World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

SUPPLEMENTATION OF GREEN BEAN SPROUTS IN THE RATION OF CROSSBRED YORKSHIRE BOARS ON SEMEN QUALITY

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Article Received on 02/09/2022 **Article Revised on 29/09/2022** **Article Accepted on 19/10/2022**

ABSTRACT

The aim of the study was to determine the semen quality of cross-bred Yorkshire boars fed with green bean sprouts. Twenty crossbred Yorkshire boars were divided into two groups, namely the first group which received feed without green bean sprouts (NGBS) and the second group which received rations containing green bean sprouts (GBS). Semen from the two groups of boars was then collected and observed for quality in time intervals: 0 hours; 12 hours; 24 hours; 48 hours; 60 hours; and 72 hours after semen collection. Each observation was repeated three times. The results showed that the group of boars that received feed containing GBS had semen with a volume, color, consistency, pH, mass movement, motility, and viability of spermatozoa that were not significantly different ($P \geq 0.05$) higher than the semen of NGBS boars. However, the concentration of spermatozoa in the fresh semen of the GBS boar group was significantly different ($P \leq 0.05$) higher than that of the NGBS boar group. The semen of the GBS boar group after dilution, had significantly higher motility and viability ($P \leq 0.05$) compared to the semen of the NGBS boar. It was concluded that the provision of green bean sprouts could improve the semen quality of crossbred Yorkshire boars.

KEYWORDS:Crossed Yorkshire boar, green bean sprouts, semen, motility.

INTRODUCTION

Pigs are classified as monogastric livestock which have the ability to convert feed ingredients efficiently if supported by feed quality that is in accordance with the needs of pigs. Pigs have prolific traits which are indicated by the ability to have many children in each birth, which ranges from 8-14 tails, and can give birth twice a year.[1] Based on data from the Directorate General of Livestock and Animal Health[2] in 2021, the national pig population reached 8,011,776 heads. This number increased by 389,052 heads or 5.1% compared to the previous year which reached 7,622,724 heads. In 2021, the province of Bali occupies the sixth position with a pig population of 409,960 heads.

Pigs developed in Indonesia come from the Landrace, Duroc, Yorkshire, Hampshire, and Berckshire breeds. Meanwhile, the largest breed of pigs developed in Indonesia is Yorkshire, which amounts to around 125,000 heads.[3] Yorkshire pork originates from England, and is a high-quality bacon-type pork. In terms of cement quality, according to the research results of[4] that the quality of Yorkshire pig liquid semen with M-Zorlesco diluent can maintain the quality of spermatozoa stored in Styrofoam boxes and refrigerators, for 42 hours with a motility percentage reaching 40-50%. However, studies using Semen Life diluents have not been reported.

In maintaining and improving the quality of cement boar, feed additives are needed which are easily available and contain sufficient vitamin E. Green bean sprouts (*Vigna radiata*) is one of the feeds that contain high enough protein and rich in vitamin E which serves to increase fertility. According to[5] that the provision of green bean sprouts as a substitute for grass can increase the productivity of fresh semen spermatozoa in ruminants. There has been no research related to the use of green bean sprouts in feed on the quality and quantity of semen on boars, so this research is interesting to do. The aim of the study was to determine the quality and quantity of cross-bred Yorkshire boar semen fed with green bean (*Vigna radiata*) sprouts.

MATERIAL AND METHODS

Animal treatments and experimental design.
This research was conducted at UD. AGET MUPU on Jalan Wisnu Desa Baru, Marga District, Tabanan

Regency, Bali Province. Twenty crossbred Yorkshire boars were divided into two groups, namely the first group which received feed without green bean sprouts (NGBS) and the second group which received rations containing green bean sprouts (GBS). Semen from the two groups of boars was then collected and observed for quality in time intervals: 0 hours; 12 hours; 24 hours; 48 hours; 60 hours; and 72 hours after semen collection. Each observation was repeated three times. The pigs used as a source of semen in this study were 20 sex adult boars, from crossbred Yorkshire, aged 3 years, in good health, and had good semen quality, ie spermatozoa concentration was more than $150x10^6$ cells/ml and spermatozoa motility was more than 60%. The crossbreed Yorkshire boar semen was collected twice a week. Cement collection was carried out in the morning, twice a week, using the manual method (glove hand method) assisted by storage tube equipment. The separation of the gelatin fraction was carried out by covering the gauze at the mouth of the tube. The cement diluent used in this study was Semen Life. The staining material used was 2% eosin stain. The use of 2% eosin stain was used to observe the viability of the Yorkshire boar crosses spermatozoa.

Boars were kept in cages with concrete floors, cage walls made of woven iron measuring: 2.2m long; 1.1 m wide; and 0.6 m high. Each boar cage was equipped with a feeder and an automatic water nipple. The feed given to the boar contains 18% crude protein and 3150 kcal/kg metabolized energy derived from the complete Master 1031 feed with a total feeding of 4 kg/head/day, with an additional 1% GBS.

Observation of cement quality

The volume of fresh semen was checked from the cement boar that was accommodated using a scaled container tube, while the color of fresh semen was assessed by looking directly at the pig semen that has been accommodated in the reservoir tube (in general the color of cement is cloudy white, milky white, cream, yellowish cream, to grayish white). Consistency of cement: the tube containing the cement boar was shaken slowly and the level of viscosity was seen, with three assessment criteria, namely: watery, medium, and thick. Acidity (pH): to determine the acidity level of cement boar was done by using litmus paper. The boar cement was taken using a dropper, then the cement was dripped onto litmus paper, then the resulting color was matched with the color change in the existing standard. Spermatozoa mass movement: examined using a light microscope with a magnification of 10x10, with the assessment being: very good (+++); good (++); enough (+); and less (-).

Spermatozoa concentration: presented in units of 10^6 cells/ml and the calculation of spermatozoa using the hemocytometer method.[6] Percentage of spermatozoa motility: using a glass object covered with a cover glass and observed using a light microscope with a magnification of 10x40. Percentage of motility can be assessed subjectively by comparing motile spermatozoa that move forward (progressive) with non-progressive (linear). The rating given was from 0% (not motile) to 100% (all motile). Viability of spermatozoa: viability percentage was calculated using 2% eosin dye. Examination was carried out under a microscope. Spermatozoa were counted at least 200 cells from 10 fields of view. Live spermatozoa will not absorb color (transparent), on the contrary, dead ones will absorb red color on the head of spermatozoa.

The data obtained were analyzed with SPSS version 26 in 2020 and if there was a significant difference ($P \leq 0.05$) followed by Duncan's test.

RESULTS

The results of the evaluation on fresh semen, is an initial inspection of cement which is used as the basis for determining the feasibility of cement to be further processed. Cement examination was carried out in the laboratory at room temperature of 20-22°C. The results showed that the semen obtained from 8 storages had a fairly good quality, was voluminous with spermatozoa motility above 60% and spermatozoa concentration above $150x10^6$ cells/ml. The average yield of 8 times the semen storage on crossbred Yorkshire boars is presented in Table 1 and the semen quality of cross-bred Yorkshire boars after dilution is presented in Table 2.

Table 1: Characteristics of fresh semen from crossbred Yorkshire boars.

Characteristics of cement	Avarage value		Standart
	NGBS	GBS	
Volume (ml)	318.75±14.32	323.75±16.90	150-200*
Colour	Milky white	Milky white	Milky white **
Consistency	watery	watery	watery **
Mass movement	(+++)	(+++)	(+++)**
Degree of acidity (pH)	7.54±0.42	7.63±0.59	7.3-7.8*
Spermatozoa concentration (10^6 cell/ml)	351±6.91[a]	378±8.29[b]	200-300*
Spermatozoa motility (%)	70±0.01	70±0.01	50-80*
Spermatozoa viability (%)	78.55±2.15	79.93±2.69	70-90*

Note: ([a],[b]) Different letters were significantly different ($P \leq 0.05$); (*) Standart[7]; Ax *et al.*[8]; (**) Standar[9]

The average volume of semen per ejaculate obtained during the study was 318.75±14.32 (NGBS) and 323.75±16.90 (GBS) which were statistically not significantly different (P≥0.05). The results of this study are higher than the results of research by[10], namely the volume of cement boars ranged from 200-250 ml, and 240-250 ml[11], and the volume of cement boars ranged from 100-300 ml.[12]

Table 2. Semen quality of cross-bred Yorkshire boars after dilution.

Cemen quality	Avarage value	
	NGBS	GBS
Spermatozoa concentration (10^6 cell/ml)	282±3.13	289±3.63
Spermatozoa motility (%)	70±0.01	70±0.01
Spermatozoa viability (%)	78.01±2.42	78.87±2.60

The color of cement is closely related to the concentration and consistency (thickness) of the cement. The higher the concentration of spermatozoa, the higher the consistency and color density of the semen. Normal boar semen is watery in consistency and milky white in color, due to the presence of riboflavin, a secretion of the vesicular gland. The average color of the cement obtained in this study, both the cement produced by the NGBS group and GBS group boars, which was milky white with a watery consistency, and the mass movement (+++) were statistically not significantly different (P≥0.05). This is in accordance with what was reported by[10] that the color and consistency of the cement in the boar depended on the fraction accommodated, namely the prespermatozoa fraction was watery with a white-gray color, and the spermatozoa-rich fraction was like non-condensed milk. milky-nonviscous) with creamy white color.

Physiological values of acidity (pH) of fresh semen obtained during the study were in the range: 6.5-8.0 with averages: 7.54±0.42 (NGBS) and 7.63±0.59 (GBS) statistically showed no significant difference (P≥0.05). This result is in line with the results of[13] study, namely the average pH of cement boars: 7.4±0.2 and in line with the results of[7] study, namely: 7.3-7.8.

Spermatozoa concentration is very important to determine the quality of spermatozoa. Concentration, volume and percentage of motility of spermatozoa can describe the degree of dilution. The spermatozoa concentrations obtained in this study were: 351±6.91x10^6 cells/ml (NGBS) and 378±8.29x10^6 cells/ml (GBS) which were statistically significantly different (P≤0.05). The concentration of spermatozoa obtained in this study was higher than the normal concentration according to[7,10], which ranged between: 200-300x10^6 cells/ml.

Progressive spermatozoa motility is the movement of spermatozoa that move forward. The motility of the fresh boar spermatozoa in this study was 70% the same as the motility of the spermatozoa in the GBS boar group, which was 70% statistically not significantly different (P≥0.05). In line with the research of[7] that the motility of spermatozoa ranged between: 50-80%. The results of microscopic examination showed that the viability of spermatozoa, namely 78.55% ± 2.15 (NGBS) and 79.93% ± 2.69 (GBS) were statistically not significantly different (P≥0.05). This result is not much different from the opinion of[7] which shows that the viability of spermatozoa ranges from: 70-90%.

The cement used in this study came from twenty boars which were divided into two groups NGBS and GBS. All treatments showed the same pattern of decrease in the percentage of motility and the percentage of live spermatozoa in semen, both in the semen produced by the NGBS and GBS boars with observation time every 12 hours (Table 3).

Table 3: Average percentage of motility and viability in spermatozoa from crossbred Yorkshire boars at different observation times.

Variables	Obervations (hours)	Treatments		SEM
		NBGS	BGS	
Spermatozoa motility (%)	0	70.000^{Aa1}	70.000^{Aa}	0.509
	12	65.000^{Ba}	65.000^{Ba}	
	24	65.000^{Ba}	65.000^{Ba}	
	36	60.625^{Ca}	63.125^{Bb}	
	48	56.875^{Da}	60.000^{Cb}	
	60	51.875^{Ea}	58.125^{Cb}	
	72	46.875^{Fa}	53.750^{Db}	
Spermatozoa viability (%)	0	78.057^{Aa}	78.786^{Aa}	0.775
	12	75.344^{Aba}	75.988^{Aba}	
	24	73.179^{Ba}	74.651^{Ba}	
	36	69.936^{Ca}	72.056^{BCa}	

	48	67.774^{Ca}	69.946^{Ca}	
	60	63.914^{Da}	67.687^{CDb}	
	72	61.162^{Da}	64.657^{Db}	

Notes

[1] Values with different letters in one row (lowercase) and in one column (capital letters) were significantly different (P≤0.05).

The results showed that the cement produced by NGBS and GBS boars showed significant differences (P≥0.05) at 36 hours of storage with the percentage of motility of: 60.625%±0.509 and 63.125%±0.509. After being stored for 36 to 72 hours, there was no significant difference (P≥0.05) in the percentage of motility between the cement produced by NBGS and GBS group boars. The percentage of live spermatozoa (viability) in semen also decreased after 12 hours of storage.

DISCUSSION

Boar semen is voluminous, has a large volume of ejaculate, but has a low concentration of spermatozoa. The large volume was due to the ejaculated semen consisting of several fractions, namely pre-spermatozoa, sperm-rich, and post-spermatozoa. The pre-spermatozoa fraction did not contain spermatozoa, only in the form of gelatin from the bulbourethral glands (Cowper's glands) which accounted for 20% of the total semen volume. The sperm-rich fraction contained 20-30% spermatozoa at a concentration of 600 - 1000 x 106 cells/ml[11], and the post-spermatozoa fraction contained fluid from other accessory glands, namely the prostate gland and vesicular gland. According to[14] the factors that affect the volume of semen when accommodated are variations in age, level of stimulation, frequency of ejaculation, and quality of feed.

The production of spermatozoa is a continuous process and is not affected by the frequency of ejaculation, but the frequency of ejaculation that is too frequent in a relatively short time tends to decrease libido, semen volume and sperm concentration per ejaculate. According to[15] semen production in male animals after reaching the optimum point will decrease with increasing age.

The color of the semen obtained in this study was generally milky white with a watery consistency. This is in accordance with what was reported by[10] that the color and consistency of the cement boar depends on the fraction accommodated, namely the pre-spermatozoa fraction is watery with a gray white color, and the sperm-rich fraction is like non-condensed milk. milky-nonviscous) with creamy white color.

The consistency of the fresh semen obtained was runny, with very good mass movement (+++). The results obtained are in accordance with the opinion of[9] which states that pig semen has a milky white color and the consistency of the semen is watery. Semen color is closely related to the consistency and concentration of spermatozoa. Solid colored semen shows consistency and a high concentration of spermatozoa.[16]

The physiological value of the acidity (pH) of fresh semen obtained during the study was in the range of 6.5-8.0. This result is in line with the results of[13] study, namely the average pH of pig semen is 7.4±0.2; pH 7.3-7.8[7] and pH 7.2-7.5.[14] If there is a decrease in pH, the metabolism and motility of spermatozoa will also decrease.[13] Johnson et al. [14] added that a pH below 7.2 will decrease the motility of spermatozoa. Differences in physiological pH values can be caused by differences in race, environment, and buffer differences.[17] This is the basis for making diluent solutions because the pH of the solution can affect the viability of spermatozoa.

The factors that influence the difference in sperm motility are pH, temperature and feed quality.[13,14,18] Spermatozoa motility will decrease gradually in fresh boar semen with a pH below 7.2.[14] According to[19] heat stress can affect cement quality. Boars exposed to 34.5^{0}C for 8 hours had lower spermatozoa motility than boars exposed to 23^{0}C. Decreased motility of spermatozoa is also caused by selenium deficiency in feed.

Spermatozoa motility correlates with the viability or viability of spermatozoa. The number of live spermatozoa is always higher than motile spermatozoa, because not all live spermatozoa move progressively.[20] The viability of spermatozoa was observed by looking at the color of the head of the spermatozoa. The live spermatozoa appear transparent, while the dead spermatozoa appear red (Fig. 1).

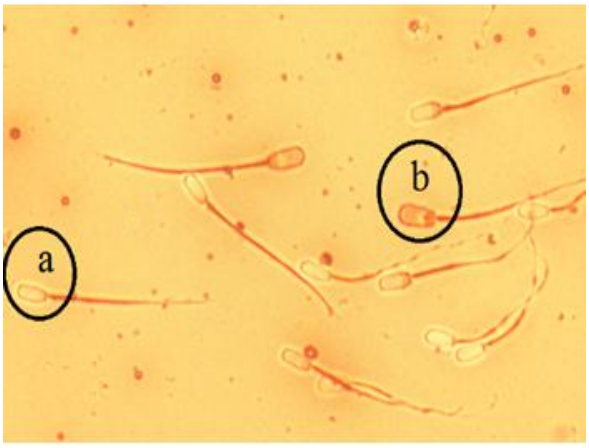

Figure 1: Staining of spermatozoa using 2% eosin: live spermatozoa (a) and dead spermatozoa (b).

Based on the results of the study, it can be seen that storage time can affect the decrease in motility of

spermatozoa. During storage, spermatozoa use carbohydrates obtained from semen plasma or diluents to be converted into energy through the process of glycolysis. The longer storage time will cause the accumulation of lactic acid, resulting in a decrease in the pH of the semen and inhibiting the motility of spermatozoa.[13] The percentage of spermatozoa viability decreases along with the decrease in spermatozoa motility.[16] The cause of cell death is due to DNA (Deoxyribo Nucleic Acid) being damaged due to the influence of free radicals. DNA damage can occur, due to oxidative stress which causes the formation of free radicals to increase, and has an impact on the occurrence of apoptosis.[21]

CONCLUSION

It was concluded that the provision of green bean sprouts could improve the quality of semen on cross-bred Yorkshire boars and was able to maintain the quality of cement stored for up to 72 hours.

ACKNOWLEDGEMENTS

The author would like to thank the director of UD. Aget Mupu for the boar and cage facilities, and the head of the Reproduction Laboratory, Faculty of Animal Husbandry, for the laboratory facilities.

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