The Effect of Goat Milk Yoghurt Casein Tablet on Dioxin Residue Level in Wing and Thigh Meat of Coob Broiler Chicken

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Abstract. Chicken meat consumption, especially for broiler chicken, is increasing with time. Chicken meat is preferred by the community because it is easily digestible, relatively cheap, and a good source of animal protein. Latest rumor on the safety of animal derived food is the presence of dioxin compound. Dioxin is a dangerous chemical produced during incineration of household and industrial waste. Food is the main source and pathway for dioxin to enter the body. Most dioxin consumption come from beef, chicken meat, fish, egg, and milk. This research utilized Complete Randomized Sampling (CRS) with 18 Cobb strain broiler chickens as research animals which were divided into 3 groups: negative control group, positive control group, and treatment group. Research animal were given 3 goat milk yoghurt casein tablet/chicken/day (casein dosage was 750 mg of tablet weight) diluted in Reverse Osmosis (RO) water and 2,3,7,8-TCDD 50 ng/ml/1 kg feed dosage for 21 days. Wing and thigh meat samples were analyzed for dioxin residue by UV spectrophotometer in 300 nm wavelength. Residue level data was statistically analyzed by One Way Analysis of Variance (ANOVA) test followed by Tukey’s Honestly Significant Test (HSD). The result showed a drop of dioxin residue level in the wing and thigh of treatment group. The result was obtained by comparing with the average residue level of positive control.

1. Introduction
Animal derived food product is required for human health as functional protein source for growth. Animal protein is especially important as it contains amino acid closest to amino acid compound essential for human, thus making it easily digestible and efficient [1]. Meat producing chicken, collectively called broiler, is still among the commodities used by the community to fulfill their animal protein needs. Meet producing chicken is young male or female chicken age less than eight weeks in certain weight with rapid growth and wide chest as well as good meat reserve [2]. With the improvement of community welfare, income level, and education level, food safety become important to obtain safe and healthy food. Availability of healthy and safe food source is the main key in obtaining good nutrition level [3]. Safety in this context is interpreted as free from microbes, chemical, or contamination that may be hazardous for health [3]. At the end of 1960s, world’s attention on foreign substance residue or xenobiotics in animal derived food products was still lacking, for during that time attention was focusing on pesticide residue problem in fruits and
vegetables. However, after DDT, dieldrin, tetracycline, hormone, and other drugs compound were discovered in animal products, the issue started to garner attention [3]. In several countries, namely Japan, United States, Belgium, and Germany, dioxin contamination in food sources was found and caused serious impact to human health.

A study conducted 2,3,7,8-TCDD detection in beef obtained from slaughterhouses in Yogyakarta, Klaten, and Kupang [4]. The study reported that 2,3,7,8-TCDD residue level in beef obtained from Yogyakarta and Kupang slaughterhouses were over the maximum residue level set by the government of Indonesia while no 2,3,7,8-TCDD residue found in beef from Klaten slaughterhouse. 2,3,7,8-TCDD is a non-polar lipophilic compound with long half-time which allows it to accumulate in food chain while indirectly originated from incineration of plastic waste, textile industry waste, metal industry waste, paper industry waste, vehicle and factory smoke, and pesticide. 90% of 2,3,7,8-TCDD compound contamination in human are caused by intake of contaminated food product such as meat, egg, and milk [5] [6]. Yoghurt is a product produced from fermented milk with lactic acid bacteria started. Casein as bioactive peptide source available in relatively high amount, reaching up to 80% of milk protein, consists of \( \text{as1-casein}, \text{as2-casein}, \beta\text{-casein}, \ \text{and\-casein} \). During fermentation process of milk turning into yoghurt, casein hydrolysis by lactic acid bacteria produced bioactive peptide with various biological function. Goat milk yoghurt contains bioactive peptide comprised of 16 amino acid and exhibits antioxidant activity [7]. The many benefit of goat milk yoghurt casein as multifunctional bioactive peptide can reduce the level of free radical. Thus, this research utilized goat milk yoghurt casein tablet then observed the 2,3,7,8-TCDD residue level in the wing and thigh of broiler chicken.

2. Methods

This research was conducted in community chicken coop farm in Karangploso region, Malang, and Chemical Instrumentation Laboratory, Faculty of Mathematics and Natural Science, University of Brawijaya. Tools used in this research were chicken coop, research animal feeding and drinking container, stomach intubation tool, weight scale, gloves, tissue, mixer, plastic wrap, mask, hot plate, vortex, separation funnel, refrigerator, aluminum foil, magnetic stirrer, spectrophotometer UV-Vis 9 (UV-1601 Shimadzu), and glass apparatus commonly used in laboratory environment. Research materials used in this research were goat milk yoghurt casein tablet, male Cobb strain broiler chicken, standard feed, dioxin (2,3,7,8-TCDD), filter paper, toluene diluent brand p.a., technical toluene, corn oil, and distilled water.

2.1. Sample Preparation

Cobb strain chicken research animal aged 14 days were acclimatized for 7 days and divided into three groups: I (negative control group), II (positive control group), and III (therapy control group). On age 21, animals were given treatment 2,3,7,8-TCDD per oral via feed, with dosage 2,3,7,8-TCDD 50 ng/mL/1 kg feed. Animals were treated by goat milk casein yoghurt tablet 750 mg of weight from goat milk yoghurt casein tablet (3 tablets of goat milk yoghurt casein tablet/chicken/day) diluted in RO water, administered per orally by intubation. Treatment period was 21 days. At the end of research treatment, wing and thigh meat sample were taken for 2,3,7,8-TCDD residue level analysis by UV spectrophotometer.

2.2. TCDD Residue Level Measurement in Broiler Chicken Wing and Thigh Meat

Standard curve for 2,3,7,8-TCDD was first created. The 2,3,7,8-TCDD residue level in wing and thigh meat was determined by mincing the wing and thigh meat samples and then having them ground. Technical toluene was added, mixed, and homogenized by using magnetic stirrer for 2 hours in 8000 rpm. The mixture was left for 3 hours and then filtered by Whatman 21 paper. This process was repeated two times with the same procedure. The filtrate obtained was processed for 2,3,7,8-TCDD residue level measurement by UV spectrophotometer in 300 nm wavelength.
2.3. Data Analysis
Data analysis was performed quantitatively by statistical test One Way ANOVA by using Microsoft Office Excel and SPSS 16.0 software for windows. Analysis was proceeded with HSD test to determine the significant difference in the administration of goat milk yoghurt casein tablet against 2,3,7,8-TCDD residue level with α = 5%.

3. Result and Discussions
3.1. The Result of Dioxin Residue in the Wing and Thigh Meat of Broiler Chicken
2,3,7,8-TCDD residue level in the wing and thigh meat of broiler chicken was determined by utilizing spectrophotometer in ultraviolet area measured in λ 300 nm. Standard curve was made to determine 2,3,7,8-TCDD residue level in the wing and thigh meat. The standard curve results obtained equation line y = 1.69 + 0.0408 with R² = 0.9806. 2,3,7,8-TCDD residue level in the wing meat of broiler chicken exposed to 2,3,7,8-TCDD in 50 ng/mL/1 kg feed dosage and given 750 mg casein therapy from goat milk yoghurt casein tablet for 21 days could be seen in Table 1. The 2,3,7,8-TCDD residue level of broiler chicken thigh meat could be seen in Table 2.

Table 1. Average Dioxin Residue Level in the Wing Meat of Broiler Chicken

| Treatment Group       | Average Dioxin Residue Level (mg/L) (X±SD) | Dioxin Residue Level Increase (%)** | Dioxin Residue Level Decrease (%)* |
|-----------------------|-------------------------------------------|------------------------------------|-----------------------------------|
| Negative Control      | 0.0144±0.0064a                            | -                                  | -                                 |
| Positive Control      | 0.0611±0.0044c                            | 76.432                             | -                                 |
| Therapy Control       | 0.0418±0.0107b                            | 31.587                             |                                   |

Annotation: a data % of dioxin residue level decrease in the wing meat from therapy control group was obtained by comparing it with the average residue level of positive control. Different notation showed significant difference (p<0.05).

Table 2. Average Dioxin Residue Level in the Thigh Meat of Broiler Chicken

| Treatment Group       | Average Dioxin Residue Level (mg/L) (X±SD) | Dioxin Residue Level Increase (%)** | Dioxin Residue Level Decrease (%)* |
|-----------------------|-------------------------------------------|------------------------------------|-----------------------------------|
| Negative Control      | 0.0181±0.0185a                            | -                                  | -                                 |
| Positive Control      | 0.0611±0.0145c                            | 70.376                             | -                                 |
| Therapy Control       | 0.0397±0.0035b                            | -                                  | 35.024                            |

Annotation: a data % of dioxin residue level decrease in the thigh meat from therapy control group was obtained by comparing it with the average residue level of positive control. Different notation showed significant difference (p<0.05).

Research result showed that exposure to 2,3,7,8-TCDD 50 ng/mL/kg feed done on broiler chicken caused 2,3,7,8-TCDD residue in chicken wing meat as shown by negative control 2,3,7,8-TCDD residue level of 0.0144 mg/L or 14.4 ng/mL. An increase of dioxin residue level in negative control compared to positive control as much as 76.43% could be seen with dioxin residue level from positive control being 0.0611 mg/L or 61.1 ng/mL. Meanwhile, treatment of 759 mg casein from goat milk yoghurt casein tablet target showed decrease of residue level compared to positive control with therapy control group achieving 31.58% decrease and 2,3,7,8-TCDD residue level of 0.0418 mg/L or 41.8 ng/mL which is significantly different (p<0.05). After 2,3,7,8-TCDD enters the body and passed cell wall, it would bind with AhR in cytosol and moved into nucleus. In the nucleus, AhR will bind with 2,3,7,8-TCDD, forming a dimer with protein Aryl hydrocarbon Receptor Nuclear Translocator (ARNT). As TCDD-AhR-ARNT complex compound, they bind to certain DNA element, which is Dioxin-Responsive enhancer Elements (DRE). This compound complex would then work as transcription factor in DRE and induce Cytochrome p450 (CYP1A1 and CYP1B1) which will cause oxidative stress [8]. Goat milk yoghurt casein tablet has antioxidant content, such as β-casein and as2-casein, which can suppress free radical produced by 2,3,7,8-TCDD, thus preventing the accumulation of 2,3,7,8-TCDD in the tissue.
2,3,7,8-TCDD residue level in thigh meat in negative control was 0.0181 mg/L or 18.1 ng/mL, higher than negative control level in wing meat. The presence of dioxin residue in each broiler negative control is possibly caused by indirect exposure to 2,3,7,8-TCDD through the air or contaminated feed before direct exposure treatment to 2,3,7,8-TCDD. Animal feed is commonly obtained from rural region with several production path, allowing the possibility of being contaminated with dioxin. In the animal feed, dioxin was found in 1-5 µg/kg 2,3,7,8-TCDD concentration. Other type of dioxin was caused by environmental contamination from plastic waste incineration, factory smoke, textile, paper, and metal industrial waste, vehicle waste, and pesticides contaminating food sources and animal feed such as chicken feed, catfish feed, swine feed, and cow feed [10].

Administration of 750 mg casein therapy from goat milk yoghurt casein tablet can reduce 2,3,7,8-TCDD residue level with significantly different result (p<0.05) in broiler thigh meat as shown by therapy control 2,3,7,8-TCDD residue level of 0.0397 mg/L or 39.7 ng/mL. If compared to positive control 2,3,7,8-TCDD residue level of 0.0611 mg/L or 61.1 ng/mL, a decrease of 35.02 % was observed. Food sources drived from animal has more risk of being contaminated by 2,3,7,8-TCDD for 2,3,7,8-TCDD is naturally easily soluble in fat (lipophilic) [8]. [12] reported that 90% of 2,3,7,8-TCDD compound exposure in human is from intake of contaminated food. Intake of food such as contaminated meat, egg, and milk is the main source of 2,3,7,8-TCDD intoxication in living organism. Thus, the higher the level of 2,3,7,8-TCDD contamination in the food chain, the higher the amount accumulated in the body from food source residue and may one day cause health problems [4].

Based on Table 1. and Table 2., the administration of casein therapy in 750 mg casein dosage by goat milk yoghurt casein tablet for 21 days may lower 2,3,7,8-TCDD residue level in the wing and thigh meat of broiler chicken, showing significantly different treatment effect between negative control, positive control, and therapy control (p<0.05). This means goat milk yoghurt casein tablet function as antioxidant. One of antioxidant activity is as radical scavenger and cation chelator which can prevent lipid oxidation. Milk fermentation process by lactic acid bacteria (LAB) can release bioactive peptide from main milk protein. Antioxidant peptide originating from milk consists of five to ten hydrophobic amino acid, namely proline, histidine, tyrosine, or tryptophan. They exist in milk casein and may function as scavenger or prevention of toxin and free radical creation and preventing lipid peroxidation process. Lactic acid bacteria (LAB) in goat milk yoghurt casein tablet can reduce ROS accumulation caused by xenobiotics compound such as 2,3,7,8-TCDD during food digestion process and could degrade superoxide anion and hydrogen peroxide [13].

4. Conclusion
Therapy of 750 mg goat milk yoghurt casein tablet can prevent the increase of 2,3,7,8-TCDD residue level and able to lower 2,3,7,8-TCDD residue level which was significantly different (p<0.05) in the wing and thigh meat of broiler chicken.

Acknowledgement
Gratitude is given to “Badan Penelitian dan Pengabdian Masyarakat” (Research and Community Service Agency), Faculty of Mathematics and Natural Science, University of Brawijaya, Malang, for the research grant “Hibah Guru Besar” Year 2020 and Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, University of Brawijaya, Malang, for the facilities during the course of this research.

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