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Chronic dietary exposure to a glyphosate-based herbicide in broiler hens has long-term impacts on the progeny metabolism.

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Short title: **Effects of hen exposure to glyphosate-based herbicides on the offspring**

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ABSTRACT

Glyphosate-based herbicides (GBH) are the most commonly used herbicides in agriculture. Several studies reported possible adverse effects on human and animal models after a GBH exposure. However, the effects of a temporary maternal exposure on the progeny have been poorly documented, especially in avian models. We investigated the effects of a hen chronic dietary exposure to a GBH on the progeny, obtained during the period following the withdrawal of GBH from the diet. Hens were exposed to a GBH via their food for 6 weeks, after which the GBH was removed from their food. Eggs from these hens were collected 3 weeks after the GBH was withdrawn for one week. We monitored the growth performances, metabolic parameters and behavior from the progeny of the hens (Ex-GBH chicks, n = 186) and compared them with those of unexposed control-hen progeny (CT chicks, n = 213). Ex-GBH chicks were more likely to explore their new environment than CT chicks during the open field test. In addition, they had an increased fattening and blood triglycerides level, while their food consumption was similar to CT-chicks. Quantitative PCR on the chemerin system and *FASN* in chicks livers indicate a transcriptional activity in favor of fatty acid synthesis, and lipidomic analysis on chicks abdominal adipose tissue reveal a global increase in monounsaturated fatty acid and a global decrease in polyunsaturated fatty acids. Seven genes involved in the synthesis of fatty acids were identified with the open access LIPIDMAP software, and their disturbance in Ex-GBH chicks was confirmed via qPCR. Taken together, these results suggest that the progeny of hens temporarily exposed to a GBH are more likely to fatten, even with a balanced diet. The removal of GBH from their contaminated environment would therefore not be sufficient to completely restore their health, has it could induce transgenerational effects.

Introduction

Glyphosate Based Herbicides (GBH) are commonly used in conventional agriculture and are spread on all types of crops. They are non-specific herbicides mainly composed of 36–48 % of Glyphosate (Gly), water, salt, and other coformulants such as polyoxyethylene tallow amine (POEA), a hydrophilic ethoxylated alkylamine, and of contaminants such as heavy metals or polycyclic aromatic hydrocarbons (PAHs), and quaternary ammonium (Defarge et al., 2018; Mesnage et al., 2019). Gly acts on the shikimate signaling pathway, which produces aromatic amino acids in plants and in some microorganisms only (Schönbrunn et al., 2001). In plants and animals, Gly is metabolized into CO_2 and aminomethylphosphonic acid (AMPA) by the enzyme glyphosate oxidoreductase (Mesnage et al., 2015). After its use in fields, Gly can be still found in soil and water, where its half-life is from 2 to 197 days and 91 days, respectively (Muñoz et al., 2021). Since animals do not use the shikimate pathway to produce amino acids, Gly is not supposed to have any adverse effect on their health. However, several studies have shown that GBH formulations can induce tissue damage (Jasper et al., 2012; Larsen et al., 2014), act as endocrine disruptors in various models (Walsh et al., 2000; Romano et al., 2010; Gill et al., 2018), and induce developmental issues in rats brain (Cattani et al., 2021). The use of GBH is therefore very controversial, since scientific organizations have drawn contrasting conclusions about its dangerousness and recent studies have shown that human populations are widely exposed to it (Grau et al., 2022).

Recent studies have been performed on poultry, because these animals are easily exposed to GBHs through their diet. Long-term exposure to GBH, with a concentration of Gly below to the one that causes observable adverse effects (NOAEL; 100 mg/kg/ body weight/day (European Food Safety Authority (EFSA))) in males Japanese quails showed that Gly and AMPA were found within the liver and reduced the plasma concentrations of testosterone at puberty (Ruuskanen et al., 2020a). Moreover, GBH seem to have epigenetic effects, with long terms effects on the offspring when the parents are exposed (Ruuskanen et al., 2020b). This last study highlighted that Gly residues were found within the eggs and that GBH caused both lipid damages within the brains of embryos and a reduced embryonic development. Studies about the direct *in ovo* exposure of chicken embryos to GBH reported that this herbicide had negative impacts on embryo development. Indeed, results showed that GBHs induced embryonic mortality (Szabó et al., 2018), reduced the hatching rate (Fathi et al., 2019), disrupted cytochrome P450 enzymes within the liver and the small intestine (Fathi et al., 2020) and increased reactive oxygen species (ROS) production (Fathi et al., 2019, 2020). An observational study performed in Denmark in breeding companies of broiler breeder egg production highlighted a negative association between residues of Gly commonly found in food for adults and egg hatchability. However, no association was found between residues of Gly in the food and the laying rate (Foldager et al., 2021). Results from our laboratory demonstrated that chickens exposed to GBH through their diet (46.8 $mg.kg^{-1}.day^{-1}$ glyphosate) were not affected in terms of behavior or metabolism, for both males and females (Serra et al., 2021; Estienne et al., 2022). However, males exhibited a reduced sperm motility after 5 weeks of exposure in association with higher plasma testosterone and estradiol levels (Serra et al., 2021). For females exposed to GBH at a concentration similar to males, we noticed an accumulation of Gly within the egg yolk, resulting in severe early embryonic mortality and a delayed embryonic development in survivors that were abolished two weeks after the end of GBH exposure (Estienne et al., 2022). For the same animals, we also showed possible disturbances of the cecal microbiota associated with plasma oxidative stress and accumulation of glyphosate in metabolic tissues in response to dietary GBH exposure. Luckily, this concentration of GBH did not impair growth and most of these effects on the phenotypes were reversible (Fréville et al., 2022).

We decided to go further in our investigations by analyzing the putative long terms effects of maternal GBH exposure on the progeny. Indeed, in Serra et al. (2021) we demonstrated that chicks obtained from rooster exposed to GBH had a higher food consumption, body weight, subcutaneous adipose tissue content and epigenome modifications. Also, the animals had higher plasma chemerin levels in their blood plasma, which could indicate reduced fattening processes, since it has been shown that chemerin is negatively correlated with fattening in hens (Estienne et al., 2020). In our study in female, all chicks died during the female exposure to GBH (Estienne et al., 2022). Therefore, the aim of the present study was to investigate the impact of GBH on the progeny obtained during the period following the withdrawal of GBH from the diet, when the embryo mortality within the Ex-GBH group was similar to the control group. The aim of this study was to determine if maternal GBH exposure had long-term effects on the progeny, even after the exposure arrest. For that, we proceeded to artificial inseminations of hens two weeks after the GBH exposure arrest and collected the eggs during the 4th week following the withdrawal. Eggs were then put into artificial incubation and chicks obtained from these eggs were studied until day 10 (Day 10) of age to check their behavior and metabolism.

Material and methods

Ethical issues

All experiments were approved by the Ethics Committee in Animal Experimentation of Val de Loire (CEEA Vdl) (APAFIS number 21549-2019071809504554v3). The CEEA vdl is registered by the National Committee ‘Comité National de Réflexion Ethique sur l’Expérimentation Animale’ under the number 19. All experiments were performed in accordance with the European Communities Council Directive 2010/63/UE. This study is reported in accordance with ARRIVE guidelines.

Animals

All animals (150 female chicks and 10 male chicks of the commercial breed ROSS 308) were obtained at 1 day of age from a local hatchery (Boye Accouvage La Villonniere 79310 La Boissière en Gatine, France) and reared at "Pôle Expérimental Avicole de Tours" (INRAE, Nouzilly, France) until 32 weeks-old according to the traditional breeding conditions. At 32 weeks old, hens were divided into two homogenous groups: control (n = 75) and dietary GBH exposed (n = 75). For each group of hens, animals were distributed by 5 in 15 pens, each pen with an area of 3 m^2. Roosters (n = 10) were reared together. They were included in the study for sperm collection followed by artificial insemination. The design of the experiment is summarized in **Figure 1**. Seventy-five hens were exposed for 6 weeks to GBH via the food (GBH group, 46.8 mg/kg bw/day), and 75 hens were fed with a regular diet without GBH (noted control or CT) (week 1 to week 6 of the protocol). These weeks correspond to the egg laying peak period of the animals. After that, all animals (CT hens and ex-GBH hens) were fed with a regular diet (week 7 to week 11 of the protocol). At week 7, exposure to GBH was stopped. At the beginning of week 9, hens were artificially inseminated with sperm from control roosters that were fed with regular diet (not GBH exposed). This two-week delay between exposure stop and artificial insemination was set up into the protocol to allow the total elimination of Gly in eggs yolks as shown in Estienne et al., 2022. One week after the artificial insemination and for one week (week 10), eggs were collected. At the end of this week (week 10), eggs fertilized with the sperm of CT roosters were incubated for 21 days. Before and during the whole experiment, we did not observe any significant differences between CT and GBH (week 6: CT: 3762 ± 18.9 g and GBH: 3699 ± 18.9 g) and

CT and ex-GBH (week 11: CT: 3996 ± 20.5 g and GBH: 3920 ± 31.7 g) hens in terms of body weight. All animals were killed by electrical stunning and bled out, as recommended by the ethical committee.

Diet composition of adults

Hens (32 weeks-old) received a restricted laying diet according to Hendrix Genetics recommendation. They were fed with either GBH exposed feed (n = 75) or control feed (n = 75) from the first week of protocol to week 6 (**Figure 1**). The control feed contained low measurable Gly and AMPA concentrations (0.21 mg/kg feed for Gly and undetectable levels for AMPA as determined by Phytocontrol, Nimes, France) (Estienne, 2022). The concentration of Gly in the GBH feed was 1250 mg/kg feed for Gly and 0.30 mg/kg feed for AMPA, as determined by Phytocontrol. Gallup super 60, called GBH within the text, was obtained from Axereal (Monnaie, France); it contained 360 g/L Gly (485.8 g/L isopropylamine salt). Hens were food-restricted as recommended by Hendrix Genetics. Knowing that the hens weighed on average 3.45 kg over the exposure period and their food consumption was on average 130 g/day, the concentration in the feed thus corresponded to a dose of 47 mg Gly/kg body weight/day. The diet for the GBH group consists of regular broiler diet mixed with Gallup 360 in our laboratory in accordance with the directives of the "Directions Départementales de la Protection des Populations" (Departmental Directorate for the Protection of Populations). The mix was carried out by a technician with certification for the handling of phytosanitary products "Certiphyto", as recommended by the French law. The European Food Safety Authority (EFSA) has reported a NOAEL of 100 mg/kg body weight/day for poultry, with a maximum residue level (MRL) of 2.28 mg/kg bw/d (European Food Safety Authority (EFSA), 2018). Therefore, our experiment tested a concentration of approximately 47 % of the NOAEL threshold. From week 7 to week 11, all animals were fed with the control feed (CT n = 63 and ex-GBH n = 63).

Determination of Mortality, Food Consumption, Body and Different Organ Weights in Offspring

The chicks (n = 213 and 186 chicks from CT and Ex-GBH groups, respectively) were weighed at hatching (Day 0) as well as 5 and 10 days of age (Day 5 and Day 10). At hatching, chicks were divided into 10 pens (5 pens for 42/43 chicks from CT group and 5 pens for 37/38 chicks from Ex-GBH group). Each pen had almost the same number of male and female chicks. All animals were fed *ad libitum* with the same starting diet without GBH exposure. The amount of food was recorded at days 5 (Day 5) and 10 (Day 10) for each pen. Each day, the number of dead animals was recorded, and the mortality level was calculated from hatching to Day 10. At hatching as well as Day 5 and Day 10, 20 chicks (10 males and 10 females) from each group (from CT and Ex-GBH groups) were killed, and their organs or tissues (subcutaneous adipose tissue, brain, heart, liver and digestive tract) were dissected and weighed.

Behavior (Open field test)

Reactivity to a new environment was assessed in a square arena (80 cm ×80 cm × 29 cm) made of white wood with a floor made of yellow waterproof plastic surface under dim and dispersed light conditions when chicks were 1 days old. Chicks were carried individually in a room containing no other birds alternating between sex and treatment conditions. The room used for the test was at the same temperature than the home room (35°C). Each chick was placed in the center of the arena and allowed to freely explore for 6 min. At the end of the session, the arena was cleaned with water. A digital camera was mounted directly above the arena, capturing images at a rate of 5 Hz. The images were transmitted to a PC running the Ethovision tracking system (v XT8.0, Noldus Technology, Wageningen, The Netherlands). The locomotor activity (total distance travelled), the velocity, were obtained using the Ethovision tracking system. The total number of jumps and feces was also recorded manually by an experimenter blind to the sex or treatment.

Biological samples

Blood samples from chicks were collected after slaughtering into heparin tubes on different days during the experiment (Day 0, Day 5 and Day 10 after chicks' birth). Blood samples were centrifuged (5,000 × g for 10 min at 4° C) and stored at −20° C before use for assays. Tissue samples were obtained at the same moments during the experiment (Day 0: CT n = 20 and Ex-GBH n = 20, Day 5 : CT n = 10 and Ex-GBH n = 10, and Day 10 : CT n = 20 and Ex-GBH n = 20) by dissection after slaughtering.

Gene expression analysis

Total RNA from 10 CT and 10 Ex-GBH chicks at Day 0, Day 5 and Day 10 were extracted from chicks' liver and abdominal adipose tissue using TRIzol RNA Isolation Reagents and an Ultra-Turax instrument for grinding, according to the manufacturer's recommendations (Invitrogen by Life Technologies, Villebon-sur-Yvette, France). The purity and concentrations of the obtained RNA were checked via their A260/A280 ratios using a Nanodrop machine. cDNA were obtained by reverse transcription of 2 µg of the total RNA in 20 µL of a mix containing each deoxyribonucleotide triphosphate (dATP, dTTP, dGTP, dCTP; 0.5 mM), 2 M RT Buffer, 15 µg/µL oligodT, 0.125 U of ribonuclease inhibitor and 0.05 U of Moloney murine leukemia virus reverse transcriptase (MMLV); the mixture was kept for 1 h at 37°C. Quantitative PCR was performed using a mix of 3 µL of cDNA and 8 µL of SYBR Green Supermix 1X Reagent (Bio-Rad, Marnes-laCoquette, France) with 250 nM of specific primers (Invitrogen by Life Technologies, Villebon-sur-Yvette, France) given in **Table 1**. Samples were set up in duplicate in a 384-well plate and a MyiQ Cycle Device (Bio-Rad, Marnes-la-Coquette, France) was used to apply the following procedure: incubation (2 min at 50°C), denaturation (10 min at 95°C) and 40 PCR cycles (30 s at 95°C, 30 s at 60°C, 30 s at 72°C). Relative expression of genes was related to the geometric mean of the expression of three reference genes (GAPDH (glyceraldehyde-3-phosphate dehydrogenase), ACTB (actin B) and EEF1α (eukaryotic elongation factor 1 alpha)). For each target gene, expression was calculated according to primer efficiency (E) and quantification cycle (Cq), where expression = E – Cq. Then, relative expression of the target gene to the three reference genes was analyzed.

Plasma lipid and uric acid assays

Plasma concentrations of triglycerides, uric acid, phospholipids and cholesterol were determined by enzymatic assay using specific kits from Biolabo SAS (Maizy, France): triglycerides (reference: LP80519), uric acid (reference: 80,351), phospholipids (reference: 99,105) and cholesterol (reference: 80,106, Biolabo SAS, Maizy, France). The measurements were performed according to the manufacturer’s protocol. For all these assays, the inter and intra-assay coefficient variations were <15%.

Plasma chemerin assays

Chemerin concentrations were measured by Enzyme Linked Immunosorbent Assays (ELISA) using commercial kits [chicken chemerin: MBS738819 (sensitivity 0.1 ng/mL, MyBioSource, San Diego, USA)] according to the manufacturer’s instructions. All assays were performed in 96-well plates and absorbance was measured at 450 nm using a Microplate

Reader (Tecan, Magellan, Männedorf, Switzerland). A standard curve was drawn for the determination of hormone levels. The experiment was performed following the manufacturer's protocol with an intra-assay coefficient variation of < 15%.

Lipidomic analysis

Quantitative analysis of neutral lipids: (Free Cholesterol; Cholesterol ester C16, C18 and C20 :4 ; Triacylglycerols C49, C51, C53, C55, C57, C59) and total conventional fatty acids : (c10:0, c12:0, c14:0, c15:0, c16:0, c17:0, c18:0, c20:0, c22:0, c23:0, c24:0, c14:1w5, c15:1, c16:1w7, c18:1w9, c18:1w7, c20:1w9, c22:1w9, c24:1w9, c18:2w6, c18:3w6, c18:3w3, c20:2w6 , c20:3w3, c20:3w6, c20:4w6, c20:5w3, c22:2w6, c22:6w3, c22:4w6) was performed in abdominal adipose tissue from CT chicks at Day 5 (n=5) and Ex-GBH (n=5) by the lipidomic plateform (I2MC - MetaToul - Plateau Lipidomique, Toulouse, France).

Lipidomic metabolism

The lipidomic datasets were analysed by an openly available bioinformatics tools LIPIDMAP (https://lipidmaps.org/biopan) to produce a graphical representation of the results. BioPAN takes an input file in CSV (Comma-Separated Values) format containing quantitative data. The file structure should be as follows:

- The first row contains sample labels (e.g.: 'wild_type_1', 'wild_type_2', 'control_1', 'control_2'). The file must contain at least two conditions (e.g.: 'wild_type and 'control') and two samples per condition.
- The first column contains lipid molecular species. The lipid subclasses recognised by BioPAN are provided on the website.
- The columns 2, contain molecular concentration quantification.

After loading a dataset, LipidLynxX2 is launched in the background. LipidLynxX takes the input file and converts the nomenclature of the lipid molecular species to BioPAN nomenclature. It will also equalise the structure of the lipid molecular species to the Bulk level if it not already (e.g.: PE(34:1)). Within the graphical representation, the following information is presented:

1. Groups for comparison: interest condition and control condition. Choose the groups of samples that you want to compare.
2. Type: lipid and fatty acid. Visualise the fatty acid or lipid pathways.
3. Status 1: active, most active, suppressed and most suppressed. View the pathways defined as having the selected status. The nodes of the lipids involved are stained blue with a red circle and the edges of the reactions are gray.

To search for active pathways which have changed in the samples of interest compared to the control samples, BioPAN compute a Z-score for each weighed pathway. Those with higher scores will be classified as active. The most active and the most suppressed are the most active/suppressed pathways in the active/suppressed identified pathways. That is, when there are several pathways that start with the same reactions, only one will be retained. For each step of the pathway, the Z-scores of the reactions are compared and the one with the highest Z-score will have its pathway considered as the most active/suppressed.

Statistical Analysis

The GraphPad Prism® software (version 8) was used for all analyses. All data are reported as means ± standard error of mean (SEM). Bartlett's test was run to test the homogeneity of variance, and normal distribution was verified by the Shapiro–Wilk test. We performed one t-test or one-way analysis of variance (ANOVA) to compare the different means, when appropriate. When the ANOVA indicated significant effects at $p<0.05$, the means were analyzed by using the Fisher's test. Stars (*) correspond to the unpaired t-test significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

Results

Long terms effects of hen GBH exposure on the progeny behavior

After birth, chicks at Day 0 of age were tested with an open field test to check their reactivity to a new environment. Male chicks from the ex-GBH group exhibited a significant increased traveled distance (cm), velocity (cm/sec) and number of feces when compared to the male chicks obtained from fertilized eggs from the CT group. However, the number of jumps during the test remained equal between the CT and the Ex-GBH groups. For female, results were similar with also a significant higher number of feces produced during the test by chicks from the Ex-GBH group. Finally, male and female chicks were compared altogether, and results demonstrated that chicks from the Ex-GBH group exhibited a significant increased traveled distance (cm), velocity (cm/sec), number of jumps and number of feces when compared to the chicks from the CT group (**Table 2**).

Long terms effects of hen GBH exposure on the growth, mortality and food intake of the progeny

At Day 0, Day 5 and Day 10, all the chicks from the CT and the Ex-GBH groups were weighed and chicks from the Ex-GBH group exhibited a heavier body weight when compared to the CT group at Day 5 and Day 10. Moreover, during the same period, we measured the average quantity of food consumed per chick within the 5 CT pens and the 5 Ex-GBH pens and we noticed no differences between the food consumption between the two groups. Finally, we also checked the mortality rate within the two groups during the first 10 days of life and no significant results appeared between the two groups (**Table 3**).

Long terms effects of hen GBH exposure induce a fattening in the offsprings

At Day 0, Day 5 and Day 10, tissue samples were collected and weighed from respectively 20, 10 and 20 chicks (50% of males and 50% of females). We collected the vitelline vesicle, the liver, the brain, the digestive tract, the heart, the subcutaneous adipose tissue, and the abdominal adipose tissue (except at Day 0 because it was absent). The total body weight of the chicks was also measured. Results showed that at Day 0, chicks from the Ex-GBH group have more subcutaneous adipose tissue compared to chicks from the CT group but with a similar body weight between the two groups. At Day 5, chicks from the Ex-GBH group exhibited a significant less heavy liver, digestive tract and subcutaneous adipose tissue compared to the CT group. On the other hand, we noticed a significant increase of their abdominal adipose tissue compared to the CT group. At Day 10 of age, chicks from the Ex-GBH group exhibited a significant lighter digestive tract and heart but a heavier abdominal adipose tissue when compared to the chicks from the CT group (**Table 4**). We measured the blood plasma concentrations of triglycerides, phospholipids, uric acid and cholesterol. Results showed a significant increase of triglycerides concentrations within the blood plasma of chicks from the Ex-GBH group at Day 0, Day 5 and Day 10 (**Table 5**) whereas the other parameters remained similar for both groups at the same ages (**Table 5**). We also determined plasma chemerin concentration since we have previously shown that it was negatively associated to the fattening in hen. As shown in **Table 6**, plasma chemerin concentrations were significantly decreased in chicks from Ex-GBH group compared to CT group at Day 0, Day 5 and Day 10.

Lipidomic measures in abdominal adipose tissue in the offsprings

We then focused on the lipidomic metabolism of chicks at Day 5 from CT (n=5) and Ex-GBH (n=5) animals by measuring the relative abundance of several lipids within the abdominal adipose tissue. Results showed decreased amounts of pentadecylic, palmitic, palmitoleic, hexadecatrienoic, linoleic, alpha linoleic, eicosadienoic, eicosapentaenoic and docosapentaenoic acids within the abdominal adipose tissue of Ex-GBH chicks compared to CT chicks (**Figure 2**). The relative abundance of polyunsaturated and the ratio between PUFA/SAFA (PolyUnsaturated Fatty Acids/Saturated Fatty Acids) were also decreased within this group compared to the CT group. On the other hand, the relative abundance of monounsaturated and the ratios between MUFA/PUFA (MonoUnsaturated Fatty Acids/ PolyUnsaturated Fatty Acids) and MUFA/SAFA (MonoUnsaturated Fatty Acids/ SAturated Fatty Acids) were increased in the Ex-GBH group when compared to the CT group (**Figure 2**). For other lipids with non-significant differences, data are given in a table (**Supplemental data 1**). These results were analyzed by an openly available bioinformatics tools LIPIDMAP (https://lipidmaps.org/biopan) to produce a graphical representation of the results and to predict the relative expression of enzymes involved within the lipids synthesis pathway. The predicted pathway indicates an expected down expression of *ELOVL3* and *FADS2* genes and an over expression of *SCD1*, *ELOVL6*, *FADS1* and *ELOVL5* genes in Ex-GBH group when compared to CT group (**Figure 3**). To confirm these expected results, we proceeded to an analysis of these gene expression by RT-qPCR within the adipose tissue of chicks from both groups at the same age (Day 5). Expressional results confirmed the predicted data by BIOPAN, RT-qPCR results showing a sub-expression of *ELOVL3* and *FADS2* genes and an over-expression of *SCD1*, *ELOVL6*, *FADS1* and *ELOVL5* genes in Ex-GBH adipose tissue when compared to CT group (**Figure 4**). We next determined which of the following gene expression (*SCD1*, *ELOVL6*, *FADS1*, *ELOVL5, ELOVL3* and *FADS2*) was more associated with the amount of abdominal fat tissue. Once CT and Ex-GBH data were pooled, the

Pearson correlation coefficient (r) between the amount of abdominal fat tissue and ELOVL3 ; FADS2 ; SCD1 ; ELOVL6, FADS1 and ELOVL5 gene expression was -0.58 (P=0.007), -0.58 (P=0.007), 0.59 (P=0.006) ; 0.72 (P=0.0003), 0.73 (P=0.0002), 0.68 (P=0.001), respectively. Thus, the correlation between the amount of abdominal fat content and FADS1 gene expression was the highest and most significant.

Expression of chemerin and chemerin receptors and Fatty Acid Synthase (FAS) in liver of the offsprings

Since lipogenesis takes place primarily in the liver and chemerin is a hepatokine, that is known to regulate lipid metabolism, we further investigated the chemerin system expression within the liver of Ex-GBH and CT chicks at Day 0, Day 5 and Day 10. It appeared that *chemerin* expression was higher within the liver of the CT chicks at Day 0 and Day 5 compared to the Ex-GBH group. For *CMKLR1* expression, its expression was also significantly increased within the liver of CT chicks at Day 10 only and results were similar for *CCRL2* expression. Finally, *GPR1* expression appeared significantly increased within the liver of the chicks from the Ex-GBH group (**Figure 5**). We also measured the expression of Fatty Acid Synthase (FASN) involved in the lipogenesis. Results showed *FASN* expressions were significantly lower and higher within the liver of Ex-GBH chicks at Day 5 and Day 10, respectively (**Figure 5**).

Discussion

In the present study, we showed for the first time that a maternal dietary exposure of a glyphosate-based herbicide (GBH) in chicken could affect the behavior and lipid metabolism of the offspring. These effects were associated to a variation of lipid composition in abdominal adipose tissue and some actors of lipid metabolism in liver including fatty acid synthase and chemerin system.

Indeed, behavioral measurements performed in chicks on Day 0 revealed that chicks from Ex-GBH group moved faster and farther than chicks from CT group. In the literature, GBH and other pesticides or endocrine disruptors have been already shown to alter the locomotion activity of the animals. In bees (Tetragonisca angustula), GBH increased the locomotion time (Sousa Prado et al., 2023). One explanation is that GBH like neonicotinoids, carbamates, pyrethroids, and organophosphates could alter sodium transport, compete with acetylcholine, and consequently collapse the nervous system causing poisoning symptoms such as hyperexcitation (Soderlund et al., 2002. In mice, in utero exposure to Mixture N1 (a mixture of phthalates, pesticides and bisphenol A) results in behavioral changes in adults, including an increase in distance moved in the Open field test (Repouskou et al., 2020). In our study, the Ex-GBH animals produced more feces than the CT group. Active animals are considered to be more emotionally reactive than inactive animals (Pelhaitre et al., 2012). In other words, animals from Ex-GBH group would feel less fear in response to a new environment than CT animals. A previous study suggested that GBHs could increase anxiety in mice, by triggering dysbiosis in their gut microbiome (Aitbali et al., 2018). In Fréville et al. (2022), we showed disturbances of the cecal microbiome in exposed and formerly exposed hens. We also showed that these disturbances correlate with a reduction in short-chain fatty acids levels, and that this reduction could possibly induce mental disorders and depressive-like behavior (Cattani et al., 2017; Silva et al., 2020). However, here, we observed what rather looks like a reduction in anxiety. Another mechanism would therefore better explain our observations.

We also observed an increase in body weight at Day 5 and Day 10 in Ex-GBH chicks, which was not explained by an increase in the food intake by the chicks. Still, Ex-GBH chicks exhibited a heavier abdominal adipose tissue, and this was coherent with a higher triglycerids concentration in their blood plasma from Day 0 to Day 10. A study on cockerels brought similar results after dietary administration of 125 mg/kg/day of glyphosate for 15, 30 and 45 days (Hussain, 2019). The lipidomic profile of a chicken may depend on several factors, including the diet. During fattening, chicks accumulate an increased amount of triglycerides and fatty acids in their body (hence the increased amount of triglycerides in chicks plasma), which can be stored in fatty tissue. This can lead to an increase in the saturated fatty acid and unsaturated fatty acid content of the chick's body fat. In hens, the fatty acid profile is greatly influenced by the one from the diet, to which it tends to look like. Moreover, polyunsaturated fatty acids (PUFA) mainly originate from the alimentation (Villaverde et al., 2006). In our case, CT and Ex-GBH animals received the very same diet, of which they ate similar amounts each day. Lipidomic analysis of chicks abdominal adipose tissue reveals that monounsaturated fatty acids abundance is globally increased and polyunsaturated fatty acids abundance is globally decreased in Ex-GBH animals. It is well known that when PUFA are supplemented in the diet, there is decreased abdominal fat (Crespo and Esteve-Garcia, 2001) compared to supplementation with saturated or monounsaturated fats. These data may be explained by results from studies suggesting that PUFA suppress fat synthesis in birds (Sanz et al., 2000). Even if the increase in fat in adipose tissue is mainly due to hepatic lipogenesis in chicken, we can suggest that the higher MUFA/PUFA ratio could promote fattening in Ex-GBH animals by reducing fat synthesis.

A recent study performed on human serum reports disturbances of the lipidomic profile following to an occupational exposure to glyphosate leading to potential severe health effects (Zhang et al., 2023). Beside the effect for the health of the chick itself, a change in fatty acid content in farm animals must be taken seriously, as the relative amount of saturated, mono and polyunsaturated fatty acids must range in the proper interval for a safe and healthy human consumption (Milićević et al., 2014). Open-source software LIPIDMAP allows to make predictions on the expression levels of enzymes involved in the synthesis of fatty acids. Several enzymes were predicted to be affected (ELOV3 and FADS2 were predicted to be downregulated, and FADS1, SCD1, ELOVL2, ELOVL1, 2, 3, 5 and 6 were predicted to be upregulated), which was confirmed by the qPCR analysis on chicks abdominal adipose tissue. After Pearson correlation analyses, we showed that correlation between the amount of abdominal fat content and FADS1 gene expression was the most significant.

Considering that the moment when the eggs were collected is far enough from the parental exposure for the eggs to be considered non-contaminated by glyphosate residues (Estienne et al., 2022), we make the assumption that the regulation of these enzymes is done by a transgenerational mechanism such as an epigenetic reprogramming, which is known for being a potential link between the exposure to environmental pollutants and metabolic diseases (Lian et al., 2023). Moreover, a previous study from our laboratory showed epigenetic modifications in GBH-exposed roosters spermatozoa (Serra et al., 2021). Interestingly, the progeny from these roosters also had higher growth performances and fattening than its unexposed control. Similar modifications could possibly therefore occur in female reproductive cells. The hypothesis that the absorption of fatty acids in the digestive tract would be modified by the parental exposure remains to be explored in future studies as well.

The accumulation of lipids could also be the result of a disruption of liver functions, as it has been reported that glyphosate exposure could lead to fatty liver diseases (Mesnage et al., 2017). We measured the mRNA expression of the chemerin system in chicks liver. Chemerin is an adipokine but also a hepatokine involved in several metabolic processes. In chicken, chemerin injections are negatively correlated with body weight (Estienne et al., 2021) and plasma chemerin is negatively associated to fattening in hen (Estienne et al., 2021). *Chemerin* mRNA level was reduced in Ex-GBH animals during Day 0 and Day 5, but not on Day 10. Its main receptor *CMKLR1* mRNA expression was reduced, just like *CCRL2* which is another receptor for chemerin. Therefore, it seems that the expression of the chemerin system is reduced in Ex-GBH animals which would explain their increase in body weight and fattening. However, the higher expression of *GPR1* mRNA in Ex-GBH animals during Day 10 does not support this hypothesis. We also measured a higher expression of *FASN* mRNA expression in Ex-GBH animals liver at Day 10, which is consistent with their higher adiposity, since FASN stimulates fatty acid synthesis (Anderson and Hammes, 1984). However, this was not true at Day 5 where FASN expression was lower in Ex-GBH animals suggesting that the activity of FASN could be increased in this condition. It therefore seems that several key regulators of fattening are affected by the maternal exposure to the GBH.

Conclusions

The present study demonstrates that a exposure to a GBH in hens has behavioral and metabolic outcomes on the progeny. We show that chicks whose the mothers are exposed to a GBH do not have the appropriate fear response to the discovery of a new environment. We also show that despite a normal alimentation, they gain more body weight and they are fatter than chicks whose parents would not have been exposed. Moreover, the fatty acids composition of their abdominal adipose tissue is disturbed, which could have bad consequences on their health as growing animals and later as adults.

Declaration of interest

None.

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Author contributions

AE, MF, OB, CR, LC, FC, PF and JD contributed to the overall approach and design of experiments. AE, MF and JD performed statistical data analysis. LC and FC contributed to the analyses of behavior. PG and MC took care of animals. AE, OB and CR performed RT-qPCR. AE, MF and JD wrote the manuscript. All authors critically revised the manuscript and approved the final version.

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Figure legends

Figure 1. Experimental design. The timeline is represented in weeks (W) and days (Day). One hundred fifty 32-weeks-old hens ROSS 308 were included in the study. Seventy-five GBH hens were exposed for 6 weeks to GBH via the food (46.8 mg/kg bw/d), and seventy-five CT hens were fed with a regular diet without GBH. Then GBH was removed from the diet and animals were followed for 5 more weeks (W7 to W11) and named Ex-GBH group. At week 9, hens were artificially inseminated with sperm from control roosters. The next week and for 1 week (W10), eggs were collected daily and were artificially incubated for 21 days at the end of the week collection. At birth, all chicks (n = 213 for CT and n = 186 for Ex-GBH) were counted and weighed, sex was determined, and chicks from each group were slaughtered to collect samples and weigh organs at Day 0, Day 5 and Day10. At Day 0, some chicks were randomly selected to participate to an open field test to measure their reactivity to a new environment.

Figure 2. Lipidomic measures within the abdominal adipose tissue of chicks at Day 5 of age. The amount ofseveral lipids (µg/mg adipose tissue) were determined and results for thosewith significant differences between CT (n=5) and Ex-GBH (n=5) groups are presented as diagrams (black bars for CT chicks and grey bars for Ex-GBH chicks). Stars (*) correspond to the unpaired t-test significance ($p<0.05$) corresponding to the comparison between CT andEx-GBH chicks. * p<0;05; ** $p<0.01$. MUFA : MonoUnsaturated Fatty Acids; PUFA : PolyUnsaturated Fatty Acids; SAFA : SatUrated Fatty Acids.

Figure 3. Prediction of the level expression of enzymes within the pathway of synthesis of the lipids. The lipidomic datasets were analyzed by an openly available bioinformatics

tools LIPIDMAP (https://lipidmaps.org/biopan) to produce a graphical representation of the results. The figure is presented as the comparison of Ex-GBH data versus CT data. The genes in red are expected to be down regulated whereas the genes in green are expected to be over expressed in the Ex-GBH group.

Figure 4. Expression of enzymes involved within the lipidomic pathway. A total of 20 chicks were randomly selected at Day 5 within the CT (n=10) and the Ex-GBH (n=10) groups and were euthanized to collect samples of abdominal adipose tissue. A. *ELOVL3*, B. *FADS2*, C. *SCD1*, D. *ELOVL6*, E. *FADS1* and F. *ELOVL5* relative expression in the adipose tissue of chicks from the CT and the Ex-GBH groups quantified by RT-qPCR. Values are expressed as mean ± standard errors of means. * Indicates significant differences between the CT and Ex-GBH groups for unpaired t-test with significance at $p < 0.05$.

Figure 5. Expression of chemerin and chemerin receptors and Fatty Acid Synthase (FASN) in the liver of CT and Ex-GBH groups of chicks. A total of 20 chicks were randomly selected at Day 0, Day 5 and Day 10 within the CT and the Ex-GBH groups and were euthanized to collect samples of liver tissue. A. *Chemerin*, B. *CMKLR1*, C. *GPR1*, D. *CCRL2* and E. *FASN* relative expression in the liver tissue of chicks from the CT and the Ex-GBH groups (n=20) quantified by RT-qPCR. Values are expressed as mean ± standard errors of means. * Indicates significant differences between the CT and Ex-GBH groups for unpaired t-test with significance at $p < 0.05$.

Table 1. List of primers sequences used for qRT-PCR.

Gene	Forward	Reverse
CCRL2	CACGCAGTGTTTGCTTTAAAAGC	CAACAGCCCACGTGACAATG
Chemerin	CGCGTGGTGAAGGATGTG	CGACTGCTCCCTAAAGAGGAACT
CMKLR1	CGGTCAACGCCATTTGGT	GGGTAGGAAGATGTTGAAGGAA
ELOVL3	TGGAGCAGAGCTTCAACGAG	CGCAGCTTGTAGCCTCTCTT
ELOVL5	AGCTACCTGGATGTTTGGCT	TCCGCATGTACTTTGGTCCT
ELOVL6	CATGTCCGTGTTGACTTTGC	AGCACACCATGTCCTTGTAGG
FADS1	TTGGGATTACGCTGCTCTCC	CTTGGCTACCCGGATGTCAG
FADS2	GCGAGAAAGGAGAGGAGTCCG	TCAGGTTGTGCTTCTGGATCTC
FASN	AGCCAAGGTCATTCTTTC	TTTCAATGATCCAAATCCAGATA
GPR1	ACCTGCCTGAGGAAGAAGAA	AAAGGCCAGTGGAAGCCCAT
SCD1	GTTCTCCCGTGGGTTGATGT	ACCTTAGGGCTCAATGCCAC
GAPDH	ACGGATTTGGTCGTATTGGG	TGATTTTGGAGGATCTCGC
EEF1a	AGCAGACTTTGTGACCTTGCC	TCACATGAGACAGACGGTTGC
β-actin	ACGGAACCACAGTTTATCATC	GTCCCAGTCTTCAACTATACC

Table 2. Long terms effects of maternal GBH exposure on the progeny behavior. Results of the open field test to check the behavior of CT (n=7 for males and n=8 for female, total n=15) and Ex-GBH (n=7 for males and n=8 for female, total n=15) groups. The distance (cm), the velocity (cm/sec), the number of jumps and the number of feces were recorded. Values are expressed as mean ± standard errors of means.. The p value shown corresponds to the unpaired t-test significance (*p < 0.05; **p < 0.01; ***p < 0.001).

Behavior	**Males**			**Females**			**All**		
	CT (n = 7)	**Ex-GBH** (n = 7)	**p.value**	**CT** (n = 8)	**Ex-GBH** (n = 8)	**p.value**	**CT** (n = 15)	**Ex-GBH (n = 15)**	**p.value**
Distance (cm)	**1331±152.7**	**1890±132.9***	**P < 0.05**	**1199±114.5**	**1904±231.7***	**P < 0.05**	**1265±93.7**	**1897±129.1*****	**P < 0.001**
Velocity (cm/sec)	**4.43±0.51**	**6.30±0.44***	**P < 0.05**	**3.99±0.38**	**6.34±0.77***	**P < 0.05**	**4.21±0.31**	**6.32±0.43*****	**P < 0.001**
Jumps (n)	0.37±0.26	1.50±0.57	NS	**0.37±0.37**	**1.50±0.44***	**P < 0.05**	**0.50±0.22**	**1.81±0.36****	**P < 0.01**
Faeces (n)	**0.12±0.12**	**0.62±0.18**	**P < 0.05**	0.37±0.18	0.87±0.23	NS	**0.25±0.11**	**0.75±0.14***	**P < 0.05**

Table 3. Phenotypical measures on CT and Ex-GBH chicks during their 10 firsts days of growth. Chicks from the CT group (n=213) and from the Ex-GBH group (n=186) were weighed (g) at Day 0, Day 5 and Day 10. Animals from CT and Ex-GBH were divided into 5 pens. Their daily food consumption (g) was estimated at Day 5 and Day 10. The percentage of mortality was calculated during this period for both groups. Values are expressed as mean ± standard errors of means. The p value shown corresponds to the unpaired t-test significance (*p < 0.05).

Chicks weight (g)	**CT (n = 213)**	**Ex-GBH (n = 186)**	**p.value**
Day 0	44.19±0.21	44.57±0.22	NS
Day 5	**107.1±0.80**	**112.5±0.79***	**P < 0.05**
Day 10	**171.5±1.39**	**176.1±1.59***	**P < 0.05**
Food eaten (g)	**CT (n =5)**	**Ex-GBH (n =5)**	**p.value**
Day 5	2.67±0.30	2.38±0.11	NS
Day 10	2.74±0.39	2.38±0.08	NS
Mortality (%)	**CT**	**Ex-GBH**	**p.value**
	1.76±0.09	1.80±0.05	NS

Table 4. Phenotypical measures on CT and Ex-GBH chicks at Day 0, Day 5 and Day 10. Chicks were randomly selected at D0 (n=20 for the CT group and n=20 for the Ex-GBH group), 5 (n=10 for the CT group and n=10 for the Ex-GBH group) and 10 (n=20 for the CT group and n=20 for the Ex-GBH group) and were weighed and euthanized to collect their vitelline vesicle (only at Day 0), liver, brain, digestive tract, heart, subcutaneous adipose tissue and abdominal tissue (only at Day 5 and Day 10). Tissues were also weighed. Values are expressed as mean (Ratio organ weight/ total body weight (bw)) ± standard errors of means. The p value shown corresponds to the unpaired t-test significance (*p < 0.05; **p < 0.01; ****p < 0.0001). NA: Not detected.

	Day 0			**Day 5**			**Day 10**		
	CT (n = 20)	Ex-GBH (n = 20)	p.value	CT (n = 10)	Ex-GBH (n = 10)	p.value	CT (n = 20)	Ex-GBH (n = 20)	p.value
Chick (g)	44.79±0.49	44.94±0.45	NS	112.1±3.29	114.4±4.10	NS	168.3±4.89	179.4±5.02	NS
Vitelline vesicle/bw	6.46±0.29	5.93±0.28	NS	NA	NA		NA	NA	
Liver/bw	2.62±0.05	2.66±0.06	NS	**3.20±0.10**	**2.79±0.09***	**P < 0.01**	3.19±0.08	2.93±0.11	NS
Brain/bw	2.02±0.06	2.01±0.06	NS	1.04±0.04	0.99±0.04	NS	0.77±0.02	0.75±0.02	NS
Digestive tract/bw	12.22±0.24	12.22±0.14	NS	**24.44±0.80**	**22.20±0.64**	**P < 0.05**	**18.87±0.26**	**16.53±0.39******	**P < 0.0001**
Heart/bw	0.76±0.02	0.80±0.02	NS	0.75±0.04	0.65±0.05	NS	**0.69±0.02**	**0.62±0.02***	**P < 0.05**
Subcutaneous adipose tissue/bw	**0.44±0.06**	**0.68±0.07***	**P < 0.05**	**0.48±0.07**	**0.27±0.03***	**P < 0.05**	0.43±0.03	0.46±0.04	NS
Abdominal adipose tissue/bw	NA	NA		**0.24±0.02**	**0.49±0.06****	**P < 0.01**	**0.37±0.02**	**0.47±0.04***	**P < 0.05**

Table 5. Metabolites concentration within the blood plasma of chicks from the CT and the Ex-GBH groups. Chicks were randomly selected at Day 0, Day 5 and Day 10 (n=20 for the CT group and n=20 for the Ex-GBH group) to collect a sample of their blood that was used to measure triglycerides (g/L), phospholipids (g/L), uric acid (mg/L) and cholesterol (g/L) plasma concentrations. Values are expressed as mean ± standard errors of means. The p value shown corresponds to the unpaired t-test significance (****p < 0.0001).

Triglycerides (g/L)	CT (n = 20)	Ex-GBH (n = 20)	p.value
Day 0	**0.23±0.006**	**0.40±0.023******	**P < 0.0001**
Day 5	**0.28±0.012**	**0.42±0.026******	**P < 0.0001**
Day 10	**0.36±0.019**	**0.46±0.009******	**P < 0.0001**
Phospholipids (g/L)	CT (n = 20)	Ex-GBH (n = 20)	p.value
Day 0	0.93±0.04	0.93±0.06	NS
Day 5	0.79±0.01	0.85±0.04	NS
Day 10	0.94±0.03	0.89±0.03	NS
Uric acid (mg/L)	CT (n = 20)	Ex-GBH (n = 20)	p.value
Day 0	21.56±1.55	19.79±1.44	NS
Day 5	21.08±1.29	21.02±1.36	NS
Day 10	22.49±1.04	20.69±0.90	NS
Cholesterol (g/L)	CT (n = 20)	Ex-GBH (n = 20)	p.value
Day 0	0.78±0.03	0.76±0.23	NS
Day 5	0.36±0.01	0.38±0.01	NS
Day 10	0.39±0.01	0.41±0.01	NS

Table 6. Plasma chemerin concentrations (ng/mL) in chicks from the CT and the Ex-GBH groups. Chicks were randomly selected at Day 0, Day 5 and Day 10 of age (n=20 for the CT group and n=20 for the Ex-GBH group) to collect a sample of their blood that was used to measure chemerin. Values are expressed as mean ± standard errors of means. The p value shown corresponds to the unpaired t-test significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

Chemerin (ng/mL)			
	CT (n = 20)	Ex-GBH (n = 20)	p.value
Day 0	3.26 ±0.61	1.70±0.023***	$P < 0.001$
Day 5	15.42±2.87	8.33±1.94**	$P < 0.01$
Day 10	21.91±2.92	15.38±1.84*	$P < 0.05$

Supplemental data 1. Lipidomic measures within the abdominal adipose tissue of chicks at D5. The amountof several lipids were determined and results for those with no significant difference between CT and Ex-GBH groups are presented in this table as µg/mg adipose tissue. USFA: UnSaturated Fatty Acids; SAFA : SAturated Fatty Acids.

Lipids	**CT (n = 5)**	**Ex-GBH (n = 5)**	**p.value**
Myristic acid	2.3±0.4	1.3±0.4	NS
Margaric acid	0.6±0.07	0.5±0.04	NS
Stearic acid	32.7±4.2	28.8±1.5	NS
Arachidic acid	0.08±0.007	0.10±0.012	NS
Lignoceric acid	2.1±0.4	1.8±0.3	NS
Myristoleic acid	0.17±0.02	0.16±0.01	NS
Oleic acid (C18_1w7)	119.0±7.5	129.1±3.9	NS
Gadoleic acid	0.63±0.12	0.53±0.05	NS
Hexadecatrienoic acid (L_C16_3)	0.25±0.03	0.19±0.02	NS
Alpha linoleic acid (3w6)	0.57±0.09	0.46±0.05	NS
Eicosadienoic acid	0.72±0.04	0.59±0.08	NS
Dihomo-gamma-linolenic acid	0.80±0.08	0.62±0.04	NS
Arachidonic acid	4.4±0.6	3.6±0.3	NS
Adrenic acid	0.99±0.12	0.85±0.04	NS
Saturated	163.5±14.4	136.9±13.1	NS
Total	396.6±31.9	384.9±30.6	NS
USFA/SAFA	1.43±0.04	1.85±0.19	NS

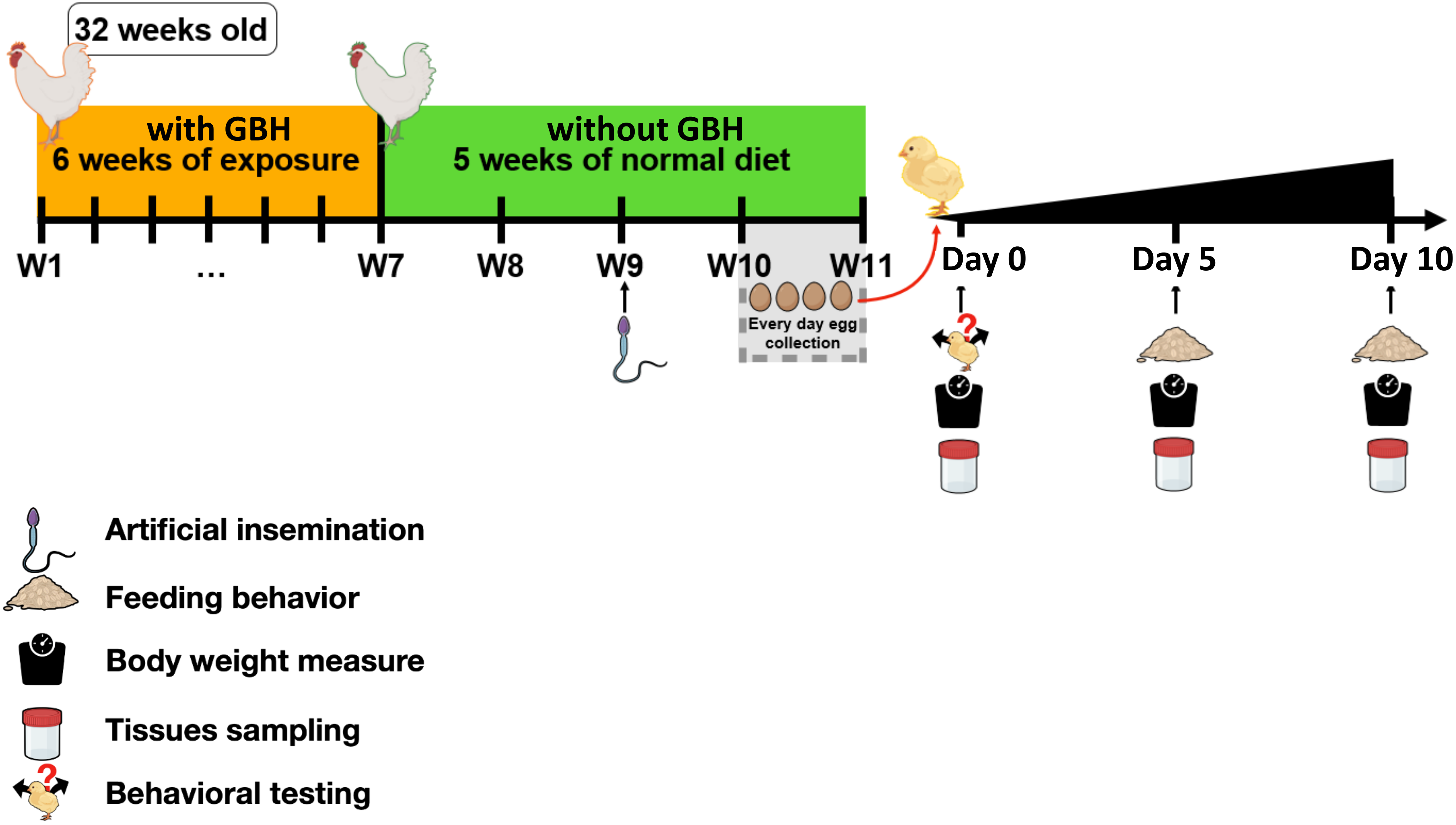
32 weeks old
with GBH
6 weeks of exposure
without GBH
5 weeks of normal diet
W1
…
W7
W8
W9
W10
W11
Every day egg collection
Day 0
Day 5
Day 10
Artificial insemination
Feeding behavior
Body weight measure
Tissues sampling
Behavioral testing

Figure 1

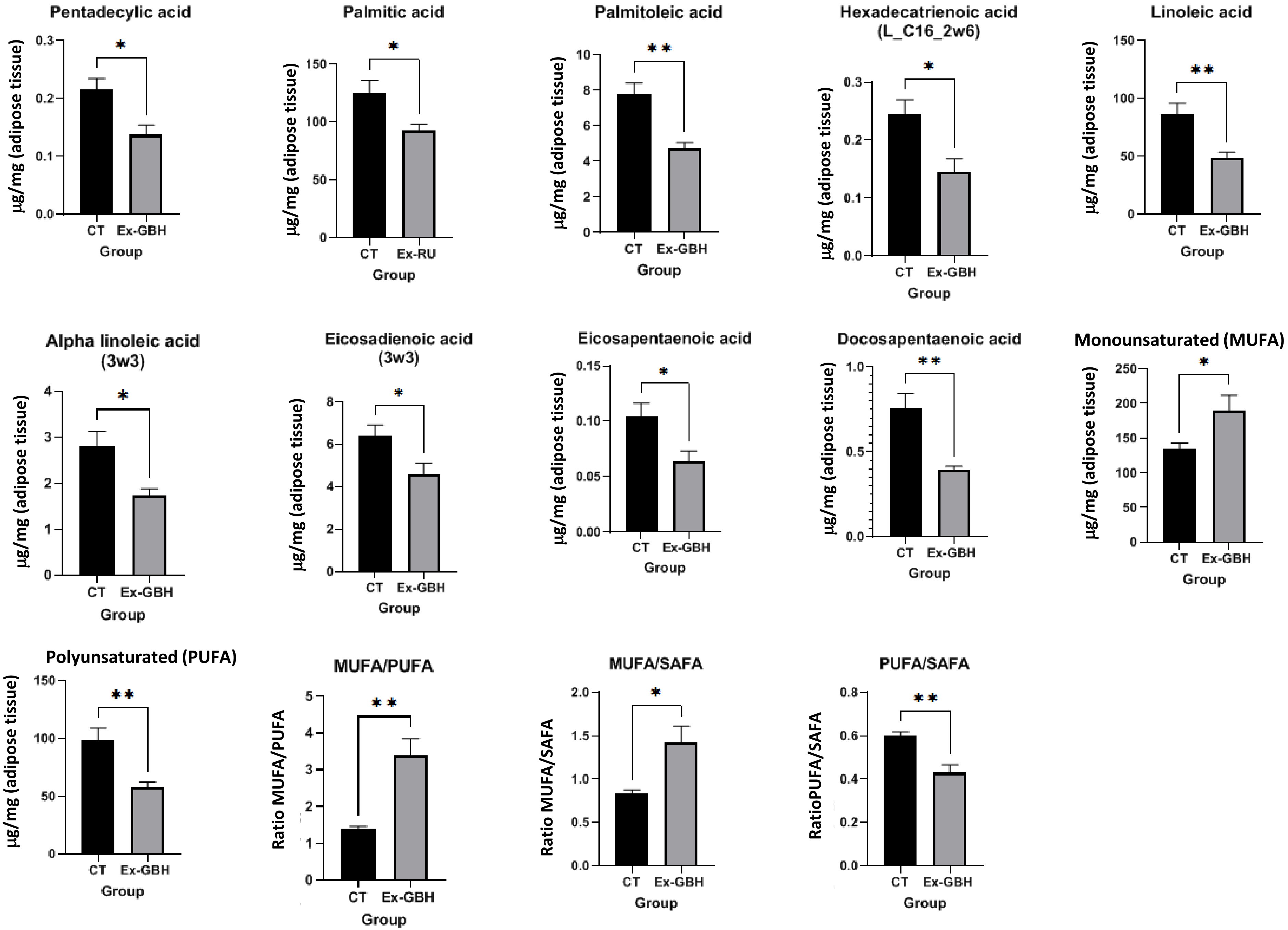
Pentadecylic acid
µg/mg (adipose tissue)
0.0
0.1
0.2
0.3
*
CT
Ex-GBH
Group
Palmitic acid
µg/mg (adipose tissue)
0
50
100
150
*
CT
Ex-RU
Group
Palmitoleic acid
µg/mg (adipose tissue)
0
2
4
6
8
10
**
CT
Ex-GBH
Group
Hexadecatrienoic acid (L_C16_2w6)
µg/mg (adipose tissue)
0.0
0.1
0.2
0.3
*
CT
Ex-GBH
Group
Linoleic acid
µg/mg (adipose tissue)
0
50
100
150
**
CT
Ex-GBH
Group
Alpha linoleic acid (3w3)
µg/mg (adipose tissue)
0
1
2
3
4
*
CT
Ex-GBH
Group
Eicosadienoic acid (3w3)
µg/mg (adipose tissue)
0
2
4
6
8
*
CT
Ex-GBH
Group
Eicosapentaenoic acid
µg/mg (adipose tissue)
0.00
0.05
0.10
0.15
*
CT
Ex-GBH
Group
Docosapentaenoic acid
µg/mg (adipose tissue)
0.0
0.5
1.0
**
CT
Ex-GBH
Group
Monounsaturated (MUFA)
µg/mg (adipose tissue)
0
50
100
150
200
250
*
CT
Ex-GBH
Group
Polyunsaturated (PUFA)
µg/mg (adipose tissue)
0
50
100
150
**
CT
Ex-GBH
Group
MUFA/PUFA
Ratio MUFA/PUFA
0
1
2
3
4
5
**
CT
Ex-GBH
Group
MUFA/SAFA
Ratio MUFA/SAFA
0.0
0.5
1.0
1.5
2.0
*
CT
Ex-GBH
Group
PUFA/SAFA
RatioPUFA/SAFA
0.0
0.2
0.4
0.6
0.8
**
CT
Ex-GBH
Group

Figure 2

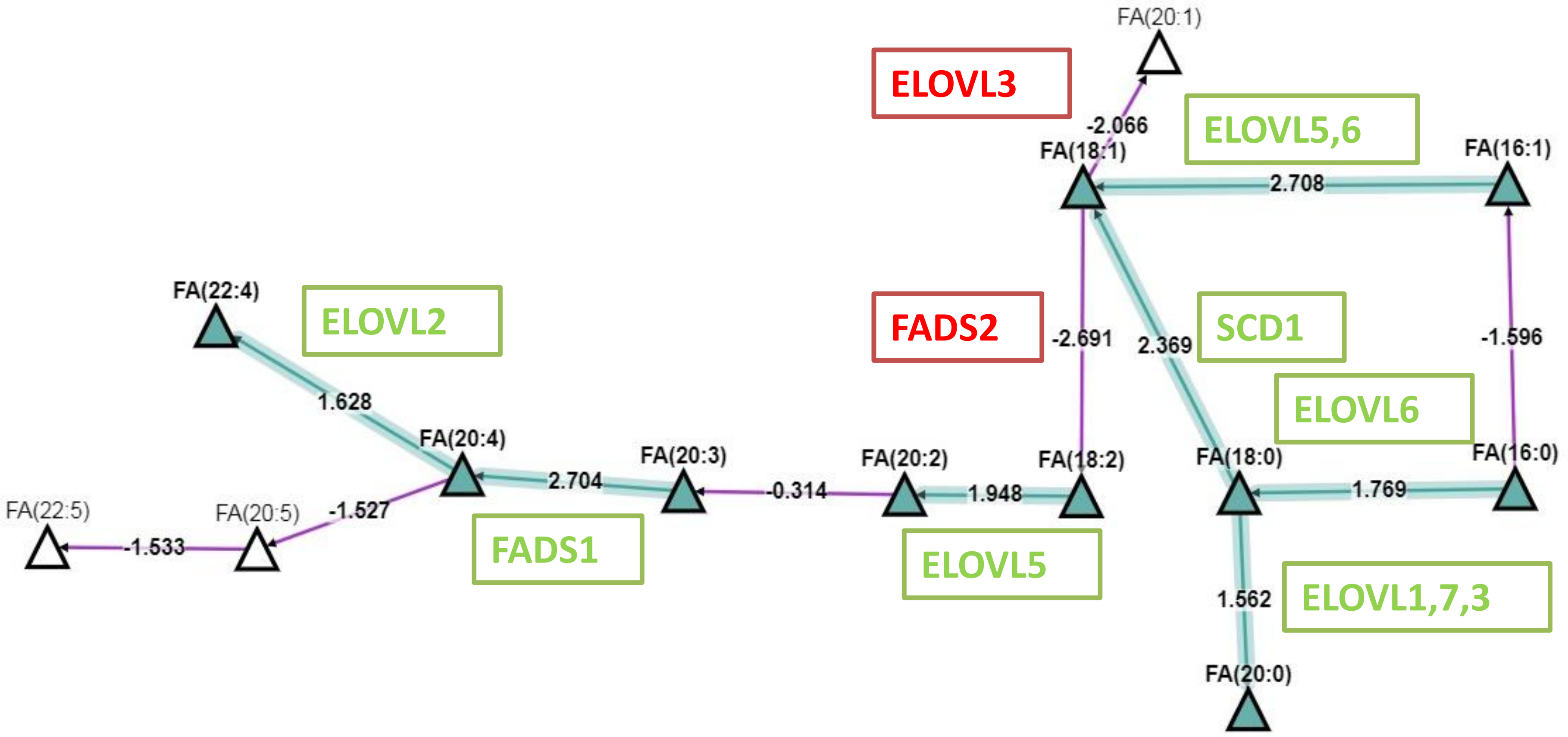
FA(20:1)
ELOVL3
-2.066
ELOVL5,6
FA(18:1)
FA(16:1)
2.708
FA(22:4)
ELOVL2
FADS2
-2.691
2.369
SCD1
-1.596
ELOVL6
1.628
FA(20:4)
FA(20:3)
FA(20:2)
FA(18:2)
FA(18:0)
FA(16:0)
2.704
-0.314
1.948
1.769
FA(22:5)
FA(20:5)
-1.527
-1.533
FADS1
ELOVL5
1.562
ELOVL1,7,3
FA(20:0)

Figure 3

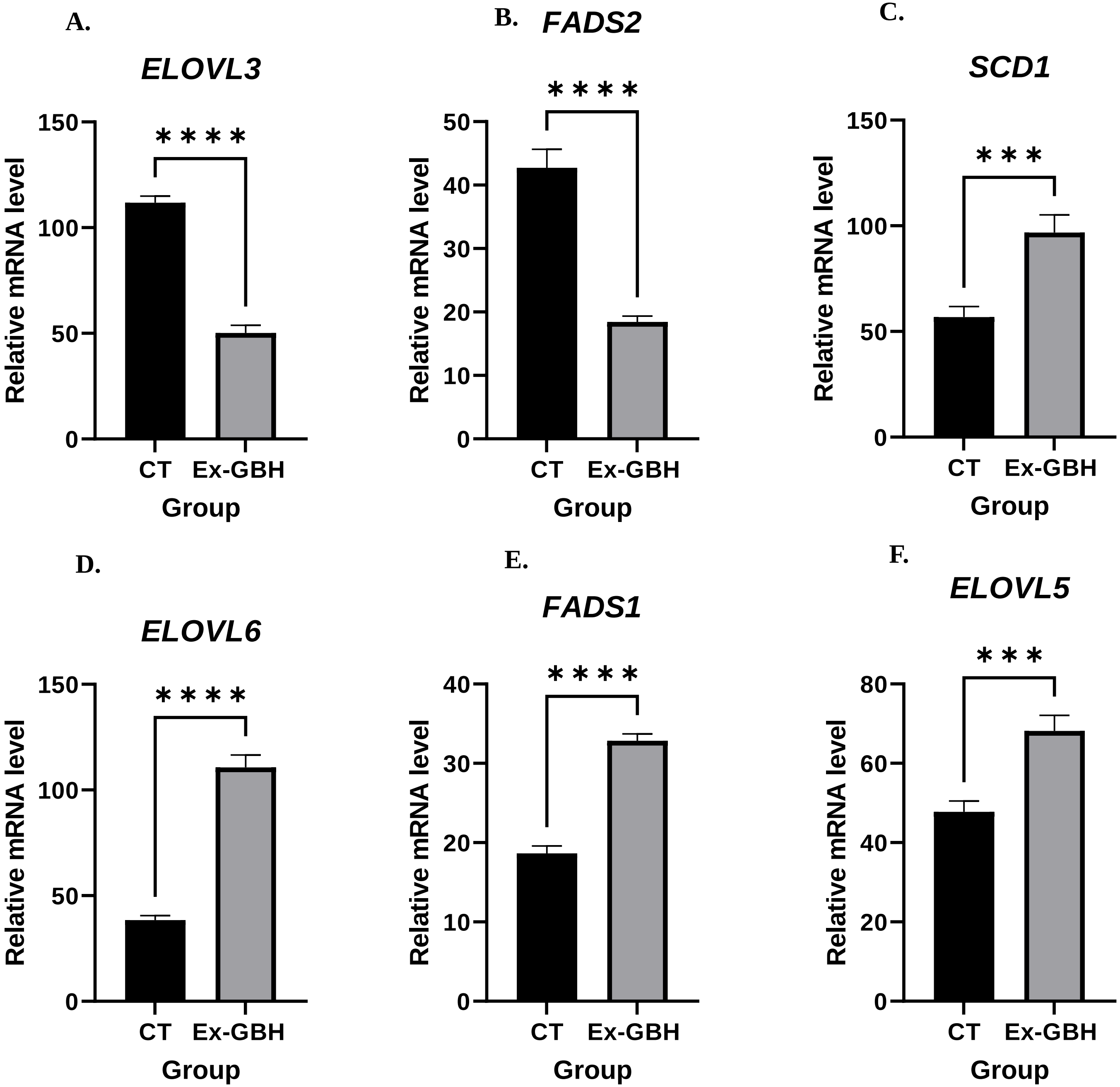A.
ELOVL3
150
100
50
0
Relative mRNA level
****
CT
Ex-GBH
Group
B.
FADS2
50
40
30
20
10
0
Relative mRNA level
****
CT
Ex-GBH
Group
C.
SCD1
150
100
50
0
Relative mRNA level
***
CT
Ex-GBH
Group
D.
ELOVL6
150
100
50
0
Relative mRNA level
****
CT
Ex-GBH
Group
E.
FADS1
40
30
20
10
0
Relative mRNA level
****
CT
Ex-GBH
Group
F.
ELOVL5
80
60
40
20
0
Relative mRNA level
***
CT
Ex-GBH
Group

Figure 4

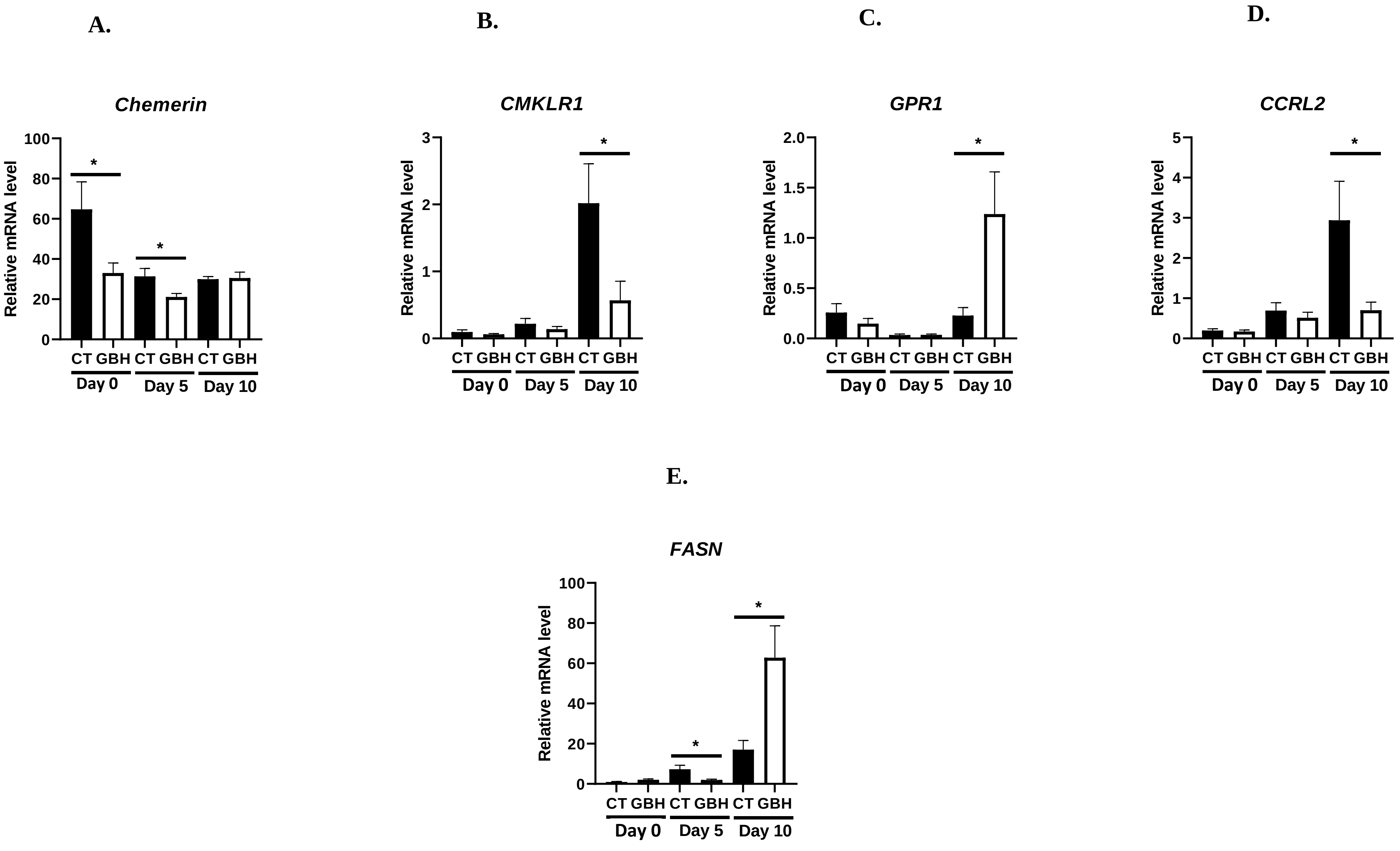
A.
Chemerin
Relative mRNA level
100
80
60
40
20
0
*
*
CT GBH CT GBH CT GBH
Day 0
Day 5
Day 10
B.
CMKLR1
Relative mRNA level
3
2
1
0
*
CT GBH CT GBH CT GBH
Day 0
Day 5
Day 10
C.
GPR1
Relative mRNA level
2.0
1.5
1.0
0.5
0.0
*
CT GBH CT GBH CT GBH
Day 0
Day 5
Day 10
D.
CCRL2
Relative mRNA level
5
4
3
2
1
0
*
CT GBH CT GBH CT GBH
Day 0
Day 5
Day 10
E.
FASN
Relative mRNA level
100
80
60
40
20
0
*
*
CT GBH CT GBH CT GBH
Day 0
Day 5
Day 10

Figure 5