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EFFECT OF DIETARY SUPPLEMENTATION OF PROTECTED FATS ON POST DILUTION INTERVAL AND SEMEN QUALITY OF BEETAL BUCKS

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ABSTRACT

Artificial insemination (AI) made a great contribution to the development of the livestock industry but deleterious effects of cryo-preservation on sperm fertility are profound. Effects of dietary supplementation of rumen protected fats on the semen quality in Beetal bucks were evaluated. Sperm quality of fresh and diluted semen was assessed through dietary supplementation of protected fats in Beetal bucks. A total of 20 Beetal bucks having nearly similar age (1.5 year) and weight (22 kgs) were selected, divided into five groups having four animals each in a completely randomized design. One group was controlled and the rest of the groups were supplemented with protected fats at the rate 10, 20, 30 and 40 gm per kg dry matter intake (DMI). The Collected semen was diluted in Tris-Glucose-Citrate-Yolk extender. The sperm quality parameters like sperm volume, sperm motility, sperm concentration and individual motility were determined at intervals of 0, 1, 2, 24, and 96 hours in fresh and diluted semen. There were no significant ($p>0.05$) effect on the semen volume, concentration, mass motility and individual motility at different intervals. It is concluded from the present study that the supplementation of protected fats has no significant effect on fresh semen whereas protected fat supplementation up to 30 gm per kg DMI preserved individual motility of semen that is an indicator for quality of Beetal buck semen.

Keywords: *Semen, Protected fats, Dilution Interval, Beetal Bucks*

INTRODUCTION

Interest in the Artificial insemination (AI) of goats has increased largely in recent years. For selective breeding programs, selective male produces enough spermatozoa which is used for inseminating thousands of females per year and improving the genetic merits. The increased milk, meat and skin production could be achieved by the development of AI to utilize superior genetics.

Fertility of fresh and cryo-preserved semen is important for AI. Cryo-preservation reduces the fertility of spermatozoa (Aitken et al., 2020, Peris-Frau et al., 2020) and induces the formation of reactive Oxygen Species (ROS) which have deleterious effects on sperm function (Akar et al., 2021). During storage of semen the fertility of sperm decreases and one of the possible reasons reported is the formation of lipid peroxides in the presence of oxygen radical.

The reactive oxygen species are normally generated as a result of oxidative metabolism and are important for normal sperm function. The imbalance of ROS leads to oxidative stress during cryo-preservation which reduces the sperm fertility (O'Flaherty, 2020). The lower fertility of spermatozoa is compensated by

insemination of larger doses of spermatozoa. However, there is a need to improve the sperm quality which could resist the harmful effects of cryo-preservation and to reduce the cost by lowering the dose of spermatozoa per insemination.

The quality of the sperm plays a decisive role in fertility. Many studies have focused on developing methods to reduce the sperm damages that occur during cryo-preservation (De Luca et al., 2021). The structural integrity of the cell membrane of the spermatozoa plays a pivotal role in successful fertilization.

Sperm production appears to be responsive to improved nutrition. Dietary modification of certain feed components can improve the sperm quality such as essential fatty acids. It has been shown that infertile spermatozoa contained lower omega-3 fatty acid content than fertile. Furthermore, the importance of omega-6/omega-3 ratio for sperm motility and normal morphology has been reported (Ngcobo et al., 2021, Khoshniat et al., 2020, Castellini et al.,2020).

This research has focused on elucidating the effect of protected fats as dietary supplements on sperm quality in terms of volume, concentration, mass motility and individual motility at different post dilution intervals.

MATERIALS AND METHODS

Twenty bucks of nearly the same age (1.5 years) and weight were trained for semen collection and passed through an adaptation period of 02 weeks, Bucks were randomly divided into 5 groups (4 animals/group). All of the experimental animals were fed a basal diet of Maize fodder ad-libitum with concentrate mixture supplemented to meet nutrient requirement of the bucks on iso-caloric and iso-nitrogenous bases (NRC, 2007). One group (00) served as a control whereas others groups were supplemented with 10, 20, 30 and 40 g protected fats (commercially available) per kg Dry Matter Intake, per animal, respectively.

Semen was collected in a graduated semen collection tube twice a week from all the experimental animals through an artificial vagina. Ejaculate volume was recorded and the semen was kept at 37ºC in a water bath (Memmert, Germany) till further evaluation. Seminal volume was measured through a graduated test tube used for the semen collection. Mass motility (graded in percent) was examined through a compound microscope (Olympus CX 21, Philippine) by placing a drop of semen on a warm (37ºC) slide. The stage temperature was also kept thermostatically controlled warm. Sperm concentration (million/ml) was determined by using a haemocytometer with a counting chamber. A semen sample of 0.01 ml was diluted with 4 ml of normal saline solution (3% w/v). The number of spermatozoa was counted in 5 large squares each containing 16 small squares. The concentration of sperm per ml of semen was calculated as under:

Sperm concentration (million/ml)=(Number of spermatozoa in one chamber)×Multiplication factor×Dilution factor

Where:

- "Number of spermatozoa in one chamber" is the count of spermatozoa in a known volume of the chamber.
- "Multiplication factor" is a factor that accounts for the volume of the sample and the volume counted in the chamber.
- "Dilution factor" adjusts for any dilution made during the sperm analysis process.

For evaluation of individual sperm motility at different intervals (0, 1, 2, 24 and 96 hours), semen was diluted by using Tris-Glucose-Citrate-Yolk extender and kept at room temperature for 15 minutes and then stored at 4°C in the refrigerator.

Statistical package of Statistix 8.1 was used to perform statistical analysis according to standard procedure of analysis of variance using Completely Randomized Design (CRD) as described by Steel and Torrie (1980). Means were compared by least significant (α= 0.05) difference (Steel and Torrie, 1980).

Results

1.Fresh Semen Volume

Table 01 shows that the overall mean of semen volume of the bucks was not affected ($p>0.05$) by the protected fats supplementation in supplemented groups. The overall mean of semen volume was higher in group supplemented with 30gm kg^{-1} followed by 40 gm kg^{-1} DMI, 20 gm kg^{-1} DMI, 10 gm kg^{-1} DMI and control group having mean values 1.05 ml, 1.04 ml, 1.02 ml, 1.01 ml and 1.01 ml, respectively.

2. Fresh Semen Concentration:

The treatment did not produce any significant ($p>0.05$) effect on sperm concentration. The overall mean values of sperm concentration were numerically higher (1049.3×106/ml) in the group supplemented with 40 gm kg $^{-1}$ DMI protected fats followed by group 30 gm kg $^{-1}$ DMI, 20 gm kg $^{-1}$ DMI, 10 gm kg $^{-1}$ DMI and control group having 1046.5 (106/ml), 1043.9 (106/ml), 1046.2 (106/ml), 1041.1 (106/ml) of sperm concentration, respectively.

3. Mass Motility:

Mass motility (%) was recorded higher in the group supplemented with 30 gm kg^{-1} DMI protected fats having overall mean of 87.48%; though supplementation to all groups have no significant ($p>0.05$) effects on mass motility of the sperm. Further increase in dietary supplementation of protected fats up to 40 gm Kg $^{-1}$ DMI decreased the mass motility to 86.82%. Overall mean values recorded for the controlled group, 10 gm kg-1 DMI and 20 gm kg-1 DMI protected fats supplemented group were 85.92%, 86.14% and 86.82%, respectively.

Table 01: Effect o protected fats Supplementation on the overall mean of semen volume (ml), concentration (10^6 ml) and mass motility (%) of the Beetal bucks.

Semen quality Parameters	Dietary protected fats Supplementation levels gm Kg -1 DMI				
	0	10	20	30	40
Volume (ml)	0.96±0.03	0.99±0.03	1.00±0.03	1.02±0.03	1.00±0.03
Concentration (10^6 ml)	1041.1±6.40	1046.2±4.55	1043.9±8.37	1046.5±3.75	1049.7±7.47
Mass motility (%)	85.92±1.18	86.14±0.07	87.10±1.70	87.48±0.23	86.82±1.27

4. Individual Motility at different Interval:

As shown in Table 2 the individual motility observed at 0, 01, 02, 24, and 96 hour Interval. The investigation on individual motility at the 0-hour interval revealed no significant differences ($p > 0.05$) over the course of the experimental period in response to the treatments. At the 0-hour interval, the group receiving 40 gm Kg^{-1} DMI of protected fat supplementation exhibited the highest individual motility value (85.33%). This was followed by the groups supplemented with 30g, 20g, and 10g Kg^{-1} DMI of protected fat, which displayed motility, values of 84.75%, 84.71%, and 84.07%, respectively. Comparatively, the control group demonstrated the lowest motility of 83.98% in contrast to all treated groups.

At the 01-hour interval, there was no significant difference ($p > 0.05$) in individual motility among the control and treated groups. The group supplemented with 40 gm Kg^{-1} DMI of protected fats exhibited the highest individual motility reaching 76.57%. This surpassed the motility values of 30 gm, 20 gm, and 10 gm Kg^{-1} DMI supplemented groups, which showed motility rates of 76.17%, 76.06%, and 75.55%, respectively. The control group's individual motility was the lowest 75.24%.

There was no significant effect ($p > 0.05$) of protected fat supplementation on individual motility after 02-hours interval. However, at the 02-hours interval, the group receiving 40 gm Kg^{-1} DMI of protected fats displayed the highest individual motility, measuring 74.78%. This was followed by the groups supplemented with 30 gm, 20 gm, and 10 gm Kg^{-1} DMI of protected fat, which exhibited mean motility values of 75.69%, 74.59%, and 74.23%, respectively. The control group's individual motility at the 02-hour interval was recorded 74.23%.

Mean values of individual motility at the 24-hour did not exhibit any significant increase after supplementation of protected fats. However, higher individual motility values at the 24-hour interval were observed in the group supplemented with 30 gm Kg^{-1} DMI of protected fat, in comparison to both the control and other supplemented groups. Mean values of individual sperm motility at 24 hours interval recorded were 67.39%, 66.54%, 66.50%, 66.37%, and 66.12% for the 30 gm, 40 gm, 20 gm Kg^{-1} DMI supplemented groups, and the control group, respectively.

The mean values of individual motility at the 96-hour interval did not indicate any significant differences ($p > 0.05$). Higher values were documented in the groups supplemented with 30 gm and 40 gm Kg^{-1} DMI of

protected fat, displaying individual motility of 55.84% and 55.18% at 96 hours after dilution. The control group had the lowest motility of 54.61%, in contrast to the supplemented groups.

Table 02: Effect of Dietary protected fats Supplementation on the individual motility (%) of diluted Beetal buck semen at different intervals

Time after dilution	Dietary protected fats Supplementation levels gm Kg -1 DMI				
	0	10	20	30	40
00 hr motility (%)	83.98±0.17	84.07±0.51	84.71±0.56	84.75±0.33	85.33±0.42
01 hr motility (%)	75.24±0.44	75.55±0.06	76.06±0.46	76.17±0.72	76.57±0.40
02hr motility(%)	73.93±0.52	74.23±0.62	74.59±0.43	75.69±0.52	74.78±1.02
24hr motility (%)	66.12±0.56	66.37±0.54	66.50±0.41	67.39±0.85	66.54±0.89
96 hr motility (%)	54.61±0.67	55.01±0.49	55.16±0.51	55.84±0.75	55.18±0.39

Discussion

1. Volume

Results showed that mean semen volume was not affected by dietary supplementation of protected fats from 10-40 gm Kg^{-1} DMI. Same results are reported by Byrne et al. (2017) where there was no increase in ejaculate volume in Holstein bulls. Similarly, other studies reported no effect of fish oil supplementation on quality parameters of fresh semen in different animal species (Yuan et al. 2023).

However, increase in semen volume was reported by Khoshvaght et al. (2016) through feeding a source of omega-3 fatty acids in bulls and in ram by Abd El-Razek, Ashmawy et al. (2009). Increase in semen volume may be due to age, season and duration of experiment. Same results have been reported by Widiyono et al. (2017) where dietary supplementation of omega-3 fatty acids were considered to be corrective for seasonal influences on semen quality.

2. Concentration

The supplementation of dietary protected fats did not show any effect on the sperm concentration throughout the experiment. Similar to our results, (Rossi et al. 2019) reported that sperm concentration remained the same between control diet and fish oil diet fed to Turkey breeders. Other studies also reported no effect of fish oil supplementation on sperm concentration and other quality parameters of fresh semen in different animal species (Yuan et al. 2023).

It has been reported by Khoshvaght et al. (2016) that there was an increase in spermatozoa concentration of the horse when supplemented with fatty acids. Same findings have been reported by Elhordoy, Cazales et al. (2008) in stallions where there was an increase in sperm concentration. Estienne, Harper et al. (2008) and Gliozzi, Zaniboni et al. (2009) reported in boars and rabbits, respectively, higher sperm concentration when omega-3 fatty acids were added to diet. Duration of experiment and amount of fatty acids fed to the experimental animal can be a factor which produced different results. Finding of various studies suggests that physiological differences among various species could be another factor producing different results.

3. Mass motility

In present study, no significant ($p>0.05$) difference was recorded in mass motility. Similar results were obtained in previous reports that motion characteristics of fresh and cooled/stored spermatozoa were also not improved by treatment when stallions supplemented with long-chain omega-3 fatty acids (Harris, Baumgard et al., 2005) and rams with omega-3 fatty acids (Fair et al. 2014).

Contrary to our findings Gholami, Chamani et al. (2010) found that feeding a source of omega-3 fatty acids leads to a higher sperm concentration. Abd El-Razek, Ashmawy et al. (2009) determined that the ejaculate volume, sperm cell concentration and sperm motility in all fish oil groups were significantly ($P<0.05$) increased during treatment period than that of the control rams. Furthermore, an experiment on broiler chickens has demonstrated that feeding of long-chain fatty acids increased both sperm number and motility (Abbaspour et al., 2020). Mammals are not capable of synthesizing these essential fatty acids due to the lack

of desaturase enzymes. Mass motility is an indicator of spermatozoa concentration and total motility which depends upon the number of viable spermatozoa.

4. Semen motility at different Dilution intervals

Individual motility assessed at different intervals in fresh diluted semen did not show any significant increase in response to experimental treatment. However, numerically higher motility was observed for dietary supplementation of 30g Kg^{-1} and 40g Kg^{-1} protected fats supplementation.

Spermatozoa motility is also an indicator of intact sperm plasma membrane. In this study higher values were recorded for individual motility in fresh diluted semen after protected fat supplementation. These findings indicate that fats supplementation improved sperm plasma membrane integrity and hence individual motility was improved. The same results have been reported by Shao et al. (2009). While De Graaf, Peake et al. (2007) reported that supplement of essential fatty acids (like Oleic and linoleic acids) did not show significant effect on the motility characteristic, viability and acrosome integrity of sex-sorted spermatozoa in rams. Gholami, Chamani et al. (2010) demonstrated a better effect on sperm viability, motility and motion of spermatozoa in bulls when supplemented with omega-3 fatty acids.

Similarly, other studies like (Towhidi et al. 2008) in goat and Towhidi, Samadian et al. (2008) in sheep reported a significant ($p<0.05$) correlation between dietary omega-3 supplementation and the number of motile spermatozoa. It seems that dietary supplementation of protected fats have contributed to fatty acids contents of sperm plasma membrane and resisted oxidative stress and lipid peroxidation.

Conclusions and recommendation

It is concluded from the results of the above experiment that dietary supplementation of protected fats (30 g Kg^{-1} DMI) as a source of omega-3 fatty acids did not show any significant effect on semen quality parameters in fresh and diluted semen in Beetal bucks. However, numerically higher values in semen volume, concentration, mass motility and individual motility at different intervals in fresh semen and diluted semen were observed through dietary supplementation of protected fats. It is recommended from the present study that fats supplementation up to 30gm^{-1}DMI can improve quality of buck semen. However, further research is needed to investigate the effect of fat supplementation on fatty acids contents of sperm plasma membrane and its relation with sperm fertility and oxidative stress.

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