with respect to secondary metabolites) between crops produced by organic and conventional production methods,[8,11,20] there are very few published dietary intervention studies in which the impact of consuming crops from different production systems was studied.[15−20] Also, there are to our knowledge no factorial studies in which the impact of contrasting crop protection and fertilization regimes (used in organic and conventional farming) on animal physiological and growth parameters was quantified.

Effect of Crop Protection and Fertilization Protocols on Rat Feed Composition. The finding of higher antioxidant, but lower protein and Cd concentrations in rat feeds made from crops produced with organic rather than mineral fertilizers, confirms previous studies that concluded that composition differences between organic and conventional crops are primarily caused by differences in fertilization regimes.[8,11] For example, high nitrogen availability to crops, resulting from mineral N-fertilizer use in conventional crop production, previously was linked to a reduction in antioxidants (including polyphenols) and other plant defense-related metabolites but not carotenoids.[8] In this study, no main effects of crop protection were detected for any of the secondary metabolites analyzed in feeds, but there were significant interactions between crop protection and fertility management for total antioxidant activity and polyphenols, β-carotene, and lutein concentrations, which are parameters thought to be at least partially responsible for the health promoting effects of whole grain, fruit, and vegetable consumption.[8,12] The highest polyphenol and lutein concentrations were detected in organic feeds (those produced from compost fertilized crops under organic crop protection).

The synthesis of polyphenols (many of which are known resistance compounds in plants) was shown to be induced by foliar disease and pest damage,[44] and this could explain the interaction between fertilization and crop protection detected for polyphenols (and possibly also lutein). However, the interactions observed for β-carotene and TEAC are difficult to explain.

As expected from previous studies, CCC applications (which are used in conventional farming systems to reduce stem length and lodging risk) resulted in significant CCC-residues in cereal grains.[45] However, here we report for the first time that CCC-residues were 4 times higher in grain from CCC-treated wheat fertilized with composted manure instead of mineral fertilizer. This result may have been due to the mineral fertilizer applications causing a delay in the start of senescence (natural aging of leaves resulting in yellowing and tissue death over a 1 to 2 week period) in cereal plants, which is supported by the finding that in the NFSC experiments, senescence of wheat starts 1−2 weeks later in mineral fertilized compared to compost fertilized plots (individual results not shown). This later senescence may have allowed a greater proportion of the CCC to be metabolized by the plant. However, this hypothesis will need to be tested in future studies.

Intake of Feeds and Selected Nutrients. There are very few studies in which the intake of rat feeds made from organic and conventionally produced crops was compared. Finamore et al.[17] and Lauridsen et al.[18] reported no significant difference in the intake of feeds made from organic or conventional crops. There are, to our knowledge, no factorial studies in which the

Table 5. Effects of Crop Protection and Fertilization Regime on the Basic Physiological Parameters and Plasma Hormones Concentration[a]

	Hb (g/100 mL)	WBC (× 10^3/mm^3)	glucose (mmol/L)	leptin (ng/mL)	IGF-1 (μg/mL)	Cs (ng/mL)	Ts (ng/mL)
Crop Protection (P)							
organic ($n = 8 \times 6^b$)	16.7 ± 0.4	10.08 ± 0.48	6.42 ± 0.31	2.93 ± 0.21	1.53 ± 0.09	244 ± 14	1.84 ± 0.20
conventional ($n = 8 \times 6^b$)	17.2 ± 0.4	8.97 ± 0.87	7.07 ± 0.23	3.42 ± 0.20	1.62 ± 0.06	213 ± 14	1.23 ± 0.17
Fertility Management (F)							
organic ($n = 8 \times 6^b$)	16.8 ± 0.3	10.86 ± 0.57	7.23 ± 0.18	3.56 ± 0.22	1.87 ± 0.07	247 ± 15	1.07 ± 0.14
conventional ($n = 8 \times 6^b$)	17.1 ± 0.5	8.19 ± 0.63	6.22 ± 0.35	2.78 ± 0.19	1.28 ± 0.06	209 ± 13	1.97 ± 0.21
ANOVA *P*-Values							
P	NS	NS	NS	NS	NS	NS	NS
F	NS	0.0020	0.0007	0.0055	<0.0001	0.0428	0.0001
P × F	0.0403[a]	NS	NS	0.0016[c]	<0.0001[c]	NS	*0.0580*

[a]Data are presented as the mean ± SE with ANOVA *P*-values. Only the results for the parameters where there was a significant effect of crop protection or fertilization, or a significant interaction, are included in the table (Hb (hemoglobin content) WBC (white blood cell count), glucose, leptin, IGF-1 (insulin-like growth factor 1), Cs (corticosterone), and Ts (testosterone)); see Supporting Information for RBC (red blood cell count), PCV (packed cell volume), insulin, GH (growth hormone); *n* (number of samples) (number of field replicates × number of experimental rats). [b]For Hb and WBC, $n = 4 \times 6$. [c]See Figure 3 for details of the interaction.

Table 6. Effects of Crop Protection and Fertilization Regime on the Immune Parameters[a]

	sp-LP (× 10^3 cpm[b])	ConA-LP (× 10^3 cpm)	LPS-LP (× 10^3 cpm)
Crop Protection (P)			
organic ($n = 2 \times 6^c$)	9.94 ± 1.20	164 ± 14	1.33 ± 0.71
conventional ($n = 2 \times 6^c$)	6.64 ± 1.13	297 ± 24	2.34 ± 0.97
Fertility Management (F)			
organic ($n = 2 \times 6^c$)	11.14 ± 1.47	182 ± 12	−0.80 ± 0.63
conventional ($n = 2 \times 6^c$)	5.03 ± 0.24	278 ± 30	4.47 ± 0.39
ANOVA *P*-Values			
P	NS	NS	NS
F	0.0004	<0.0001	<0.0001
P × F	0.0074[d]	<0.0001[d]	0.0479[d]

[a]Data are presented as the mean ± SE with ANOVA *P*-values. Only the results for the parameters where there was a significant effect of crop protection or fertilization, or a significant interaction, are included in the table (LP (lymphocyte proliferation) sp (spontaneous), ConA (concanavalin A-stimulated), LPS (lipopolisaccharide-stimulated)); see Supporting Information for CRP (C-reactive protein) and IgA (immunoglobulin A). [b]Counts per minute; *n*, number of samples (number of field replicates × number of experimental rats). [c]For lymphocyte proliferations, $n = 4 \times 3$. [d]See Figure 4 for details of the interaction.

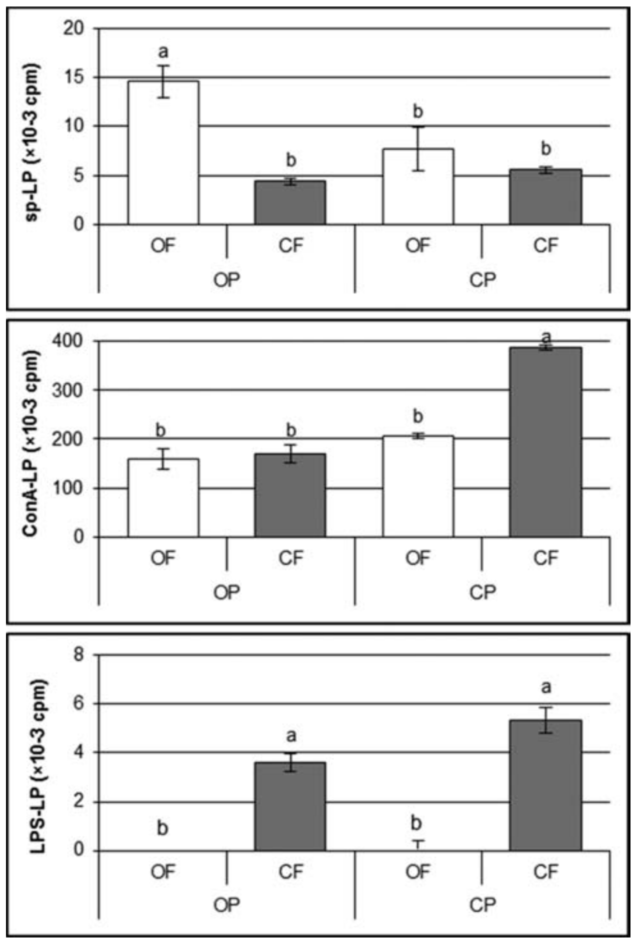

Figure 4. Effects of organic crop protection (OP) or conventional crop protection (CP) and organic fertility management (OF) or conventional fertility management (CF) on lymphocyte proliferation (sp (spontaneous), ConA, (concanavalin A-stimulated), LPS (lipopolisaccharide-stimulated)). Bars labeled with the same letter are not significantly different (Tukey's honestly significant difference test, $P = 0.05$).

relative effect, and interactions between, contrasting fertilization and crop protection regimes (used in organic and conventional farming) on feed intake was compared. However, some feed preference studies (in which rats were provided with access ad libitum to both organic and conventional feeds simultaneously) have shown that rats prefer organic over conventional crops or feeds and speculated that this preference may be due to rats sensing taste or deleterious compounds (e.g., pesticides) in conventionally produced feeds.[46,47] A more recent factorial study reported that the preference for organic wheat was linked to organic fertilization and crop protection regimes.[48] Although there were significant differences in the concentrations of CCC and Cd between feeds, there were no negative correlations between the concentrations of these potentially deleterious compounds and daily feed intake; in fact, feed intake was correlated positively with Cd concentrations in feeds. However, there was a significant negative correlation between polyphenol concentrations and feed intake, a result that supports previous studies showing that a high content of certain phenolic

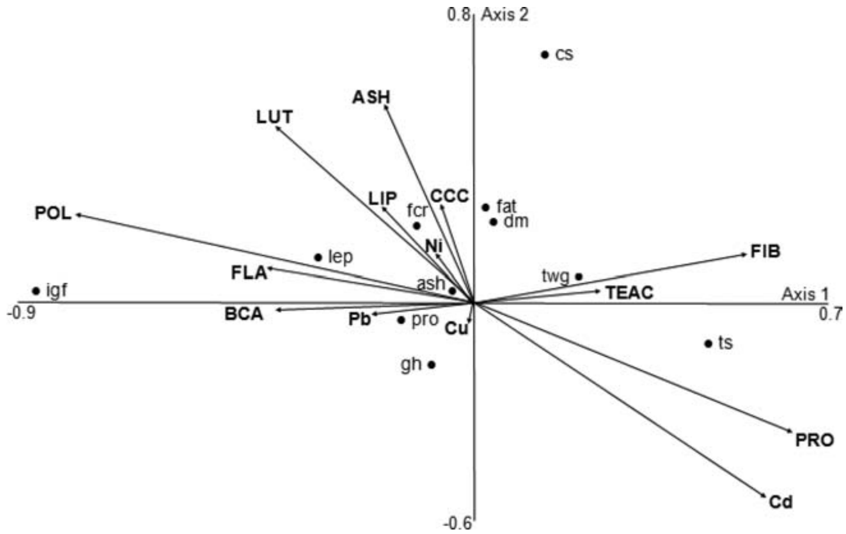

Figure 5. Biplot derived from the redundancy analysis showing the relationship between the physiological parameters (ash (body ash), cs (corticosterone), dm (body dry mass), fat (body fat), fcr (feed conversion ratio), gh (growth hormone), igf (insulin-like growth factor 1), lep (leptin), pro (body protein), ts (testosterone), twg (total weight gain)) and selected feed compounds (ASH (ash), BCA (β-carotene), CCC (chlormequat), Cd (cadmium), Cu (copper), FIB (fiber), FLA (flavonols), LIP (lipids), LUT (lutein), Ni (nickel), Pb (lead), POL (polyphenols), PRO (protein), TEAC (antioxidant activity)). Insulin was not included in the analysis as a response variable due to a large number of missing data caused by analytical equipment failure.

compounds (e.g., tannins) can limit feed intake in a number of animals.[49,50]

Rat Growth, Body Composition, Plasma Glucose, and Hematological Parameters. In the present study, experimental feeds were provided during mating, pregnancy, and lactation of the parental rats and to young rats postweaning for a 9 week period. This protocol may have increased the magnitude of physiological effects on experimental animals but makes it impossible to link the effects of contrasting feeds to specific developmental stages. Results indicate that the use of feeds made from mineral compared to organic fertilized crops results in a lower weight at weaning but to higher subsequent weight gains, indicating that effects on growth may differ depending on the developmental stage of rats. The higher growth rate of rats postweaning is likely to have resulted from the higher intake (3.4%) and protein content (approximately 9.6%) of feeds made from conventionally fertilized crops. Previous studies have shown that protein supply is a major factor affecting growth rates of experimental rats.[51] However, it is unclear what factors were responsible for the finding that rats from mothers provided with feeds made from organically fertilized crops had a higher body weight at weaning since the milk composition of lactating rats was not analyzed. This should be investigated in future studies since it could provide valuable information on the effect of organic and conventional feeds on milk composition and physiological effects, as shown previously for bovine milk.[52−54]

The finding that the use of organic fertilization resulted in lower protein content in feeds, but in higher body protein in rats, may be explained by differences in (a) the protein quality (e.g., digestibility, albumin and globulin content, and amino acid profiles) of rat feeds and (b) the growth rates or feed conversion ratio of rats. For example, there are reports that starch and protein digestibility and concentrations of nutritionally desirable albumins and globulins and certain essential amino acids (leucine and threonine) are higher in organically than conventionally produced wheat.[55−57] However, rat feeds were not analyzed for concentrations of these nutritionally relevant compounds in the study reported here.

An increase in WBC count can be an indicator of rats being exposed to a dietary or immune system challenge. For example, Hsiao et al.[58] reported that WBC increased in male Wistar rats from $6.84 \times 10^3/\mu L$ to $9.23 \times 10^3/\mu L$ following the induction of subacute peritonitis. However, although we detected a significant effect of fertilization on WBC in this study, the concentrations recorded for rats on all 4 feed types were well within the range reported by other authors for rats on balanced diets without immune systems challenge.[59]

Endocrine Parameters. Growth hormone (GH, produced mainly in the pituitary gland), insulin-like growth factor 1 (IGF-1, produced mainly by the liver), and testosterone (Ts, produced mainly in the testes) are anabolic hormones involved in the regulation of cell growth and development (GH and IGF-1) as well as male reproduction (Ts). In contrast, corticosterone (produced mainly in the adrenal cortex) is a catabolic hormone involved in the regulation of immune function and physiological responses to stress. Leptin (produced mainly in adipose tissues) is involved in the regulation of energy intake and expenditure.[60] The finding that the use of feeds made from organically fertilized crops resulted in a higher leptin, IGF-1, and corticosterone but lower testosterone indicates that feed intake or composition differences (e.g., lower protein and Cd, and higher polyphenol and CCC concentrations) resulting from contrasting fertilization practices may affect hormonal balances and the processes they regulate. This conclusion also is supported by (a) the high proportion of variability explained by feed component drivers in the RDA (where polyphenols, antioxidant activity, carotenoids, and proteins explained more than 60% of variation in hormone concentrations and rat growth and body composition parameters) and (b) previous studies which reported a significant effect of protein, polyphenol, pesticides, and deleterious metals concentrations on hormonal balances in rats.[61−63]

Strong correlations between plasma hormone concentrations and rat growth and body composition were only detected for insulin. The finding of positive correlations with total weight gain, body fat, and body dry matter confirms results from previous studies that reported positive association between insulin levels and body fat in normal weight albino Sprague–Dawley rats on macronutrient diets.[64]

Immune Parameters. Many of the immune system parameters (plasma IgA and CRP concentrations, sp-LP, ConA-LP, and LPS-LP) measured in this study are known to be affected by feed components or composition, directly or indirectly (e.g., via effects on hormonal balance).[65] For example, restricted dietary protein supply has long been known to impair immune system function in animals and humans.[66] However, this effect does not explain the interactions between fertilization and crop protection for ConA-LP detected in this study.

Lauridsen et al.[18] reported higher serum IgG concentration in rats fed organic compared to conventional feeds, but this result was unlikely to have been caused by differences in protein supply since protein concentrations were the same in organic or conventional feeds. In contrast, Jensen et al.[20] reported lower concentrations of plasma IgG in rats fed organic than conventional feeds but no significant differences for the feed protein concentrations. Finamore et al.[17] reported higher immune system reactivity (conA-LP) in protein-energy malnourished rats on organic cereal based diets when compared to that of conventional feeds with the same protein concentration. Huber et al.[19] reported that organic feeds can enhance immune system reactivity (ConA-LP and LPS-LP) and growth rates of chicken following immune challenge, even if the protein content in organic feed is 10% lower than in conventional feed. Apart from the differences in dietary requirements between chicken and rats, the fact that different crop varieties were used for the preparation of organic and conventional chicken feed may have had a confounding effect in their study.[67] It therefore remains unclear to what extent protein supply was responsible for the differences in immune system reactivity between organically and conventionally fed animals. Previous animal studies reported an immunostimulating effect of increased dietary polyphenols intake.[68,69] However, here we show that feeds made from organically produced crops, with higher polyphenol content, were associated with lower immune system responsiveness (e.g., lower ConA-stimulated lymphocyte proliferation). As suggested by Finamore et al.,[17] the observed differences could be explained by the presence of other, unidentified chemicals with immunomodulating properties in feeds. It is important to point out that a range of potentially confounding factors (e.g., differences in the bioavailability of secondary metabolites in feeds made from crops grown under contrasting agronomic regimes, effects of other dietary components on bioavailability) that may have had an effect on animal growth, body composition, and hormonal and immunological parameters were not investigated in the study reported here.[70,71] Moreover, compounds such as certain polyphenols and vitamins (e.g., vitamin C and carotenoids) are known to decrease during storage,[72] and this may also have been a confounding factor.

On the basis of the data presented, it can be concluded that crop production practices (fertilization and crop protection) that modulate the composition of feeds, resulting in significant effects on animal physiology, especially immune status. Further research should focus on identifying the physiological effects of specific compounds found to be modulated by agricultural practice (e.g., polyphenols, Cd, and proteins). This action could be achieved by selective supplementation of feeds naturally deficient in specific compounds, for example, supplementation of feed made from conventionally produced crops with polyphenols or feeds made from organically fertilized crops with proteins or Cd. To investigate potential health impacts future dietary intervention, studies should include the use of (a) immune system challenges (as described by Huber et al.[19]) or (b) animals with genetically impaired immune system function or specific disease susceptibilities.

ASSOCIATED CONTENT

Supporting Information

Description of the methods for analysis of pesticide residues in crops and flavonols, polyphenols, and carotenoids in rat feeds; results on the effects of crop protection and fertility management on the concentration of metals (Cu, Cd, Ni, and Pb) in crops (wheat, potato, carrot, and onion) (Tables 1–4) and daily intake of macronutrients, selected antioxidants, and total antioxidant activity (Table 6); main effects means, ANOVA *P*-values, and interaction figures (Figure 1, 2, and 3 sup.); and results of the feed chemical composition parameters and rat physiological parameters where there was no significant effect of crop protection or fertilization, or a significant interaction between the factors (Table 5, 7, and 8). 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

CCC, chlormequat; CF, conventional fertility management; ConA, concanavalin A; ConA-LP, concanavalin A-stimulated lymphocyte proliferation; CP, conventional crop protection; cpm, counts per minute; CRP, C-reactive protein; DSL, Diagnostic Systems Laboratories, Inc.; ELISA, enzyme-linked immunosorbent assay; FCR, feed conversion ratio; GH, growth hormone; GIT, gastro-intestinal tract; Hb, hemoglobin; IgA, immunoglobulin A; IGF-1, insulin-like growth factor 1; LPS, lipopolisaccharide; LPS-LP, lipopolisaccharide-stimulated lymphocyte proliferation; NaHCO3, sodium bicarbonate; NESC, Nafferton Factorial Systems Comparison; NH_4Cl, ammonium chloride; OF, organic fertility management; OP, organic crop protection; PCV, packed cell volume; RBC, red blood cells; RDA, redundancy analysis; RIA, radioimmunoassay; RPMI, Roswell Park Memorial Institute medium; SI, stimulation index; Sp-LP, spontaneous lymphocyte proliferation; WBC, white blood cell count

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