Survival Analysis of the Effect of Season at Calving, Lactation Number and Breeding on Days Open in Dairy Cattle

E. TAUFIK[1] and W. SURIYASATAPHORN[2]

[1]*Department of Animal Production Science and Technology, Faculty of Animal Science, Bogor Agricultural University (IPB), Bogor 16680, Indonesia. email: etaufik@yahoo.com*

[2]*Ruminant Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*

(Diterima dewan redaksi 13 Mei 2008)

ABSTRAK

TAUFIK, E. dan W. SURIYASATAPHORN. 2008. Analisis survival dari pengaruh musim saat melahirkan, jumlah laktasi dan pemuliaan terhadap masa kosong pada sapi perah. *JITV* 13(3): 214-220.

Tujuan dari kajian ini adalah untuk mengevaluasi pengaruh musim saat melahirkan, jumlah laktasi dan pemuliaan terhadap masa kosong (jeda waktu antara melahirkan anak dengan bunting kembali) pada sapi perah dengan menggunakan model Cox proportional hazards sebagai metode analisis survival. Data contoh diambil dari 143 ekor sapi perah betina dewasa dari 6 peternakan yang berlokasi di Propinsi Khon Khaen, arah Timur Laut Thailand. Data tersebut kemudian diklasifikasikan sebagai identitas peternakan (FID), identitas sapi perah betina dewasa (CID), tanggal beranak (CDA), tanggal terakhir pengambilan data kondisi sapi (LAF), persentase Holstein-Friesian dalam pemuliaan (PHF), jumlah laktasi (LAN) dan kebuntingan (1 = bunting, 0=tidak bunting). Masa kosong merupakan selisih antara LAF dengan CDA dan CDA telah digunakan untuk menentukan musim saat melahirkan. Hasil kajian menunjukkan bahwa berdasarkan Kaplan-Meier survivorship percentiles, nilai median masa kosong untuk seluruh sapi perah yang dikaji adalah 210 hari, sedangkan untuk sapi perah yang beranak di musim panas dan selain musim panas masing-masing adalah 231 dan 210 hari. Adapun nilai median masa kosong untuk sapi perah yang beranak pada laktasi pertama dan pada laktasi kedua atau lebih masing-masing adalah 226 dan 207 hari. Nilai median masa kosong untuk sapi perah dengan persentase darah Holstein-Friesian hasil pemuliaan ≤75% adalah 211 hari dan 206 hari untuk sapi dengan persentase Holstein-Friesian >75%. Hasil regresi dengan menggunakan Cox proportional-hazard terhadap data masa kosong sapi perah asal Khon Khaen menunjukkan bahwa sapi perah yang beranak di musim hujan dan musim dingin memiliki peluang masing-masing 1,28 dan 1,76 kali lebih besar untuk bunting dibanding sapi perah yang beranak di musim panas, walaupun perbedaannya tidak signifikan. Sementara itu, sapi perah yang telah mengalami masa laktasi dua kali atau lebih memiliki peluang 1,54 kali lebih besar untuk bunting dibandingkan dengan sapi perah yang baru satu kali laktasi, hanya saja peluang ini tidak signifikan secara statistik (P = 0,1725). Adapun sapi perah dengan persentase darah Holstein Friesian >75% memiliki peluang yang nyata 1,17 kali lebih besar untuk bunting dibandingkan dengan sapi perah yang memiliki persentase darah Holstein Friesian ≤75%.

Kata Kunci: Analisis Survival, Model Cox Proportional Hazard, Sapi Perah, Masa Kosong

ABSTRACT

TAUFIK, E. and W. SURIYASATAPHORN. 2008. Survival analysis of the effect of season at calving, lactation number and breeding on days open in dairy cattle. *JITV* 13(3): 214-220.

The objective of this study was to evaluate the effect of season on calving, lactation number and breeding on days open (interval between calving and conception) in dairy cattle by using Cox proportional hazards model as a survival analysis method. The data were sampled from 143 cows at 6 farms located in Khon Kaen Province, North-Eastern Thailand and classified as farm identification (FID), cow identification (CID), calving date (CDA), date at last follow up (LAF), percentage of Holstein-Friesian (PHF), lactation number (LAN) and conception (1 = concieve, 0 = not concieve). Time of days open was calculated by subtracting LAF by CDA and CDA was used to determine season of calving. The result showed that based on Kaplan-Meier survivorship percentiles, overall median days open of cattle were at 210, whereas median days open for the cow calved in summer was 231 and 204 for the cow calved in other season. Median days open for the cow calved with one lactation was 226 and 207 for the cow with two lactation and more. Median days open for the cow with percentage of Holstein-Friesian ≤75% was 211 and 206 for the cow with percentage of Holstein-Friesian >75%. The result from Cox proportional-hazard regression of days open for Khon Khaen dairy cows showed that cows that calved in rainy and winter had a greater chance of 1.28 times and 1.76 times, respectively, of becoming pregnant than those calved in summer, although the difference was insignificant and cows with lactation number two or more were marginally had 1.54 times chance to get pregnant compare to cows with one lactation number, even though this chance was not statistically significant (P = 0.1725), whereas cows with percentage of Holstein Friesian >75% had significantly greater chance 1.17 times more to get pregnant compare to those with percentage of Holstein Friesian ≤75%.

Key Words: Survival Analysis, Cox Proportional Hazard Model, Dairy Cow, Days Open

INTRODUCTION

The most common reason for culling in dairy herds is caused by poor reproductive performance (DURR, 1997; PRYCE *et al.*, 1997; SWEDISH DAIRY ASSOCIATION, 2002). Low fertility, lower production per day, higher insemination costs and higher replacement costs due to increased culling are the main variable costs related to low fertility. Cow with good fertility is characterized by short period time of days open, has a high probability of becoming pregnant when inseminated at the correct time, shows strong signs of estrus, and has the ability to carry the resulting fetus to term. Among the potential measures that can be used to describe these complex traits is days open or interval between calving and last insemination (CLI). The trait CLI is a measure that is a combination of return to cyclicity, the expression of estrus, and the ability to conceive (conception rate). If insemination dates are available, CLI can be used in breeding programs, which is the case in some countries (MARK *et al.*, 2001; SCHNEIDER *et al.*, 2005).

Survival analysis is an alternative method for analyzing reproductive traits recorded as time intervals (ALLORE *et al.*, 2001). Survival analysis or Cox proportional hazard model is a statistical method for studying the occurrence and timing of events, where the outcome variable corresponds to a measure of time elapsed from a starting point until the occurrence of a certain event (SCHNEIDER *et al.*, 2005). The length of this interval is not always known, because competing events may occur before the occurrence of the event under study. One of the main advantages of survival analysis is that it could retain the information from cows that are culled before conception or not pregnant by the time the data recording was completed. Thus, records from pregnant (uncensored) and non-pregnant (censored) cows can be treated jointly and included in the analysis, making proper use of all the available information. Within the field of fertility in dairy cattle, survival analysis has been applied to study: 1) the effects of diseases on days to conception (LEE *et al.*, 1989; HARMAN *et al.*, 1996b), 2) the relationship between body condition scoring (BCS) and postpartum reproductive efficiency (SURIYASATHAPORN *et al.*, 1998), and 3) the effect of early lactation milk yield on days open (HARMAN *et al.*, 1996a). 4) The effects of calving season and milk yield on pregnancy risk and income (FARIN *et al.*, 1994).

Terminology using in most studies on survival analysis are survival time, failed case, censored case, hazard's function, hazard ratio, life table, Kaplan-Meier curve, and Cox's proportional hazard model (SURIYASATHAPORN, 2006). At the beginning, survival analysis is involved an event of death. Nowadays, survival analysis can be used for any event. Therefore, time-event analysis or Cox's proportional-hazards models are used instead of survival analysis in some studies (SURIYASATHAPORN *et al.*, 1998).

The aim of Cox's proportional hazard model is to assess hazard ratio, HR (analogous to risk ratio or relative risk), that uses to estimate the ratio of the rates of intended event (in this study was pregnancy) at the particular value compared with both rates at a reference value, respectively. That is to say, if the pregnancy risk at a particular value was >1, it meant that the pregnancy rate of the particular value was higher than the pregnancy rate of the reference value (SURIYASATHAPORN, 2006).

According to LEE (1984), in general survivor function is very useful to explore the data and become familiar with it. However, it is becomes a bit limited when survival depends upon many covariates. To solve this problem, the hazard function was used. Hazard function (instantaneous hazard, force of mortality) denoted by λ (t), is the risk that an event will occur during a very short time interval (s (t)) at time t, given the subject did not have an event before that time. The very popular regression in survival analysis using hazard function is Cox's proportional hazard model. Proportional hazards regression is computed using the ranks of the survival times. While it is useful for studying the relationships among the covariates, it can't be used to build prediction equations.

Therefore, the goal of the analysis is to assess the HR. For categorical variable, HR is the ratio of hazard of the particular value and a reference value or the so-called baseline hazard. The assessed HR (analogous to risk ratio or relative risk), is used to estimate the ratio of the rates of event of interest at the particular value compared with the rate at a reference value.

The main objective of this study was to evaluate the effect of season at calving, lactation number and breeding on days open (interval between calving and conception) in dairy cattle from dairy farm at Khon Khaen Province, Thailand by using Cox proportional-hazard regression. In juncture, this study was also aimed to show that this type of survival analysis can be used to analyze the interaction between farm animal characteristics and their environment which is involving time and occurrence of the event of interest.

MATERIALS AND METHODS

Data collection

Data were collected from 143 cows at 6 farms located in Khon Kaen Province, north-eastern Thailand. Farm identification (FID), cow identification (CID), calving date (CDA), date at last follow up (LAF), percentage of Holstein-Friesian (PHF), lactation number (LAN) and event of interest (1 = conception, 0

= not conception) (event) were recorded by MS-EXCEL.

Outcome and explanatory variables

The outcome variable (dependent factor) in this study was survival time of days open which is the interval between the calving date (CDA) and date at last follow up (LAF). The censoring variable defined by SURIYASATAPHORN *et al.* (1998) that cow did not conceive within 184 days postpartum but still present at that point in time was used to classify event of interest (event 1 = conception, 0 = not conception). Whereas the explanatory variables (independent factors) were season at calving (summer, rainy and winter) which was determined from CDA, lactation number (Par1 and Par2) and percentage of Holstein Friesian breed in the animal (per_HF1 (≤75%) and per_HF2 (> 75%)).

Statistical analysis

Cox proportional hazards models were used to examine effects of calving season, lactation number and percentage of Holstein Friesian breed in the animal on days open (the outcome variable). Following addition and deletion of individual variables in the model, the maximum likelihood ratio statistic was examined for shifts in magnitude. The final model included the main effects of lactation number, calving season, and percentage of Holstein Friesian breed.

The other survival function calls corresponding estimate of the survivor function S(t). This method is very similar to the life table estimator apart from number at risk. This is the Kaplan-Meier estimate of the survivor function which is presented in the Kaplan-Maier curve. The Kaplan-Maier curve depicted the association between survivorship S (t) and survival time (in this study case, time = days open and event = pregnancy). From this function and curve, the median of days open for overall sample as well as for each grouping of explanatory variable was determined.

In the analysis, independent variables were treated as categorical variables rather than continuous. Lactation number (LAN) data were categorized into one lactation (par1=1 as baseline) and else (two lactation or more; par2=0), calving seasons were divided into summer (as baseline), rainy and winter. In northern Thailand, summer is between March to May (temperature: 20-38^{0}C); rainy is between June to October (temperature: 22-34^{0}C) and winter is between November to February (temperature: 11-33^{0}C). Breed of Holstein-Friesian was categorized into per_HF1 (≤75%) and per_HF2 (> 75%). The P value from Log Likelihood Chi-square of overall model will be used to assess the differences between categories in one independent variable.

The Cox proportional-hazards model was used to examine the effects of baseline and follow-up variables on the outcome variables. The goal of the analysis was to assess the hazard ratio (HR) of the particular value compared with a reference value (HR1). The assessed HR (analogous to risk ratio or relative risk in epidemiology; LEE, 1984), was used to estimate the ratio of the rates of pregnancy at the particular value compared with rates at a reference value. That is to say, if the pregnancy risk at a particular value was >1, it means that the pregnancy rate of the particular value was higher than the pregnancy rate of the reference value. Statistical software package of *Statistix 8*® was used for the statistical analysis.

RESULTS AND DISCUSSION

Kaplan-Maier Curve

The Kaplan-Maier curve for overall survival time and event from this study result is shown in Figure 1. Based on Kaplan-Meier survivorship percentiles, in overall, median days open of dairy cattle was at 210. This median of days open was higher compared to the mean of days open of dairy cattle in the northern part of Thailand which was 131 days as reported by PUNYAPORNWITHAYA and TEEPATIMAKORN (2004). But it was relatively similar to the report by SONDIPHIPOJ *et al.* (1999) that the mean of days open of pure breed Holstein Friesian imported from Canada to Thailand at Chiang Mai Livestock Research Center was 201 days.

Based on Kaplan-Meier survivor-ship percentiles, median days open for the cow calved in summer was 231 and 204 for the cow calved in other season (Figure 2). There was no significant difference between groups (summer and else) since two samples survival test by Cox-Mantel test resulted P = 0.2621.

Median days open for the cow calved with one lactation was 226 and 207 for the cow with two lactation and more (Figure 3). Based on two samples survival test by Cox-Mantel test, P=0.60581, it means there was no significant difference between groups of cow with one lactation and more.

Median days open for the cow with percentage of Holstein-Friesian ≤75% was 211 and 206 for the cow with percentage of Holstein-Friesian >75% (Figure 4).

The P-value from two samples survival test by Cox-Mantel test was P=0.6243, it meant there was no significant difference between groups of cow with percentage of Holstein-Friesian ≤75% and with percentage of Holstein-Friesian >75%.

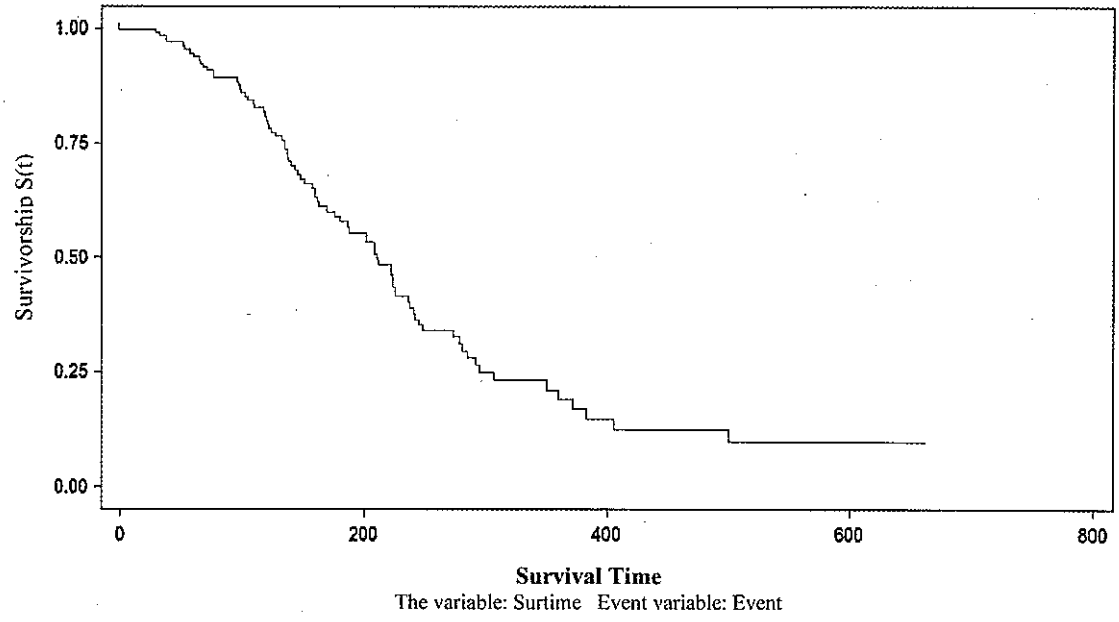


Figure 1. The Kaplan-Maier curve for survival time (day) and event for overall model

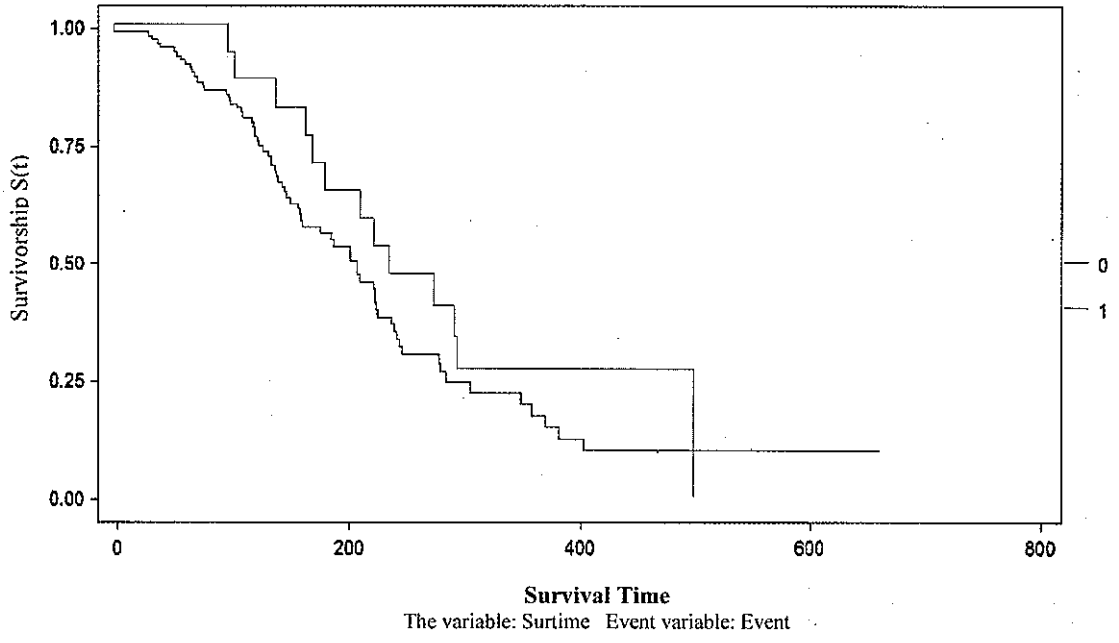


Figure 2. The Kaplan-Maier curve of survival time (day) and event for season {1 = summer as baseline, 0 = else (winter and rainy)}

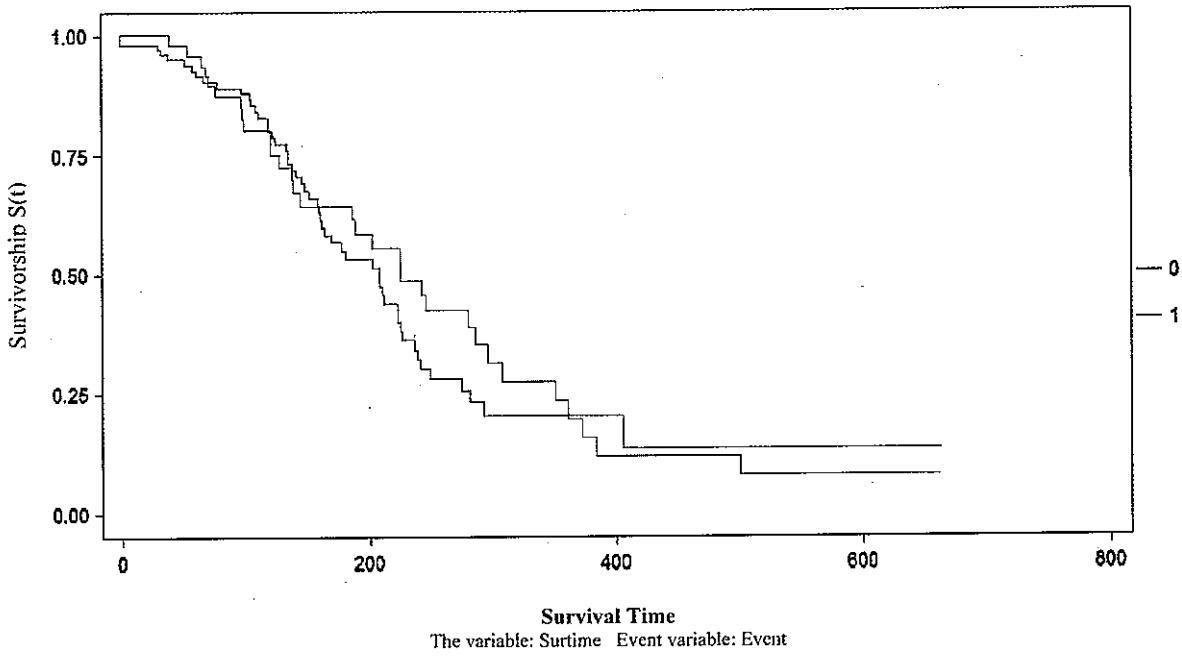


Figure 3. The Kaplan-Maier curve of survival time (day) and event for lactation number {1 = lactation 1 as baseline, 0 = else (lactation 2 and above)}

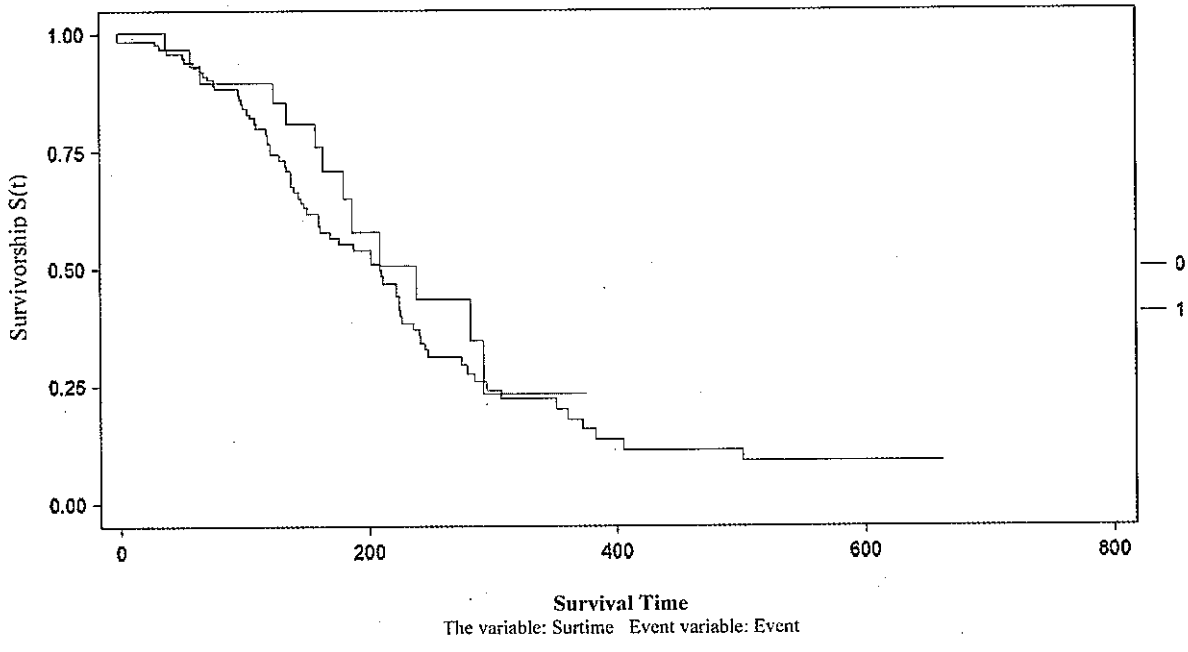


Figure 4. The Kaplan-Maier curve of survival time (day) and event for percentage of Holstein-Friesian of cow (1 = ≤75% as baseline, 0 = else >75%)

Table 1. Cox proportional hazards model of days open for Khon Khaen dairy cows

Variable	β	SE β	P	Hazards Ratio
Season at calving				
Summer	-0.01077	0.43537	0.9803	0.99
Rainy	0.24709	0.38000	0.5155	1.28
Winter	0.56794	0.38337	0.1385	1.76
Lactation number				
Par1	-0.02656	0.24399	0.9133	0.97
Par2	0.43231	0.31686	0.1725	1.54
Percentage of Holstein-Friesian				
Per_HF1 (<=75%)	-0.13132	0.000001	0.0000	0.88
Per_HF2 (>75%)	0.15468	0.000001	0.0000	1.17

Season at calving

The result of log likelihood Chi-square of overall model was showed P value = 0.2443, meaning that there was no difference between season. Compared to cows that calved in summer, cows that calved in rainy and winter season were marginally more likely to become pregnant. Cows that calved in rainy and winter had a greater chance of 1.28 times and 1.76 times of becoming pregnant than those that calved in summer, although the difference was not significant. Despite of statistical insignificancy, the research finding was consistent with report by FARIN *et al.* (1994) that cows calving in summer were less likely to become pregnant than cows that calved during the cooler months. This was due to adverse effect of temperature on reproduction performance of dairy cattle. JORDAN (2003) supported this biological phenomenon; he stated that when dairy cattle are subjected to heat stress, reproductive efficiency declines. Cows under heat stress have reduced duration and intensity of estrus, altered follicular development, and impaired embryonic development.

Lactation number

P value from log likelihood Chi-square overall model was more than 0.05 (P = 0.3687), therefore there was no significant difference between lactation numbers. Cows with lactation number 2 or more were marginally had 1.54 times chance to get pregnant compare to cows with one lactation number, even though this chance was not statistically significant (P=0.1725). This result was different with finding reported by PUNYAPORNWITHAYA and TEEPATIMAKORN (2004) that there was a significant difference between first lactation cows compare to second-third and more than third lactation cows on days open of cows in northern part of Thailand. The difference of statistical significance test result of the data from this study was likely to be associated with the difference of sample size, PUNYAPORNWITHAYA and TEEPATIMAKORN (2004) evaluated the data from 6,125 heads of cow.

But there was similarity of this study finding with the study result reported by PUNYAPORNWITHAYA and TEEPATIMAKORN (2004) that stated cows with more than one lactation had more chance to get pregnant with shorter time of days open than those with only one lactation. On the other hand this finding was in agreement with the result reported by FARIN *et al.* (1994) that there was no significant difference between lactation numbers on days open in North Carolina (USA) Holstein cows.

Percentage of Holstein Friesian (Breeding)

The result of log likelihood Chi-square of overall model gave P value = 0.6243, therefore no difference between percentage of Holstein Friesian breed composition grouping (≤75% and >75%) of cows. There was significant difference between group (P = <0.05), meaning the cows with percentage of Holstein Friesian more than 75% had greater chance 1.17 times more to get pregnant compare to those with percentage of Holstein Friesian less than 75%. It can be said that Holstein Friesian breed is significantly better (P=0.000) in reproductive performance than any other dairy cattle breeds.

CONCLUSIONS

The Kaplan-Meier survivorship percentiles data showed that in overall, median days open of dairy cattle in Khon Kaen Province of northern Thailand was 210 days. Median days open of non baseline variables in the grouping variables were less than baseline variables.

Grouping of independent variables did not give significant difference effect on outcome variables. Cows that calved in rainy and winter had a greater chance of 1.28 times and 1.76 times of becoming pregnant than those that calved in summer, although the difference was not significant. Cows with lactation number 2 or more were marginally had 1.54 times chance to get pregnant compare to cows with one lactation number, even though this chance was not statistically significant. Whereas the cows with percentage of Holstein Friesian more than 75% had significantly ($P<0.05$) greater chance 1.17 times more to get pregnant compare to those with percentage of Holstein Friesian less than 75%. It was also proved that survival analysis of Cox proportional hazards model can be applied as a tool of analysis to assess interaction between farm's environment and characteristics of animal.

REFERENCES

ALLORE, H.G., L.D. WARNICK, J. HERTL and Y.T. GROHN. 2001. Censoring in survival analysis: A simulation study of the effect of milk yield on conception. *Prev. Vet. Med.* 46: 223–234.

CHONGKASIKIT N., T. VEARASILP, K.GATPHAYAK and J.D. KABASA. 2004. The Effects of Calving Season and Calving Years on Productive and Reproductive Traits of Dairy Cattle Population in Thailand. The Proceeding of Deutscher Tropentag. 5-7 October 2004. Berlin: Humboldt-Universtät zu Berlin. Pp. 320.

DURR, J.W. 1997. Genetic and phenotypic studies on culling in Quebec Holstein cows. *Ph.D. Dissertation.* McGill Univ., Montreal, Canada.

FARIN, P.W., B.D. SLENNING, M.T. CORREA and J.H. BRIT. 1994. Effects of calving season and milk yield on pregnancy risk and income in North Carolina Holstein cows. *J. Dairy Sci.* 77: 1848-1855.

HARMAN, J.L., Y.T. GROEHN, H.N. ERB and G. CASELLA. 1996a. Event time analysis of the effect of 60-day milk production on the parturition-to-conception interval in dairy cows. *Am. J. Vet. Res.* 57: 634–639.

HARMAN, J. L., Y. T. GROEHN, H. N. ERB and G. CASELLA. 1996b. Event time analysis of the effect of season of parturition, parity, and concurrent disease on parturition-to-conception interval in dairy cows. *Am. J. Vet. Res.* 57: 640–645.

JORDAN, E.R. 2003. Effect of heat stress on reproduction. *J. Dairy Sci.* 86(E. Suppl.): E104–E114.

LEE, E.T. 1984. Statistical methods for survival data analysis. 1st ed. Wiley, New York, pp. 557.

LEE, L.A., J.D. FERGUSON and D.T. GALLIGAN. 1989. Effect of disease on days open assessed by survival analysis. *J. Dairy Sci.* 72: 1020–1026.

MARK, T., U.S. NIELSEN, J. POSO, M. GUNDEL and M. SVENDSEN. 2001. Genetic relationships among functional traits in the Nordic Holstein populations. *Interbull Bull.* 27: 64–67.

PRYCE, J.E., R.F. VEERKAMP, R. THOMPSON, W.G. HILL and G. SIMM. 1997. Genetic aspects of common health disorders and measures of fertility in Holstein Friesian dairy cattle. *Anim. Sci.* 65: 353–360.

PUNYAPORNWITHAYA, V and S. TEEPATIMAKORN. 2004. Reproductive efficiency of dairy cows in the northern part of Thailand. *Chiang Mai Vet. J.* 2: 3-8.

DEL P. SCHNEIDER, M., E. STRANDBERG, V. DUCROCQ and A. ROTH. 2005. Survival analysis applied to genetic evaluation for female fertility in dairy cattle. *J. Dairy Sci.* 88: 2253–2259

SONDHIPIPOJ, P., S. SUBROD and P. MAHINCHAI. 1999. Reproduction and production performances of imported Holstein cows from Canada. The proceedings of 37th Kasetsart University Annual Conference. 4-7 February 1999. Bangkok:Kasetsart University, pp. 237-48.

SURIYASATHAPORN, W., M. NIELSEN, S.J. DIELEMAN, A. BRAND, E.N. NOORDHUIZEN-STASSEN and Y.H. SCHUKKEN. 1998. A Cox proportional-hazards model with time-dependent covariates to evaluate the relationship between body-condition score and the risks of first insemination and pregnancy in a high-producing dairy herd. *Prev. Vet. Med.* 37: 159–172.

SURIYASATHAPORN, W. 2006. Introduction to Survival Analysis. Modul. Ruminant Clinics, Faculty of Veterinary Medicine, Chiang Mai University, Thailand.

SWEDISH DAIRY ASSOCIATION. 2002. Husdjursstatistik (cattle statistics). Svensk Mjolk, Eskilstuna, Sweden.

Jv — mfn. 1490

Analisis Residu Sulfametazin pada Produk Unggas

YULVIAN SANI dan R. WIDIASTUTI

Balai Besar Penelitian Veteriner
Jl. R.E. Martadinata 30 – Bogor

(Diterima dewan redaksi 10 April 2008)

ABSTRACT

SANI, Y. and R. WIDIASTUTI. Residue analysis of sulfametazine in poultry product. *JITV* 13(3): 221-228.

Sulfamethazine (SMZ) is a sulfonamide preparate widely used in feed to control and prevent diseases, and to promote growth. The use of sulfonamides may lead to residue formation, induce microbial resistance and suspected as a carcinogen. A serial study has been done to investigate the withdrawal pattern of SMZ in meat and liver tissues in order to reduce residue of SMZ. A total of 80 day old chicken were divided into 4 groups: (1) negative control without SMZ; (2) positive control dosing with SMZ for 35 days; (3) treatment-1 dosed with SMZ for 28 days consecutively three times per week then ceased thereafter; and (4) treatment-2 dosed with SMZ for 30 days consecutively three times per week then ceased thereafter. A field study revealed that some antimicrobials were detected in poultry meat samples, such as sulfamerazine ($\bar{X}$ = 2.52 ppb or undetected – 12.62 ppb) and sulfamethazine ($\bar{X}$ = 0.02 ppb or undetected – 0.09 ppb). An intragastric dosing of SMZ at 50 mg/kgBW did not affect growth. Both positive control and treated groups showed haemorrhagic enteritis, nottling of capsular surface of liver and pale kidneys. Pathological changes were not found in negative control. Microscopically, pathological changes in liver, intestines and kidneys were found consistently in SMZ treatment. Withdrawal time of SMZ in broilers was between 5 to 10 days. Therefore it is strongly recomended that sulfonamides treatment should be withdrawn 5 to 10 days prior to culling of birds and substitution of feed with unmedicated feed within this period to produce safe and healthy poultry product.

Key Words: Residue, Sulfametazine, Poultry, Meat, Prevention

ABSTRAK

SANI, Y. dan R. WIDIASTUTI. Analisis residu sulfametazin pada produk unggas. *JITV* 13(3): 221-228.

Sulfametazin (SMZ) adalah salah satu preparat sulfonamida yang sering digunakan dalam pakan ternak untuk pengendalian dan pencegahan penyakit serta imbuhan pakan. Penggunaan preparat sulfonamida ini dapat menimbulkan residu pada produk unggas, resistensi agen penyakit dan diduga mengandung materi karsinogen. Sebanyak 80 ekor ayam pedaging umur sehari dibagi menjadi 4 kelompok yang terdiri dari: (1) kelompok kontrol negatip tanpa pemberian SMZ selama perlakuan; (2) kelompok kontrol positip yang diberi SMZ selama perlakuan; (3) kelompok perlakuan-1 yang diberi SMZ hingga 7 hari sebelum akhir percobaan umur 36 hari; dan (4) kelompok perlakuan-2 yang diberi SMZ hingga 5 hari sebelum akhir percobaan umur 36 hari. Analisis terhadap sampel lapangan terdeteksi beberapa jenis residu antimikroba pada daging ayam, yakni sulfamerazin ($\bar{X}$ = 2,52 ppb atau tidak terdeteksi (tt) – 12,62 ppb) dan sulfametazin ($\bar{X}$ = 0,02 ppb atau tidak terdeteksi (tt) – 0,09 ppb) Pemberian SMZ sebanyak 50 mg/kgBH secara intragastrik tidak mempengaruhi pertumbuhan berat badan ayam. Pada kelompok kontrol positip dan kedua kelompok perlakuan terlihat enteritis haemorrhagika, mottling pada permukaan kapsular hati (degenerasi hati) dan kepucatan ginjal dan tidak dijumpai kelainan patologis pada kelompok kontrol negatip. Secara mikroskopis terlihat bahwa kelainan patologis pada hati, usus dan ginjal merupakan perubahan yang konsisten terhadap pemberian SMZ pada ayam. Sementara itu kelompok kontrol negatip tidak menunjukkan kelainan patologis. Waktu henti untuk SMZ pada ayam ras berkisar antara 5 – 10 hari. Oleh karena itu, untuk menghasilkan produk unggas yang aman dan sehat sebaiknya pemberian preparat sulfonamida dihentikan 5 – 10 hari sebelum ternak dipotong untuk dikonsumsi dan pergantian pakan dengan pakan tanpa bahan imbuhan pertumbuhan dalam kurun waktu tersebut.

Kata Kunci: Residu, Sulfametazin, Unggas, Daging, Pencegahan

PENDAHULUAN

Preparat sulfonamida merupakan antimikroba yang digunakan secara luas dalam pakan ternak sebagai perangsang pertumbuhan dan pencegahan berbagai penyakit bakterial dan protozoa. Sulfametazin (SMZ) adalah salah satu preparat sulfa yang digunakan untuk tujuan terapeutik dalam mengobati penyakit dan tujuan profilaktik dalam mengendalikan berbagai penyakit bakterial, serta memperbaiki efisiensi pakan dan perangsang pertumbuhan hewan (KO *et al.*, 2000; DIXON-HOLLAND dan KATZ, 1991). Seperti penisilin, preparat sulfonamida (termasuk sulfamethazin) berpotensi menimbulkan reaksi alergis, dimana sekitar

10 – 15% populasi masyarakat di Uganda menunjukkan reaksi alergis terhadap penisilin dan sulfonamida (SASANYA *et al.*, 2005). Sementara itu, sulfametazin berpotensi menimbulkan resistensi agen penyakit terhadap antimikroba bila diberikan secara berlebihan dan dilaporkan pula mengandung karsinogen (HOLMBERG *et al.*, 1984; THRELFALL *et al.*, 1994; DIXON-HOLLAND dan KATZ, 1991; SASANYA *et al.*, 2005). Sulfametazin umumnya diberikan kepada ternak sebagai antimikroba untuk mengendalikan penyakit bakterial. DIXON-HOLLAND dan KATZ (1991) melaporkan bahwa residu SMZ sering dijumpai pada sampel susu dimana tingkat kejadiannya dapat mencapai lebih dari 40% dari total sampel susu yang diamati. Terdeteksinya residu SMZ didalam susu dapat berasal dari pakan ternak yang terkontaminasi oleh SMZ (DIXON-HOLLAND dan KATZ, 1991).

Dalam lima tahun terakhir ini, keamanan pangan (*food safety*) menjadi isu penting dalam masyarakat untuk mendapatkan dan menghasilkan pangan yang sehat dan aman. Ayam merupakan bahan pangan hewani yang sangat digemari oleh masyarakat Indonesia. Salah satu upaya untuk meningkatkan produktivitasnya, perbaikan kesehatan ternak menjadi prioritas dalam industri peternakan unggas. Oleh karena itu, penggunaan obat-obatan seperti preparat sulfonamida untuk pencegahan penyakit dan perangsang pertumbuhan tidak terhindarkan lagi dalam mengoptimalkan produksi perunggasan. Namun penggunaan obat-obatan yang tidak terkendali dapat menimbulkan residu pada produk ternak dan bahkan beberapa diantaranya bersifat karsinogen sehingga memungkinkan untuk tidak memenuhi persyaratan keamanan pangan.

Residu preparat sulfa dapat diakibatkan karena penggunaan secara berlebihan yang tidak mengikuti aturan pakai, sengaja dicampur ke dalam pakan sebagai imbuhan pertumbuhan dan tidak memperhatikan waktu henti dari preparat sulfa tersebut. Residu SMZ pernah dilaporkan WIDIASTUTI dan MURDIATI (1999) terdeteksi pada sampel daging dan hati ayam ras di Jawa Barat yang masing-masingnya berkisar antara tidak terdeteksi (tt) hingga 286 ppb (daging) dan tt hingga 1.507 ppb (hati). Kandungan residu SMZ tersebut lebih tinggi dari nilai batas maksimum residu (BMR) yang ditetapkan oleh *Joint WHO/FAO Expert Committee Food Additives* (*JEFCA*) sebesar 100 ppb (COMMITTEE FOR VETERINARY MEDICINAL PRODUCT, 2004) dan Indonesia sebesar 200 ppb (STANDAR NASIONAL INDONESIA, 2000). Tingginya kandungan residu SMZ pada produk pangan asal unggas perlu mendapat perhatian mengingat bahwa SMZ bersifat karsinogenik untuk kesehatan masyarakat dan menimbulkan resistensi terhadap agen penyakit.

MATERI DAN METODE

Penelitian ini terdiri dari (1) identifikasi residu sulfonamida (sulfametazin) pada produk peternakan dan (2) patotoksisitas SMZ pada ayam potong. Tujuan penelitian ini adalah mempelajari jenis dan sumber-sumber residu sulfonamida pada daging ayam ras potong dan gambaran patologi serta mengembangkan pola minimalisasi pembentukan residu SMZ pada produk ternak unggas (ayam ras potong).

Identifikasi residu sulfametazin pada daging ayam ras potong

Identifikasi residu sulfonamida pada produk ternak dilakukan pada lokasi peternakan swasta produsen daging ayam di Parung dan Bogor, Jawa Barat. Sampel dianalisis terhadap residu sulfonamida dengan menggunakan alat deteksi *High Performance Liquid Chromatography* (HPLC).

Analisis residu sulfonamida pada sampel daging ayam ras potong

Analisis residu sulfonamida mengikuti metoda yang dilaporkan oleh HORII *et al.* (1981). Sebanyak 10 g sampel daging ayam bagian dada dipotong halus dan dihomogenkan, ditambahkan dengan 10 ml trikloroasetat 1% dan kemudian diekstraksi dengan 2 x 50 ml asetonitril. Ekstrak dikocok rata selama 20 menit, kemudian disaring dan dipisahkan lapisan asetonitril. Filtrat asetonitril dipindahkan ke dalam corong pemisah dan ditambahkan 2 x 20 ml heksan. Larutan dikocok dan lapisan heksan dibuang, selanjutnya fraksi asetonitril dipisahkan. Filtrat asetonitril dicampur dengan 10 ml asam trikloroasetat 1% dalam *n*-propanol dan dikeringkan dengan rotavapor hingga volume 1 – 2 ml. Selanjutnya filtrat pekat diencerkan dengan 2 x 10 ml asam trikloroasetat 1% dan dimasukkan ke dalam kolom berisi lapisan alumina basa (ketebalan ± 10 cm) yang terlebih dahulu telah dibasahi dengan 5 ml asetonitril dan 10 ml air. Residu di dalam kolom dibilas dengan 10 ml air dan dilarutkan dengan 2 ml trietilamin 0,1% dan dikeringkan. Untuk penginjeksian ekstrak kering ke HPLC, dilarutkan terlebih dahulu dengan 200 µl metanol.

Patotoksisitas sulfametazin pada ayam ras potong

Sebanyak 80 ekor ayam potong umur sehari dibagi menjadi 4 kelompok perlakuan yaitu: **Kelompok-1** (10 ekor) sebagai kontrol negatip tanpa SMZ tetapi hanya

menerima aquades selama percobaan; **Kelompok-2** (10 ekor) sebagai kontrol positip yang diberi SMZ secara intragastrik dengan menggunakan sonde lambung sebanyak 50 mg/kg BH sebanyak 3x per minggu. Nekropsi dilakukan setiap minggu terhadap 2 ekor ayam dari masing-masing kelompok-1 dan kelompok-2 untuk analisis residu SMZ dan perubahan patologis. **Kelompok-3** (30 ekor) sebagai kelompok perlakuan diberi SMZ sebanyak 50 mg/kg BH sebanyak 3x per minggu selama 28 hari, kemudian dihentikan 7 hari sebelum panen pada umur 35 hari; dan **Kelompok-4** (30 ekor) sebagai kelompok perlakuan diberi SMZ sebanyak 50 mg/kg BH sebanyak 3x per minggu selama 30 hari, kemudian dihentikan 5 hari sebelum panen pada umur 35 hari. Nekropsi dilakukan untuk masing-masing kelompok-3 dan kelompok-4 pada 1, 2, 3, 5, 7, 24, 48, 72, 120, 168 dan 240 jam setelah pemberian SMZ dihentikan untuk analisis residu SMZ dan pemeriksaan patologi.

Organ yang mengalami kelainan patologis (hati, ginjal, paru-paru dan saluran pencernaan) difiksasi dalam larutan *buffered neutral formalin* (BNF) 10% dan disayat halus dengan menggunakan mikrotom pada ketebalan 5 – 7 µm untuk diwarnai dengan pewarnaan rutin hematoksilin eosin (HE) dalam pemeriksaan mikroskopis di bawah mikroskop cahaya. Sampel yang terdiri dari daging paha dan dada serta jaringan hati dikoleksi pada periode yang sama saat nekropsi dilakukan untuk analisis waktu henti (*withdrawal time*) preparat SMZ.

HASIL DAN PEMBAHASAN

Identifikasi residu sulfametazin pada daging ayam ras potong

Sebanyak 6 sampel karkas ayam pedaging telah dikoleksi dari peternakan ayam komersial di Parung, Jawa Barat. Analisis residu sulfonamida terdeteksi beberapa residu preparat sulfonamida yang terdiri dari sulfamerazin ($\bar{x}$ = 2,52 ppb dengan kisaran tt – 12,62 ppb) dan sulfametazin ($\bar{x}$ = 0,02 ppb dengan kisaran tt – 0,09 ppb). Hasil analisis residu sulfonamida pada daging ayam ras pedaging terlihat pada Tabel 1.

Keberadaan residu sulfonamida di dalam produk daging ayam perlu menjadi perhatian, mengingat preparat sulfonamida dapat menimbulkan gejala toksisitas, reaksi alergis dan resistensi agen penyakit (THRELFALL *et al.*, 1994; SASANYA *et al.*, 2005). Terdeteksinya beberapa jenis residu sulfonamida ini menunjukkan bahwa penggunaan preparat sulfonamida secara tidak benar, berlebihan atau tidak mengikuti aturan pakai obat sebagaimana sampel daging ayam untuk analisis merupakan hasil panen dari peternakan unggas. Namun demikian penambahan preparat sulfa di dalam pakan sebagai imbuhan pertumbuhan juga sangat memungkinkan terjadi. Oleh karena itu, untuk mengatasi kejadian ini perlu diketahui secara pasti waktu penghentian aplikasi preparat sulfa baik dalam pakan ternak maupun untuk tujuan pengobatan dan pencegahan penyakit pada unggas.

Tabel 1. Residu preparat sulfonamida pada daging ayam ras pedaging

No. sampel	Kandungan residu (ppb)		
	Sulfadiazin	Sulfamerazin	Sulfametazin
1	Tt	Tt	Tt
2	Tt	Tt	Tt
3	Tt	Tt	0,093
4	Tt	12,62	Tt
5	Tt	Tt	Tt

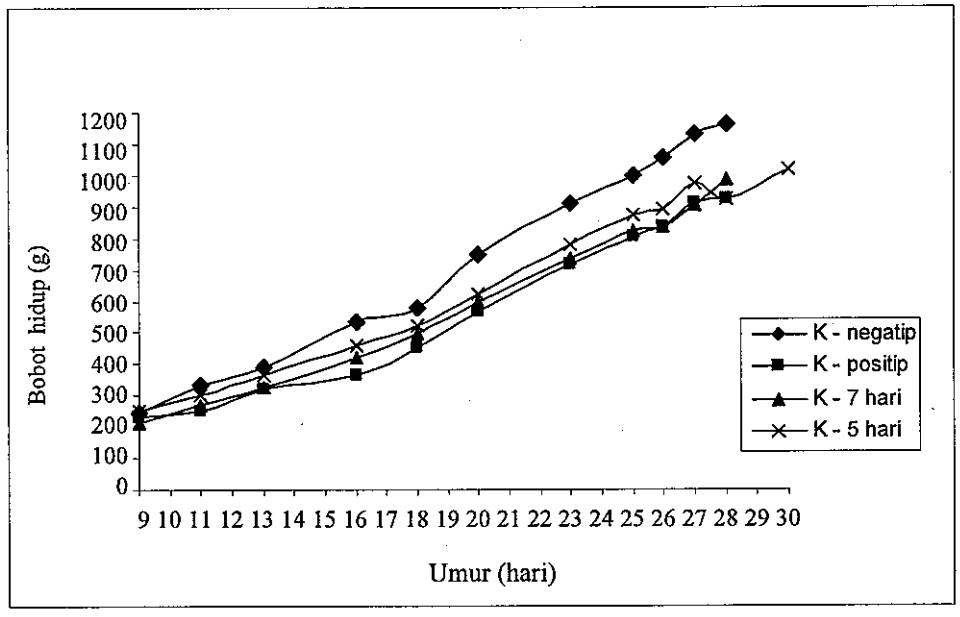
Keterangan: BMR untuk masing-masing senyawaan adalah 100 ppb (SNI, 2003)

Patotoksisitas sulfametazin pada ayam ras potong

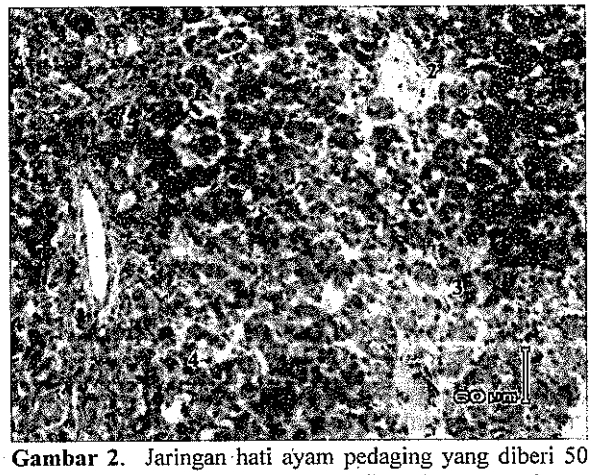
Untuk mengetahui waktu henti preparat SMZ maka sebanyak 80 ekor ayam ras pedaging umur sehari diberi preparat SMZ secara intragastrik sebanyak 3x per minggu selama 28 dan 30 hari berturut-turut sebelum pemotongan hewan pada umur 35 hari. Grafik 1 menggambarkan pertambahan rata-rata BH ayam ras potong yang diberi preparat SMZ. Pemberian SMZ sebanyak 50 mg/kgBH secara intragastrik tidak mempengaruhi pertambahan bobot hidup ayam. Hewan coba dapat tumbuh secara normal dengan kecenderungan meningkat setiap harinya. Namun tingkat pertambahan bobot hidup ayam cenderung lebih rendah pada kelompok kontrol positip dan kelompok perlakuan dibanding kelompok kontrol negatip tanpa pemberian preparat SMZ. Rendahnya pertambahan bobot hidup ayam pada kelompok kontrol positip dan kelompok perlakuan kemungkinan disebabkan karena pemberian preparat SMZ yang cukup tinggi. Dosis SMZ yang dianjurkan adalah 0,05 ml/kgBH atau setara dengan 12,5 mg/kg BH, sedangkan dalam penelitian ini diberikan 50 mg SMZ/kg BH dimana jumlah yang diberikan empat kali lebih tinggi dibanding dosis yang dianjurkan. Disamping itu, terdapat pula reaksi tubuh yang dapat mempengaruhi proses pencernaan ayam.

Selama percobaan tidak dijumpai gejala klinis yang menunjukkan ayam mengalami keracunan SMZ, terserang penyakit infeksius lainnya maupun kematian ayam. Hewan coba terlihat sehat dan normal dengan pertambahan bobot hidup yang cenderung meningkat, bulu mengkilat dan bersih, serta aktivitas yang baik. Nekropsi dilakukan pada umur 28 hari dan 30 hari sebelum akhir percobaan pada umur 35 hari. Secara

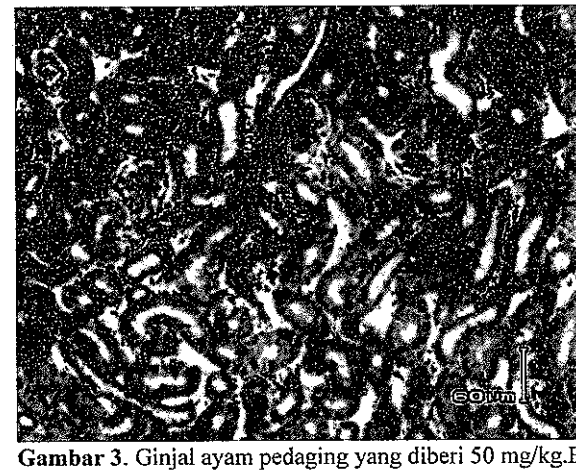
makroskopis terlihat enteritis haemorrhagika, mottling pada permukaan kapsular hati (degenerasi hati) dan kepucatan ginjal pada kelompok kontrol positip dan kedua kelompok perlakuan, sebaliknya tidak dijumpai kelainan patologis yang menunjukkan bahwa hewan mengalami keracunan pada kelompok kontrol negatip. Secara mikroskopis terlihat bahwa kelainan patologis pada hati, usus dan ginjal merupakan perubahan yang konsisten terhadap pemberian SMZ pada ayam. Hati menunjukkan perubahan berupa nekrosis sel epitel jaringan hati, dilatasi sinusoid, vakuolisasi dan infiltrasi sel mononuklear serta proliferasi sel saluran empedu. Pada saluran pencernaan terlihat nekrosis tunika muskularis, nekrosis sel epitel mukosa villi dan haemorrhagi. Sedangkan pada ginjal menunjukkan perubahan akumulasi protein cast pada tubulus, nekrosis sel epitel tubulus proximalis dan haemorhagi (Gambar 2, 3 dan 4). Kelompok kontrol negatip tidak terdapat kelainan patologis selama percobaan.



Gambar 1. Pertumbuhan rataan bobot hidup ayam potong yang diberi sulfametazin (g/hari)



Gambar 2. Jaringan hati ayam pedaging yang diberi 50 mg/kg.BH sulfametazin selama 2 minggu secara oral. 1. Traktus portal; 2. Vena sentralis; 3. Nekrosis sel hati; dan 4. Infiltrasi sel mononuklear. 40x. H.E.



Gambar 3. Ginjal ayam pedaging yang diberi 50 mg/kg.BH sulfametazin selama 1 minggu secara oral memperlihatkan nephrosis. 1. Glomerulus; 2. Tubulus proximalis; dan 3. Dilatasi tubulus. 40x. H.E.