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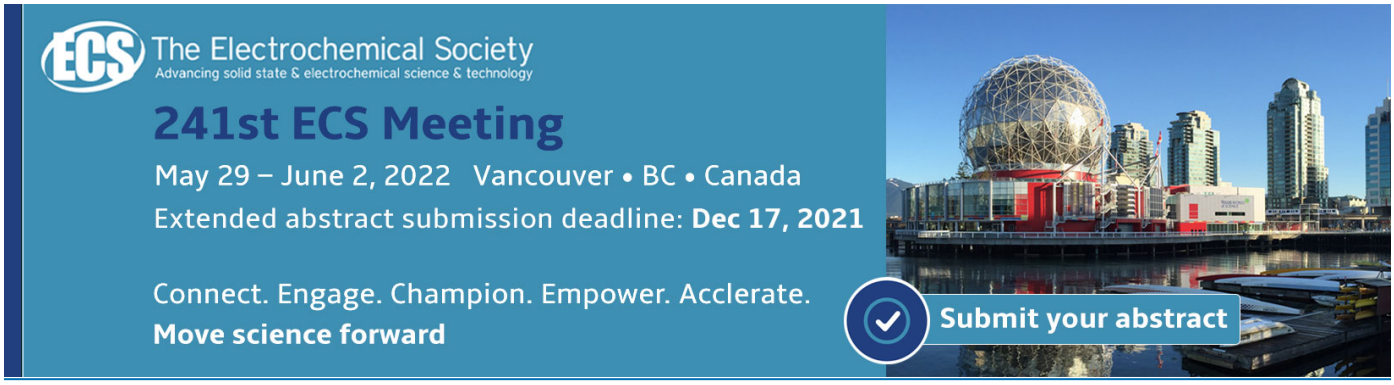

Influence of parity and live weight on the concentration of Pregnancy-Specific Protein B (PSPB) in Kedah Kelantan (KK) cattle

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Abstract. The pregnancy-specific protein B (PSPB) is the protein of binucleated cells in the ruminant's placenta. It contains several antigenic variants, which contributed to the ability to detect pregnancy from day 20 of pregnancy until parturition. In this research, the main objective is to determine the concentration of PSPB in serum and urine and their relation to Kedah-Kelantan (KK) cattle's parity and live weight. Ten cattle (n=10) were selected for this study. The blood serum (2 ml) and urine samples were collected at days 0, 24, 42, and 164 post artificial insemination (AI). All the samples were centrifuged at 3000 rpm for 15 minutes at 4ºC. The serum and urine were analyzed with competitive ELISA test kit and read at 450 nm wavelength speed. The standard OD value provided by the kit created the equation to calculate the concentration of PSPB in the serum and blood. Then, the correlation and multiple linear regression of parity and live weight were analyzed with a significant level of $P < 0.01$. Through the finding, the live weight and parity were positive correlate with PSPB in serum. The relationship between live weight and PSPB concentration in urine showed a negative correlation but there no correlation between parity and PSPB concentration in urine. The regression analysis result was 35% and 36% for PSPB concentration in serum and urine, respectively. This study suggests that cattle parity and live weight did not significantly affect the PSPB concentration.

1. Introduction

Diagnosis of pregnancy in cattle is a crucial method that farmers need to detect pregnancy in cattle. Standard methods frequently used among veterinarians and farmers are direct methods such as rectal palpation and ultrasound. These methods are beneficial in many years because of the simple action to be taken [3]. However, these methods may create a severe problem when an inexperienced operator, costly machinery usage, and lack of skill perform the techniques. The worse situation for the rectal palpation method is it may cause prenatal damage, leading directly to abortion and may not be accurate if the handling in the immense scale of cattle. On the other hand, ultrasound requires high skills and intensive practice to interpret the image. On top of that, these techniques can only detect

pregnancy at day 30 and above of gestation [4]. Several reports have shown studies on the relationship between live weight and PSPB concentration in serum [5]. However, the relationship between parity, live weight, and PSPB concentration in urine is still no work carried out. Therefore, it is sensible to modify the linear regression model to see the relationship between random independent and specific dependent variables.

Other biochemical substances are also generated in the body during pregnancy when the ovum is fertilized in the oviduct and forms a zygote, which is pregnancy-specific Protein B (PSPB). PSPB is a protein that enables the detection of pregnancy and mortality of cattle in the early stage [6]. According to Humblot et al. [7], this protein is developed at the binucleated trophoblast cell in the placenta membrane. The PSPB will be secreted continuously during the gestation period until the pregnancy is over [7]. This protein can be found in animal bodies' circulation and capable as a marker for early pregnancy detection after purification [8,9]. The PSPB is reliable for detecting pregnancy on day 20 and above after artificial insemination (AI) [10]. Balhara et al. [4] reported that PSPB is detected in the serum of pregnant cattle and the results displayed the increase in the concentration of PBPS in serum. Besides, radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) are laboratory methods that can also be used to detect pregnancy [11]. The advantage of quantifying using immunoassays such as Enzyme-Linked Immunosorbent (ELISA) and Radioimmunoassay (RIA) is high sensitivity [2]. The fundamental theories that rely upon the reaction of antigen and antibody are related to the binding process that occurred in the reaction. The reaction happens in antigen, and antibody is a useful finding for creating the new system that can be biomarker-based pregnancy detection for animals. Thus, this research aimed to observe the differences in PSPB concentration in serum and urine related to the animals' parity and live weight.

2. Materials and Methods

2.1. Sample collection

Ten (n = 10; age = 5.1 ± 1.22 year-old; parity = 1.9 ± 0.67; live weight = 258.3 ± 51.01 kg) of Kedah-Kelantan cattle were placed in a separate space at Pusat Ternakan Haiwan Pantai Timur Tersat, Kuala Berang, Terengganu Farmhouse (5° 3' 0.20148"N 103° 1' 4.36654"E). The cattle were placed in the feedlot and the feed and water were provided ad libitum. Blood samples were obtained from each cattle at the coccygeal vein and inserted with a clot activator in the red blood tube (BD Vacutainer®, US) [12]. The urine samples were collected using the perennial massage and placed in a sterile container [1]. The samples were collected at days 0, 24, 42, and 164 after artificial insemination. All the samples were centrifuged at 3000 rpm for 15 minutes at 4 ºC [13] for further analysis.

2.2. Competitive ELISA test for PSPB concentrations in blood and urine

The samples were analysed through semiquantitative analysis by the Cusabio® bovine PSPB competitive ELISA test kit. In this analysis, 50 µl of standard and samples of serum and urine were added to each well. 50 µl of HRP-conjugate and the same amount of PSPB antibody were added to the wells. The microplate well needed to incubate at 37ºC for 60 minutes. The washing process was repeated three times by using 200 µl of washing buffer. Combination of mixture Substrate A and B (100 µl) were added to each well and incubated again for 15 minutes at 37ºC. 50 µl stop solution was added to stop the reaction of binding continued to each well. The microtiter well was analysed using a microplate reader (SPECTROstar® Omega, BMG LABTECH) at wavelength speed 450 nm. The positive and negative controls were also included in this procedure [11]. The standard provided in the kit developed the equation of the Optical Density value (OD) to calculate the concentration of PSPB (ng/ml) [14].

The MMF model equation was selected to calculate the PSPB concentration through the graph of the standard due to low standard error (s = 0.11). The standard equation was followed as below:

$$y = \frac{ab + cx^{d}}{b + x^{d}}$$

These equations were used to calculate the OD value of each sample to get the PSPB concentration. The PSPB concentration can be detected at a range of 0.06 ng/ml until 24 ng/ml through this analysis. Based on the standard graph, the higher the OD level, the lower the concentration of PSPB.

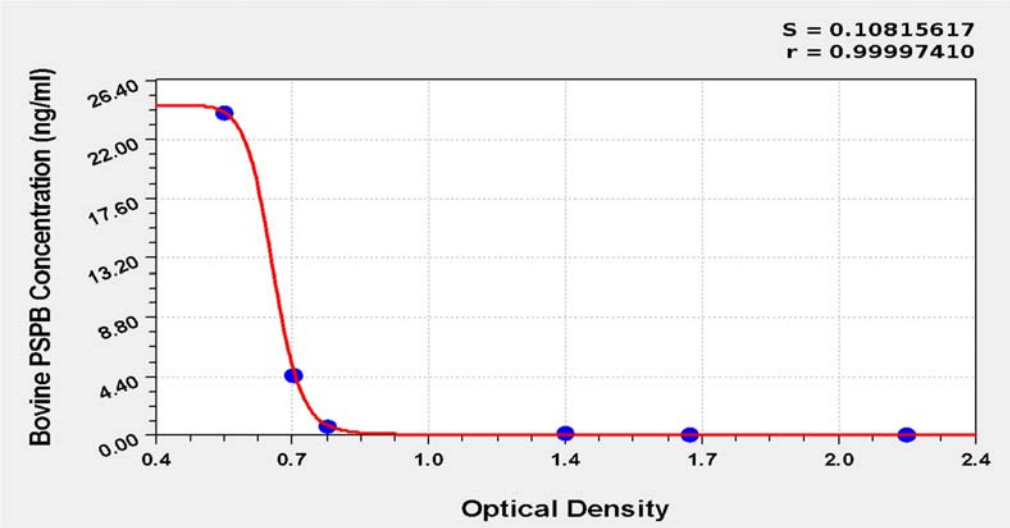

Figure 1. The standard graph of bovine PSPB concentration of bovine (ng/ml) against optical density value.

2.3. The Correlation and Regression Analysis of Parity and Body Weight

The data of parity and bodyweight of the cattle were collected for the variables. These variables data were used to make a relationship against the PSPB concentration in serum and urine by using Pearson's correlation coefficient. The two variables relationship was needed in a straight line, either an entirely negative correlation (r = -1) or an entirely positive correlation (r = +1).

To make a strong relationship, the simple linear regression analysis of parity and bodyweight was calculated to enable the interpretation relationship in PSPB concentration in serum and urine [15]. The multilinear linear regression model enables to analyze of the relationship between the dependent and independent variables. The model equation was followed as below:

$$Y=0+ 1x1 + 2x2+\ldots+ kxk+\epsilon$$

In this model, β_0 is regression constant, and β_i is partial regression coefficient. Throughout this analysis, there was enabled to analyzed one or more dependent variables against each of the independent variables. The analysis created many equations and paths that according to the dependent variables tested with the independent variables. The highest coefficient (R2) was determined and selected to make regression [16]. The independent variable, the estimated multiple regression equation was used to predict the value of dependent variables by assuming the value of error (∈) is zero. The equations of the estimated multiple regression equation were following as below:

$$\hat{y}=b0+ b1x1+b2\ x2+ b3x3$$

Pearson's correlation coefficient and regression were analyzed using the IBM SPSS Statistics 26 software with a significant level $P < 0.01$ to be accepted [17]. The regression analysis of each

dependent variable created the model's new equation through the multilinear regression model above.

3. Results and Discussion

Descriptive statistics of the PSPB concentration in serum (Y_1) and urine (Y_2), parity (X_1), and live weight (X_2) of cattle are given in Table1.

Table 1. Descriptive statistic of PSPB concentrations, parity, and live weight.

Variables (n=10)	Mean	Standard Deviation	Standard Error	Minimum	Maximum
S-PSPB (Y_1)	12.565	9.647	1.525	0.090	24.550
U-PSPB (Y_2)	23.532	3.894	0.616	1.660	24.550
Parity (X_1)	1.900	0.738	0.233	1.000	3.000
Live weight (kg) (X_2)	258.300	53.767	17.003	175.000	367.000

S-PSPB = PSPB concentration in serum, U-PSPB = PSPB concentration in urine.

The correlation coefficient of PSPB concentrations in serum and urine were examined to see the relationship between parity and live weight shown in Table 2. Based on Table 2, the correlation coefficient among variables was not significant ($P < 0.01$). The relationships between live weight and parity were moderately positive correlation with the PSPB concentration in the serum (r bodyweight =0.412, r parity = 0.406). These indicated that the higher level of live weight and parity of cattle could increase PSPB concentration in the blood serum. This finding is consistent with that of Pradhan and Nakagoshi [18], stated that a correlation has existed between the cattle's fertility and body condition at early pregnancy detection. Besides, the animal with optimal body weight has shown good fertility signs [19,20]. Pradhan and Nakagoshi [18] stated that nutrition is the most vital link to cattle production's fertility to enable the cattle body's good performance, especially in hormone production by the pituitary gland. These results further support the idea that the high dietary may increase the blood flow at the cattle's liver and gut [21]. Therefore, it can be expected that the cattle's nutrition intake is significantly impacting the development of the PSPB production in serum due to the regulation of reproductive hormone. Enhance quality feed with lower cost can be offered by replacing the feed material with agriculture by-products [22], this will lead to enhancement of the hypothalamic-pituitary-gonadal axis. Plus, these show that nutrition was one of the factors that affect the production of PSPB concentration.

The cattle's live weight indicated a moderately negative correlation in PSPB concentration in the urine biomarker. However, the correlation between parity and the PSPB concentration in urine was shown in Table 2 that there was no correlation due to the lowest value of the correlation coefficient (r = 0.040). According to Reese et al. [23], urine can become the biomarker for pregnancy indicators since it consists of many microRNAs that can create protein in the body's cells. This statement was aligned with the previous study that pregnancy hormone was excreted in the urine cow [12]. However, the serum's PSPB concentration analysis peaked the ELISA test kit's sensitivity, which is 24 ng/ml. The two-dimensional (2D) gel electrophoresis technique was suitable to use [24]. Rawat et al. [25] stated that urine is a biological fluid rich with many proteomics types that act as abundant protein.

Table 2. Correlation between PSPB concentration (serum and urine) and bodyweight and parity.

Variables	Bodyweight	Parity	S-PSPB	U-PSPB
Bodyweight	1	-0.044	0.412	-0.601
Parity		1	0.406	0.040
S-PSPB			1	0.005
U-PSPB				1

According to Table 3, the multiple linear regression analysis of parity (t_{xs}=1.395; t_{xu}=0.043, P>0.05) and bodyweight (t_{xs}=1.411; t_{xu}= -1.986, P>0.05) were shown not effected in the PSPB concentrations in serum and urine. These were shown that the protein development regulation was not directly dependent on the cattle's live weight and parity. Most previous studies focused on the nutrition that affects hormone changes and protein development [26,27]. Table 3 explained the regression coefficient that analyzed the independent variables and dependent variables relationship in PSPB concentration. Table 3 shows the intercept point in the linear graph of regression for serum PSPB concentration (b_0=-9.286) and urine PSPB concentration (b_0=42.388). The regression coefficient of a constant of the urine was significantly related between the independent variables (P<0.05) through this analysis. However, serum PSPB concentration's regression coefficient was not significantly related to the independent variables (P>0.05). The standard error of the constant-coefficient value of urine PSPB concentration was larger than serum PSPB concentration. Hence, the average distance of data points from the regression line of PSPB concentration in urine is larger than the PSPB concentration in serum.

Table 3. Regression analysis for PSPB concentration.

Dependent variables	Independent variables	Unstandardized		Standardized	t-value	P
		Beta	Standard Error	Beta		
S-PSPB (Y_1)	Constant	-9.286	6.467	-	-1.436	0.194
	Parity (X_1)	2.171	1.557	0.425	1.395	0.206
	Bodyweight (X_2)	0.030	0.021	0.430	1.411	0.201
U-PSPB (Y_2)	Constant	42.388	12.298	-	3.447	0.011
	Parity (X_1)	0.128	2.960	0.013	0.043	0.967
	Bodyweight (X_2)	-0.081	0.041	-0.600	-1.986	0.087

The coefficient analyzed was presented the relation of the independent variables against the dependent variables. The PSPB concentration in serum and urine coefficient value (b_{1serum}=2.171; b_{2serum}=0.030; b_{1urine}=0.128; b_{2urine}=-0.081) were placed in the Table 3. The analysis's residue value can explain the relationship between independent and dependent variables through the data given. The residual values of the independent variables related to the PSPB concentration in serum are hugely different from the predicted value given. Figure 2 and 3 displayed the scatterplot graph of the regression residual of PSPB concentration in serum and urine. Based on the graph in Figure 2 and 3, the scatterplot showed a high and low result than the parallel line of point zero. Throughout this scatterplot also proved the scatterplot of residual was not scattered near the zero lines. If the center of the residual on zero that is meant that the prediction is correct. In Table 4, the R2 of PSPB concentration in serum and urine were 35% and 36.1% while F ratios for Y1 and Y2 were 1.885 and 1.980.

Table 4. Regression model for PSPB concentration.

Model	R^2	Adj R^2	Standard Error	R^2 Change	F	P
S-PSPB (Y_1)	0.350	0.164	3.443	0.350	1.885	0.221[b]
U-PSPB (Y_2)	0.361	0.179	6.547	0.361	1.980	0.208[b]

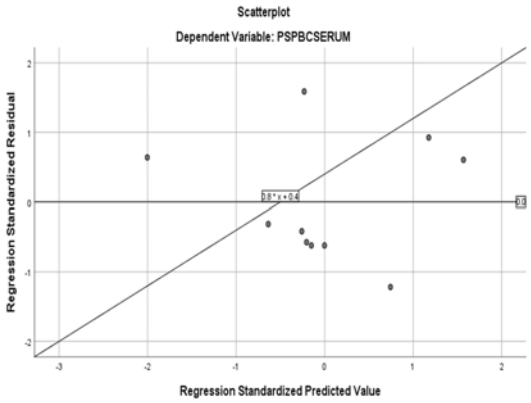

Figure 2. The scatterplot graph of regression standardized residual of PSPB concentration in serum.

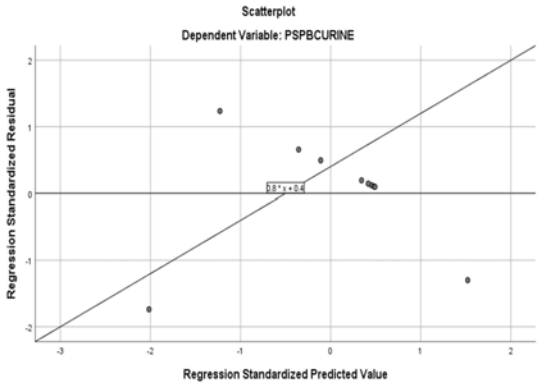

Figure 3. The scatterplot graph of regression standardized residual of PSPB concentration in urine.

4. Conclusion

In conclusion, the parity and live weight of cattle do not affect the PSPB concentration in serum and urine. These can be seen when parity and live weight were analyzed using the Pearson coefficient correlation that showed the results were not significant. Through the multiple linear regression analysis, the value of R2 value gives a good indicator supporting the statement above since the correlation analysis was not significant. However, this model can be improved by focusing on seeing the relationship between body measurement and embryonic mortality towards the PSPB concentration.

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