REDUCTION OF DIOXINS CONTAMINATION IN CHICKEN EGG AND TISSUES BY ACTIVATED CARBON AS A FEED ADDITIVE

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Introduction

Dioxins (PCDDs, PCDFs and DL-PCBs) are unintentionally generated by-products from many industrial processes, pesticide formulation, and municipal incineration. Due to great chemical stability and fat-solubility, they can accumulate in animal and human bodies through the food chain. More than 90% of the dietary intake of dioxins is taken place through food consumption. In particular, fish and animal products have been reported to be the principal routes for human exposure to dioxins. World Health Organization (WHO) established a tolerable daily intake (TDI) of 1 - 4 pg-TEQ/ kg/ bw in 1998 (Leeuwen et al., 2000). From the late 1990s, several serious incidents of dioxin contamination in foodstuffs in Europe and North America were caused by dioxin-contaminated feed in the livestock industry. More recently, at the end of 2010, feedstuffs for chicken and pig in Germany were contaminated by dioxins, which had far-reaching negative impacts around the world (Bundesinstitut fur Risikobewertung, 2011). To prevent health hazards caused by dioxins, it is essential to control them during food production and to ensure food safety. There are extensive monitoring programs in place to identify potential contamination entering into the food chain. Screening methods such as cell-based reported gene assays and immunoassays are provided fast and cost effective detection of dioxins in wide range of samples. However, those methods may not provide 100% guarantee for reduction of dioxins in all feed and animal products. Previous studies in laboratory animals have shown that using several dietary supplements to inhibit the absorption of harmful substances and promote excretion (e.g., activated charcoal, cholestyramine, dietary fiber, and chlorella) are effective means to prevent accumulation and health hazards (Yoshimura et al., 1986; Takenaka et al., 1991; Takekoshi et al., 2005). Therefore, our study was planned to find a suitable method to prevent chicken meat and eggs from being polluted by dioxin-contaminated feed. In this study, a chicken exposure study using activated carbon as a feed additive was implemented based on the results of previous studies (Iwakiri et al., 2007). In addition, plasma biochemistry, histopathological examination, measurement of fat-soluble vitamins in eggs, and other biological measurements were conducted to evaluate the hens' health and the nutrients in the eggs.

Materials and methods

Twenty-four hatchlings (White leghorn layers) were emplyed for the expsoure experiment at the National Institute of Animal Health, Tsukuba, Japan. All of the chicks were fed with a basal chick feed in stages 1 (0–4 weeks) and 2 (5–10 weeks) with a recomended feed. They were then divided into four groups of six hens each, and were housed individually. During stages 3 (11–20 weeks) and the laying period (stage 4, 21–30 weeks), two groups were then fed with either the basal feed (Control group) or basal feed containing activated carbon (Control+C group). Another two groups were fed with either the contaminated feed with dioxin standard (DXN group) or the contaminated feed containing activated carbon (DXN+C group). The "control" groups were prepared to confirm whether there were any negative effects due to the addition of dioxins. The activated carbon additive in the feed made up 0.5% (w/w) with reference to Iwakiri et al (2007).

During the experiment, the whole body weights of laying hens were measured once a week. After 30 weeks, each hen was dissected; blood plasma samples were immediately collected, and parts of the breast muscle and abdominal fat were stored at -20 °C to analyze the dioxins. The plasma biochemical parameters such as fatty acids, cholesterols and enzymes were analyzed using an auto-analyzer. The following tissues were fixed in 10% phosphate-buffered formalin and processed for histological examination: esophagus, crop, glandular stomach,

gizzard, duodenum, liver, pancreas, small intestine, cecal tonsil and rectum. During egg-laying, all of the eggs were collected daily; after weight measurement, the whole raw eggs without the shell were mixed five by five in order into a polypropylene sample tube and then stored at -20 °C for analysis. Details of dioxin extraction in breast muscle, abdominal fat and eggs were given in elsewhere (Fujith et al., in press). The purification for dioxin analysis was performed fully automated, which can carry out accurate and rapid purification for extracts (Fujita et al., 2009). The criteria for quality dioxin analysis was conducted based on the Japanese industrial standard (JIS K0311:2005). Data were analyzed by the ANOVA/Tukey's multiple comparisons test to assess statistical significance in plasma biochemical parameters among groups. Student *t*- test was used to evaluate vitamin levels in eggs between DXN group and DXN+C group. This experiment was conducted according to the guidelines for animal experiments of the National Institute of Animal Health, Tsukuba, Japan.

Results and discussion:

The dioxin concentration (ΣPCDDs, PCDFs and DL-PCBs) in normal chicken feed was ranged from 23 to 55 pg /g wet ww which was accounted for 0.0037 to 0.023 pg TEQs/g. The dioxin concentrations in dioxins added feed was 190 to 270 pg/g ww which was accounted for 5.7 to 6.5 pg TEQs/g, respectively.

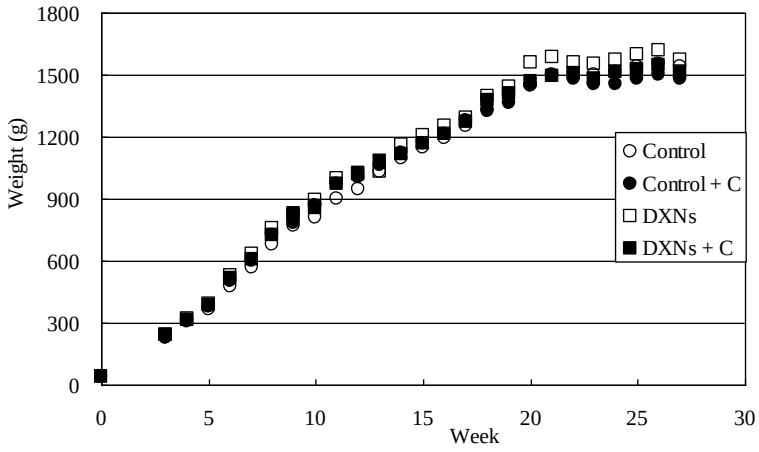

Figure 1. Mean body weight gain (g) of chicken

The growth rate of each feeding group during whole experiment is given in Figure 1. There was no weight difference among control and activated carbon fed groups. The results also agreed with the normal growth for general poultry industry. None of the diet groups showed any significant differences of egg production, egg weight, lipid content, egg laying rate, and plasma biochemical results (data not showing, Fujitha et al., in press). Moreover, based on the histopathological diagnoses of the dissected hens, no significant lesions in the digestive system or liver were seen relative to those from vehicle controls. Hence, layers health was unaffected by the concentrations of dioxins and addition of activated carbon in the feed during the exposure period.

Fig. 2 shows the temporal trend of dioxins accmulation in eggs; data points are analytical composites of five egg samples in laying order. In the starting of the DXN group, the mean total dioxins concentration was 53.4 pg TEQs /g fat. Then dioxins concentrations gradually increased with the time and reached a steady state after about 1 month (corresponding to "egg laying number; 26-30" shown in Fig. 2). During the steady state period, the average concentration was 76.2 pg TEQs/g fat. On the other hand, the concentrations of the DXN+C group were stationary and stayed at around 1.93 pg TEQs/g fat on average, which is far below the maximum EU level of 6 pg TEQs/g fat.

The dioxins concentrations in the breast muscle, abdominal fat, and mixed eggs laid from first laid egg to thirtieth are shown in Fig 3. Although the highest concentration for the tissues were in abdominal fat followed by the eggs and then muscle, the congener profiles were similar in all tissues. The accmulation of dioxins (TEQs) in breast muscle, abdominal fat, and mixed eggs were reduced by 96.4, 93.2 and 95.2% in DXN+C group,

respectively, compared those in DXN only group. Moreover, PCDD/PCDF, non-ortho-PCB, and mono-ortho-PCB congeners were decreased by more than 90%, 84%, and 53%, respectively, regardless of the type of tissue. In out earlier study with broilers, a significantly decrease (>99%) of dioxins accumulation (TEQs) in fat and meat were found, with out any health impairment of chickens, due to addition of 0.5% activated carbon to the feed (Guruge et al., 2007)

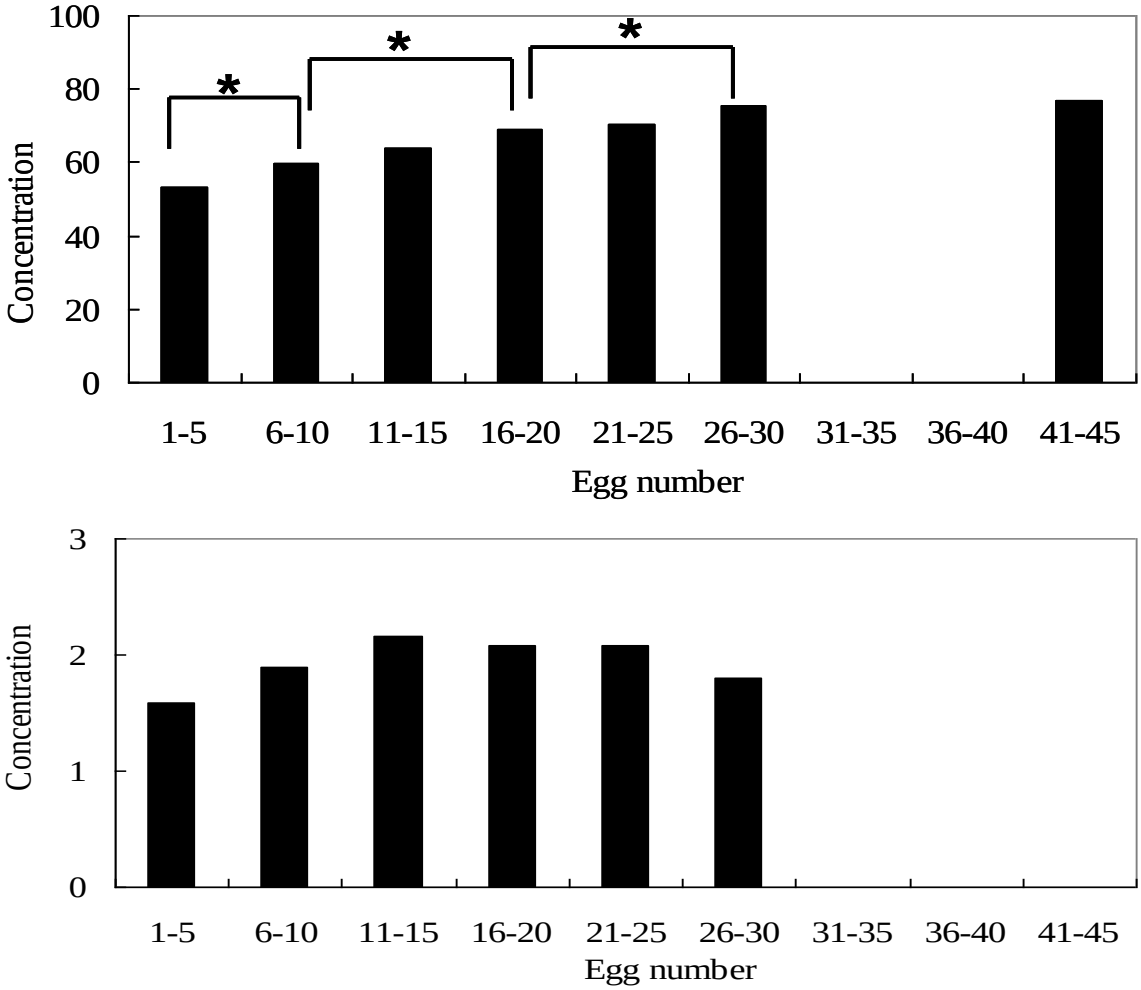

Figure 2. Accumulation trend of mean dioxin concentrations (pg TEQs/g fat wt) in eggs (upper: DXN group, lower: DXN+C group). All data points are represented pooled composites of five egg samples in a laying order (n=6). Asterisk indicates significant differences ($p < 0.05$, Student's t-test).

The above results clearly shows that the absorption of dioxins ingested from feed by the intestine can be inhibited by the addition of activated carbon into the contaminated feed. However, the nutritional effects causes by the addition of activated carbon need to be determined to ensure that this technology is practical. In this study, retinol, β-carotene, tocopherols and cholecalciferol were determined in the egg at steady state. These compounds are fat soluble vitamins that cannot be produced in the body and are absolutely essential for its growth. The concentrations of γ-tocopherol and α-tocopherol in DXN+C group were significantly reduced by about 40%. Although quality parameters such as fatty acids, the oxidative stability, and minerals were not significantly influenced by activated carbon (Kawashima et al., 2009; Usydus et al., 2009), retinol content was associated

with a reduction in color parameters. Hence, a practical application of this new technique may require compensating for the vitamin shortfall caused by the addition of activated carbon.

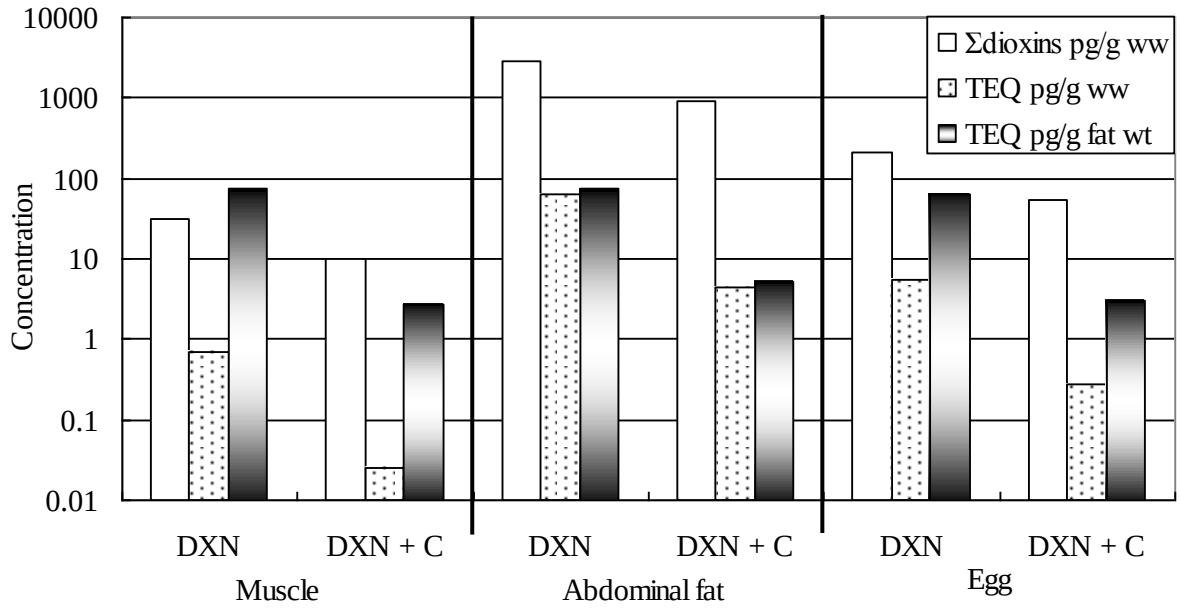

Figure 3. Mean accumulation of dioxins in muscle, abdominal fat and egg of hens (1^{st} to 30^{th} egg). (Σ dioxins: total of PCDD/DFs + DL-PCBs, DXN: dioxin fed group, DXN+C: dioxin and carbon absorbent fed group)

In conclusions, comparison to the dioxin exposure group, the DXN+C group showed a significant decline of the dioxin bioaccumulation into the egg. This reduction due to activated carbon was also observed in the muscle and abdominal fat. The reductions were compound- and congener-dependent, irrespective of the type of tissues. Although fat soluble vitamin concentrations in the eggs of the DXN+C group showed lower trends compared to those in DXN group, the addition of activated carbon into animal feed could obviate the remote potential for accidents causing unintentional food pollution with dioxins.

References:

1. Bundesinstitut fur Risikobewertung, 2011. BfR Opinion Nr. 002/2011, 26 January 2011
2. Fujita H, Honda K, Hamada N, Yasunaga G, Fujise Y, 2009. Chemosphere 74, 1069-1078.
3. Fujita H, Iwakiri R, Honda, K., Guruge KS, Yamanaka N, Tanimura N, 2012. Chemosphere, in press.
5.Iwakiri R, Asano R, Honda K, 2007. Organohalogen Compd. 69, 2391-2394.
6. Kawashima S, Iwakiri R, Honda K, 2009. Chemosphere 75, 788-794.
7. Leeuwen FXR, Feeley M, Farland W, Younes M, 2000. Chemosphere 40, 1095-1101.
8. Takekoshi H, Suzuki G, Chubachi H, Nakano M, 2005. Chemosphere, 59, 297-304.
9. Takenaka S, Morita K, Takahashi K, 1991. Fukuoka Acta Med. 82, 310-316.
10. Usydus Z, Szlinder-Richert J, Polak-Juszczak L, Malesa-Ciecwierz M, Dobrzanski Z, 2009. Chemosphere 74, 1495-1501.
11. World Health Organization, 1999. WHO Technical Report Series 883.
12. Yoshimura H, Kamimura H, Oguri, K., 1986. Chemosphere 15, 219-227.