Production Profile of Laying Hens Without the Use of Antibiotic Growth Promoters in Bali, Indonesia

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Abstract

In Indonesia, the prohibition of Antibiotic Growth Promoter (AGP) is regulated in Law no. 18 of 2009 in conjunction with Law no. 41 of 2014 concerning husbandry and animal health. It is also regulated by the Ministry of Agriculture Regulation no. 14 of 2017 concerning the classification of veterinary drugs. This prohibition aims to prevent antibiotic resistance and ensure food safety of animal origin. Therefore, this research examines the production profile and health of laying hens that do not use AGP in the breeding process. This was conducted based on a field survey by measuring the health status and performance of hens. The measured health parameters included morbidity, mortality, haematological profile, antibody titers for Newcastle Diseases (ND) and Avian Influenza (AI), as well as intestinal macropathology. Meanwhile, the measured production performance included daily feed intake, age at first egg laying, age at peak egg production, hen day percentage (%HD) above 90%, peak production duration above 90%, and average egg weight. Laying hens aged 30 to 49 weeks were used as the animal models, and they belong to breeders in three locations, namely Penebel Tabanan, Kayuambe Bangli, and Pesedahan Karangasem. Furthermore, the data were descriptively analyzed. The results showed the production profile of laying hens that did not receive growth promoter antibiotics in the feed experienced intestinal wall problems in the form of necrotic enteritis, normal haematological status, seropositive ND and AI antibody titers, as well as decreased egg production.

***Keywords**: production peformance; laying hens; blood profile, intestinal macropathology; antibody titer.*

1. Introduction

The use of antibiotics as feed additives (Antibiotic Growth Promoter/AGP) has been prohibited by the Indonesian government through Law no. 18 of 2009 in conjunction with Law no. 41 of 2014. The prohibition is also regulated by the Ministry of Agriculture Regulation No. 14/2017 on registration and distribution of animal feed which officially took effect on January 1, 2018. This prohibition is reasonable because some antibiotics used in feed cause side effects for people who often consume meat or eggs (Hao et al. 2014). The potential danger of meat or eggs containing antibiotic residues is in the form of poisoning and antimicrobial resistance or can cause a pathological immune response (Mund et al. 2017).

Laying hens are one of the most important products of animal origin in Indonesia. In production, about 60-70% of the total cost of laying hens is from the feed sector, hence many breeders make efforts to increase the feed efficiency to meet their nutritional needs (Huda et al. 2019). Furthermore, hens are threatened by the presence of bacterial disease agents that interfere with the absorption of nutrients and overall health (Clavijo and Flórez 2018). AGP is one of the solutions previously used by breeders in improving the production performance of laying hens. However, with this prohibition, breeders need to use other materials that have no impact on human health (Huda et al. 2019).

Reports stated that the AGP ban had an impact on the livestock industry in Indonesia. The productivity of laying hens is reported to decrease by up to 30% (Gumilar and Natalia 2018). Consequently, breeders need to adapt to the enactment of these regulations, especially those open houses where bacterial contamination is likely to occur (Wan et al. 2021). Meanwhile, in Bali, there has never been a report on the hens' production and health after the ban on the use of AGP.

2. Materials and Methods

a. Ethical approval

This research was approved by Ethical Commission at Faculty of Veterinary Medicine Udayana University with No. B/163/UN14.2.9/PT.01.04/2022.

b. Research design

This is observational research conducted in three locations which are the center of laying hens in Bali, namely, in Penebel Sub-district at Tabanan, Kayuamba at Bangli, and Pesedahan at Karangasem Regencies. At each location, 10 breeders were randomly interviewed, and samples of 5% hens were each taken for health checks. The number of chickens kept by each farmer ranged from 35,000-75,000 with relatively the same chicken farm conditions, namely the Hyline strain, a mixed feed consisting of corn, bran, concentrate and premix without using AGP.

c. Research sample

Two types of samples were collected, including breeders and laying hens. The data on the hens' performance included feed consumption, age of early and peak production, percentage of peak production, length of peak production, and egg weight. Meanwhile, health data included morbidity, mortality, examination of blood condition, antibody titers against Newcastle Diseases and Avian Influenza viruses, as well as the examination of digestive organs in anatomical pathology. The hens' samples used were Hy-line layer phase strains with an age range of 30 to 49 weeks.

d. Data collection technique

The interviews were guided using a structured questionnaire, and the hen samples were randomly taken from each farm. The blood samples were collected and anticoagulant was added for routine haematological examination purposes, while serum was used for antibody titer testing. The small intestine was taken through the necropsy process.

e. Haematology profile and antibody titer

Complete blood count was performed using an Auto Hematology Analyzer (RT-7600, Rayto Life and Analytical Sciences Co., Ltd). The red blood cells profile includes the following parameters: total erythrocytes, hemoglobin, hematocrit, and erythrocyte index. The erythrocyte index includes mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Examination of white blood cells includes total and differentiated leukocytes including heterophils, lymphocytes, eosinophils, monocytes, basophils (Julendra et al. 2010; Lutfiana et al. 2015). Meanwhile, the antibody titers detected were Newcastle Diseases and Avian Influenza using the Haemagglutination Inhibition (HI) method (Kencana et al. 2021).

f. Digestive organ health examination

Necropsy was performed on all the hen samples. The small intestine was opened, examined pathologically and anatomically, and the changes found were recorded. Macropathological examination was carried out at the location of the farm.

g. Data analysis

Research data including production performance, complete blood count, ND and AI antibody titres, and intestinal macropathology of laying hens were carried out descriptively.

3. Results

The results of the study showed that laying hens fed without AGP experienced delays and decreased production, as presented in Table 1. Observations for morbidity and mortality are shown in Table 2. Complete blood count results showed normal values for the erythrocyte and leukocyte profile (Table 3 and Table 4). The results of the ND and AI antibody titer tests showed a protective seropositive, as presented in table 5.

Table 1. Laying Hens Production Performance without AGP

Production Parameters	Sample Origin			Tierzucht, 2021	Remark
	Tabanan n= 55,000	Karangasem n= 35,000	Bangli n= 75,000		
Average age of early production (weeks)	21	21	20	18-19	Late

Average age of peak production (weeks)	28	28	26	24-26	Late
Average percentage of peak production (%HDP)	90.5	90	91	94-97	Decreased
Average time of peak production (weeks)	45	46	47	49	Decreased
Average consumption per head/day (Grams)	125	120	125	110-120	Increased
Average egg weight (Grams)	50.8	50.7	50.9	47.2 (Small) 47.4 -54.2 (medium) 54.4 -61.4 (Large)	Good

Note: n is population of laying hens.

Table 2. Morbidity and Mortality of Laying Hens Produced without AGP

Production Parameter	Sample Origin		
	Tabanan	Karangasem	Bangli
Morbidity (%)	8	7	8
Daily Mortality (%)	0.03	0.036	0.035

Table 3. Blood profile of laying hens produced without AGP

Production Parameter	Sample Origin			Mean	Smith and Mangkoewidjojo, 1988	Remark
	Tabanan N =20	Karangasem N = 20	Bangli N= 20			
RBC ($x10^6/\mu L$)	2.97	2.37	3.0	2.78	2.0-3.2	Normal
WBC ($x10^3/\mu L$)	84.3	83.7	85.3	84.43	16-40	High
Hb (Gr%)	9.57	9.5	9.6	9.56	7.3-10.9	Normal
PCV (%)	24.79	25.68	30.80	27.1	24-43	Normal
MCH (pg)	39.79	39.5	39.6	39.63	33-47	Normal
MCV (fL)	112.1	112.7	113.3	112.7	90-140	Normal

Table 4. Leukocyte differential profile of laying hens produced without AGP

Production Parameter	Sample Origin			Mean	Standard	Des.
	Tabanan N= 100	Karangasem N = 100	Bangli N=100			
Heterophile (%)	30.47	35.20	32.50	32.72	9-56	Normal
Eosinophils (%)	1.93	1.87	1.2	1.67	0-7	Normal
Lymphocytes (%)	55.83	60.12	56.80	57.58	24-84	Normal
Monocytes (%)	15.1	15.8	17.6	16.2	0-30	Normal
Basophils (%)	0	0	0	0	0-30	Normal

Table 5. ND and AI antibody titers of laying hens produced without AGP

Production Parameter	Sample Origin			Mean	Standard	Des.
	Tabanan N =100	Karangasem N = 100	Bangli N =100			
Newcastle Disease	2^9	2^9	2^9	2^9	$>2^4$	Protective
Avian Influenza	2^8	2^8	2^8	2^8	$>2^4$	Protective

4. Discussion

The achievement of the production target is highly dependent on the timeliness of achieving bwt, frame size, and the target production period both at the beginning and peak. This is also supported by the quality of day-old chick, digestive tract health, and the growth of hens (pullet) (Tona et al. 2005). In the starter phase, there is a massive cell division called hyperplasia. With the occurrence of hyperplasia, organ development becomes rapid and should be supported by nutritional intake and health (Wang et al. 2017). The health problems at this stage will hamper their development, which will disrupt the production process.

This starter phase is a period of growth and formation of laying hen's frames. Even though some literature mentioned the 0–4-week starter phase as the first critical period, that does not mean the next starter phase is safe from being critical (Ardana 2012). This phase has a decisive role in the success of egg production, which is around 50-90% of the pullet maintenance success (Do et al. 2022). After the pullet phase, it will continue to the grower phase which has 3 critical times, namely at 6-7, 12, and 14 weeks of age. In the grower phase, technical activities should be carried out (production or maintenance, health, and food or nutrition) to achieve standard bwt (Ardana 2012). The health of the digestive tract should also be maintained to achieve optimal growth (Tajudin et al. 2021). At 14 weeks, the reproductive organs and medullary bones develop rapidly. This medullary bone stores calcium (Ca) reserves for the formation of egg shells. In this period, adequate calcium and vitamin D intake is very necessary (Alfonso-Carrillo et al. 2021).

The results showed the age of early production to be 20-21 weeks as seen in Table 1. This early production age from hens without AGP was found to have a delay of 1-2 weeks. Normally, hens lay their first eggs at 18-19 weeks, where the production rate only reached about 3.8% (Hy-Line 2018) Theoretically, the delay occurred due to low calcium and vitamin D intake, and other causes such as poor pullet quality, namely having experienced enteritis, lack of nutrition and drinking water, lack of lighting, stress, and infection. Digestive tract enteritis due to infection will interfere with nutrient absorption, including calcium and vitamin D (Pan and Yu 2014; Alfonso-Carrillo et al. 2021). This research provides the cause of the delay in laying eggs. Before the ban on the use of AGP in feed, breeders revealed that the start of egg production could be achieved earlier than the standard, namely 17 weeks of age and 18 weeks at the latest.

The delay in the age of egg production affects the achievement of peak production age. This research found that in hens without AGP, the peak production age was reached at an average of 28 weeks with %HDP: 90-91% (Table. 1). Normally, peak production is reached at 25-26 weeks, and %HDP is around 93-96% (Hy-Line, 2018). Laying hens are said to have reached peak egg production when production reaches 90%, or within five weeks there is no further increase. The results showed that the age to reach peak production experienced a decline of about 2-3 weeks compared to the standard %HDP and production decreased by 2-5% (Tab. 1). Many factors can be the cause, one of which is digestive tract infection. The gastrointestinal tract is infested by micro-organisms from the outside and becomes a warm refuge for complex anaerobic bacteria. As the poultry grows, these micro-organisms become very diverse until they reach a relatively stable but dynamic state (Pan and Yu 2014).

Previous research on poultry has proposed that the gastrointestinal tract of broiler chickens is infected by about 600-800 bacteria species (Torok et al. 2011). This may also occur in laying hens. A large number of bacteria in the intestines can cause dysbacteriosis and necrotic enteritis, leading to impaired intestinal function, especially the provision of nutrients and absorption of nutrients which in turn interferes with egg production. This condition can be treated with antibacterial drugs, organic acids, or probiotics. Because the administration of AGP is prohibited, the hens used as objects may have dysbacteriosis and/or necrotic enteritis. The necropsy shows the hens used in this research got some necrosis in the digestive tract. This disease could be responsible for the delay in the age of peak egg production compared to the standard and shorter peak length. The results showed the peak production time was up to 45-47 weeks. Normally, the peak production time is up to 49 weeks (Hy-Line 2018). This will be detrimental to breeders because optimal productivity cannot be achieved. Furthermore, after passing the peak production, it will slowly decrease by around 0.4%-0.5% per week. The short duration of peak production accelerates the phase of late production. Hence, breeders need to adopt alternatives to restore the performance of laying hens after the ban on the use of AGP (Hidayat et al. 2018; Sinurat et al. 2019; Rahmawati and Irawan 2021).

Feed consumption of the hens obtained an average of 125g/head/day as presented in Table 1. This exceeds the 5g/head/day from the Lohman Brown Management Guide standard, namely consumption during the production period of 110-120 grams/head/day (Tierzucht 2021). Excess feed consumption can be caused by poor food digestion due to pathogenic agents that interfere with the digestive process. Conversely, when the development of pathogenic bacteria in the digestive tract can be inhibited, then the nutrients will be fulfilled and feed consumption is lower. Many gastrointestinal bacteria are known, but several different viruses have also been identified as causing infections in poultry, including rotavirus, coronavirus, enterovirus, adenovirus, astrovirus, and reovirus.

Furthermore, several other unknown viruses have been associated with gastrointestinal disease in poultry based on electron microscopy examination of feces and intestinal contents. Viral infections of the gastrointestinal tract are known to have a negative impact on production and are likely to contribute to the development of other diseases (Guy 1998).

To inhibit the development of pathogenic bacteria, feed additives such as probiotics and prebiotics can be administered. Lourens-Hattingh and Valjoen (2001) explained that Lactobacillus spp. and Bifidobacterium spp. produce several beneficial metabolites for digestion, namely lactic acid, hydrogen peroxide, and bacteriocin. These metabolites have the ability to inhibit pathogenic bacteria, hence the availability of nutrients increases. The increased nutrient in the digestive tract provides the benefit of lower feed consumption with stable egg production. Adequate nutrition for the hens will affect the amount of production, size, and weight of eggs (Afikasari et al. 2020).

The average egg weight was 50.8g as shown in Table 1, which is classified as medium size. The egg size consists of small size with an average weight of 47.2g, medium with 47.2-54.2g, large with 54.4-61.4, and jumbo with 61.5g. Even ISA (2015) published that the standard egg weight of laying hens strain Isa Brown aged 28-30 weeks is 62g. The factors that affect egg size include the level of sexual maturity, protein adequacy, amino and linoleic acids, genetics, age of chickens, drugs, and other food substances (Mancinelli et al. 2008). Therefore, feed of good quality can produce large eggs, while amino acid deficiency can result in decreased production and size.

In general, the health of laying hens can be measured from their morbidity rate, daily mortality, and blood condition (total erythrocytes, total leukocytes, hemoglobin levels, hematocrit, and leukocyte differential values) (Ardana 2012; Lutfiana et al. 2015), ND antibody titers (Agustin and Ningtyas 2021) and AI (Kencana et al. 2021), as well as the pathology of the small intestinal wall (Ardana 2012). Digestive tract disease is an important concern for the poultry industry because apart from causing a decrease in egg production, increasing morbidity and mortality, it is also harmful to human health. Therefore, during the pullet period, enteritis should be avoided because the pullet has complex stages of body development according to its age period, starting from the starter and growers' phase, hence it should not be disturbed (Puriastuti et al. 2019).

The survey revealed that morbidity during production ranged from 7-8% and the daily mortality rate ranged from 0.03-0.036% (Tab. 2). The breeders stated that the morbidity rate depends on the situation and condition of the disease in the cage. When there is an infectious disease attack, such as Snot, CRD, Fowl Cholera, E. coli, or infection with ND, AI, EDS, and IB viruses, the morbidity rate can increase to 50-80%. However, this incident is very rare.

The morbidity rate in laying hens produced without AGP at the sampling location seemed very small (Tab. 2), but serious pathological problems were found in the small intestine. This can be seen in several hens that appear healthy and then necropsied to observe the pathological condition, it turns out that there is inflammation of the small intestine even to ulceration called necrotizing enteritis. This incident can be related to data that indicate an increase in feed consumption as presented in Table 1, and a decrease in production performance due to intestinal disturbances. Intestinal health depends on maintaining a balance between the host, gut microbiota, gut environment, and nutrients

(Carrasco et al. 2019). This balance can be significantly affected by factors such as poultry rearing, feed quality, and the livestock environment. Several diseases that interfere with small intestinal health include dysbacteriosis, and necrotic enteritis caused by toxins produced by Clostridium perfringens (Torok et al. 2011).

The blood examination in Table 3 shows that laying hens in the production phase reared without using AGP are known to have a normal blood profile. They still get sufficient nutrition to form erythrocytes and are supported by an adequate cage environment. Also, there is adequate temperature and oxygen which are sufficient to meet the needs of binding hemoglobin. The cage temperature where the blood samples were taken ranged from 19-25°C with a humidity of 65-66%. According to North and Bell (1990), the comfortable temperature for hens is 18.3-23.9°C. This condition may cause normal total erythrocytes, hemoglobin levels, and PCV values.

However, the total leukocyte value showed a significant increase (Tab. 3). This supports the incidence of inflammation in the small intestine. This inflammation will be followed by an increase in the production of inflammatory cells (Lubis et al. 2021). The increase and decrease in the number of leukocytes in the circulation reflect their responsiveness in preventing the presence of disease and inflammatory agents (Fournier and Parkos 2012). This indicates that hens in the laying phase that were reared without AGP had a bacterial infection. This is supported by the results of pathological examinations of the small intestine which found enteritis at the time of carcass surgery. However, regarding the heterophile profile as an indicator of bacterial infection, it is still in normal condition (Tab. 4). The high total leukocytes are caused by prolonged stress conditions because they are in the battery cage (Alabi et al. 2014). Therefore, further research needs to be conducted to determine the causes of the high total leukocytes. Table 4 shows that the increase in total leukocytes was not followed by a significant increase in one leukocyte type. The proportion of leukocyte cell types is still within normal values.

The ND and AI antibody titers of the samples showed good protection, all of which have very high antibody titers, namely 29 for ND and 28 for AI (Tab. 5). The antibody titer is obtained from vaccinations regularly. Breeders reported that the ND vaccination program for hens in production was carried out every three months, preceded by the administration of deworming drugs. The AI vaccination program was carried out at 16 weeks and repeated every 15-20 weeks. Likewise, Nurcholis et al. (2009) stated that efforts to prevent ND and AI diseases were carried out by increasing biosecurity and regular vaccinations.

5. Conclusions

In conclusion, the production profile of laying hens produced without AGP experienced a decrease in the age of early and peak production, the percentage and period of peak production, and an increase in daily feed consumption, as well as good egg weight (>50g). The haematological profile was normal, except for an increase in the total leukocyte count. However, there was damage to the structure of the small intestine in the form of necrotic enteritis. Hence, antibody titers are protective against ND and AI diseases.

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Conflict of Interest

All authors declare no conflict of interest in this research

Author contribution

All researchers have participated actively according to their respective duties since the beginning of the study until publication.

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