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Effect of organically and conventionally produced diets on jejunal gene expression in chickens

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Using a nutrigenomics approach we studied the response of second-generation chickens at a transcriptional level to organically grown feed ingredients compared with conventionally grown feed ingredients. Both diets consisted of the same amounts of ingredients, the only difference was the production method. Gene expression was analysed in jejuni using whole genome chicken cDNA arrays. After analysis, forty-nine genes were found to be differentially regulated between chickens fed on the different diets, independent of their genetic background. Of these forty-nine genes, seven genes were involved in cholesterol biosynthesis. Genes involved in cholesterol biosynthesis were higher expressed in jejuni from organically fed birds. Other genes found to be regulated were involved in immunological processes, such as B-G protein (part of chicken major histocompatibility complex), chemokine ah221, and the immunoglobulin heavy chain. Using quantitative PCR the effect of genetic background on the differential expression of genes was studied. Differences in gene expression existed between animals fed different diets as well as between different chicken lines. This indicated that diet and genetic background influence the transcriptional response of the jejunum. This is the first time that significant differences in gene expression were shown between animals on diets with organically or conventionally produced ingredients.

Organic feed: Diet: Nutrigenomics: Chickens

Organic food production is characterised by the absence or limited use of synthetic herbicides, pesticides, insecticides, growth regulators and livestock feed additives. In addition, only organic fertilisers are used such as animal or green manure, and long crop rotation is applied[1,2]. In recent years, many consumers have turned to organic foods, expecting organic products to be healthier than conventionally grown products. The composition of organically grown ingredients has been studied extensively[3,4]; however, studies on the effects of consumption of organic dietary components are limited and have not led to conclusive results concerning (beneficial) effects on health.

Nowadays, it is generally accepted that food consumption has an impact on gene expression, the metabolome and finally human health[5]. Recently, several publications described a relationship between diet (components) and gene expression that relates to difference in disease incidence. For example, the low incidence of certain cancers in the Mediterranean area was suggested to be caused by the Mediterranean diet[6]. Menendez *et al.* [7] showed that olive oil, one of the main ingredients of the Mediterranean diet, inhibits the expression of the human epidermal growth factor receptor 2 (HER2) oncogene. Inhibition of this gene does not only exert protective effects against the risk of breast cancer, but can also protect against further progression of disease. Another study demonstrated that rats fed on fresh broccoli for 1 month showed improved ventricular function of the heart and reduced myocardial infarct size[8]. Broccoli appeared to rescue the cardiomyocytes through regulation of gene expression that led to activation of the survival pathway. Due to novel techniques such as genomics, metabolomics and proteomics, the molecular responses to diets or dietary components can be studied on a whole-genome level, thereby providing insight into the complex interplay of diet and physiology[9]. Summarised, the literature describes that diet (components) influences disease status and physiology. However, the gene expression responsible for those influences is mainly unknown.

In the present study we describe a nutrigenomics approach to see whether second-generation chickens respond differently at a transcriptional level to identically composed diets, from either certified organically or conventionally grown feed ingredients. Gene expression is studied in the jejunum, since the gut is the first contact of diet (components) with the

Abbreviations: C, randomly bred chickens control line; H, chickens with a high antibody response; L, chickens with a low antibody response; qPCR, quantitative PCR; SREBP, sterol regulatory element binding protein.

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host, and the gut strongly influences the general condition of the host. It has been shown that maternal diet influences gene expression in the intestine of offspring, suggesting a role for epigenetic modification of the DNA[10]. Hollingsworth *et al.*[11] recently showed that a gestational diet indeed influenced allergic airway inflammation through epigenetic programming[11], confirming that diet can influence the immune status of both mother and child. Therefore, it was decided to feed two generations of chickens to maximise the effects. Mother hens and chickens were fed the described diets, adapted to their age. Gene expression was studied in the jejunum of second-generation chickens. The described study was part of a larger study, where different physiological parameters were measured and the feed was extensively analysed[12]. An overview of the experimental set-up and results is given elsewhere[13]. In our experiment forty-nine genes were found to be differentially expressed between the different diet groups. Several of these genes were involved in cholesterol biosynthesis. Using quantitative PCR (qPCR) the effect of genetic background on the differential expression of genes was studied. In summary, we show evidence that a diet from organically grown feed ingredients induces different genes in chicken jejunum tissue compared with a diet from conventionally grown feed ingredients. To our knowledge this is the first time that clear differences in gene expression are shown due to organically grown feed ingredients.

Materials and methods

Animal experiment

Institut de Sélection Animale (ISA) Brown Warren medium heavy layer hens were divergently selected for twenty-five generations on their primary antibody response to the multi-antigen sheep erythrocytes at 5 d after immunisation at age 35 d. Two selected chicken lines were established: chickens with a high antibody response (H) and chickens with a low antibody response (L). Also a control line of randomly bred chickens was included (C) resembling the parental stock of origin[14]. The first experimental generation consisted of seventy-one hens and twenty-two roosters that were housed in groups until age 8 weeks, after which they were housed individually. Until 11 weeks, chickens were fed normal commercial feed. From 11 weeks, the chickens were fed *ad libitum* either organically grown chickenfeed, or conventionally grown chickenfeed. Both feeds consisted of wheat, barley, triticale, peas, maize and soya from neighbouring farm pairs of conventional and certified organic farms with the same basic soil and climatic conditions and preferably the same variety of produce. Feed composition for the different age groups is summarised in Table 1. Via artificial insemination, the second generation was raised. Six groups of twenty-six second-generation chickens were formed: fifty-two H chickens, fifty-two L chickens and fifty-two C chickens that were fed *ad libitum* either organically or conventionally grown feed according to Table 1. In contrast to the first generation, the second generation was housed in groups of six animals, two hens from each line. The runs were spacey and enriched to secure optimal natural behaviour and physiological reactions of animals. The immune system of second-generation chickens was triggered by injecting keyhole limpet haemocyanin (KLH) at week 9. Animals were killed at week 13 by cervical dislocation. Tissue samples from several organs were taken and snap-frozen in liquid N_2. The animal experiment was approved by the ethical committee of Wageningen University, Wageningen, The Netherlands, in accordance with the Dutch law on animal experiments.

Table 1. Composition of chickenfeed

Ingredient (%)	Starter diet (0–6 weeks)	Grower diet (7–17 weeks)	Layer diet (from 18 weeks)
Maize	20	20	25
Wheat	30	26·42	25·23
Barley	5	10	5
Triticale	12·05	0	0
Soyabeans, heated	0	10·17	19·87
Soya flakes	10·16	20	0
Peas	10	10	10
Potato proteins	7	0	2·5
Monocalcium phosphate	1·13	0·73	1·01
Fx Layers Premix	1	1	1
Fat of plant origin	1·5	0	0·52
Salt	0·07	0·09	0·06
Chalk	1·64	1·16	7·65
Broken shells	0	0	2
$NaCO_3$	0·09	0·08	0
Methionine	0·11	0·04	0·15
Total	99·75	99·69	99·99

RNA isolation

RNA was isolated from the jejunum of individual chickens using the Trizol method as described by Van Hemert *et al.*[15]. Tissue was ground under liquid N_2 in a pestle and mortar. A small volume of ground tissue was dissolved in 1 ml Trizol (Invitrogen, Carlsbad, CA, USA), and homogenised. The RNA was extracted after the addition of 1/5 volume of chloroform. Subsequently, the RNA was precipitated with isopropanol, washed and dissolved in diethylpyrocarbonate-treated water. RNA concentration and quality were determined using the Nanodrop (Thermo Scientific, Waltham, MA, USA), as well as by gel electrophoresis. RNA (10 μg) from four to six individual chickens of the same line was pooled, after which RNA quality and quantity were checked again.

Hybridisation of microarrays

A quantity of 5 μg of each pooled RNA sample was labelled and hybridised using the Micromax TSA Labelling and Detection Kit (Perkin Elmer, Wellesley, MA, USA) according to the instructions of the manufacturer with modifications as described by Van Hemert *et al.*[15]. On each slide pooled RNA from animals fed organically was compared with pooled RNA from animals fed conventionally. Each sample was labelled twice, once with Cy3 and once with Cy5 (dye-swab). A single spotted chicken 20K oligo-array (ARK Genomics, Roslin, Midlothian, UK) was used. Hybridised microarrays were scanned using the Scanarray scanner and

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software (Perkin Elmer). Spot detection was done using GenePix Pro (Molecular Devices, Sunnyvale, CA, USA). A customised in-house developed R-based normalisation procedure was performed to fit the data[16]. Subsequently, data were analysed using significance analysis of microarrays[17]. Microarray data are available in the ArrayExpress database (www.ebi.ac.uk/arrayexpress) under accession number E-MEXP-1798.

Quantitative PCR analysis

cDNA was synthesised using a Superscript II transcriptase kit (Invitrogen) according to the manufacturer's instructions. Then, a quantity of 200 ng RNA from individual chickens was diluted 10-fold and 0·5 μg random hexamers were added. The reaction mixture was incubated at 70°C for 10 min. Quantities of 4 μl transcription buffer, 2 μl 0·1 M-dithiothreitol, 1 μl transcriptase, 1 μl deoxynucleotide triphosphates (dNTPs) (2 mM each), 1 μl RNAsin (Promega, Madison, WI, USA) and 8 μl water were added. The reaction mixture was incubated at 37°C for 50 min, followed by 70°C incubation for 10 min. Primers were designed using Primer Express 3.0 software for Real-Time PCR (Applied Biosystems, Foster City, CA, USA) based on the gene sequence that is represented by the oligonucleotide found to be regulated on the microarray. Primer sequences are listed in Table 2. cDNA (2 μl) or colony material was used in a PCR reaction mix containing 5 μl buffer, 1 μl Expand High Fidelity Taq polymerase (Roche, Basel, Switzerland), 1 μl dNTPs (10 mM each), 1 μl forward primer (10 μM), 1 μl reverse primer (10 μM), 1 μl $MgCl_2$ (2 mM) and 38 μl water. The PCR program was as follows: 96°C for 5 min, forty times (94°C for 1 min, 58°C for 1 min, 72°C for 30 s), 72°C for 7 min. PCR products were analysed on agarose gels. In the case of colony PCR, the reaction was started with 96°C for 10 min to lyse the bacteria. PCR products were purified from agarose gel using a QIAEX II gel extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Purified PCR products were cloned into TOPO4 using a TOPO® TA Cloning Kit for Sequencing (Invitrogen). Cloned fragments were transformed to *Escherichia coli* TOP10 cells (Invitrogen) according to the manufacturer's protocol. *E. coli* containing TOPO4 insert was grown in Luria–Bertani (LB) medium containing ampicillin (100 μg/ml) and kanamycin (50 μg/ml) overnight. Isolation of plasmid DNA was performed using the QIAprep Spin miniprep kit (QIAgen) according to the manufacturer's instructions.

cDNA was diluted ten times for qPCR analysis. Each reaction contained 12·5 pmol forward primer, 12·5 pmol reverse primer and POWR SYBR Green PCR Master Mix (Applied Biosystems) according to the manufacturer's instructions. qPCR was performed using an ABI7500 (Applied Biosystems). The amount of *28S* was measured to control for variation in RNA yield and RT reaction conditions. In each run a standard curve was incorporated consisting of a vector (TOPO4) containing the cloned gene fragment. In this way both the gene expression and the external control gene expression could be related to a standard curve. The efficiency of the PCR reaction was 90–100 % for all reactions (slope standard line between −3·3 and −3·6). The standard line consisted of 10-fold dilutions of the control vector. For each reaction negative water controls were included. Analysis was performed using the ABI7500 Software (Applied Biosystems). Statistical analysis on data was performed using independent Student's *t* tests.

Results

Gene expression in chicken intestine after two different diets

Feed analysis has shown that the energetic value of both feeds was similar (Table 3). Consistent differences existed in protein content, which was higher in conventional feed, whereas crude fat and ash contents were higher in organic grower and starter feed, respectively (Table 3). After 13 weeks, conventionally fed animals cumulatively consumed about 80 g more feed compared with organically fed animals (3686 g *v.* 3607 g). This difference in feed intake was statistically significant at age 12 and 13 weeks. There were differences in body weight of the animals. At hatching all animals showed similar weights between 32 and 35 g. At 13 weeks, in general, L-line animals gained most weight. Conventionally fed L-line animals reached a weight of 1209 ± 12 g and organically fed L-line animals reached 1209 (SD 21) g. H-line animals on the contrary were the lightest at the end of the experiment, where conventionally *v.* organically fed H-line animals reached 1050 (SD 15) and 1048 (SD 18) g. Only among C-line animals did significant differences exist in body weight between the diets: conventionally fed C-line animals reached 1241 (SD 27) g, whereas organically fed C-line animals reached an end weight of 1098 (SD 23) g. Extensive description of the analysis of the animal growth and feed intake is described elsewhere[13].

To analyse the effect of diet on gene expression, all three chicken lines (H, C and L) were analysed as one group, to

Table 2. Primer sequences

Gene name	Accession no.	Primer sequence
Hb α chain	AY016020	Forward: TGCCAACACAGAGGTGCAA Reverse: GGGTCTCGGCGCCATAC
Acetoacetyl-CoA synthase	NM_001006184	Forward: AGCTGCTGGCACTCCTGAA Reverse: TCCTCCACCTTCGGAATCC
Isopentenyl-diphosphate delta isomerase 2	XM_418561	Forward: TGTGCAGAAGGATGTAACGCTTA Reverse: CGAGGCTTTGTCTAGAAGTTGCT
28S	DQ018756	Forward: CAAGTCCTTCTGATCGAG Reverse: TCAACTTTCCCTTACGGTAC

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Table 3. Feed analysis

	Second generation			
	Starter		Grower	
Nutrient	Conventional	Organic	Conventional	Organic
Energy (kJ/kg)	14 882	14 729	15 102	15 231
Protein (g/kg)	164	151	199*	176
Crude fat, by acid hydrolysis (g/kg)	42	42	53	62*
Carbohydrates, total (g/kg)	620	624	574	585
Raw fibre (g/kg)	34	36	39	39
Moisture (g/kg)	123	119	120	122
Ash content (g/kg)	51	64†	54	55

* ≥ 10 % higher than the conventional or organic feed.
† ≥ 20 % higher than the conventional or organic feed.

minimise genetic background influence, with diet as the only variable. After data analysis, forty-nine genes were found to be significantly at least 3-fold regulated due to the different diets. Of those forty-nine genes, twenty-eight genes were expressed higher in chickens fed conventionally grown ingredients, whereas twenty-one genes were expressed higher in chickens fed organically grown ingredients. The false discovery rate of those genes was 6·6 %. The top fifteen genes of up- and down-regulated genes, containing the genes with the strongest fold induction, are listed in Table 4.

Quantitative PCR analysis of regulated genes in individual chickens of different chicken lines

To study the effect of genetic background on gene expression, expression of three diet-induced genes was studied in individual animals of different genetic background using qPCR. One gene that was higher expressed in chickens fed on conventionally grown feed ingredients was selected (Hb α chain) and two genes that were higher expressed in chickens fed on organically grown feed ingredients were selected (acetoacetyl CoA synthase and isopentenyl-diphosphate delta isomerase 2). Data were analysed using two parameters: diet (organically fed *v.* conventionally fed) and genetic background (H-, C- and L-lines). Figure 1 shows that differences in gene expression between chicken lines as well as between diets were found. The unselected C-line animals showed gene expression patterns similar to the results of the microarray. However, animals from the selected lines (H- and L-lines) showed different gene expression patterns. The H-line animals showed a higher expression of acetoacetyl-CoA synthase and isopentenyl-diphosphate delta isomerase 2 in organically fed animals, which is comparable with the microarray results. L-line animals on the other hand showed a higher expression of acetoacetyl-CoA synthase in conventionally fed animals, whereas isopentenyl-diphosphate delta isomerase 2 expression was not regulated at all. Hb α chain expression was found to be higher in conventionally fed animals of the C-line and L-line, but in H-line animals Hb α chain expression was higher in organically fed animals.

Discussion

In the present study we demonstrated transcriptional differences between the jejunum of chickens fed on two diets, identically composed out of organically grown or conventionally grown feed ingredients. Forty-nine genes were differentially expressed at least three-fold between chickens on the different diets. Of those forty-nine genes, seven genes were directly or indirectly involved in cholesterol biosynthesis. qPCR analysis revealed differences in the way genes are regulated between the different chicken lines. Thus feed regulates gene expression independently of genetic background of the chickens, but the genetic background influences to what extent feed regulates gene expression.

Table 4. Genes that are regulated at least 3-fold in the jejunum of chickens fed conventionally grown feed ingredients compared with chickens fed organically grown feed ingredients independent of genetic background*

Homology	Fold induction	*q* value
Hb α chain	4·8	6·6
CCLi10	4·6	6·6
No homology	4·6	6·6
Chemokine ah221	4·2	6·6
Genome Hit Contig 1336.1	4·2	6·6
NDR-2 (weakly similar)	3·9	6·6
Early response to neural induction	3·9	6·6
Nuclear receptor (NroB2)	3·8	6·6
Insig-1	3·7	6·6
Immunoglobulin heavy chain	3·6	6·6
F-Box/IRR repeat protein 3A	3·6	6·6
Cytochrome P450	3·6	6·6
HGFL	3·5	6·6
Thrombospondin receptor (CD36)	3·5	6·6
No homology	3·4	6·6
Soluble carrier family 1	−3·2	6·6
α2-Macroglobulin precursor α2	−3·3	6·6
Genome Hit Contig 190.26	−3·3	6·6
No homology	−3·3	6·6
No homology	−3·5	6·6
No homology	−3·6	6·6
No homology	−3·9	6·6
Hydroxysteroid (17β) dehydroxygenase	−4·8	6·6
Hypothetical protein	−4·9	6·6
B-G protein precursor/MHC 3-G antigen	−5·1	6·6
C4 methyl sterol oxidase	−5·2	6·6
Isopentenyl-diphosphate delta isomerase 2	−5·4	6·6
Acetoacetyl-CoA synthase	−5·6	6·6
Squalene mono-oxygenase	−9·3	6·6
Genome Hit Contig 41.179	−10·9	6·6

NDR, Nodal-related; Insig-1, insulin-induced gene 1; HGFL, hepatocyte growth factor-like protein; MHC, major histocompatibility complex.
* The top thirty regulated genes out of forty-nine are shown.

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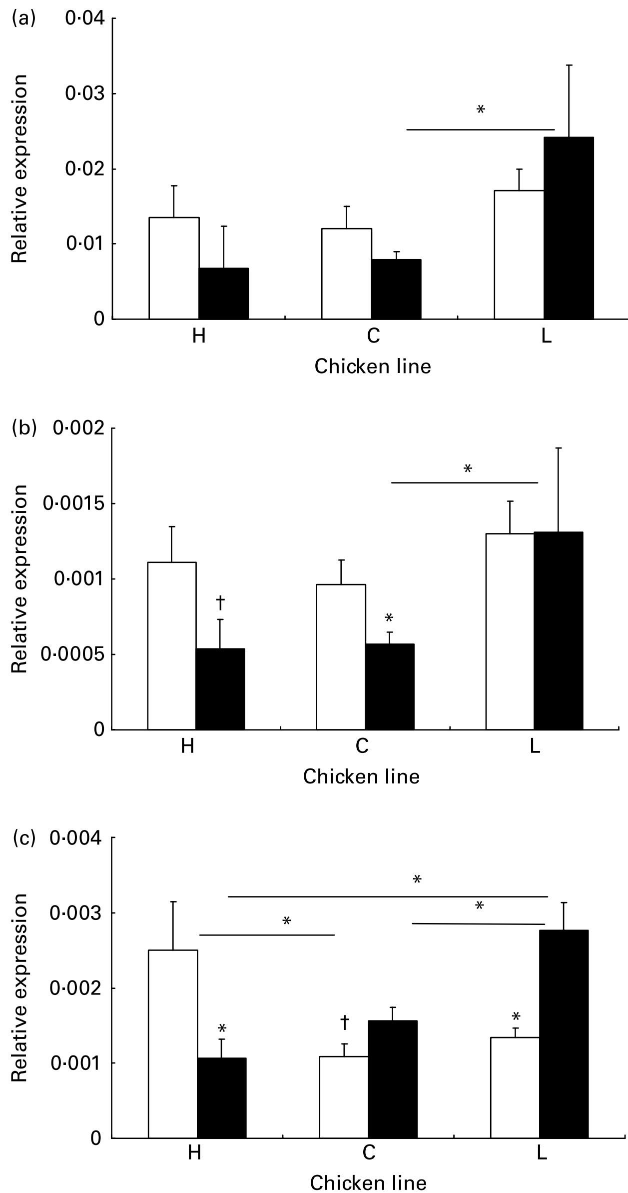

Fig. 1. Effect of genetic background on diet-induced gene expression studied by quantitative PCR on three diet-regulated genes: acetoacetyl-CoA synthase (a), isopentenyl-diphosphate delta isomarase 2 (b) and Hb α chain (c). Three chicken lines were included: H-line chickens with a high specific antibody (agglutinin) response to sheep erythrocytes; L-line chickens with a low specific antibody (agglutinin) response to sheep erythrocytes; C-line control animals of randomly bred chickens. (□), Chickens fed on organically grown feed ingredients; (■), chickens fed on conventionally grown feed ingredients. Values are means of four to six chickens, with standard errors represented by vertical bars. * $P<0·05$; † $P<0·1$.

Our microarray data yielded forty-nine regulated genes between chickens fed on the different diets with a false discovery rate of 6·6 %. Compared with other microarray experiments this is a low number of regulated genes with a low statistical power. The clustering of regulated genes in the pathway of cholesterol biosynthesis strengthens the power of our microarrays. However, considering the fact that both chicken groups are healthy chickens in good condition, fed on the same feed ingredients, this small difference in gene expression was expected. It can be debated how comparable both feeds actually were. Although it was attempted to compose diets of the same ingredients, with the same energetic value, it is clear that differences existed between the feeds as shown in Table 2. In the present study, it was decided to collect ingredients from neighbouring conventional and organic farms. Since both agricultural systems have their own varieties suitable for their specific system, it had to be accepted that different varieties were used[18–20]. Besides, it is known in both conventional and organic farming that large differences exist between farms. Still, this approach was chosen because the full system of either conventional or organic farming is represented, both systems using their own specialised variables. Differences in gene expression can thus be attributed to differences between farming systems.

Seven genes that were differentially regulated between the two feed groups, independent of genetic background, are directly or indirectly involved in cholesterol biosynthesis. Figure 2 shows a schematic representation of cholesterol

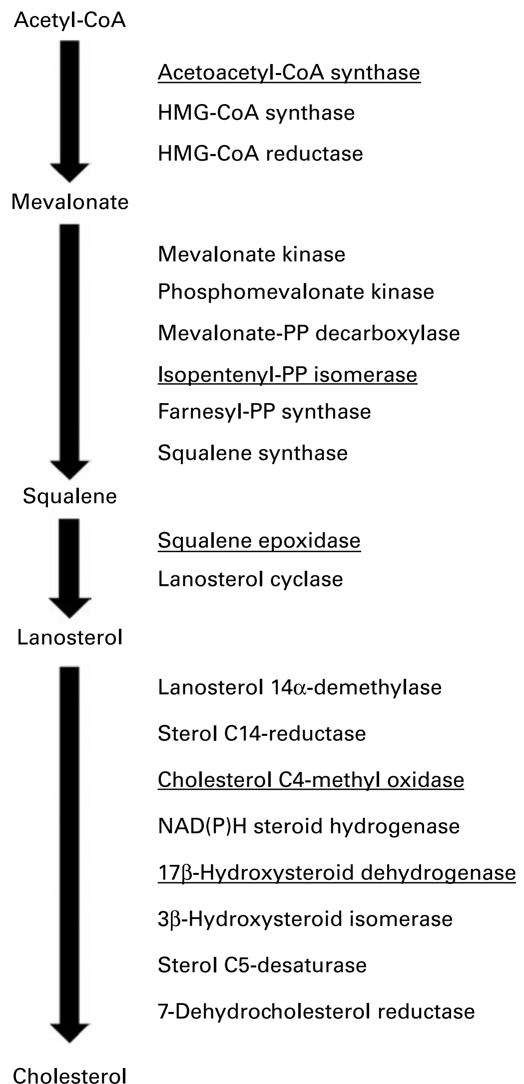

Fig. 2. Schematic representation of cholesterol biosynthesis freely adapted from Espenshade & Hughes[21]. Underlined genes were found to be lower expressed in chickens fed on a diet of conventional ingredients. HMG, hydroxy-3-methylglutaryl; PP, diphosphate.

biosynthesis. Five genes directly involved in this pathway were found to be lower expressed in the chickens fed on conventional ingredients (underlined in Fig. 2). Two other regulated genes were indirectly involved in cholesterol synthesis. Insulin induced gene 1 (*insig-1*) and P450 were both higher expressed in chickens fed conventionally. Insig-1 is a key regulator in cholesterol synthesis that forms a complex with sterol regulatory element binding protein (SREBP) and SREBP-cleavage activating protein (SCAP) in the presence of cholesterol. When this complex of SREBP–SCAP–Insig-1 exists, SREBP is repressed in its transcription activation of cholesterol synthesis (for a review, see Espenshade & Hughes[21]). Overexpression of *insig-1* will thus repress cholesterol synthesis. P450 is directly involved in steroid synthesis, but also acts as a negative feedback mechanism to shut down cholesterol biosynthesis (for a review, see Espenshade & Hughes[21]). Up-regulation of *p450* will therefore result in down-regulated cholesterol synthesis. In conclusion, regulation of these seven genes involved in cholesterol biosynthesis led to less cholesterol synthesis in the jejunum of chickens on the conventionally grown feed.

Cholesterol synthesis is tightly regulated by several factors. The strongest regulator of cholesterol biosynthesis is circulating blood cholesterol itself. However, no differences were found in circulating cholesterol levels between animals from the two diet groups[13]. Feed analysis revealed that the organically grown diet contained more crude fat compared with the conventional grower diet (Table 2). This difference in constitution between the two diets could lead to differences in cholesterol biosynthesis. Conventional feed on the other hand contained slightly more calculated phytosterols compared with organic feed (data not shown). A correlation exists between phytosterols and cholesterol metabolism that among others acts through the SREBP pathway[22]. Phytosterols lead to lower cholesterol levels, so the differential regulation of the cholesterol biosynthesis pathway could also be attributed to this difference in feed composition. All these factors together might explain the differences observed in cholesterol synthesis.

Although the genes in the cholesterol pathway are higher expressed in the organically fed chickens, these chickens had lower body weight. We do not know, however, if the end-product of the cholesterol pathway is cholesterol or, for example, steroid hormones. Therefore it is hard to relate body weight to the observed differential gene expression.

Taken together, microarray results showed that cholesterol synthesis is differentially regulated between the two feed groups. The exact trigger for this regulation is unknown.

Three other diet-regulated genes seem to be involved in immunological functions: chemokine ah221, B-G protein precursor and immunoglobulin heavy chain. Chemokine ah221 (homologous to human macrophage inflammatory protein α1; MIP-A1) is higher expressed in conventionally fed chickens. This chemokine is involved in innate immunity and promotes chemotaxis of T lymphocytes. B-G protein precursor is higher expressed in organically fed chickens. B-G protein precursor is part of the major histocompatibility complex of the chicken, and is strongly correlated with disease resistance in chickens[23]. Immunoglobulin heavy chain is higher expressed in conventionally fed chickens. Although one would expect the immunoglobulin light chain to be higher expressed as well, only little is known of B-cell development in the avian gut. Therefore, it is hard to interpret overexpression of just the immunoglobulin heavy chain. The regulated expression of genes involved in immunity at least indicates that there are immunological differences between the different diet groups of chicks. This observation is confirmed by differences in specific and innate cellular and humoral immune responses in birds fed organically and conventionally grown feed, described elsewhere[13]. The synergistic relationship between diet and the immune system had been already described in the 1960s (for a review, see Scrimshaw[22]). Recently, the field of epigenetics seems to explain at least part of those dietary effects. *In utero* exposure to a methyl-rich diet can enhance the severity of allergy airway disease in the offspring through changed methylation of specific genes[11]. Since in the present study design, both maternal and offspring animals were fed the same diet, epigenetic changes in the genome of the offspring due to differences in diet of the mother hens cannot be excluded. The resulting changes in gene expression may therefore already have been induced in maternal animals and subsequently transferred to the offspring. To test this hypothesis, epigenetic studies on both generations are required. It is hard to predict if the differential expression of immunological genes might have an effect on disease resistance or health in either one of the groups. Further research including a challenge experiment with a pathogen, as well as connected clinical observations on the animals, are necessary to draw conclusions regarding the effects of these regulated genes.

To investigate the effect of genetic background on the genes that were regulated by diet, three independent chicken lines were separately investigated by qPCR. The chickens studied originated from lines that as a consequence of genetic selection differ in almost every aspect of innate as well as specific immune responsiveness[24–27]. Such lines enable estimation of advantageous or negative effects of diet and health risks with respect to genetic background. Gene expression of three differentially expressed genes, found by microarray analyses, that were diet dependent was analysed. qPCR analysis on individual chickens revealed that the three chicken lines used in the present study did not behave uniformly. Two out of three genes were regulated in all three lines. However, those genes were higher expressed in conventionally fed animals of the one line, whereas they were higher expressed in organically fed animals of the other line. Expression of the third gene was regulated in two out of three lines, but not regulated in the third line. These data show that besides dietary effects, the genetic background of chickens can also affect the transcriptional response to diet (components).

In the present study we describe that there are transcriptional differences in the jejunum of chickens that were fed different diets. Forty-nine genes were differentially regulated between chickens fed a diet from organically grown feed ingredients, compared with a diet from conventionally grown ingredients. Although differences in mRNA expression levels are not necessarily correlated to protein expression levels or physiological effects, it is the first time that significant differences in gene expression were shown between animals on identically composed diets from conventional and organic origin. Based on our data it is impossible to predict

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the implication of those differences, let alone decide which diet is more healthy or beneficial for the chickens.

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A. R. initiated the project at the Central Veterinary Institute and revised the manuscript. A. G. conducted and analysed the microarray experiments and drafted the manuscript. W. S. assisted with the animal experiment and conducted qPCR analyses. H. P. genetically selected the chicken lines that were used in the present study. L. V. described the feed analysis. M. H. is the project leader of the project 'Organic, More Healthy?', and assigned part of this project to the Central Veterinary Institute.

The authors state that there are no conflicts of interest.

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