Fresh, Chilled and Post-thawed Semen Characteristics of Dorper Rams Fed with Different Concentrations of Protein and Selenium

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Received: 09 May 2022
Accepted: 08 August 2022

Abstract

This study aimed to determine the effects of different levels of protein and selenium (Se) supplementation on semen characteristics of fresh, chilled and post-thawed Dorper rams. Seventeen rams between 15 to16 months of age were used and fed on either 180 g of protein (P1) or 270 g of protein (P2); supplemented without Se (S0) or with1mg/kg of body weight; of Selemax 2000 (S1). Semen was collected using an artificial vagina and cryopreserved using a Tris-egg yolk extender. All fresh, chilled and post-thawed semen samples have been evaluated for their quality. The wave motion was observed under a phase contrast microscope and sperm motility was analysed using a CEROS sperm analyser. Eosin-nigrosin stain was used to determine sperm viability and abnormalities, while Pope's stain was to determine sperm acrosome intact. The HOST solution was to determine sperm membrane integrity. The results showed wave motion score of fresh semen in P1 was higher ($P<0.05$) than in P2. Meanwhile, S1 showed an increment ($P<0.05$) in wave motion score, total motility and acrosome integrity of fresh semen compared to S0. However, there was no significant effect on the quality of chilled semen fed with different CP and Se. Nonetheless, S1 did show lower ($P>0.05$) head abnormalities in post-thawed semen than S0. It was concluded that Dorper ram fed with 180g per kg/DM of protein is recommended in maintaining reproductive performances while organic Se supplementation (1 mg/kg Se) did improve head damages in fresh and post-thawed Dorper ram semen.

Keywords: Fresh, chilled and post-thawed semen, ram, protein, selenium.

Introduction

Dorper sheep were imported into this country in 2008 because of their potential for high meat production and adaptability in various climates, this breed may help increase the meat and livestock industry (Nurulhuda *et al*., 2014). The adaptability and growth performances of imported Dorper sheep had been studied by others (Julie Marzlinda *et al*., 2012). However, there is still a lack of information on the reproductive performances

of Dorper ram under Malaysian climate as well as the effects fed with local feed.

Cryopreserved semen in sheep is more difficult compared to other farm animals (Banday *et al*., 2017). Fresh and chilled semen is commonly used during the artificial insemination of sheep due to increasing sperm survival in the ewe reproductive tract (Lima *et al*., 2010). However, during the cooling process, semen has the potential to expose to oxidative damage, which is associated with a decrease in sperm motility and fertility and a further decrease in the frozen semen (Kheradmand *et al*., 2006).

Nutrients play the main function in the development of male reproduction. Studies have shown that protein influences growth and reproductive performances in male animals (Rios-Rincon *et al*., 2014) and ram fed with high protein increases semen production, quality and testosterone concentration (Fernandez *et al*., 2004). Organic Se supplement in diets will enhance reproductive performances (Ali *et al*., 2009) and at 0.01mg/kg DM of Se can increase the volume, semen concentration and sperm viability of ram (Marai *et al*., 2009). Understanding the effects of different levels of protein intake and Se as a supplement may optimize Dorper ram reproductive performances. Therefore, this study aims to determine the effects of different protein levels and Se supplementation on semen characteristics of fresh, chilled and post-thawed Dorper rams.

Materials and Methods

Animal Housing

Nineteen Dorper ram were aged between 15 to 16 months with an average live weight of 50.8 ± 2.19 kg selected and trained for semen collection. During the early feeding trial, two rams had to remove from this trial due to injury and health issues. Therefore, seventeen Dorper ram were used in this study and was randomly divided into four groups and allocated into individual pens (1.2 m x 1.5 m). Clean water was available ad libitum. This study was conducted at Ladang 2, MARDI Kluang, Johore (GPS coordinates: 1°57'26.1"N 103°21'35.0" E).

Experimental animals and dietary treatments

Rams were fed with a diet consist either two different levels of CP and with or without organic Se in a 2x2 factorial design. Diets given were formulated according to the protein requirement for maintenance of ram (P1: 180 g per kg/DM) or 20% above CP requirement (P2: 270 g per kg/DM) with energy maintained at 12.5 MJ ME/kg DM as shown in Table 1 (NRC, 1981). Rams were fed based on 3% of body weight and dietary treatments contained a 50% formulated diet and 50% rice straw as a fibre source. Calculated chemical compositions (% DM basis) of dietary treatments are shown in Table 1. Both CP groups were further divided into two groups, either not supplemented with Se (S0) or supplemented with Se (S1). Rams in the S1 group were given 1 mg/kg of body weight of organic Se-enriched yeast (Selemax 2000, Biorigin, Brazil) and S0 as the negative control. Feed was given as two equal meals at approximately 09:00 and 16:00 hr daily and a feeding trial was carried out for 140 days.

Semen collection

To evaluate the effects of the feeding trial, semen was evaluated after the eighth week because the spermatogenesis in ram takes 49 days. Semen evaluation was done once a week for four weeks. Semen was collected using an artificial vagina (AV) and a non-oestrogenised ewe was used as a teaser. Semen was evaluated at the National Animal Embryo Centre Laboratory (NAEC), MARDI Kluang, Johore for wave motion, sperm

motility, viability, normality, abnormalities, membrane integrity and acrosome integrity

Table 1. The percentage of feed ingredients in formulated diets for 180 g of protein (P1) and 270 g of protein (P2) content and chemical composition of dietary treatments

Feed ingredients (%)	P1	P2
Soybean meal	41.23	66.11
Rice bran	8.50	8.07
Broken rice	37.76	13.94
Molasses	1.88	1.78
Ground corn	8.48	8.05
Crude Palm Oil	1.54	1.46
Monocalcium phosphate	0.16	0.15
Salt	0.15	0.15
Premix vitamin*	0.15	0.15
Calcium carbonate	0.15	0.15
Total	100	100
Calculated chemical composition (DM basis) of dietary treatments		
Crude protein (g)	180.00	263.41
Energy (MJ ME/kg)	12.50	12.50

P1: Low crude protein (12% CP); P2: High crude protein (18% CP); DMI: Dry matter intake*Vit A: 50000i.u, Vit D3: 8000i.u,Vit E: 8mg, Manganese: 1400mg, Ferrous: 300mg, Copper: 50mg, Cobalt: 10mg, Iodate: 20mg, Phosphorus: 1000mg, Salt 5500mg, Calcium: 1300mg, other contents: Formic acid, Citric acid, Malic acid, Tartaric acid, Lactic acid and Orthophosphoric acid.

Determination of wave motion and sperm motility

Immediately after ejaculation, approximately 10 µl of fresh semen was observed under a phase-contrast microscope (Euromax, Holland) for wave motion and was scaled ranging from zero to five. Meanwhile, a 10 µl semen sample was diluted with 2-3 drops of 0.9% sodium chloride (NaCl) intravenous infusion solution (B. Braun, Malaysia) and approximately10 µl of diluted semen was placed on a slide covered with a glass coverslip (22 mm x 22 mm). Semen samples were observed and evaluated under a phase-contrast microscope (CX41, Olympus, Japan) at 600x magnification and sperm motility (total and progressive) was analysed using a sperm analysis system (Hamilton Thorne–CEROS: Version 12, USA). The settings for motility analysis are shown in Table 2.

Table 2. Settings of Hamilton-Thorne Ceros Version 12 for sperm analysis

Parameters	Values
Frame rate (Hz)	60
Number of frames	45
Minimum contrast	60
Minimum cell size (pixels)	5
Cell size (pixels)	5
Cell intensity	55
Path Velocity (VAP) (µm/s)	75
Straightness (STR) (%)	80
Static VAP cut-off (µm/s)	21.9
Static VSL cut-off (µm/s)	6

Source from Hamilton-Thorne Ceros Version 12 Manual

Determination of sperm viability and abnormality

Approximately 10 µl of semen sample was used to stain with a drop of eosin-nigrosin stain (1 g of Eosin Y Disodium salt (E6003, Sigma-Aldrich, USA) and 5 g of Nigrosin water-soluble (N4754, Sigma-Aldrich, USA) in 100 ml of 3% Sodium Citrate Tribasic Dihydrate (S4641, Sigma, USA). Then, the sample mixtures were smeared on a glass slide and the percentage of sperm viability and abnormalities were evaluated. A total of 100 spermatozoa have been observed under a phase-contrast microscope (CX31, Olympus, Japan) at 400x magnification. The percentage of sperm viability was carried out by observing the viable sperm (sperm head with

no stain or white colour) and nonviable (with red stain) while sperm abnormality was determined by observing normal and abnormal sperm (head, midpiece, and tail of sperm) (Speight *et al*., 2012).

Determination of sperm membrane integrity

Hypo-osmotic swelling test (HOST) was used to determine the membrane integrity of sperms. The hypo-osmotic solution consists of 0.735 g of Sodium Citrate Tribasic Dihydrate (S4641, Sigma, USA) and 1.351 g of D-(-)-Fructose (F3510, Sigma, USA), in 100 ml distilled water. Approximately 20µl of semen sample was placed in a 0.5 ml microtube (Eppendorf®, Germany) containing 100µl hypo-osmotic solution and incubated for 60 min at 37°C. After incubation, 10µl of the mixture was smeared on a glass slide and a total of 100 spermatozoa were observed under a phase-contrast microscope with 400x magnification (CX31, Olympus, Japan). The percentage of membrane integrity was carried out by identifying the unswollen and swollen tail of the spermatozoa (Qiu *et al.,* 2011).

Determination of sperm acrosome integrity

Approximately 10 µl of semen sample was stained using Pope's stain (1 g Rose Bengal sodium (R4507, Sigma-Aldrich, USA), 1 g Fast green FCF (F7252, Sigma-Aldrich, USA) in a 100 ml of buffer solution (60 ml Citrate Phosphate buffer and 40 ml absolute methanol)). The sample mixture was spread on a glass slide and 100 spermatozoa were observed under a phase-contrast microscope (CX31, Olympus, Japan) at 400x magnification. To determine the percentage of acrosome integrity, sperm head with blue stained has an intact acrosome or normal acrosomal, while abnormal acrosome had patchy staining, white or pink cap: indicate membrane damage, no blue staining or clear sperm head showed loose membrane or missing acrosomes (Spindler *et al*., 2004).

Semen freezing

Tris-egg yolk extender was prepared by using 37.86g Trizma Base (T1503, Sigma, USA), 21.15 g Citric Acid (C1909, Sigma, USA), 0.5 g Streptomycin Sulfate (S6501, Sigma, USA) in 1 L distilled water. The egg yolk was added with 1:4 ratios and the solution was centrifuged at 3000 rpm for 10 min (Kubota 2100, Japan). Later, 1% of D-(-)-Fructose (F3510, Sigma, USA) and 6.8% Glycerol (G5516, Sigma, USA) of total supernatant solution were added.

Fresh semen from three rams of each group with 70% motility or more will proceed with the freezing process and be repeated three times (Memon *et al*., 2012). Fresh semen samples collected were diluted using a Tris-egg yolk extender into a final concentration of 100x106 sperm/ml and placed in a cold room at 4°C for 4 hr. After equilibration, chilled semen was evaluated (sperm motion, viability, abnormality and membrane integrity). Samples were aliquot into labelled 0.25 ml straws (IMV, France) and sealed straws were placed on a straw rack. Then, straws were placed 4 cm above the surface of the liquid nitrogen in a polystyrene box to expose to liquid nitrogen vapours (-120°C) for 3 min. Finally, straws were plunged into liquid nitrogen (-196°C) for storage. Post-thawed semen was evaluated after being thawed in a water bath at 37°C for 3 sec.

Statistical Analysis

A completely randomised design (CRD) was used in this study. The normality of data was analysed using PROC UNIVARIATE of Statistical Analysis System (SAS) programme version 9.3 (SAS Institute Inc., Cary, NC, USA). All variables were transformed accordingly using logarithmically transformed (wave motion) and arcsine transformation (total and progressive motility, sperm viability, abnormalities, membrane and acrosome integrity of semen). Data were

analysed using PROC GLM of Statistical Analysis System (SAS) programme version 9.3 (SAS Institute Inc., Cary, NC, USA) and Duncan's Multiple Range Test (DMRT) was used to compare means at the significance level of 0.05. Variables with no interaction between CP and Se were presented according to the main factors (CP and Se).

Results and Discussion

Characteristics of fresh semen

This study showed no interaction (P>0.05) between the effect of different CP levels and Se supplementation on all parameters of fresh semen studied (Table 3). The result showed that the ram fed with P1 was significantly higher (P<0.05) for wave motion compared to P2. Nonetheless, both CP levels did not influence the sperm motility, viability and morphology of fresh semen. However, P1 of fresh semen did tend to have a higher value on sperm motility, viability and normality and a lower value of sperm abnormalities than P2.

Meanwhile, results of sperm membrane and acrosome integrity tended to have higher values on P2 assessed to P1. Ram fed with Se supplement (S1) did show a significant increment (P>0.05) in wave motion, total motility and acrosome integrity compared to S0. However, there were no significant differences (P>0.05) in sperm progress motility, viability, normality, abnormalities and membrane integrity. In addition, results tended to show higher values in progress motility, sperm viability and normality did show while lower values in abnormalities in S1 compared to S0.

In this study, both CP and Se significantly influenced (P<0.05) wave motion scores where P1 showed 11.68% higher than P2 and S1 was 11.68% higher than S0. Similarly, Al-Haboby *et al*. (1999) reported that Awassi ram fed with lower daily CP showed a significant increment in wave motions compared to the higher CP given. In contrast with findings by Piagentini *et al*. (2017) where different levels of Se (0, 5, 10 and 15 mg/kg Se) did not influence wave motions of ram semen. Meanwhile, sperm motility of fresh semen was not significantly affected by different levels of CP (Table 3) and this was similar to findings by Jibril *et al*. (2011) and Elmaz *et al*. (2007). Nonetheless, P1 did show the tendency to have a higher value in total and progressive motility than S0 (Table 3) which is parallel with findings by Elmaz *et al*. (2007) where feeding protein higher than the requirement showed negative effects on sperm motility. However, the results showed total motility in S1 was significantly higher (P<0.05) compared to S0 while Se did not influence sperm progress motility (Table 3). This is in contrast to Piagentini *et al*. (2017) reports, which showed that different levels of Se (0, 5, 10 and 15 mg/kg) did not affect the total motility of rams. Therefore, better sperm motions will increase the chances of fertilization in the female reproductive tract (Del Olmo *et al*., 2013).

Although there were no effects (P>0.05) on viability when fed with different CP and Se, sperm viability produced was more than 58% which is an acceptable value for live sperm viability (WHO, 2010).

Nonetheless, rams fed with P1 tended to have a 4.64% higher value in viability compared to P2 while rams in S1 had a 5.91% higher value than those in S0 (Table 3). These results were similar to Jibril *et al*. (2011) findings, where sperm viability values in low (12%) and medium (15%) CP levels were 0.48% and 3.26% higher than rams fed with high CP (18%). This may occur due to excess levels of protein in feed and causing excess urea consumption that could lead to negative effects on semen quality (Jibril *et al*., 2011; Fernandez *et al*., 2005; Kaur and Arora, 1995). Meanwhile, results for rams fed with

Se supplement (0.01 mg/kg DM) contradicted Marai *et al*. (2009) findings, where Se supplementation did significantly increase sperm viability (86.5%) as compared to the control (80.17%).

Table 3. Effects of different levels of protein and selenium supplementation on wave motion, sperm motility, viability and morphology (mean±SEM) of Dorper ram fresh semen

Diets	P1	P2	S0	S1	*P* value[1]		
					P	S	P*S
Wave motion (Score 1-5)	4.59 ±0.13^{a}	4.11 ±0.23^{b}	4.00 ±0.25^{y}	4.64 ±0.12^{x}	0.03	0.01	0.31
Total Motility (%)	75.50 ±2.93	72.06 ±3.06	68.25 ±3.22^{y}	78.50 ±2.59^{x}	0.31	<0.01	0.23
Progressive Motility (%)	49.13 ±2.48	44.86 ±3.03	44.81 ±2.98	48.69 ±2.66	0.25	0.28	0.85
Sperm viability (%)	71.45 ±2.23	68.28 ±2.00	67.66 ±2.42	71.66 ±1.77	0.24	0.14	0.13
Sperm morphology							
Normality (%)	66.53 ±2.58	57.72 ±3.55	58.78 ±3.81	64.61 ±2.65	0.05	0.18	0.77
Head abnormalities (%)	7.34 ±2.59	11.39 ± 3.55	11.06 ±3.81	8.08 ±2.65	0.05	0.14	0.18
Midpiece abnormalities (%)	11.59 ±1.56	12.17 ±1.61	11.66 ±1.96	12.11 ±1.26	0.84	0.90	0.15
Tail abnormalities (%)	14.53 ±1.17	18.72 ±1.28	18.50 ±1.14	15.19 ±1.29	0.40	0.58	0.34
Membrane integrity (%)	50.83 ±4.07	52.59 ±3.59	53.42 ±4.16	50.27 ±3.46	0.59	0.61	0.06
Acrosome integrity (%)	88.94 ±1.98	90.11 ±1.75	85.59 ±2.17^{y}	93.20 ±1.26^{x}	0.93	<0.01	0.30

$^{ab\ xy}$Means with different superscripts within the same row (main effect) significantly differ at $p< 0.05$. [1] P, S, P*S: Effect of protein, selenium and its interaction, respectively, N: number of samples, SEM: Standard error of means, P1: 180 g/kg DM; P2: 270 g/kg DM; S0: without Se supplementation; S1: with Se supplementation (1 mg/kg of Bwt).

Other studies showed that sperm morphology characteristics were associated with male fertility (Ghorbani *et al*. 2018). This study showed CP level did not significantly influence (P>0.05) sperm morphology of fresh semen and is in agreement with Fernandez *et al*. (2005). On the contrary, Jibril *et al*. (2011) and Al-Haboby *et al*. (1999) showed abnormalities of the head, midpiece and tail sperm were significantly affected by feeding different levels of CP. The Dorper rams fed with Se did not affect (P>0.05) sperm morphology of fresh semen except in the S1 group in this study. The sperm acrosome integrity in the S1 is significantly higher (P<0.05) than S0. The results were supported by Marai *et al*. (2009), the acrosome damage of sperm from Egyptian Suffolk rams fed with Se showed significantly decreased. Se supplement fed to Dorper rams did not alter sperm abnormality and the membrane integrity and it was also reported in Sanjabi

rams (Ghorbani *et al.*, 2018). The sperm normality tends to be higher for P1 and S1 (6.63% and 5.83%, respectively) compared with P2 and S0 (4.19% and 3.31% respectively) (Table 3). The lower abnormalities of the sperm head (27%) and tail (18%) were observed in S1 compared to S0. These increments of Se dosage could probably reduce sperm abnormalities as reported by Shi *et al*. (2010), the increment of Se (0, 0.5, 1.0 and 2.0 mg/kg) linearly decreases sperm abnormalities.

However, both CP and Se supplements given in feed showed 38 % of total sperm abnormalities and can be considered low fertility ram (more than 15% sperm abnormalities) (Ramakrishnan, 2005). These high values of sperm abnormalities occur may be due to untreated rice straw fed to ram, which causes changes in the semen pH (El-Azab *et al*., 1998).

Characteristics of chilled semen

Results in Table 4 showed that chilled semen had no interactions on all parameters of semen characteristics between different CP levels and Se supplementation. Meanwhile, different levels of CP and Se as the main factors did not show any significant effects in all parameters of chilled semen at 4°C. However, semen of rams fed with P1 did tend to have higher values in sperm motilities and membrane integrity compared to P2. The semen of rams fed with Se (S1) tends to show a higher value in total and progressive motility, sperm viability and membrane integrity while a lower value on head and midpiece abnormalities compared to S0. Fresh and chilled ram semen was often used as an alternative for AI to increase the survival of sperm in the female reproductive tract (Lima *et al*., 2010). It is because sheep have low chances of successful fertilization in artificial insemination due to the difficulties in transferring semen into the cervix of the ewe (Lima *et al*., 2010). Paulenz *et al*. (2002) compared the usage of fresh semen, the motility and morphological integrity of cooled ram semen usually decrease and are accompanied by a failure to survive in the reproductive tract when this reduces fertility. Furthermore, there is still a lack of findings regarding chilled ram semen and the effects on semen quality fed with different levels of CP or Se supplementation.

In this study, acrosome integrity of chilled and post-thawed semen could not be observed due to agglutination in staining, and a similar situation was reported by Zambelli and Cunto (2006). Agglutination occurs may be due to the presence of egg yolk in the extender. Results in P1 tended to show a higher value on total and progressive motility, sperm normality and a lower value in midpiece and tail abnormalities compared to higher CP requirements (P2). A similar pattern was observed in fresh semen (Table 3). Other studies agreed that higher protein levels in diets have negative effects on the quality of semen (Jibril *et al.*, 2011; Fernandez *et al.*, 2005). In this study, the total motility of chilled semen was within range (70-80%) compared to other studies (Yotoz *et al*., 2019; Paulenz *et al*., 2002). Meanwhile, Dorper rams fed with S1 showed higher values of 0.97%, 6.15% and 4.92%, respectively, on the percentage of viability, normality and membrane integrity compared to S0 (Table 4). This showed similar patterns to Shi *et al*. (2010) where semen characteristics of bucks had higher values when fed with Se-enriched yeast (0.5-2.0 mg/kg) compared to the control group (0.043mg/kg). Despite no increments in the quality of Dorper rams chilled semen, this study showed higher values compared to findings by Memon *et al*. (2011) on bucks (sperm motility: 68.7%, membrane integrity: 57.2%) and O'Hara *et al*. (2010) on rams with sperm viability was a range between 55.7 % to 68.0 %.

Table 4. Effects of different levels of protein and selenium supplementation on sperm motility, viability and morphology (mean±SEM) of Dorper rams chilled semen at 4°C

Diets	P1	P2	S0	S1	*P* value[1]		
					P	S	P*S
Total Motility (%)	81.50 ±2.34	79.12 ±2.13	77.00 ±2.83	83.17 ±1.58	0.61	0.05	0.19
Progressive Motility (%)	16.36 ±1.25	15.80 ±1.37	15.00 ±1.36	17.00 ±1.23	0.54	0.26	0.89
Sperm viability (%)	55.40 ±2.58	57.61 ±2.65	55.96 ±2.94	56.93 ±2.31	0.60	0.66	0.71
Sperm morphology							
Normality (%)	29.69 ±2.54	27.14 ±2.89	28.89 ±3.18	28.25 ±2.28	0.23	0.73	0.37
Head abnormalities (%)	10.69 ±2.01	9.46 ±1.58	12.39 ±2.30	8.13 ±1.30	0.70	0.10	0.47
Midpiece abnormalities (%)	18.13 ±1.32	18.32 ±1.75	19.43 ±1.63	17.16 ±1.40	0.91	0.32	0.62
Tail abnormalities (%)	41.50 ±2.70	44.43 ±3.30	38.75 ±2.81	46.47 ±2.96	0.53	0.09	0.41
Membrane integrity (%)	59.50 ±3.10	57.89 ±3.68	56.18 ±3.53	61.10 ±3.17	0.77	0.34	0.44

[ab] Means with different superscripts within the same row differ significantly ($p< 0.05$); [1]P, S, P*S: Effects of protein, selenium and its interaction, respectively. N: number of samples, SEM: Standard error of means, P1: Low protein (180 g/kg DM); P2: High Protein (270 g/kg DM); S0: without Se supplementation; S1: with Se supplementation (1 mg/kg Bwt).

Therefore, further studies on AI using Dorper ram chilled semen is needed to determine its potential to produce higher pregnancy and lambing rate. As shown by Fernandez-Abella *et al*. (2003), the ewes that were AI with chilled ram semen (stored at 5°C for 24 hr) resulted in high pregnancy (52%) and lambing (45.3%) rates.

Characteristics of post-thawed semen

Results of this study also indicate that post-thawed semen had no interaction (P>0.05) with different CP levels and Se supplementation for all parameters tested (Table 5). The different levels of CP showed no significant effects (P>0.05) on sperm motility, viability, and morphology of post-thawed semen. The differences in Se supplementation also showed no significant influence (P>0.05) on all parameters except head abnormalities. S1 showed significantly decreased (P<0.05) head abnormalities in post-thawed semen compared to S0. While P2 tended to have a higher value on sperm motility and viability and P1 showed a tendency to have a higher value in sperm normality. Ram fed with S1 tended to have higher values in motility, viability and lower values in midpiece abnormalities (Table 5).

During the freezing process, osmotic effects, intracellular ice formation and exposure to oxidation contribute to sperm damage or abnormality (Banday *et al*., 2017).

Table 5: Effects of different levels of protein and selenium supplementation on sperm motility, viability and morphology (mean±SEM) of Dorper rams post-thawed semen

Diets	P1	P2	S0	S1	*P* value[1]		
					P	S	P*S
Total Motility (%)	47.16 ±3.59	47.50 ±3.20	45.11 ±3.44	49.25 ±3.38	0.63	0.80	0.87
Progressive Motility (%)	8.38 ±1.00	8.50 ±1.13	7.96 ±1.10	8.84 ±1.02	0.96	0.74	0.87
Sperm viability (%)	24.97 ±1.89	27.11 ±2.55	26.65 ±2.01	25.43 ±2.37	0.65	0.46	0.97
Sperm morphology							
Normality (%)	33.86 ±3.30	32.25 ±3.30	34.65 ±3.85	31.74 ±2.82	0.58	0.70	0.77
Head abnormalities (%)	11.21 ±1.76	11.57 ±1.99	14.38 ±2.24[a]	8.87 ±1.40[b]	0.86	0.02	0.64
Midpiece abnormalities (%)	16.03 ±1.56	13.82 ±1.60	15.2 3±1.88	14.71 ±1.34	0.25	0.94	0.54
Tail abnormalities (%)	38.90 ±3.21	42.36 ±3.60	35.73 ±3.31	44.68 ±3.29	0.48	0.08	0.45

[ab] Means with different superscripts within the same row differ significantly ($p< 0.05$); [1]P, S, P*S: Effects of protein, selenium and its interaction, respectively. N: number of samples, SEM: Standard error of means, P1: 180 g/kg DM; P2: 270 g/kg DM; S0: without Se supplementation; S1: with Se supplementation (1 mg/kg of Bwt).

In addition, cryopreserving ram sperm is rather difficult to compare with other farm animals and there is still a lack of studies on post-thawed semen of ram affected by feeding trials. In this study, Dorper rams showed a higher value in sperm total motility (40.1%) compared to findings by Mahmuda *et al*. (2015), however, has lower results in sperm viability and normality (40.6% and 70.9% respectively). This high sperm damage possibly occurred due to the untreated rice straw given had increased ram semen pH and its abnormalities as reported in El-Azab *et al*. (1998). Though chilled semen of Dorper rams fed with Se supplement had no significant findings, results in post-thawed semen in Dorper ram fed with Se supplement (S1) showed a significant decrease in head abnormalities or sperm by 38.3% decrease (Table 5). The significant result may be related to the improvement as shown in acrosome integrity in fresh semen (Table 3). In addition, post-thawed semen in S1 tended to have a higher value in all parameters compared to S0 which shown similarity trend in fresh and chilled semen. These slight increments are possibly due to the presence of Se increased GSH-Px activities, which helps prevent sperm damage as reported by Martins *et al*. (2015) and Kendall *et al*. (2000). Ganabadi *et al*. (2010) stated that Se as a supplement may affect semen quality from the early stages of spermatogenesis of rams. Further studies are necessary to determine the suitability of Se as organic supplementation and the potential to increase the quality of post-thawed semen of Dorper ram.

Conclusion

This study indicated that Dorper ram fed with higher protein concentration had similar quality in semen characteristic with required

CP. Meanwhile, the usage of organic Se (1 mg/kg of body weight) as supplementation showed lower acrosome damage in fresh semen and decreasing in head abnormalities in post-thawed semen in Dorper ram semen. Therefore, higher protein in the diet did not improve the quality of Dorper ram semen. Meanwhile, fed with organic Se showed the potential to improve head damage and further research is needed to implement its benefits in Dorper ram semen.

Acknowledgement

The authors are grateful to all the staff in the Department of Animal Science, Universiti Putra Malaysia and the National Animal Embryo Centre, MARDI Kluang for their technical support and assistance throughout this study.

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