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Accuracy of Various Doses of Sulphate Acid (H_2SO_4) in early Pregnancy Detection in Friesian Holstein Dairy Cows

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Abstract— *This study aims to find out the accuracy of doses and time in early detection of pregnancy using sulfuric acid (H2SO4) concentrated in FH dairy cows. A total of 600 urine samples came from 120 FH dairy cows that had been artificially inseminated on the 24th day, the 34th day, the 44th day, the 60th day. Urine samples are taken in the morning from 06.00 – 10.00. Concentrated doses of Sulphate Acid (H2SO4) are divided into 3 including P1 (0.1 ml), P2 (0.5 ml) and P3 (1.0 ml) according to treatment. The observed variables are gas fluorescence in the solution, discoloration of the limes pink solution, and the timing of changes in the solution. To ensure the pregnancy is carried out rectal palpation examination day 60 after artificial insemination. The collected data is analyzed with the Chi-Square test. Chi-Square test analyst results showed that sulphate acid (H2SO4) dose treatment is no different (P >0.05) in detecting early development. The optimal dose of use of Sulphate Acid (H2SO4) concentrated in detecting early production of friesian holstein milk cows of age is achieved at a dose of 0.5 ml with an accuracy of 83.33 % - 95.23 %, which indicated the formation of air bubbles (fluorensce) and pink in the duration of time, with a duration of 9-45 seconds. Conclusion Early pregnancy detection technology method "Concentrated Sulphate Acid (H2SO4) can be used to detect cow pregnancy 24 days after insemination.*

Keywords— ***early pregnancy, Sulphate Acid (H_2SO_4), Rectal Palpation, FH dairy cows.***

I. INTRODUCTION

Dairy cows are one of the mainstay businesses of the livestock sub-sector that is prospective in agribusiness activities. The development of the livestock business has a positive impact on job creation and promises cash income, to motivate farmers to play an active role in agribusiness activities to increase their family income (Kaliky and Hidayat, 2006). Dairy cows are very potential cattle to be developed because it is by the needs of Indonesia today. The success of efforts to increase the productivity of dairy cows depends on the population. Real conditions in smallholder cause slow population development and low milk production, among others, due to high reproductive disorders in female dairy cows.

Assessment of reproductive efficiency in dairy cows in the field shows the length of the distance between the mother gave birth to bunting back (Days Open) (Atabany et. al. 2017; One of the causes of Days Open is due to the failure of early detection of domestication in dairy cows after artificial insemination (AI). Pregnancy is a series of immunologic and endocrinological changes in the effort to produce children in the womb from fertilization to normal birth. As the pregnant ages, some conception hormones, especially estrogen and progesterone, increase (Frastantie et al., 2019).

Early detection of pregnancy in cows is very important to be reviewed in terms of the economy, this will affect the income of breeders. By knowing whether the cattle are bunting or not in a faster and more accurate time, farmers can take further action faster when knowing the cow is bunting i.e. improving the feed for the maintenance of the farm. On the other hand, early detection of pregnancy is necessary in terms of identifying livestock that is pregnant or not pregnant immediately after in the artificial insemnation (AI), in this case when knowing the cattle are not pregnant , it will be immediately examined by veterinarians or officers Asisiten Reproductive Engineering so that the production time lost due to infertility can be suppressed. According to Jainudeen and Hafez, (2000) in general early diagnosis of pregnancy is necessary in the case of 1. identifying cattle that are not bunting immediately after mating or AI so that production time lost due to infertility can be suppressed with proper handling, 2. as consideration if livestock should be sold or culled, 3. to reduce costs on breeding programs using expensive hormonal techniques and 4. economical livestock management. The purpose of the method of pregnancy examination is to determine the status of the pregnant with 100% accuracy, and do not have false positives or false negatives, determine the pregnancy as early as possible, determine the age of the pregnant, determine the ability to survive the pregnant and determine the sex of the fetus and can succeed in a short time.

The most common farm check in cows is done by cows again in the way what happens is that the mountain of lust / estrus returns after mating both naturally and naturally AI i.e. under the guise of (Non Return to Estrus), but to this method to the fastest, the age of the pregnancy, cost, to stop speed and diagnosis. Further on the use of non-return rate (NRR) in the case of silent heat (quiet berahi) is often interpreted livestock is considered bunting when not pregnant (Susilawati et al. 2013), on the contrary there are cases of early pregnant livestock but the symptoms of berahi, because has not been preached in rectal palpation, then by the inseminator will AI and this condition can be charged embryo death (Vernunft et al., 2013). Until now, the method of rectalization palpation will know the early pregnancy is quite accurate, namum can only be done at the age of 60 days pregnancy and competent experts (Romano et al. 2011). According to Cingi et al. (2012), rectal palpation where stressed livestock are squealed with antioxidant status (TAS). In addition, the power to use non-invasive pregnancy reforms has become increasingly popular since the principle of animal welfare (WSPA, 2007). Related to that, the examination of pregnancy using urine samples on livestock that no one has been slaughtered after 24 days in the AI and alleged pregnant can be an option so early pregnancy detection methods, so it is the same as the principle of animal welfare. One way to delay for early pregnancy in dairy cows by means of concentrated sulphuric acid (H2SO4) coupled with urine is an inexpensive and easy-to-do alternative, without specific skill skills. Everyone ca try cow pregnancy with this method, only need to be careful at this time using concentrated sulphate acind (H2SO4) because of its harsh nature and can irritate the skin.

At the beginning of implantation, the placenta is the first embryonic organ to develop (Cross, 2006) and produces estrogen hormones in the form of estrone (E1; 3-hydroxy-Δ1,3,5(10)-estratrien-17 one), estradiol 17-α (E2-α; Δ 1, 3, 5 (10)-estratriene-3, 17 α¬-diol) and estradiol 17-β (E2-β; Δ 1, 3, 5 (10)-estratriene-3, 16 β -diol) excreted through urine (Veenhuizen et. al. 1960). According to Mellin and Erd (1965), the highest estrogen content found in cow urine is E2-α. The content of E2-α in urine can react with sulphuric acid (H2SO4) producing fluorescence that has been applied to horses (Equus caballus) (Cuboni, 1935) and Camel (Camelus bactrianus) (Fedorova et al. 2015). From the basis of the theory, early pregnancy testing using concentrated sulphuric acid (H2SO4) can be done after the implantation process. Estrone sulphate is the largest derivative of estrogen produced by conception and can be measured in maternal plasma, milk, or urine in all livestock species. Estrone sulphate can be detected in plasma earlier in pigs (day 20) and horses (day 40), compared to sheep and goats (day 40 to 50) or cows (day 72).

The purpose of this study was to test: 1). The optimal dose of H2SO4 to detect early fertilization of dairy cows, 2). The time needed for fluorescence reaction between H2SO4 and E2-α content in urine and 3). Accuracy of the H2SO4 method for detecting early pregnancy in dairy cows. The results of this research are expected to be one of the contributions to the development of livestock and become a source of information about the development of reproductive technology, especially in the detection of early plantations in dairy cows. Also, breeders can detect early pregnancy using H2SO4 in an easy, cheap, fast, and accurate way to obtain efficient cow maintenance. Therefore, researchers are interested in testing early pregnancy detection methods using concentrated sulphuric acid (H2SO4) in FH dairy cows.

II. MATERIAL AND METHODS

A total of 600 urine samples taken in the morning (07.00 - 08.00 WIB), derived from 120 FH cows divided into 4 groups, namely on the 24th, 34th, 44th, and 60th days post-AI, then stored refrigerator at 5°C, tested vulnerable time 1-5 days after collection. The test was done by mixing 1 mL of urine into 5 mL aquadestilata and then homogenized, then from the urine solution was taken 1 ml and added sulfuric acid (H2SO4.) concentrated concentration of 97% as much as 0.1 ml, 0.5 ml, and 1.0 ml. The test results are divided into two, namely 1). pregnant (produces a fluorescence reaction and a confle pink run 2). No pregnant (does not produce fluorescence reaction) and no shade of turn pink (Modification of Cuboni method, 1935).

Rectal Palpation

Rectal palpation was performed on the 60th day after the AI used gloves that had been given pelican gel. Then the finger is draped into the sphincter muscle and enters according to the response of the rectal organs of the cow. After passing through the sphincter muscles are carried out carefully the release of stools to prevent air from outside entering the rectal organs. After rectal cleaning, palpation is performed on the cervix, uterus, and ovaries for five minutes each (Romano et al. 2011). If the cervix is felt hard, the uterus there will be embryos, and the ovaries their corpus luteum then it is concluded that the cattle were pregnant, if the opposite then the livestock is not pregnant

Sulphuric Acid H2SO4 Concentrated

The study used experimental methods with 3 (three) treatments that were repeated 5 times each. The treatment is a concentrated dose of sulphuric acid (H2SO4.) consisting of R1 = 0.1 mL, R2 = 0.5 mL and R3 = 1.0 mL.

R1: Cow urine dose of 1 ml + aquadest dose of 5 ml + sulphate acid (H2SO4) dose of 0.1 ml.

R2: Cow urine dose of 1 ml + aquadest dose of 5 ml + sulphate acid (H2SO4) dose of 0.5 ml.

R3: Cow urine dose of 1 ml + aquadest dose of 5 ml + sulphate acid (H2SO4) dose of 1.0 ml.

The observed variables are pregnancy with the following indications:

1. Percentage of The number of positive FH dairy cows bunting by pointing to fluorenscence reaction and discoloration of the lime pink solution after the addition of sulphuric acid (H2SO4.) in each treatment, calculated using stopwatch 1.
2. Duration of fluorenscence reaction and discoloration of the lime pink solution after the addition of sulphuric acid (H2SO4.) in each treatment, calculated using a stopwatch
3. Accuracy of the sulphate acid method (H2SO4.) compared with rectal palpation examination results on the 60th day after artificial insemination.

The response of the pregnancy test is calculated as follows:

Percentage response of bunting positive cows by H2SO4 method:

$$\frac{\Sigma \text{ positive pregnant cow with H2SO4 method}}{\Sigma \text{ total of cows tested for pregnancy}} x100\%$$

Percentage response of positive pregnant cows by rectal palpation method:

$$\frac{\Sigma \text{ positive pregnant cow with rectal palpation method}}{\Sigma \text{ total of cows tested for pregnancy}} x100\%$$

The accuracy level of H2SO4 for detecting pregnancy is by comparing the results of the H2SO4 method of pregnancy test with rectal palpation method with the following formula:

Accuracy of H2SO4 method:

$$\frac{\Sigma \text{ positive pregnant cow with H2SO4 method}}{\Sigma \text{ positive pregnant cow with rectal palpation method}} x100$$

Data analysis

The data obtained were analyzed using descriptive analysis and the Chi-Square test. This test was carried out to observe the difference between the results of the detection of pregnancy in urine collected from cows that are artificial insemination. The chi-square formula is as follows (Steel and Torie, 1990):

$$\chi^2_{hit} = \sum \frac{(O_i - E_i)^2}{E_i}$$

χ^2hit = Sum of Chi squares

O = observed frequency

E = expected frequency

χ^2hit results compared to χ^2table at 5% confidence level. If x hit is greater than χ^2table 5%, then there is a difference between the observed frequency and the expected one. If x hit is smaller than χ^2table 5%, then there is no noticeable difference between the expected frequency and the observed one.

III. RESULTS AND DISCUSSIONS

Percentage of Sulphate Acid Method Test Results (H2SO4) Concentrated

Pregnancy is a process of immunological and endocrinological changes in the effort to produce children in the womb from fertilization to normal birth. As the pregnant ages, some conceptual hormones, especially estrogen and progesterone, increase (Frastantie et al., 2019). Detection of pregnancy is a very important thing to do after the cattle are mated. Early detection of pregnancy will provide more information about the success of marriage so that it can be evaluated for failure immediately. Faster evaluation will be able to improve reproductive efficiency (Karen et al. 2004). However, such efforts require detection of pregnants that have high accuracy, easy to use, cheap and harmless to livestock. The results of early pregnant detection research in FH dairy cows tested using concentrated sulphate acid liquid (H2SO4) in all treatments gave a positive response to all samples at the age of 21 har, 34 days, 44 days and 60 days. The response of urine samples to various doses of Concentrated Sulphuric Acid (H2SO4) is presented in Table 1.

Table 1. Urine Sample Response To Various Doses of Sulphate Acid (H2SO4) Concentrated

H2SO4 Doses		**0.1 mL**	**Time**	**0.5 mL**	**Time**	**1.0 mL**	**Time**
Pasca AI (Day)	cows (Head)	fluorensce pink color (head/%)	Start to end time (second)	fluorensce pink color (head/%)	Start to end time (second)	fluorensce pink color (head/%)	Start to end time (second)
24	30	19 head	60-180	20 ekor	24-54	20 ekor	11-32
		63.33%		66,00%		66,00%	
34	30	21 head	60-180	22 ekor	20-46	23 ekor	10-29
		70.00%		73.0%		83,3%	
44	30	22 head	60-180	25ekor	9-41	23 ekor	8-24
		73.33%		83.3.0%		83.0%	
60	30	22 head	60-180	23 ekor	Jul-41	22 ekor	7-22
		73.33%		83.3.0%		86.6 %	
Mean		63.9%	60-180	66,0%	15- 46	80.0 %	9 - 27

Table 1. Indicates that the results of pregnancy detection using concentrated Sulfuric Acid (H2SO4) with different doses each for P1 (0.1 ml); resulted in a positive response to urine samples of 24 days, 34 days, 44 days, and 60 days after the AI resulted from consecutive pregnancies 63.0%, 70.0% and 73.33% and 73.33 %, respectively, with an average of 63.33%. As for the duration of time from the formation until the end of the fluorenscence gas bubble from the base of the tube ascends to the surface and the discoloration of the solution to a turning pink is 60 - 180 seconds. In the treatment of R2 (0.5 ml) and R3 (1.0 ml) of urine samples 24 days, 34 days, 44 days after AI results in the same pregnancy rate, but the duration of time in R3(1.0ml) is faster than R1(0.1ml) and R2(0.5ml). The difference in duration of time is suspected in the treatment of R1 (0.1 ml), the temperature of Sulphuric Acid (H2SO4) concentrated dose of .0.1 ml does not reach the optimal point so the working power of burning estrone sulfate takes a relatively long time. Furthermore, the R3 (1.0ml) treatment of urine post AI 60 days sample resulted in the highest percentage of pregnancy compared to R1(0.1 ml) and R2(0.5ml) treatments. This condition is suspected as the gestational age increases, the concentration of estron sulfate will increase.

In this study it was found that there were urine samples that showed no reaction to administration (H2SO4, this is thought to be related to Estrone Sulfate with the chemical formula C 18 H 22 O 2. has a melting point of 254.5 ° C

(490 ° F) and a type weight of 1.23. At high temperatures estrone is flammable and the products of estrone combustion are carbon monoxide (CO) and carbon dioxide (CO2). (Helen Varney, Jan M. Kriebs, Carolyn L. Gegor, 2004).

The results of this study showed that the treatment of P2 (0.5 ml) in 44 days post AI with a percentage of 90.0 % with a time of 7-41 seconds is an efficient and effective result in the early pregnancy test method Sulphuric Acid (H2SO4) concentrated, where between the use of 0.5 ml and 1.0 ml (H2SO4) concentrated produces the same pregnancy accuracy. while the average obtained for all urine samples with a dose of H2SO4) concentrated is 85.83% with a time of 15-34 seconds. According to Mellin and Erd (1965), the highest estrogen content was found in cow urine in the form of E2-α. The content of E2-α in the urine and can react with sulfuric acid (H2SO4) producing fluorescence. Manalu et al. (2000a) further state that an increase in the number of follicles, corpus luteum, and placenta causes an increase in secretions from the glands producing pregnancy and mammogenic hormones such as estradiol and progesterone during pregnancy.

This study found the presence of urine samples that did not act on the administration of sulphuric acid (H2SO4) concentrated, but when tested with rectal palpation it turned out that the cow was pregnant. It is suspected that the concentration of Estron Sulfate in the cow sample is relatively small, so it is not detected by concentrated Sulphuric Acid (H2SO4). This is in line with Ginther et al,. (2013) state that the work of hormones in addition to being influenced by blood hormone levels, also depends on the state of receptors in the target cells and the carrier proteins in the blood. On the other hand, urine samples taken from various feeding procedures are thought to affect the formation of the hormone estron sulfate produced is not optimal. Nutritional deficiency affects the function of the anterior pituitary resulting in the production and secretion of follicle-stimulating hormone (FSH) and low luteinizing hormone (LH), which causes the ovaries to not develop or experience hypofunction (Yendralisa, 2013). This condition causes follicle growth does not run normally, so the production process of conceptual hormones is not optimal. In this case, when the cow is experiencing a lack of nutrients it is suspected that the hormone estron sulfate that is formed is relatively inadequate so that when reacted with H2SO4 does not respond. Estrogen concentration is low during early pregnancy and increases in the middle and end of pregnancy. In horses, estrogen levels are quite high during mid-pregnancy. The main source of this estrogen is placenta. Estrogen experiences a progressive increase in the uterine bloodstream during pregnancy. Estrogen cooperates synergistically with progesterone on the development and preparation of the mammae glands for milk syntheses after birth. Placental lactogen also seems to have a role in the development of mammae glands as well as its role in regulating the growth of fetuses (Lestari, T D, 2006).

Accuracy of Pregnancy Test Sulphuric Acid Method (H2SO4) Concentrated

The pregnancy diagnosis rectal palpation method has been used for a long time with accurate results, and the results can be immediately known (Broaddus and de Vries, 2005). The accuracy of early pregnancy detection in FH dairy cows using concentrated sulphuric acid (H2SO4) ingredients with different doses in urine samp 24 days, 34 days, 44 days, and 60 days after AI day obtained by compared to the results of pregnancy test rectal palpation method presented in Table 2.

Table 2. Accuracy of Pregnancy Diagnosis of Sulphate Acid Method (H2SO4) Concentrated against Rectal Palpation

H2SO4 Doses		**0.1 mL**		**Accuracy**	**0.5 mL**		**Accuracy**	**1.0 mL**		**Accuracy**
Post AI (Day)	Cows (Head)	H2SO4	Rectal Palpation	(%)	H2SO4	Rectal Palpation	(%)	H2SO4	Rectal Palpation	(%)
24	30	19 head 63.33%	18 head 60%	80 .0	20 head 66%	18 head 60%	83,33	20 head 66%	18 head 60%	83,33
34	30	21 head 70.00%	20 head 66.7 %	86.6	22 head 73.0%	20 head 66.7 %	90.0	23 head 83,3% /	20 head 66.7 %	90.0
44	30	22 head 73.33%	21 head 86,60%	86.6	25 head 83.3.0%	21 head 86.6%	92.0%	23 head 83.0% /	21head 86,60%	90.5

60	30	22 head 73.33%	21 head 86,60%	90 .47	23 head 83.3.0%	21 head 86.6%	95.23	21 head 86.6	21 head 86,60%	95.23

Detection of pregnancy using concentrated sulphuric acid (H2SO4) was strengthened after all samples of cows were examined with rectal palpation techniques at 60 days gestation. Comparison of pregnancy diagnosis results in Sulphuric Acid (H2SO4) Concentrated R1 (0.1 ml), with rectal palpation, gave a positive response to various gestational ages of different cow urine samples, namely on the 24th day, 34th day, 44th day, and 60th day resulted in consecutive accuracy rates of 80.0%, 83.33%, and 83.33%. Lucy et al. (2011) state that the theoretical basis of examination of pregnant cows is the contained estrogen hormones that are secreted through urine. At the beginning of pregnancy, estrogen hormone concentrations slightly begin to rise by the time the pregnant age begins to age. At the end of 4 months gestation, the cow will excrete 10 X folds of estrogen hormone in its urine compared to after childbirth (Jabour et al. 1993; McG Agro et al. 1994).

The results of Chi-Square analysis (Table 2), showed that the 3rd (three) concentrated Sulphuric Acid (H2SO4) treatment of urine samples on the 24th day, day 34, day 44, and day 60, compared to the palpation per rectal test on the 60th day was no different from the real (P>0.05). This indicates that various doses of concentrated Sulphuric Acid (H2SO4) can be applied in early pregnancy detection in FH dairy cows. An early pregnancy test of the sulphate acid method (H2SO4) concentrated against urine samples 24 har, 34 days, 44 days, and 60 days after AI is an effective method to diagnose early pregnancy in cows. However, to ensure the results of pregnancy examinations are required confirmation examination by using ultrasound at a minimum gestational age of 25 days (Fricke 2002). This condition is in line with Cross, (2006), that at the beginning of pregnancy (implantation), the placenta is the first embryonic organ to develop and produce estrogen hormones in the form of estrone (E1; 3-hydroxy- Δ1,3,5(10)-estratrien-17 one), estradiol 17-α (E2-α; Δ 1, 3, 5 (10)-estratriene-3, 17 α¬-diol) and estradiol 17-β (E2-β; Δ 1, 3, 5 (10)-estratriene-3, 16 β-diol) are excreted through urine. At the beginning of pregnancy, estrogen hormone concentrations slightly begin to rise by the time the pregnant age begins to age. At the end of 4 months gestation, the cow will excrete 10 X folds of estrogen hormone in its urine compared to after childbirth (Jabour et al. 1993; McG Agro et al. 1994). According to Jainudeen and Hafez (2000), the diagnosis of early pregnancy is necessary in the case of 1) identifying cattle that are not pregnant immediately after marriage or AI; 2) as consideration if livestock should be sold or culled; 3) reduce costs on breeding programs that use expensive hormonal techniques, and 4) application of economical ration management.

Concentrated Sulphuric Acid (H2SO4) early pregnancy detection technology certainly provides an opportunity to know which cows are pregnant at an early gestational age after insemination or mating naturally. The use of sulphuric acid (H2SO4) concentrated in the detection of pregnancy of FH dairy cows can be done early and without risk. Pregnancy detection tests only require cow urine to detect the pregnancy. Besides, the detection time of pregnancy is very short only takes 45-60 minutes in its implementation

Early pregnancy detection technology method "Concentrated Sulfuric Acid (H2SO4) can be used to detect a cow's pregnancy 24 days after insemination. Further detection of early pregnancy in FH dairy cows can improve reproductive efficiency so that mother cows that are known to be not pregnant can be re-mated immediately. So that it can shorten the empty or dry period. For mother cows that have been early known to be pregnant can be immediately maintained better to maintain and save pregnancy until birth safely. Farmers can also improve the reproductive efficiency of cows and reduce production costs.

IV. CONCLUSION

The optimal dose of Sulphuric Acid (H2SO4) concentrated in detecting early pregnancy in FH dairy cows is achieved at a dose of 0.5 ml with an accuracy of 83.33 % - 95.23 %, indicated the formation of air bubbles (fluorensce) and pink in a duration of 9-45 seconds that is, the beginning of gas formation is 9 seconds after urine is realized with H2SO4) concentrated and ends at the 45th second. More research is needed to apply a concentrated mechanism of sulphuric acid l (H2SO4) by measuring the concentration of estrone and estradiol 17 alpha in plasma.

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