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4	Anti Early Embryonic Protein (EEP) for Pregnancy Test by Microtiter Strip in Dairy Cows	2019
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6	Antisperm Antibody in Repeat Breeder Friesian Holstein Cows at KPSP Setia Kawan Nongkojajar, Tutur District, Pasuruan, Indonesia.	2019
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Surabaya, 3 April 2023

Wakil Dekan III,

Prof. Dr. Mustofa Helmi Effendi, drh., DTAPH

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ASEAN  
University  
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PROCEEDING



*10<sup>th</sup> Congress and International Conference  
of Indonesian Society for Microbiology*

Surabaya, November 19<sup>th</sup>-21<sup>th</sup>, 2009

**Recent Advances of Microbiology  
in Health, Bio-Industri, Agriculture and Environment**

**Editors :**

Fedik Abdul Rantam  
Koesnandar  
Soewarno  
Purwati  
Eryk Hendrianto

Wm  
✓

# PROCEEDING



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### **Editors :**

Fedik Abdul Rantam

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**Proceeding**

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**Editor :**

Fedik Abdul Rantam, Koesnandar, Soewarno, Purwati, Eryk Hendrianto

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Surabaya, November 19<sup>th</sup>, 2009

## OPENING REMARK CHAIRMAN OF ORGANIZING COMMITTEE

Microbiology-based industrial development to date is a fundamental need in the development of health and welfare of the whole community. Microorganisms are the foundation exploration and engineering that have a competitive and productive, so has the value of a significant advantage. Basic of this development based on the role of molecular biotechnology that utilizes microorganisms. The microorganism is use for industrial, environmental remediation, diagnostic, therapeutic materials, materials for vaccine and gene therapy vectors.

Congress of this proceeding are :

Theoretically Molecular exploration can be developed and applied in the industrial world for that health is a critical point of this approach to creating superior products based on microorganisms.

This Proceeding has discussed of about the latest developments bioindustri role in the development of microbiology, health, therapeutic, food technology, agriculture and environment. The focus of the discussion which will be discussed, lactic acid, enzymes, vaccines, diagnostics, immunotherapy, probiotics, food and environment-based bioindustri and microbiology.

PERMI in espousing the construction industry based on these microorganisms in cooperation with the International Microbiology society (IMS), the Association of lactic acid, Institute of Tropical Disease (ITD) as a center of medical biotechnology, Airlangga University, which is a tropical disease research center that focuses on education, research and service in health field. Related to those things need to be addressed so that the development of scientifically-based industrial technology on microorganisms are the main targets in the development of biotechnology that leads to its role in the bioindustri, health, food security and environment.

Surabaya, November 19<sup>th</sup> 2009

Chairman of the Committee,



Prof. Dr. Fedik A. Rantam, DVM

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# UNNATURAL FORCED MOULTING IN THE LAYING HEN AS CAUSE OF ZOONOSIS FROM *SALMONELLA ENTERITIDIS*

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## Abstract

The aims of this reseach is to give information to the farmers and people about the bad consequences of consuming the poultry products that use unnatural forced moulting. The research was carried out by examining *Salmonella enteritidis* bacteria in the laying hens feces after treatment.

The laying hens devided in three groups and treatment with unnatural forced moulting, dietary feed carried out during 2, 3 and 4 days, and the another group given natural forced moulting treatment by 200 ìg/ml each hen á Prl single dose compared with the group without dietary feed and á Prl.

The result showed that unnatural forced moulting with dietary feed during 2, 3 and 4 days decrease laying hen immune function. It was founded *Salmonella enteritidis* in laying hens feces after treatment. The *Salmonella enteritidis* can be the causal factor of zoonosis for human.

**Key words :** Unnatural forced moulting, natural forced moulting, laying hen,  $\alpha$  Prl, *Salmonella enteritidis*, zoonosis



## Introduction

Problem of stopping egg production when laying hens enter molting period cause many loss of benefits, economically and time (Safitri, 2005, Safitri, 2008). First molting period of laying hens is at 22<sup>nd</sup> – 24<sup>th</sup> months. The duration of this period is 60 -75 days (Darmana and Sitanggang, 2002, Sudarso and Siriwa, 2002). If there is no action against this period, it will take longer time for laying hens to produce again eggs, about 80-100 days (Marhiyanto, 2000, Indarto, 1989, Juli, 1982).

Bell and Kunney (2003) and Avma (2003) found that to overcome molting period in United States, it has been done natural forced molting and unnatural forced molting. The forced molting method carried out in 3 ways : dietary feed, giving low nutrient feed (protein, calcium, and natrium), use of drugs and methalibure, chlordamine, high dose iodium, aluminum, and zinc.

In Indonesia, it has been done many efforts to overcome molting period : dietary feed (unnatural forced molting). It was carried out for about 30 days (Safitri, 2008, Barton, 2003, Sainsbury, 1995). Long unnatural forced molting can cause stress to laying hens, decrease of immune function, and laying hens can be attacked easily by diseases (Poultry, 2003, Alodan and Mashalay, 1999).

One of the diseases follows unnatural forced molting is Salmonellosis caused by *Salmonella enteridis* (Sukamto and Hendarti, 2008, Fact, 2001, Webster, 1999). Salmonellosis is infectious disease and zoonosis to human. Since 2000, it has been banned the use of unnatural forced molting by dietary feed (Avma, 2003).

Butcher and Miles (2002) found that unnatural forced molting decreased T cell in blood. The decrease of T cell can cause the immune decrease and increase the sensitivity to the disease, especially Salmonellosis. Turner and Bagnara (1998), Knobil et al (1988) and Hafez (2000), found high prolactin hormone at molting hens. Safitri (2005) and Safitri et al (2006) found an alternative to overcome molting period by natural method to arabic hens and laying hens (use of  $\alpha$  Prl). It is effective to resist molting period and make laying hens produce again eggs.

The present research is carried out to give information about the method to overcome molting period by natural forced molting as substitute of unnatural forced molting method.

## Materials and methods

Fifty laying hens in molting period were grouped and placed in cage randomly by five treatments (each treatment with 10 repetitions):

- PO (control) : ten laying hens in molting period without natural forced molting and without unnatural forced molting
- P1 : ten laying hens in molting period were treated by natural forced molting, by giving single dose of  $\alpha$  Prl 200  $\mu$ g/mL each laying hens

- P2 : ten laying hens in molting period were treated by unnatural forced molting, by dietary feed for 2 days consecutive without feed and only drink given
- P3 : ten laying hens in molting period were treated by unnatural forced molting, by dietary feed for 3 days consecutive without feed and only drink given
- P4 : ten laying hens in molting period were treated by unnatural forced molting by dietary feed for 4 days consecutive without feed and only drink given

In the fifth day, it has been checked *Salmonella* in 50 laying hens feces. The method was by taking one colony round shaped and transparent from MCA media, then growth at SSA media, and put at 37°C for 24 hours. Identification test were done to the colony. To ensure that the colony growth at SSA media were *Salmonella enteritidis*, biochemical tests were done to various media (TSIA, SIM, citrate, urea, and sweets).

#### **Triple Sugar Iron Agar (TSIA) Media**

The growth of *Salmonella* in TSIA media was done by taking *Salmonella* from pure culture by sterile needle then put it in the bottom of agar, and then media was putted at 37°C for 24 hours. The growth in this media is to evaluate the ability of *Salmonella* to ferment glucose, sucrose, and the formation of H<sub>2</sub>S and gas (Cappucino and Sherman, 1983).

#### **Sulfide Indol Motility (SIM) Media**

The growth in SIM media was done by taking *Salmonella* from pure culture then put it in the half part of agar and the put the media at 37°C for 24 hours. Observation was done by adding chloroform and Kovach reagent, both at the same quantity. Positive result was shown by indol circle (red).

#### **Citrat Agar**

The growth in citrate agar was done by streaking in media. The objective of this growth is to identify whether *Salmonella* uses carbon from citrate. Positive result was shown by the change of color to blue and negative result was shown by the change of color to green.

#### **Urea Agar**

Pure culture was taken to streak in media. The objective of this test to identify whether *Salmonella* produces enzyme that can hydrolyze urea to ammonia. The positive result was shown by the change of media to red color.

- P2 : ten laying hens in molting period were treated by unnatural forced molting, by dietary feed for 2 days consecutive without feed and only drink given
- P3 : ten laying hens in molting period were treated by unnatural forced molting, by dietary feed for 3 days consecutive without feed and only drink given
- P4 : ten laying hens in molting period were treated by unnatural forced molting by dietary feed for 4 days consecutive without feed and only drink given

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#### **Urea Agar**

Pure culture was taken to streak in media. The objective of this test to identify whether *Salmonella* produces enzyme that can hydrolyze urea to ammonia. The positive result was shown by the change of media to red color.

## Sweets

The growth in sweets media was done by taking Salmonella the put it in media. The objective of this growth is to identify whether Salmonella ferments sweets (Anonymous, 1999).

## Experiment Design and Statistical Analysis

In this present research, complete random design is used. The results were analyzed by Analysis of Variant (ANOVA) and least significant difference 5% level test if there was the different result (Steer and Torrie, 1995).

## Results and Discussion

The results of experiments are shown in the table 1.

Table 1 Identification of Salmonella in different treatment samples

Repetition	Treatment				
	Control	P1	P2	P3	P4
1	-	-	+	+	+
2	-	-	-	+	+
3	-	-	+	+	+
4	-	-	+	+	+
5	-	-	+	+	+
6	-	-	-	+	+
7	-	-	+	+	+
8	-	-	+	+	+
9	-	-	+	+	+
10	-	-	-	+	+
Sum	0 <sup>a</sup>	0 <sup>a</sup>	7 <sup>b</sup>	10 <sup>c</sup>	10 <sup>c</sup>

- : negative, no Salmonella found

+: positive, Salmonella found

The positive results in the table shown that there were Salmonella enteriditis in the tested feces. It is identified by biochemical test by TSIA, SIM, Citrat agar, urea agar, and sweets methods as shown in table 2.



Table 2. Identification of *Salmonella enteritidis* in different media

Media	Result
TSIA	+
Base	+
Acid	+
H <sub>2</sub> S	+
Gas	+
SIM	-
Motility	-
Indol	-
H <sub>2</sub> S	+
Citrate	-
Urea	-
Glucose	+
Lactose	-
Manitol	+
Maltose	+
Sucrose	-

According to statistical analysis, one way ANOVA, it was found there was no significant difference ( $p < 0,01$ ) between results of control and natural forced molting ( $\alpha$  Prl 200 mg/L each hen). Between results of control and P1,P2,P3, there was significant different ( $p < 0,01$ ), then it is necessary to analyze with least significant difference in order to determine the most important treatment that influence the existence of salmonella.

Butcher and Miles (2002) found that dietary feed will decrease T cell in blood circulation. By this decrease, it will decrease immune reaction and increase the sensitivity to certain disease. The disease that occurs especially is *Salmonella enteritidis*. According to Fact (2001), Webster (1999), this disease is very dangerous because it is zoonosis for human.

*Salmonella enteritidis*, the cause of Salmonellosis, is 1-2,5 micron length and 0,3-0,5 micron width, negative gram, non motile, without spore formation, and no capsule. The optimum growth of *Salmonella enteritidis* is at 37°C and in *Salmonella shigella* agar forms segmented colony, clear, and transparent (Hagan and Bruner, 1981).

Salmonellosis is infectious disease that spreads all over the world. Mammalian can be infected by this disease: rabbit, human (Ressang, 1984).

Salmonellosis spreads via oral wit feed, inhalation of polluted air (feather, dust, infected feces). Another way of spreading is congenital via egg (Hosfad, 1984). Factors that enhance the spreading are bad air circulation system, bad sanitation system, bad feed supply, another disease that may give synergic effect (Anonymous, 1981).

Salmonella enteritidis reaches optimum growth at 37°C and aerobic or anaerobic facultative. It can produce H<sub>2</sub>S, acid and gas from glucose, ferments manose, but can not ferment lactose. Maltose can be fermented rarely, sucrose not fermented. Salmonella enteritidis does not have urease enzyme and can not use carbon from citrate (Hofad, 1984, Kingscote, 1989).

Isolation of Salmonella enteritidis needs selective media. These media are Salmonella Shigela Agar (SSA), Mac Concey Agar (MCA), and Endo Agar. The growth of Salmonella enteritidis in SSA is manifested by colony formation which is smooth, round, transparent, and without color. Diameter of colony is 1 to 4 mm. Salmonella enteritidis in TSIA media gives the change of red color (base), yellow (acid), H<sub>2</sub>S formation (black), gas formation (burst medium). The growth of Salmonella enteritidis in SIM manifested by white color (non motile). Black formation shows H<sub>2</sub>S and negative inol test (Jackson and Simmon, 1981, Hofstad, 1984).

The present research gives information to farmers, and people the danger of poultry products produced by unnatural forced molting to overcome molting. Information can be presented in case of Salmonella enteritidis, found in laying hens feces.

## Conclusion

Unnatural forced molting by dietary feed to overcome molting can cause case of salmonella enteritidis which is zoonosis to human.

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