UNIVERSITI PUTRA MALAYSIA

PLASMA BOVINE GROWTH HORMONE (bGH) IN BUFFALOES AND ITS ASSOCIATIONS WITH SELECTED PHENOTYPES

NUR HUSNA ATIKA BINTI AZHAR

PLASMA BOVINE GROWTH HORMONE (bGH) IN BUFFALOES AND ITS ASSOCIATIONS WITH SELECTED PHENOTYPES

BY

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It is hereby certified that we have read this project paper entitled "Plasma Bovine Growth Hormone (bGH) in Buffaloes and Its Associations with Selected Phenotypes" by Nur Husna Atika binti Azhar and in my opinion; it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4901 – Project

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In the name of Allah, The Most Graceful and The Most Merciful

To

Beloved parents

Azhar Ahmad & Halimahtun Sa`diah Jap

Siblings

Muhammad Azfar Halimy Azhar

Nur Husni Athirah Azhar

Muhammad Afiq Hafizi Azhar

Families & friends

Lecturers and staff

Lovable little furry and scaly friends

&

All animals alive in this small yet cruel world

Thank you very much

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In the name of Allah, The Most Graceful and The Most Merciful

Hereby, I would like to express my sincere appreciation and deepest gratitude to my supervisor, Dr. Mohd Shahrom Salisi and my co-supervisor, Dr. Mark Hiew Wen Han for their encouragement, supervision, patience, dedication, comments and suggestion. Special thanks for Prof. Ariff Omar and Dr. Rosnina for their kindness, time, guidance and knowledge throughout this project. I would like to thank my project mates, Muhammad Naim and Nur Rashidah for their assistance and hard work in this project, post-grad students; Mr. Fariz Nicholas and Mr. Amirul Faiz, for their help and knowledge, patience and guidance for ELISA and the analysis. I would also like to express my gratitude to the director of The Department of Veterinary Services and Animal Industry of Sabah, Dr Punimin Abdullah (Assistant Director of Genetic Resources Division) and En Jonny Engkias (AVO) and all his staffs at Buffaloes Breeding and Research Centre, DVSAI, Telupid Sabah. For my parents and family, Mr. Azhar bin Ahmad and Mrs. Halimahtun Sa`diah binti Jap for their support and blessing, my best friends; Ainul Riza, Nurul Husna, Nur Aisyah and Nurul Farliana for their willingness to be with me through rise and fall. For Dr. Arif, thank you for your support, kindness and assistance throughout this project.

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LIST OF ABBREVIATIONS

bGH	bovine Growth Hormone
ELISA	Enzyme-linked Immunosorbent Assay
FPV	Faculty of Veterinary Medicine
GH	Growth Hormone
ITIS	Integrated Taxonomy Information System
SE	Standard Error
UPM	Universiti Putra Malaysia

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir

PLASMA HORMON PERTUMBUHAN LEMBU (bGH) DALAM KERBAU DAN KAITANNYA DENGAN FENOTIP TERPILIH

Oleh

Nur Husna Atika Binti Azhar

2016

Supervisor: Dr. Mohd Shahrom bin Salisi

Co-supervisor: Dr. Mark Hiew Wen Han

Empat puluh kerbau dengan fenotip yang berbeza telah digunakan di dalam kajian ini. Fenotip yang dikaji adalah baka, jantina, berat badan semasa, umur, berat lahir dan umur matang. Sampel darah diperolehi dari urat coccygeal ke dalam tiub ethylenediamine tetraacetic asid (EDTA). Plasma diperolehi selepas pengemparan pada 3000 rpm selama 15 minit. Kepekatan plasma hormon pertumbuhan lembu telah dinilai menggunakan kit komersial dari Novateinbio

Bovine Growth Hormone ELISA Kit yang menggunakan prinsip kuantitatif ELISA sandwich. Data-data pada fenotip terpilih dikumpulkan daripada rekod ladang daripada tahun 2006 hingga 2015 secara retrospektif. Dari segi baka, tiada perbezaan yang signifikan (p>0.05) antara Swamp dan Murrah Cross untuk kepekatan plasma hormon pertumbuhan lembu. Tiada juga perbezaan yang signifikan (p>0.05) antara kerbau betina dan jantan untuk kepekatan plasma hormon pertumbuhan lembu. Dalam kumpulan umur, tiada perbezaan yang signifikan (p>0.05) di antara kumpulan kerbau muda, dewasa dan tua untuk kepekatan plasma bGH. Pekalikorelasi (r) di antara kepekatan plasma hormon pertumbuhan lembu, berat badan, umur, berat lahir dan umur matang telah diuji di mana kepekatan plasma hormon pertumbuhan lembu telah dikaitkan secara negative dengan berat badan (r = -0,069), umur (r = -0,111), berat lahir (r = -0,141) dan umur matang (r = -0,062). Kesimpulannya, dalam kajian ini, kepekatan plasma hormon pertumbuhan lembu tidak menunjukkan sebarang kaitan dengan fenotip yang dipilih.

Kata Kunci: Kerbau, hormon pembesaran, fenotip, ELISA

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the requirement for VPD 4999 – Final Year Project

BOVINE GROWTH HORMONE (bGH) IN BUFFALOES AND ITS ASSOCIATION WITH SELECTED PHENOTYPES

By

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2016

Supervisor: Dr. Mohd Shahrom bin Salisi

Co-supervisor: Dr. Mark Hiew Wen Han

Forty buffaloes with different phenotypes were used in the study. The phenotypes were the breed, sex, current body weight, age, birth weight and age at puberty. Blood samples were collected from the coccygeal vein into ethylenediamine tetraacetic acid (EDTA) tubes. Plasma was harvested after centrifugation at 3000 rpm for 15 minutes. Concentrations of plasma bovine growth hormone (bGH) were determined by quantitative sandwich ELISA using commercial kits from Novateinbio Bovine Growth Hormone ELISA Kit. The data on selected phenotypes were collected from the farm records retrospectively between 2006 and 2015. Breeds wise, there was no significant difference ($p>0.05$) among Swamp and Murrah Cross in plasma bGH concentration. There was also no significant difference ($p>0.05$) among female and male buffaloes in plasma bGH concentration. In age groups, there was no significant difference ($p>0.05$) among young, adult and old buffaloes in plasma bGH concentration. The correlation coefficient between (r) between plasma bGH concentration, body weight, age, birth weight and age at puberty were tested which plasma bGH concentration was negatively correlated with body weight ($r = -0.069$), age ($r = -0.111$), birth weight($r = -0.141$) and age at puberty ($r = -0.062$). Thus, in this study, plasma bGH concentration did not showed any association with selected phenotypes.

Key Words: Buffaloes, growth hormone, phenotypes, ELISA

1.0 INTRODUCTION

Integrated Taxonomy Information System (ITIS) documented that buffaloes belong to the mammalian class of the family of Bovidae. Buffalo can be classified into two main species of buffalo which are the Asiatic buffalo (*Bubalus bubalis*) and the African buffalo (*Syncerus caffer*) (Iannuzzi & Meo, 2009). The domestic water buffalo (Bubalus bubalis) has two subspecies which are the river buffalo and the swamp buffalo. The river or dairy type had different breeds for example Murrah and Nilli Ravi. According to a journal on the current status and challenges in buffalo production in Malaysia, in year 2004, the total population of buffaloes in Malaysia is 138 098 heads and 95% of this population was swamp buffaloes (Mazni *et al*., 2006).

Visceral and skeletal growth, protein, lipid and carbohydrate metabolism of an animal was controlled by hormonal secretion. One of the hormones that responsible for these mechanisms is growth hormone (GH). It was the hormone that stimulates growth, especially the polypeptide hormone that is secreted by the anterior pituitary gland (Studdert *et al*., 2011). Level of GH was found to have association with growth performance. Animals with high level of GH showed enhanced growth performance. Hence, endogenous GH level is suited to be a tool to select animals as high level of endogenous GH helps to explore superior growth traits of the animal (Wickramaratne *et al*., 2010).

For quantifying the concentration of GH in blood, hormone assays involves many methods and the most frequently used method is Radioimmunoassay (RIA). Other non-radioactive method which is the enzyme immunoassay method (EIA) or enzyme-linked immunosorbent assay (ELISA) which can be considered beneficial in terms of analytical, operational, and clinical outcomes (Sachidhanandam *et al*., 2010). ELISA was claimed to have short duration of time needed for result interpretation which can be done in less than 24 hours (Mishra *et al*., 2007). The study of the association between the concentration of bGH and the selected phenotypes in buffaloes will provide information on the profile of the bGH in buffaloes. The profile of plasma bGH concentration can be use as an aid in selection of buffaloes as animals with high level of GH concentration exhibited enhanced growth performance. On the other hand, there is limited study done on the association of certain phenotypes with bGH in buffaloes.

The study of the association between the concentration of bGH and the selected phenotypes in buffaloes will provide information on the profile of the bGH in buffaloes. The profile of plasma bGH concentration can be use as an aid in selection of buffaloes as animals with high level of GH concentration exhibited enhanced growth performance. On the other hand, there is limited study done on the association of certain phenotypes with bGH in buffaloes.

The objective of this study is to determine the concentration of bovine GH (bGH) in buffaloes and to investigate the association between the concentration of bGH and the selected phenotypes in buffaloes.

2.0 LITERATURE REVIEW

2.1 Buffaloes (*Bubalus bubalis*)

Buffaloes belong to the mammalian class of the family of Bovidae, which in turn belong to Artiodactyla order according to Integrated Taxonomy Information System (ITIS). In the world, there are two main species of buffalo which are the Asiatic buffalo (Bubalus bubalis) and the African buffalo (Syncerus caffer)(Iannuzzi & Meo, 2009). According to Ianuzzi & Meo (2009) also stated that the Asiatic buffalo (B. bubalis) which also known as water buffalo has two main subspecies; the river buffalo and the swamp buffalo. River buffalo has a karyotype 2n = 50 and FN = 60 and the swamp buffalo has a karyotype 2n = 48, FN = 58.

Barker (2014) stated that there are a number of specialized breeds of river buffalo. Despite of that, the breeds are primarily used for milk production. River buffalo milk can be used to produce traditional mozzarella cheese. The cheese originating in Italy and its flavor is highly prized (Barker, 2014). Their milk production capacity is far higher which from 1000 to 2000 kg per lactation in range and it is varies among strains and countries. The body weight of river buffalo is heavier compare to swamp buffalo, which the mature weight is approximately 1100 kg for males and 550 kg for females. River buffaloes are adaptable to wide range of environmental conditions. The best milk breeds of buffaloes are of the river type and they are mostly enclosed to areas where the summer temperature rises above 46 °C and the winter temperature may fall below 4 °C in India and Pakistan (Marai & Haeeb, 2010).

According to Barker (2014), for swamp buffaloes, they are mostly phenotypically homogenous throughout their distribution which is physically similar to each other. Thus, there are no recognized breeds for swamp buffalo. They are used for the purpose of draught and meat. The swamp buffalo, which their largest distribution is found in the rice-growing countries of Southeast Asia, is not adaptable in comparisons to river buffalo as the ancestors were found only in swampy and marshy areas and in hot climates (Marai & Haeeb, 2010). They are categorized as semi-aquatic animals since during the hottest part of the day (between 10.00 a.m. to 2.00 p.m.), they are partly submerged in natural swamps or self-made wallows. They must have almost unlimited access to water to keep cool. Body weight of swamp buffaloes is lighter which are approximately 700 kg for males and 500 kg for females. For milk production capacity, they produce a lower amount which between 430 to 620 kg of milk per lactation (Webster & Wilson, 1986).

2.2 Bovine Growth Hormone (bGH)

GH is a substance which stimulates the growth, specifically the polypeptide hormone that is secreted by the anterior pituitary gland. It directly influences protein, carbohydrate and lipid metabolism, and controls the rate of skeletal and visceral growth (Studdert *et al*., 2011). It is obligatory for growth and development of the animal. GH also relate closely in the sexual differentiation and pubertal maturation. For female, it involves in the process of gonadal steroidogenesis, gametogenesis and ovulation (Hull & Harvey, 2001). On the other hand, for male, it engages in the process of gonadal steroidogenesis and gametogenesis (Hull & Harvey, 2000). The effects of GH on target organs may be direct or mediated by other molecules such as somatomedins which is also known as insulin-like growth factors or IGFs (Castigliego *et al*., 2011).

2.3 Enzyme-linked Immunosorbent Assay (ELISA)

Enzyme immunotests which the enzyme immunoassay (EIA) and enzyme-linked immunosorbent assay (ELISA) are the quantitative analytical method that demonstrate antigen-antibody reactions by the changes of color obtained by using an enzyme-linked conjugate and enzyme substrate which serve to identify the presence and the concentration of desired molecules in biological fluids (Hornbeck, 2001). ELISA is classified into two general headings which are homogeneous enzymatic immunoassay methods and heterogeneous enzymatic immunoassay methods (O'Kennedy *et al*., 1990).

O'Kennedy *et al*. (1990) stated that for homogeneous enzymatic immunoassay methods, when the enzymes bound to the antibody, they become inactivated. Since the enzymes are inactivated, there is no washing stage involved in this method. Washing is the process where the antigen is separated from the medium. Homogeneous enzymatic immunoassay methods accounted for measuring small quantities substances (O'Kennedy *et al*., 1990). Despite the advantage of easy to be done, this method is expensive and has low sensitivity (Aydin, 2015).

Heterogeneous enzymatic immunoassay methods are frequently used compared to homogeneous enzymatic immunoassay methods. In this method, the antigen-antibody complex is adhered to the walls of the experiment tubes. Washing procedure is involved in this method which anything other than the antigen-antibody complex is removed from the medium by this procedure to avoid interference of any molecule in the medium after the binding of the antigen and the antibody. This method regularly used since it is more sensitive than the homogeneous method (O'Kennedy *et al*., 1990).

ELISA is a heterogeneous immunoassay technique employed to detect specific antibodies and soluble antigens. There is an array of ELISA types produced to refine the specificity of measurement for the structure and the characteristics of substances tested are not always the same (O'Kennedy *et al*., 1990). Aydin (2015) classified the types of ELISA into four which are direct ELISA, indirect ELISA, sandwich ELISA and competitive ELISA.

The advantages of ELISA test are sensitive, specific and rapid result. It includes wide area of application as it is easy to be of used and fast. Engvall (1971) mention that this test had been frequently used in peptide and protein analyses. On the other hand, there is no requirement for two serum samples thus it is more practical. It is almost as sensitive as Radioimmuno assay (RIA) and no special equipment needed and no radioactive involved (Engvall & Perlmann, 1971). However, in comparison of reliability, ELISA has low reliability compare to RIA (Revoltella *et al*., 1998).

GH concentration in blood is quantifiable and there are many assay systems had been developed for this purpose which one of them is ELISA. ELISA can be used as a tool for quantifying GH level in buffaloes. It was claimed to had advantage of short duration of time needed for interpreting the result which in less than 24 hours. (Mishra *et al*., 2007).

2.4 GH-Phenotype Associations

Anabolic processes for example cell division, skeletal growth and protein synthesis can be either directly or indirectly stimulated by GH (Gluckman *et al*., 1987). GH concentration was found to have association with breed (Shingu et al., 2001), sex (Irvin & Trenkle, 1971), body weight (Mears & Schaalje, 1993), age (Sarkar *et al*., 2008) and birth weight (Purchas *et al*., 1970). However, there were inadequate research and study done to investigate the association of current GH concentration in grown animal with age at puberty and birth weight of the animal.

2.4.1 GH and Breed

In study of other bovine species which was cattle, it was reported that different breed exhibited different GH concentration. Keller *et al*. (1979) had found that throughout all sampling times for GH, Angus had higher rations in comparison to Hereford. The breed that larger in size and had faster rate of growth for example Simmental, was a higher GH secretor compared to breed that smaller in size and had slower rate of growth for example Hereford (Ohlson *et al*., 1981). The findings by Ohlson *et al*. (1981) was proved by Shingu *et al*. (2001) in the reasearch of GH profiling in growing Japanese Black heifers which is a beef type, in comparison with Holstein heifers which is dairy type. Japanese Black cattle which are a smaller and slower-growing breed had been found to have lower basal GH level and GH secretory function than the respective values for Holstein heifers (Shingu *et al*., 2001).

2.4.2 GH and Sex

In a study that involved cattle, male cattle had been reported to have a bit higher plasma GH concentration compared to female cattle (Irvin & Trenkle, 1971).Trenkle (1971) also stated that heifers had lower plasma GH concentration than bulls. Rate of clearance GH from circulation in heifers were faster compared to bulls which contributes to lower plasma GH concentration (Trenkle, 1971).

2.4.3 GH and Age

Keller (1979) stated that GH concentration changes according to advancing ages over all sampling times. As the animal aged, the concentration of plasma GH decreased. Mondal& Prakash (2004) reported that the ratio between plasma GH and body weight decreased significantly as the animal aged. Secretion of GH per unit body weight in younger animals was higher than older animal (Mondal & Prakash, 2004) which proved the findings reported in other bovine species by Trenkle (1971) and Keller (1979). This finding matched the report on kinetics of Holstein steer calves plasma GH which there was higher basal plasma GH in younger calves (Mears & Schaalje, 1993). Other than that, in an investigation to determine plasma GH concentrations in female yak and its relations with age, it was found that the plasma GH decreased with age (Sarkar *et al*., 2008). Sarkar *et al*. (2008) claimed that it was consistent with the findings in other bovine species despite the decreasing pattern in yak was not uniform.

2.4.4 GH and Body Weight

Mondal & Prakash (2004) related the association between plasma GH and body weight in study of changes in plasma GH in buffaloes (*Bubalus bubalis*) during growth. As the body weight of the animal increased, the plasma

GH per 100 kg showed declining trend in the study. The same findings also reported in study of plasma GH concentrations in female mithun (*Bos frontalis*) in relation to body weight (Mondal *et al*., 2007). In addition, Mears & Schaalje (1993) reported that there was a trend in Holstein steer calves which the basal plasma GH concentration and GH half-life decreased as the body weight of the animal increased. In addition, in a study that involved yak (*Poephagus grunniens L*.), it was found that the plasma GH decreased with body weight (Sarkar *et al*., 2008).

2.4.5 GH and Birth weight

There are insufficient research and study done to investigate the association of current GH concentration in grown animal with birth weight of the animal. However, there was study that investigated plasma GH concentration in bulls from birth to one year old of age. Plasma GH concentration was found higher at birth compared to any other age of the bull (Purchas *et al*., 1970).

2.4.6 GH and Age at puberty

There are inadequate research and study done to investigate the association of current GH concentration in grown animal with age at puberty of the animal. However, there was a study conducted by Haldar & Prakash (2005) that investigated patterns of GH in buffalo heifer pre-puberty, at puberty and post-puberty. Plasma GH concentration had high positive correlation with body weight in buffalo heifers that approaching puberty. In reports on cattle, it stated that, a month prior to puberty of the cattle, there was a gradual increment of mean GH concentrations to peak level (Jones *et al*., 1991).

3.0 MATERIALS AND METHODS

3.1 Study area and animals

This study was conducted at the Buffalo Breeding and Research Center in Telupid, Sabah, Malaysia. The farm is owned by Department of Veterinary Services and Animal Industry, Sabah. There were two breeds reared in this farm, Murrah Crossbred and Swamp. According to the latest data collected on 31st December 2015, the center has total population of 399 buffaloes which consist of 162 breeding cows, 24 breeding heifers, 61 heifers, 26 heifers calves, 12 stud bulls, 88 young bulls and 26 bull calves. All breeder buffaloes were managed extensively.

3.2 Blood sampling

Blood samples were collected from total 10 males buffaloes and 30 female buffaloes by venipuncture of the coccygeal vein. The buffaloes were restrained in a crush. The area for blood collection was swabbed using 70% alcohol. Blood was collected using 18-gauge needle. Approximately, 10 ml of blood was drawn out into EDTA tube. The blood collected was centrifuged at 3000 rpm for 15 minutes. The plasma was then harvested and stored at -20°C pending analysis.

3.3 Data collection

Data were collected retrospectively via farm records between 2006 and 2015. The parameters collected included the breed, sex, current body weight, age, birth weight and age at puberty of the buffaloes.

3.4 Bovine GH (bGH) analysis

Bovine GH (bGH) analysis was carried out at the Biochemistry laboratory at Faculty of Veterinary Medicine, Universiti Putra Malaysia. BGH level was measured using a commercial ELISA kit; Novateinbio Bovine GH, GH ELISA Kit. The concentrations of the hormone were expressed in ng/ml. The standard range was 3.12 to 100 ng/ml. The sensitivity of this kit was 1.0 ng/ml. The kit applied quantitative sandwich immunoassay principle. The sample was diluted in dilution ratio 1:10 as indicated in a journal on ELISA for measuring GH in buffaloes by Mishra (2007). For ELISA reading interpretation, a standard curve was constructed according to the standard values provided by the supplier and the standard values obtained from the ELISA itself. The reading from ELISA reader was the optical density (O.D.) of the samples. Average optical density was located at the x-axis and a horizontal line is extended to the standard curve. At the point of intersection, a vertical line is drawn to the y-axis to determine bGH concentration.

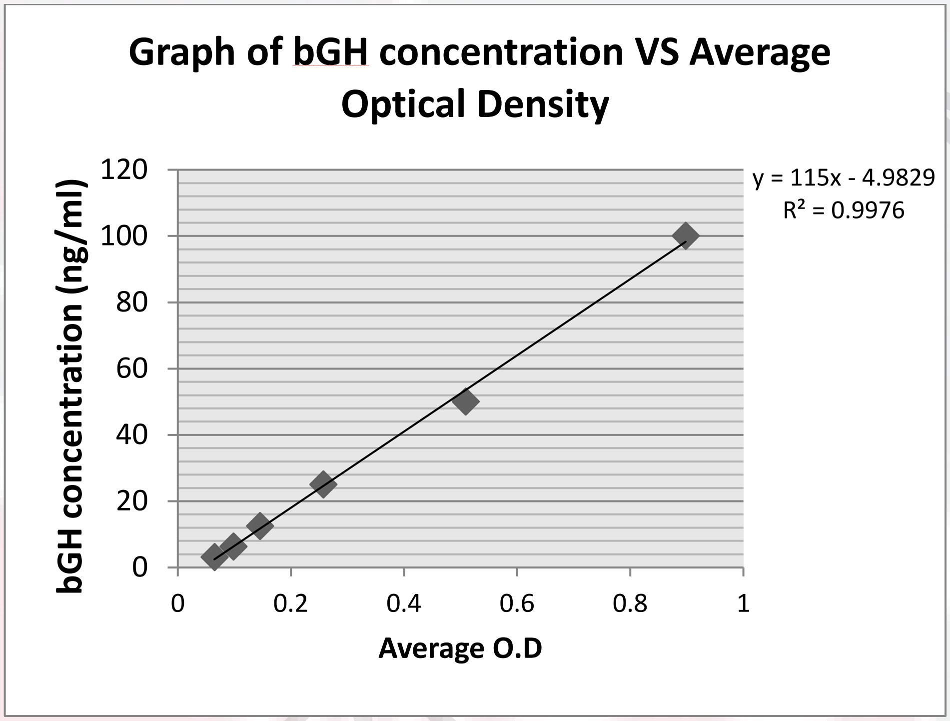
3.5 Statistical analysis

Statistical analysis was performed using IBM SPSS version 22. All data collected on breed, sex, current body weight, age, birth weight, age at puberty and bGH concentration of the buffaloes were tabulated and then analyzed. Non parametric tests; Mann-Whitney U test and Kruskal-Wallis H test were performed to determine the significant difference among the mean groups of the parameters. The association of the parameters was analyzed using Spearman's Rank Correlation Coefficient. Correlation coefficient were correlated at $p<0.05$. The values were expressed as mean (x) followed by one standard error (± SE) of the mean.

4.0 RESULT

A total of 40 blood samples of buffaloes were collected, processed to harvest the plasma and assayed using quantitative sandwich ELISA for measuring plasma bovine GH (bGH) concentration. For ELISA reading interpretation, a standard curve was constructed according to the standard values provided by the supplier and the standard values obtained from the ELISA itself as indicated in Figure 1. The reading from ELISA reader was the optical density (O.D.) of the samples. Average optical density was located at the x- axis and a horizontal line is extended to the standard curve. At the point of intersection, a vertical line is drawn to the y-axis to determine bGH concentration.

Figure 1: Graph of bGH concentration and Average Optical Density.



Distribution of plasma concentration of bGH and selected phenotypes; current body weight, age, birth weight and age at puberty was tabulated in Table 1.

Table 1: Distribution of plasma bGH concentration and selected phenotypes of buffaloes (Mean ± SE).

Parameters	Mean ± SE	Range
Plasma bGH concentration (ng/ml)	17.69 ± 1.15	7.01 - 36.39
Body weight (kg)	439.80 ± 20.18	223 – 736
Age (years)	3.75 ± 0.58	1 – 12
Birth weight (kg)	33.10 ± 0.85	25 – 43
Age at puberty (years)	1.64 ± 0.14	0 – 3

SE= Standard error, n= 40

Distribution of mean of plasma bGH concentration according to breed is tabulated in Figure 2. From the figure, it was observed that mean plasma bGH concentration for Swamp breed was 17.92 ng/ml and for Murrah Cross breed was 17.56 ng/ml. The distribution of mean of plasma bGH concentration according to sex is tabulated in Figure 3. From the figure, it was observed that mean plasma bGH concentration for female buffalo was 18.58 ng/ml and for male buffalo was 15.00 ng/ml. Next, the distribution of mean of plasma bGH concentration according to age group which was divided into three groups; young, adult and old is tabulated in Figure 4. From the figure, it was observed that mean plasma bGH concentration for young buffalo was 18.28 ng/ml, for adult buffalo was 18.32917 ng/ml and old buffalo was 15.79 ng/ml.

Figure 2: Mean plasma bGH concentration in Swamp and Murrah Cross breed.

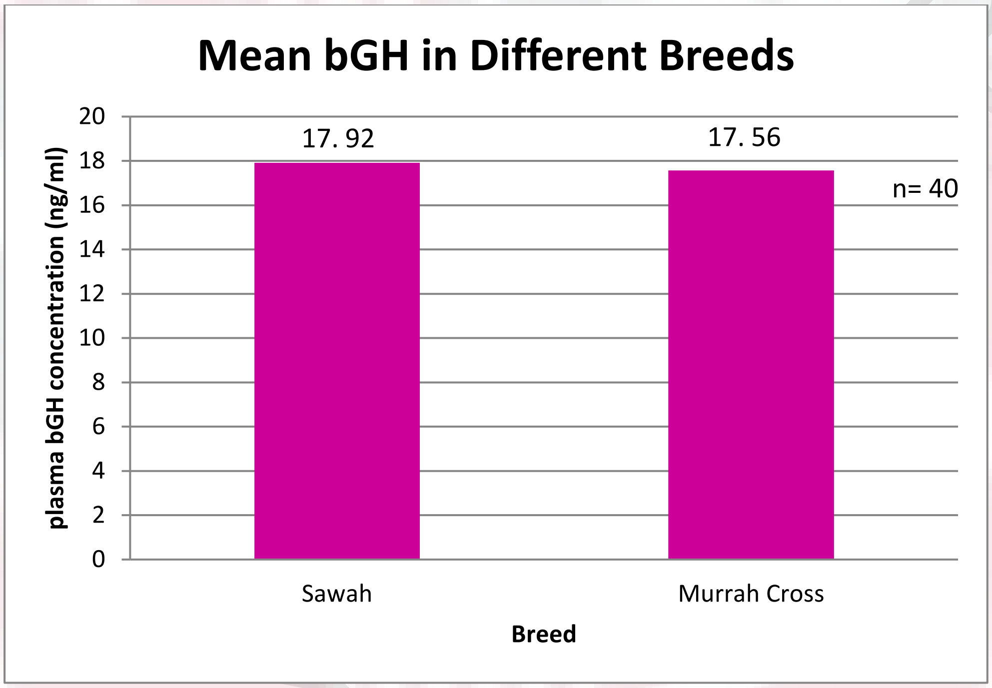


Figure 3: Mean plasma bGH concentration in male and female buffaloes.

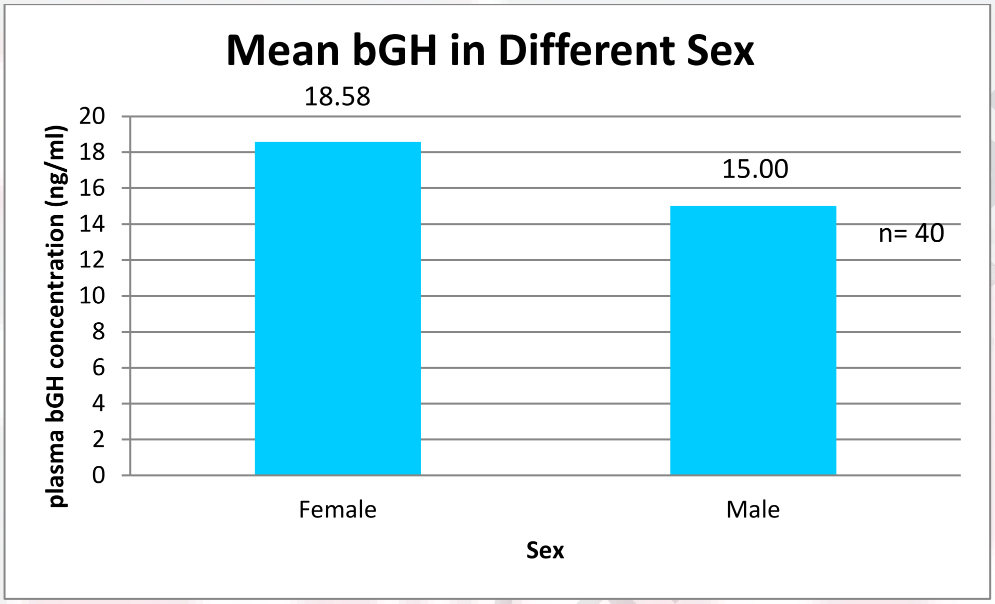
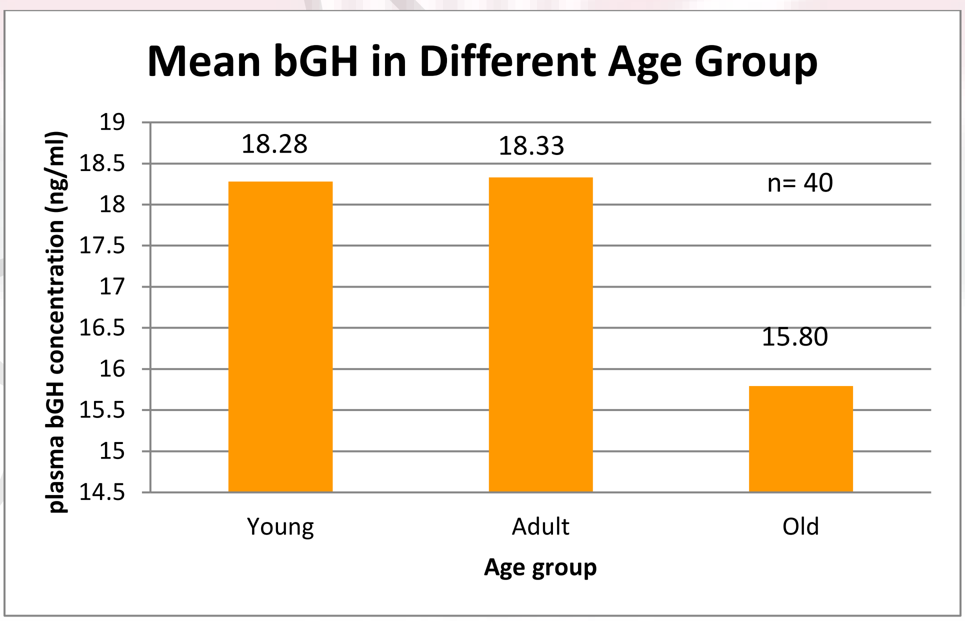


Figure 4: Mean plasma bGH concentration in age groups; young, adult and old buffaloes.



The mean difference of plasma bGH concentration among different breeds and sexes were tested using Mann-Whitney Test. Breeds wise, there was no significant difference (p>0.05) among Swamp and Murrah Cross in plasma bGH concentration. There was no significant difference (p>0.05) among female and male buffaloes in plasma bGH concentration. Meanwhile, mean difference of plasma bGH concentration among different age groups was tested using Kruskal-Wallis Test. In age groups, there was no significant difference (p>0.05) among young, adult and old buffaloes in plasma bGH concentration.

Table 2: Distribution of plasma bGH concentration among selected phenotypes of buffaloes (Mean ± SE).

Parameter		n	Mean ± SE	Range	P-value
Breed	Swamp	14	17.92 ± 1.86	8.81–31.79	0.977
	Murrah Cross	26	17.56 ± 1.48	7.01–36.39	
Sex	Female	30	18.58 ± 1.38	8.81–36.39	0.179
	Male	10	15.00 ± 1.81	7.01–27.82	
Age group	Young	7	18.28 ± 2.96	9.39–31.79	0.587
	Adult	23	18.33 ± 1.57	10.27–36.39	
	Old	10	15.79 ± 2.08	7.01– 27.82	

Means differ significantly (p<0.05)

The correlation coefficient between (r) between plasma bGH concentration, body weight, age, birth weight and age at puberty were tested and presented in Table 3. Spearman's rho reveals plasma bGH concentration is negatively correlated with body weight (r = -0.069), age (r = -0.111), birth weight(r = -0.141) and age at puberty (r = -0.062).

Table 3: Correlation between plasma concentration of bGH and body weight, age, birth weight and age of puberty of buffaloes.

	Plasma bGH conc.	BWT	Age	Birth WT	Age at puberty
Plasma bGH conc. (ng/ml)	1.000				
Body weight (kg)	-0.069	1.000			
Age (years)	-0.111	0.810	1.000		
Birth weight (kg)	-0.142	-0.096	-0.249	1.000	
Age at puberty (years)	-0.062	0.585*	0.734*	0.260	1.000

* Correlation is significant at the 0.05 level (2-tailed)

5.0 DISCUSSION

This study was conducted to determine the concentration of bovine GH (bGH) in buffaloes and to investigate the association between the concentration of bGH and the selected phenotypes in buffaloes, therefore to select buffaloes with excellent growth performance as a breeding animals.

The concentration of bGH obtained in this study was between 8.81 – 31.79 ng/ml and it varied among the buffaloes sampled according to their phenotypes. In comparisons, a study on the concentration of bGH in buffaloes by Mishra *et al*. (2007) reported values between 7.0 – 17.0 ng/ml. This difference might be influenced by the sampling difference; the current study‘s samples were from buffaloes with varies phenotypes. Mishra *et al*. (2007), focused only on female dairy buffaloes. There was also no association between plasma concentration of bGH and the selected phenotypes in buffalo. This outcome was influenced by several factors including hormonal behavior, breed of buffaloes and sampling time.

Grigsby & Trenkle (1986) reported that GH was secreted in bursts or pulses rather than at constant rates and this pulsative pattern result in a wide fluctuation of plasma GH concentrations throughout the day. The none association between plasma concentration of bGH and the selected phenotypes maybe due to the fact that sampling was only done once and blood samples were collected over a period of time (morning to evening) and the buffaloes might have different secretion rates throughout the sampling process.

The mean difference of plasma bGH concentration among different breeds and sexes were tested using the Mann-Whitney Test. In different breeds, there was no significant difference (p>0.05) among Swamp and Murrah Cross in plasma bGH concentration and this was consistent with the report by Trenkle and Topel (1978) which compared plasma GH Angus-sired steers and Charolais-sired steers of different weights and different energy intakes. The breed had no significant effect on circulating hormone concentration as there were no differences found in plasma GH (Trenkle, 1970; Irvin & Trenkle, 1971). However, it was possible that the outcome of the study was due to it being done only in cattle with small differences in type. In contrast, Shingu *et al.* (2001) reported that GH concentration was found to have an association with breed. It was reported that purebreed cattle of different sizes and growth rates exhibited significant difference in bGH concentration. In the current study, the buffaloes sampled in Telupid were mostly crossed, thus the outcome was consistent with reports by Trenkle & Tropel (1978), Trenkle (1970) and Irvin & Trenkle (1971).

There was no significant difference (p>0.05) among female and male buffaloes in plasma bGH concentration. This finding was inconsistent with the one reported in a cattle study by Irvin & Trenkle (1971), whereby males were reported to have slightly higher plasma GH concentration than females. Heifers have faster GH clearance rate from circulation which contributes to their lower plasma GH concentration compared to bulls (Trenkle, 1971).

The mean difference of plasma bGH concentration among different age groups was tested using Kruskal-Wallis Test. For age groups, there was no significant difference ($p>0.05$) among young, adult and old buffaloes in plasma bGH concentration and was consistent with findings by Irvin & Trenkle (1971) whereby no differences in plasma concentrations of GH could be related to age of the animals. Age did not significantly influence plasma GH levels in either males or females. In the study, the average level of GH of all the animals at 371 days was slightly lower compared to younger animals. However, this decrease was not consistent within all the breeds (Irvin & Trenkle, 1971). In a study to determine plasma GH concentrations in female yak and its relations with age, it was found that the plasma GH decreased with age (Sarkar *et al*., 2008) which is in contrast to findings from this current study. Result from this study did not agree with the report on kinetics of Holstein steer calves plasma GH whereby there was higher basal plasma GH in younger calves (Mears & Schaalje, 1993). Keller (1979) also stated that GH concentration changed according to advancing ages over all sampling times as the animal aged, the concentration of plasma GH decreased.

The correlation coefficient between (r) plasma bGH concentration, body weight, age, birth weight and age at puberty were tested. Spearman's rho revealed that plasma bGH concentration was negatively correlated with body weight (r = -0.069), age (r = -0.111), birth weight(r = -0.141) and age at puberty (r = -0.062). The finding was found to be in contrast with reports by Mears & Schaalje (1993) wherby GH concentration was found to have association with body weight and birth weight (Purchas *et al*., 1970). However, there is inadequate research done to investigate the association of current GH concentration in grown animal with age at puberty and birth weight.

For sampling time, in order to associate bGH concentration and birth weight, blood should be sampled right after the calf was born and the study by Purchas *et al*., (1970) revealed that plasma bGH concentration was found to be higher at birth compared to any other age of the bull. In addition, same principle should be done to associate bGH concentration and age of puberty, which the blood should be sampled at time of puberty of the buffalo. Jones *et al*. (1991) reported that a month prior to puberty, there was a gradual increment of mean bGH concentrations to peak levels and Haldar & Prakash (2005) stated that plasma bGH concentration had a high positive correlation with body weight in buffalo heifers approaching puberty

6.0 CONCLUSION AND RECOMMENDATIONS

This study showed that the concentration of plasma bGH obtained in this study was between 8.81 – 31.79 ng/ml. This concentration varies in buffaloes sampled according to their phenotypes However, there were no significant difference ($p>0.05$) among breed, sex and age group in plasma bGH concentration. Thus, it can be concluded that there was no association between the concentration of plasma bGH and the selected phenotypes in buffaloes in this study.

Results from this study warrant future studies where more samples are suggested to be taken. In addition, all the samples should be taken with at least one constant parameter for example same ages or uniform body weight. In addition, in future studies, it is recommended that the pulsative behavior of bGH secretion to be taken into consideration and samples should be obtained periodically. Based on this current study, it was observed that phenotypes; breed, sex, current body weight, age, birth weight, age at puberty and plasma bGH concentration of the buffaloes were not suitable to be the marker for selection of buffaloes. This might be influenced by the variations of plasma bGH concentration among buffaloes. Thus, the study should be advanced to the genetic level on bGH gene polymorphism as genetic markers be used as a tool for best buffaloes selection.

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8.0 APPENDICES

APPENDIX 1: Buffaloes management.

Buffaloes are managed extensively in paddock in Telupid Buffalo Breeding and Research Center Sabah, Malaysia

APPENDIX 2: ELISA Procedure

Sample Collection and Storage

Plasma – Collect plasma using EDTA as an anti coagulant. Centrifudge samples for 15 minutes at 3000 rpm at 2 – 8 °C within 30 minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use.
2. Wash Solution – Dilute 25 mL of Wash Solution concentrate (20x) with 475 mL of deionized or distilled water to prepare 500 mL of Wash Solution.

Assay Procedure

1. Prepare all the Standards before starting assay procedure. It is recommended that all Standards and Samples should be added in duplicate to the Microtiter plate.
2. a) Assign standard wells, sample wells on the assay plate/ strip.

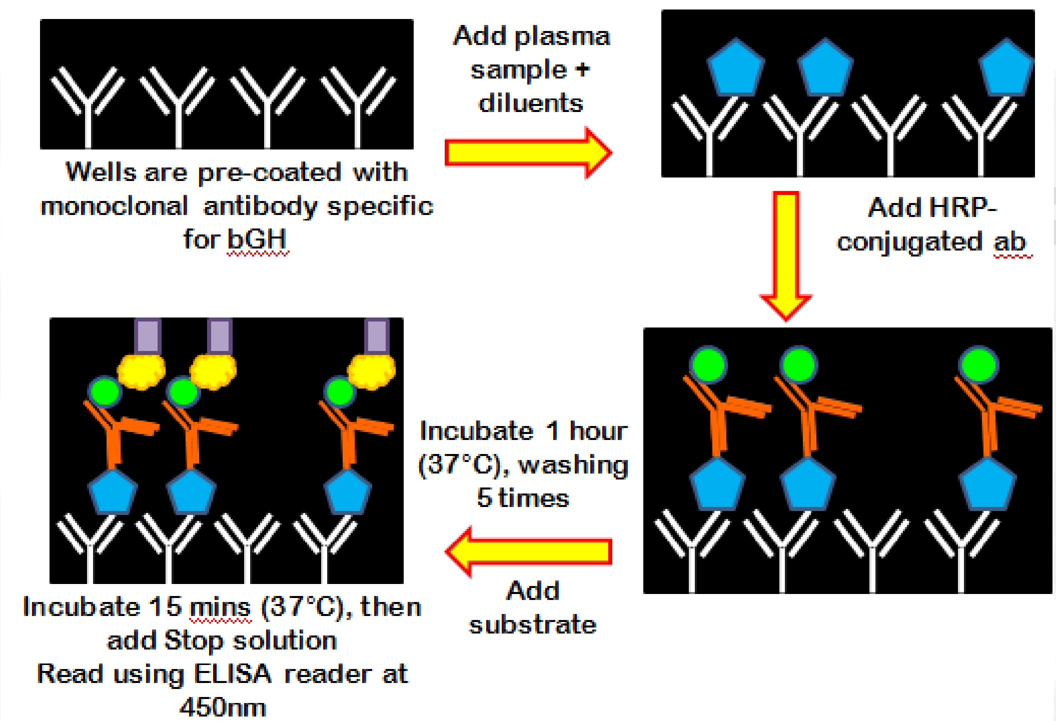
   b) Add Sample Diluent only at 50 µl/well to 2 Standard wells (for duplicate) serving as the zero standard ;take the Standards and agitate gently then add the pre-diluted standard at 50 µl/wellto other standard wells following the sequence of S1 tp S6.

   c) Add sample at 50 µl/well to sample wells.

3. Add 100 µl of Conjugate to each well. Mix well. Cover and incubate the plate for 1 hour at 37°C.
4. Wash the Microtiter Plate.

   Automated Washing: Wash plate FIVE times with diluted wash solution (350-400 µl/well/wash) using an auto washer. After washing, dry the plate . It is recommended that the washer be set for a soaking time of 10 seconds and shaking time of 5 seconds between each wash.
5. Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, subsequently. Cover and incubate for 15 minutes at 37 °C. Protect the plate from light.
6. Add 50 µl Stop Solution to each well. Mix well.
7. Read the Optical Density (O.D) at 450 nm using a microtiter plate reader immediately.

APPENDIX 3: Flow Chart of Quantitative Sandwich ELISA


Add plasma
sample +
diluents
Wells are pre-coated with
monoclonal antibody specific
for bGH
Add HRP-
conjugated ab
Incubate 1 hour
(37°C), washing
5 times
Add
substrate
Incubate 15 mins (37°C), then
add Stop solution
Read using ELISA reader at
450nm