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SHORT COMMUNICATION
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On-farm Non-Invasive Pregnancy Diagnosis of Doe (*Capra Hircus*) using Barium Chloride

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ABSTRACT

The application of reproduction-related biotechnologies, such as artificial insemination in goats, cannot proceed on farms where production records are not available. One of the requirements in adopting AI is early diagnosis of pregnancy, which will facilitate estrus synchronization and avoid the risk of abortion. Both laboratory assays and diagnosis are available. However, they require laboratory equipment and a highly skilled technical person. The use of non-invasive pregnancy detection using barium chloride as a test solution is evaluated. The efficiency of determining pregnancy in 36 heads of 1-year-old Philippine Native goats was carried out at different concentrations (1.5, 2.5, 3.5, and 4.5g). The time consumed before the formation of precipitate (sec) and the quality of the precipitate were evaluated. The result shows that regardless of $BaCl_2$ concentration, pregnancy can be detected. The results obtained show that treatments with higher concentration of $BaCl_2$, requires shorter period for precipitate formation is shorter as compared to treatments with lower concentration. The difference between periods consumed among treatments is highly significant ($P<0.05$) from Week 1 to Week 7 of the study, with Treatment 4 recording the shortest time consumed for the precipitate to occur. In terms of visibility of the precipitate, there was no significant difference between treatment means 7 days after breeding. However, as the pregnancy progresses, the difference between the treatment means ($P<0.05$) was significant. Treatment 4 developed more visible precipitate as compared to the other treatments, and this observation was noted throughout the study. Therefore, utilizing BaCl2 to detect early-stage pregnancy in goats is an effective, low-cost procedure with the same accuracy as ultrasound diagnosis. According to this study, 4.5g $BaCl_2$ is a better visual indicator for non-invasive pregnancy diagnosis.

Keywords: Goat, Pregnancy diagnosis, Non-invasive method, Barium chloride

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INTRODUCTION

Farm records are an important document in livestock production. Using this document, the farmer can track the production status of the farm. However, in most backyard farms in the Philippines, written data are not available (Orden et al., 2023), which makes farmers more dependent on their memory when making decisions related to farm management (Sharma & Dubey, 2019). The absence of the record also limits the farm from adopting advanced technologies, such as artificial insemination (AI). Estrus synchronization is a crucial component of artificial insemination (AI). Through this intervention, successful pregnancy outside breeding season is allowed (Ustuner et al., 2023), leading to an increase in ovulation rate (Habeeb & Kutzler, 2021). However, continuous administration of hormones for this purpose can substantially decrease estrus and fecundity rates of goats (Sun et al., 2023). Without proper administration, it can lead to abortion, impeding herd productivity and causing significant economic losses for farmers (Esmaeili et al., 2025). Thus, the adoption of pregnancy detection can be an important intervention in any reproduction management.

Diagnosis of pregnancy or cyesiognosis is regarded as an important component in animal production. Early pregnancy detection in goats is crucial for effective reproductive management (Lalrintluanga & Dutta, 2009). Considering that physical manifestation during the early stage of gestation is not observable, several laboratory tests have been developed (Purohit, 2010) to improve early

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diagnosis of pregnancy in ewes and does (Lone et al., 2016). When pregnancy is diagnosed, proper feeding is provided which is important in maintaining health and growth of the animals (Gautam et al., 2023). If missed, it can cause reduction of production efficiency, impaired production management, and higher risk of pregnancy-related metabolic disorders (Green et al., 2005).

Early pregnancy detection is available through the use of blood plasma or serum progesterone (Boscos et al., 2003; Capezzuto et al., 2009; Lone et al., 2016). This technique is done 21 days after breeding with recorded accuracy rate of 75–86% in identifying pregnant and 90–100% in classifying dry goats (Khadiga et al., 2005). Aside from blood serum, milk progesterone can also be used with an accuracy of 100% when test is done 19 days after mating (Jack et al., 2012). Moreover, the application of laboratory methods such as hormone profiling (Khadiga et al., 2005) and biomarkers, particularly pregnancy-associated lycoproteins (PAGs), is currently gaining popularity (Tadeo et al., 2021). PAGs are antigens that are secreted by the placenta and distributed to the maternal bloodstream from the time of implantation (Cruz et al., 2024). Lofranco et al. (2024) reported that efficiency of PAG-ELISA in distinguishing pregnant from non-pregnant goats as early as 23 days post-breeding.

Aside from these invasive techniques, ultrasonography is becoming an important tool for monitoring reproductive in animal production (Medan et al., 2004; Medan & Abd ElAty, 2010; Erdogan, 2012). The main criteria for positive diagnosis of pregnancy in goats are the visualization of anechoic cross sections of the uterine lumen (embryonic vesicle), embryo/fetus or placentomes in the amniotic fluid (Kumar et al., 2015; Suguna et al., 2008).

Doppler ultrasonography is widely utilized due to its ease of use. Doppler is a scanning device that detects pregnancy by examining blood flow in the uterine or umbilical arteries and fetal heartbeats (Wani et al., 2003). The technique is applicable 25 days after mating via the transrectal approach, but its accuracy improves as the animal approaches full-term pregnancy (Serin et al., 2010; Gonzales-Bulnes et al., 2010). Despite the promising results that this diagnosis can guarantee for raisers, these methods require specialized equipment and technical skills (Medan et al., 2004; Amer, 2010).

One method that has been explored for pregnancy detection in goats is the use of barium chloride ($BaCl_2$) (Roberts, 2023). Studies have proven that the method works in detecting pregnancy in ewes, pigs, sows, and cattle (Ndu et al., 2000). This non-invasive pregnancy detection is carried out by the presence of progesterone in the animal's body fluid. Physiologically, progesterone is secreted continuously to support the development of the embryo. This particular hormone will react with $BaCl_2$, as shown by the presence of precipitate (Lalrintluanga and Dutta, 2009). Technically, $BaCl_2$ is an inorganic compound and one of the most common water-soluble salts of barium. When urine is added to $BaCl_2$, it combines with sulphate radicals present in urine and a precipitate of barium sulphate is formed. This method will not require highly technical skills, as compared to when a visual image or an acoustic method is used. Furthermore, this method is can be applied on field-level, thus the application of synchronization to doe subjected to AI can be more efficient. Therefore, this study was conducted to determine the effectiveness of different $BaCl_2$ concentrations in detecting early-stage pregnancy in does.

MATERIALS & METHODS

The study was conducted using 36 heads of Philippine native does at age one-year old. Every morning at 5:00 to 6:00, a teaser buck was used to identify the doe's in-heat. Once in-heat does were identified, breeding proceeded.

Different levels of $BaCl_2$ (1.5g, 2.5g, 3.5g, and 4.5g) were diluted in 100mL of distilled water and served as test solutions. Urine samples were collected from does early in the morning. The collected urine was mixed with $BaCl_2$ at the same volume. The presence of precipitate is an indication of pregnancy, while a precise dilution shows a negative result. The time (in seconds) consumed before precipitation developed and the visibility of the precipitate was graded (1- Milky or cloudy, 2- Thin precipitate, and 3- Thick precipitate). This non-invasive pregnancy test was conducted during the early stage of pregnancy or until the embryo reached 8 weeks of pregnancy. The data collected were subjected to Analysis of Variance (ANOVA) using the Statistical Tool for Agricultural Research (STAR) Program.

RESULTS

Formation of Precipitation

The response of $BaCl_2$ to urine samples in terms of precipitation formation is presented in Table 1. It can be noted that there is a positive reaction from $BaCl_2$ throughout the experiment, which is an indication of a breeding. Moreover, it can also be noted that regardless of the $BaCl_2$ concentration, it has a positive reaction to progesterone.

The table constantly shows that for treatments with a higher concentration of BaCl2, the period required for precipitate formation is shorter as compared to treatments with a lower concentration. The difference between treatment means is highly significant ($P<0.05$) from Week 1 to Week 7 of the study, with Treatment 4 recording the shortest time required for the precipitate to occur. On the other hand, Treatment 1 recorded the longest period before precipitation is developed.

The data presented further suggests that as pregnancy progresses, there is no significant difference on the period consumed for the formation of precipitate between treatment, inferring that regardless of $BaCl_2$ concentration, the volume of progesterone hormone is enough to be detected.

Visibility of the Precipitate

In terms of the visibility of the precipitate, the data shows that there is no significant difference between treatments 7 days after breeding (Table 2). The precipitate remains milky throughout the entire treatment period. As the pregnancy progresses, a significant difference is noted between the treatment means ($P<0.05$). Treatment 4 developed more visible precipitate as compared to the other treatments, and this observation was pointed out throughout the study (Fig. 1).

Table 1: Formation of precipitation (in seconds)

Treatments	Pregnancy Stage (weeks)							
	1	2	3	4	5	6	7	8
Treatment 1- 1.5% $BaCl_2$	9.33^{a}	7.22^{a}	5.78^{a}	4.78^{a}	3.89^{a}	3.44^{a}	2.22^{a}	1.33
Treatment 2- 2.5% $BaCl_2$	7.44^{ab}	5.89^{ab}	4.33^{b}	3.33^{b}	2.78^{b}	1.89^{b}	1.56^{b}	1.22
Treatment 3- 3.5% $BaCl_2$	5.89^{bc}	4.22^{bc}	3.22^{b}	2.44^{b}	2.22^{b}	1.67^{bc}	1.22^{b}	1.11
Treatment 4- 4.5% $BaCl_2$	3.56^{c}	2.44^{c}	1.78^{c}	1.33^{c}	1.22^{c}	1.00^{c}	1.00^{b}	1.00
ANOVA	**	**	**	**	**	**	**	ns
LSD 0.05%	2.04	2.04	2.04	2.04	2.04	2.04	2.04	-
CV (%)	38.35	39.54	32.99	34.41	33.49	36.45	42.46	32.37

Means with the same letter are not significantly different; ns- not significant; **- highly significant at 0.01% level

Table 2: Visibility of precipitation

Treatments	Pregnancy Stage (weeks)							
	1	2	3	4	5	6	7	8
Treatment 1- 1.5% $BaCl_2$	1.44	1.67^{b}	1.78^{b}	1.89^{c}	1.78^{c}	1.78^{b}	1.89^{b}	2.11^{b}
Treatment 2- 2.5% $BaCl_2$	1.56	1.67^{b}	1.78^{b}	1.78^{c}	1.78^{c}	1.89^{b}	2.00^{b}	2.22^{b}
Treatment 3- 3.5% $BaCl_2$	1.78	1.89^{b}	2.33^{a}	2.44^{b}	2.44^{b}	2.67^{a}	3.00^{a}	3.00^{a}
Treatment 4- 4.5% $BaCl_2$	1.67	2.56^{a}	2.67^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}	3.00^{a}
Anova	Ns	**	**	**	**	**	**	**
LSD 0.05%	-	2.04	2.04	2.04	2.04	2.04	2.04	2.04
CV (%)	35.44	28.26	26.16	19.54	19.60	21.11	21.10	21.53

Means with the same letter are not significantly different; ns- not significant; **- highly significant at 0.01% level

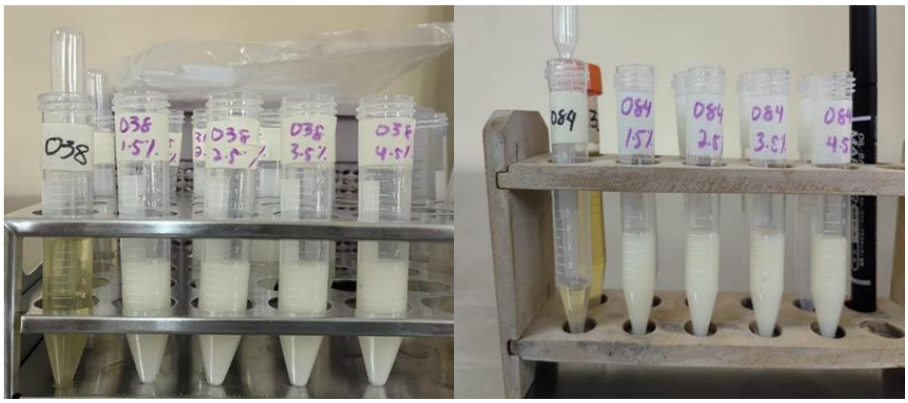

Fig. 1: Precipitate formed from reaction of urine and $BaCl_2$.

DISCUSSION

Barium chloride ($BaCl_2$) is a chemical compound that has been used in various fields, including medicine and agriculture. In the field of reproductive management in goats, $BaCl_2$ is a potential tool for early pregnancy detection (Roberts, 2023). Progesterone is released to prepare the uterus for pregnancy. The presence of progesterone can metabolize sulfate conjugates, such as pregnanediol sulfate. These sulfate metabolites are then excreted in the urine and bile. The urine of animals contains sulfate radicals that, when combined with BaCl2, form barium sulphate (Balbin et al., 2020). This method will not require highly technical skills, as compared to visual image or acoustic methods. Furthermore, the diagnosis can be applied at the field level, warranting safe synchronization of does subjected to artificial insemination (Dana et al., 2020).

The results of the present study further demonstrate the efficacy of $BaCl_2$ at the early stage of pregnancy. However, for easier detection of the reaction, a higher concentration of BaCl2. According to this study, 4.5g BaCl2 yields the most visible precipitate at a shorter period. The increased concentration of BaCl is an improvement to the recommended rate by Ohazurike (1990) at 2%. Furthermore, the result presented is in accordance with the study conducted by Balbin et al (2020), showing high accuracy rates in determining pregnant and dry does.

Conclusion

Early-stage pregnancy can be detected in goats using barium chloride ($BaCl_2$), regardless of concentration. However, based on the results obtained, 4.5% $BaCl_2$ concentration provides the accurate visualization of an effective pregnancy diagnosis.

DECLARATIONS

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Conflict of Interest: The authors declare no conflict of interest.

Data Availability: The data that support the findings of this study are openly available in the University Library of Isabela State University, Echague, Isabela, 3309 Philippines.

Ethics Statement: The study was conducted in accordance to Philippines' Republic Act No. 8485 known as the Animal Welfare Act of 1998. In particular, the experiment was carried-out following the guidelines of the Philippine National Standard- Code of Good Animal Husbandry Practice (GAHP) for Goat (60:2008).

Author's Contribution: Hannah Michelle D. Catalonia gathered data from a laboratory experiment; while Aubrey Joy M. Balbin contributed data analysis and manuscript writing. Furthermore, Jonathan N. Nayga and Mark Joker L. Marcos assisted in finalizing the paper.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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