Aflatoxin contamination in broiler feed from small-scale farms in Sidenreng Rappang Regency, South Sulawesi, Indonesia

Aminah Hajah Thaha¹, Muhammad Nur Hidayat¹, Khaerani Kiramang¹, Ali Mustopo¹, Arsul Saputra¹, Syamsiah¹, Mirsa Varadina Yafi¹, Dini Marmansari², Erdi Purwanto² and Fitriah Idris²

¹Faculty of Science and Technology, Universitas Islam Negeri Alauddin Makassar
Jl. H. M. Yasin Limpo No. 36, Kabupaten Gowa, Sulawesi Selatan, 92111, Indonesia
²Veterinary Public Health Laboratory, Balai Besar Veteriner Maros
Jl. Dr. Ratulangi, Kabupaten Maros, Sulawesi Selatan, 90514, Indonesia

Email: amina.hajah@uin-alauddin.ac.id

Abstract. This study aims to determine the aflatoxin contamination in broiler feed obtained from several small-scale farms (range 1,000-10,000) in Sidenreng Rappang Regency, Indonesia. The study was conducted by measuring the sample using the ELISA method to determine the aflatoxin level and observe potential aflatoxin contamination in broiler farms. The results showed 30.6% of broiler feed samples contain aflatoxin more than 50 ppb, it was above of Standar Nasional Indonesia (SNI) 01-6366-2000. The potential for aflatoxin contamination in broiler farms includes the temperature of the feed warehouse, the humidity of the feed warehouse, storage time, feed ingredient content, and feed warehouse model.

1. Introduction
The animal feed consists of various mixtures of organic materials which aim to meet the nutritional needs required for the growth, development and reproduction of livestock. The feed is the largest component of all production costs in the poultry business, reaching 77% [1]. It is important to ensure the quality and quality of feed from the various threats to the physical, biological and chemical. One of the microorganisms which can reduce the quality and the quality is a fungus that can produce dangerous toxins, e.g. *Aspergillus flavus*, to produce aflatoxin [2].

Aflatoxin is a natural metabolite produced by *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxin distributed almost all over the world. In 2019, Indonesia has range 24.89-29.50 °C of temperature and 70.43-86.73% high humidity [3], this condition is optimum for contaminating Aspergillus. Several Aspergillus fungi are found in hot temperature and high humidity, especially at temperatures of 27-40 °C (80-104 °F) and relative humidity 85%. Aflatoxin can contaminate raw material pre and postharvest. The feed is easy to be contaminated by Aflatoxin, and it will be accumulated in food from an animal product such as eggs, meat and milk. This compound is also more stable and resistant during food processing [4].

Aspergillus flavus produces Aflatoxin-B1, the secondary metabolite and most dangerous compared to other mycotoxins. Based on the International Agency for Research on Cancer (IARC) classification, Aflatoxin-B1 belongs to the group 1 compound that is carcinogenic in humans and the most toxic...
aflatoxin [5]. Besides being carcinogenic, aflatoxin is also genotoxic, hepatotoxic in humans, and nephrotoxic and immunosuppressive in animals. Immunosuppressive in animals have an indirect effect, such as failure of vaccination, so the prevention of diseases become ineffective.

South Sulawesi Province is located in the Eastern Indonesia Region that contributes an average of 3.00% in 3 years (2017-2019) of the national broiler population and have the largest population outside the island of Java [3]. These data indicate that the need for broiler feed is also large in South Sulawesi. To add to the latest information regarding aflatoxin contamination in various animal feed ingredients in Indonesia, especially on small-scale broiler farms, it is necessary to research to obtain the latest contamination data.

2. Materials and methods

The research was conducted against 36 samples from 6 small-scale broiler farms in the same area selected by a cross-sectional study design. Cross-sectional research is a study where variables risk factors and their effects are observed at once and simultaneously [6]. Data was also collected by observation and interview in practice feed handling and potential aflatoxin contamination comparison with aflatoxin level. Aflatoxin level was measured by enzyme-linked immunosorbent assay (ELISA) method developed by Veterinary Public Health Laboratory, Balai Besar Veteriner Maros.

A total of 20 grams of sample was prepared, then added 100 ml of 70% methanol then shaken for 3 minutes, then let stand. The supernatant was taken and filtered with Whatman Paper no. 1 and adjusted the pH to 6-8. Standard solutions are 0, 4, 10, 20 and 40 ppb. Furthermore, readings are carried out with an ELISA Reader with a wavelength of 450 nm; a wavelength of 630 nm is used for differentiation.

3. Result and discussion

This research found level contamination of aflatoxin in 6 small-scale broiler farm was 36,1 ppb; 44,4 ppb; 49,7 ppb; 47,6 ppb; 18,9 ppb; and 50,4 ppb (Table 1). Aflatoxin was found in feed on all scale of the farm. It was higher in farm VI compared to other farms. Sidenreng Rappang Regency is located 10 m - 3,000 m above sea level, with temperature per year ranging from 26-31ºC and humidity ranging from 63-89% [7]. This condition can trigger the growth of Aspergillus fungus both in feed ingredients and broiler chicken feed, so the results of the analysis using the ELISA method on all commercial broiler feed samples show the presence of aflatoxin levels as a secondary metabolite of Aspergillus fungi.

Although farm VI has higher aflatoxin levels than other farms, in fact, aflatoxin levels that exceed the Indonesian national standard of 50 ppb are found in almost all feed used by broiler farms. It is known that 66.7% of feed originating from farm III has a content of more than 50 ppb, while on-farm V, there are no aflatoxin levels (0%) of more than 50 ppb. The observation results show that farm III has a feed warehouse with a closed model, while farm V has a feed warehouse with an open model. Poor warehouse and feed handling models are thought to be among the causes of high levels of aflatoxin-contaminated feed. The closed feed warehouse model generally uses a roof made of thatch so that it is easy to leak, and the floor is made of bamboo. It is made of cardboard, is not well ventilated, dirty, and the warehouse does not control temperature and humidity (tools not available). The ideal warehouse should avoid direct sunlight. The feed is stored in a clean area, protected from rain, and the temperature is above the optimal range for mould growth which can trigger aflatoxin contamination [8].

Table 1. Result of Analysis Aflatoxin Contamination in Broiler feed.

| Farm | Average Aflatoxin Levels (ppb) | Aflatoxin Value Range (ppb) | Aflatoxin levels> 50 ppb (%) |
|------|--------------------------------|-----------------------------|-----------------------------|
| I    | 36.1                           | 22.8-50.5                   | 33.3                        |
| II   | 44.4                           | 17.5-70.1                   | 16.7                        |
| III  | 49.7                           | 37.1-57.0                   | 66.7                        |
| IV   | 47.6                           | 37.7-71.2                   | 16.7                        |
| V    | 18.9                           | 11.4-30.1                   | 0.0                         |
| VI   | 50.4                           | 19.2-80.0                   | 50.0                        |
This study also shows that there is an effect of the type of feed during the maintenance period on aflatoxin contamination, namely starter and finisher feed. This can be seen from the aflatoxin levels in the starter feed more than 50 ppb, which was 38.89% higher than the aflatoxin levels in the finisher feed, which was 22.22% of the total samples tested. Aflatoxin levels of more than 50 ppb in starter feed were found in most farms except on-farm V, while in finisher feed, aflatoxin levels of more than 50 ppb were found in farms III, IV, and VI. This is thought to be influenced by the length of time the feed is stored; the longer the feed is stored, the higher the aflatoxin levels in the animal feed. The maintenance period for the starter is longer, namely 20-21 days, so farmers in the field generally keep more feedstock for a long time, without controlling temperature and humidity in the warehouse. High humidity causes the absorption of moisture from the air so that the feed becomes moist and affects the increase in moisture content [9].

Aflatoxin contamination has not been a serious concern for the government and farmers because its effects are not seen directly to livestock. Based on the results of this study, are expected to be able to make policy such government efforts to address the feed in the form of an increase in broiler breeders extension and increase farmers knowledge about feed biosecurity on broiler chicken farms.

4. Conclusions
As much as 30.6% of the total feed samples obtained in small-scale broiler farms (range 1,000-10,000 head) in Sidenreng Rappang Regency, Indonesia, have aflatoxin levels that exceed the standards set by the Indonesian National Standard (50 ppb).

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