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Effects of omega-3 fatty acids on fresh bull semen quality: A systematic review and meta-analysis

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Abstract: Polyunsaturated fatty acids (PUFAs), including omega-3, are known to yield health benefits to mammals, including bulls and human beings. This meta-analysis was done to determine the influence of omega-3 fatty acids (OMFA) on bull semen quality. The research question formulated for this meta-analysis was centred on the Population-Intervention-Comparison-Outcome (PICO) framework. To determine the influence of omega-3 fatty acids on semen quality, a meta-analysis was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. The different effects were analysed using a random effects model. The variations among the included studies were tested using heterogeneity (I^2). Semen volume (MD = 0.28, 95% CI: 0.13; 0.44), sperm concentration (MD = 71.12, 95% CI: 15.97; 126.26), live sperm (MD = 11.93; 95% CI: 7.30; 16.55), sperm motility (MD = 9.24, 95% CI: 7.39; 11.09) and plasma membrane integrity (MD = 11.60, 95% CI: 6.30; 16.89) increased dramatically in the omega-3 fatty acid-supplemented group. Notably, the progressive motility (MD = 1.19, 95% CI: –3.24; 5.61) was the only sperm parameter showing a non-significant influence when omega-3 fatty acids were supplemented. It was then concluded that the omega-3 fatty acid supplementation can be used to improve most of the semen parameters in bulls.

Keywords: bull fertility; fatty acid supplementation; nutritional modulation; polyunsaturated fatty acid; reproductive efficiency

INTRODUCTION

The human population continues to increase exponentially and it is assumed to reach 9.1 billion by 2050. According to Bradford (1999), this growing human population will demand increased food production, including animal-based products such as meat. Therefore, global food production should rise by about 70–100% compared to the current food production (Hamad and Tayel 2025). Moreover, the demand for meat, dairy, fish, and vegetables is expected to grow faster than that for

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other types of food (Makkar 2018). This occurs in a period when climate change is influencing food production, hence, the majority of underdeveloped and developing countries are left below the breadline.

Livestock play a substantial function in securing food and generating income through fertiliser production and meat production (Smith et al. 2013). They increase food quantity available for human feeding, used as a basis of income through formal and informal trade, and used as a source of protein for balanced nutrition (Reynolds et al. 2015). However, the conception of food between animals and human beings has been identified as a challenge (Idamokoro 2023), hence the recent use of wild animals as a source of protein. Nevertheless, the recent COVID-19 pandemic suggested that the consumption of wild red meat might pose a threat to human beings (Gonzalez et al. 2020). Therefore, improving the domestic livestock productivity remains vital alongside other alternative strategies, such as crop production and promoting other protein sources. Omega-3 fatty acids have been used to enhance semen quality in bulls (Perumal et al. 2019), sheep (Ngcobo et al. 2023), and other livestock.

Meat consumers are transitioning to healthier eating habits in their diets, which include omega-3 fatty acid-rich meat and other dietary sources and supplements contributing to health benefits, such as the reduction of coronary heart disease, autoimmune disorders, and inflammatory conditions (Bodur et al. 2025; Mujic et al. 2025). Omega-3 fatty acids (OMFA) are unsaturated fatty acids with a double bond at the third carbon within their carbon chain (Ngcobo et al. 2021). Omega-3 fatty acids improve semen quality in bulls due to the high concentration of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). However, OMFA cannot be manufactured *de novo* in mammals due to the lack of delta-12 and delta-15 carbon (Ngcobo et al. 2021); hence, their dietary supplementation is necessary (Ngcobo et al. 2023). Fish oil has been the main source of OMFA in bulls and it has been attested to improve semen quality in bulls (Zakariapour Bahnamiri et al. 2019). Nevertheless, feeding fish oil to livestock increases the danger of overfishing, particularly in developing countries (Pham et al. 2023). For example, Bennett et al. (2021) observed that food security and the use of fish products such as fish oil are the factors leading to overfishing in low-income countries. Furthermore, about 3.6–19 million tonnes of marine stocks are overfished, leading to the economic loss of about $83 billion in developing countries (Sumaila et al. 2012). This alone can increase the competition between human beings and animals for fish products and lead to overfishing.

To reduce competition between animals and humans for certain foods, studies are now using other complementary sources of OMFA, such as flaxseed oil. This is vital since ruminants cannot synthesise alpha-linolenic acid *de novo*. The use of plant-based diets in livestock may reduce greenhouse gas (GHG) emissions. However, there has been no meta-analysis confirming the effect of OMFA sources on semen quality in bulls. Therefore, this review aims to evaluate the effect of omega-3 fatty acids on the semen quality of bulls.

METHODOLOGY

Study eligibility criteria

The research question formulated for this meta-analysis was based on the Population-Intervention-Comparison-Outcome (PICO) framework. The population in this meta-analysis involved cattle bulls (Israel Holstein, Nili Ravi, Holstein, Mithun, and Simental bulls) from a diverse genetic background (Israel, Pakistan, Iran, Egypt, India, Germany, and China). The interventions were the dietary use of flaxseed oil (FLAX), fish oil (FO), OMFA, and linseed oil (LO). These interventions were compared with other groups fed standard diets.

Literature search strategies

The Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) were used in this meta-analysis (Figure 1). One hundred fourteen articles were gathered from different publishers such as Google Scholar, Scopus, PubMed, ScienceDirect, and Springer. These publishers are listed under Web of Science (WoS). Literature was searched in the above search engines using "flaxseed oil effect on bull semen quality"; "fish oil effect on bull semen quality"; "omega-3 fatty acid effect on bull semen quality"; "effect of linseed oil on semen quality from bulls"; and "effect of alpha-linolenic acid on semen quality in bulls".

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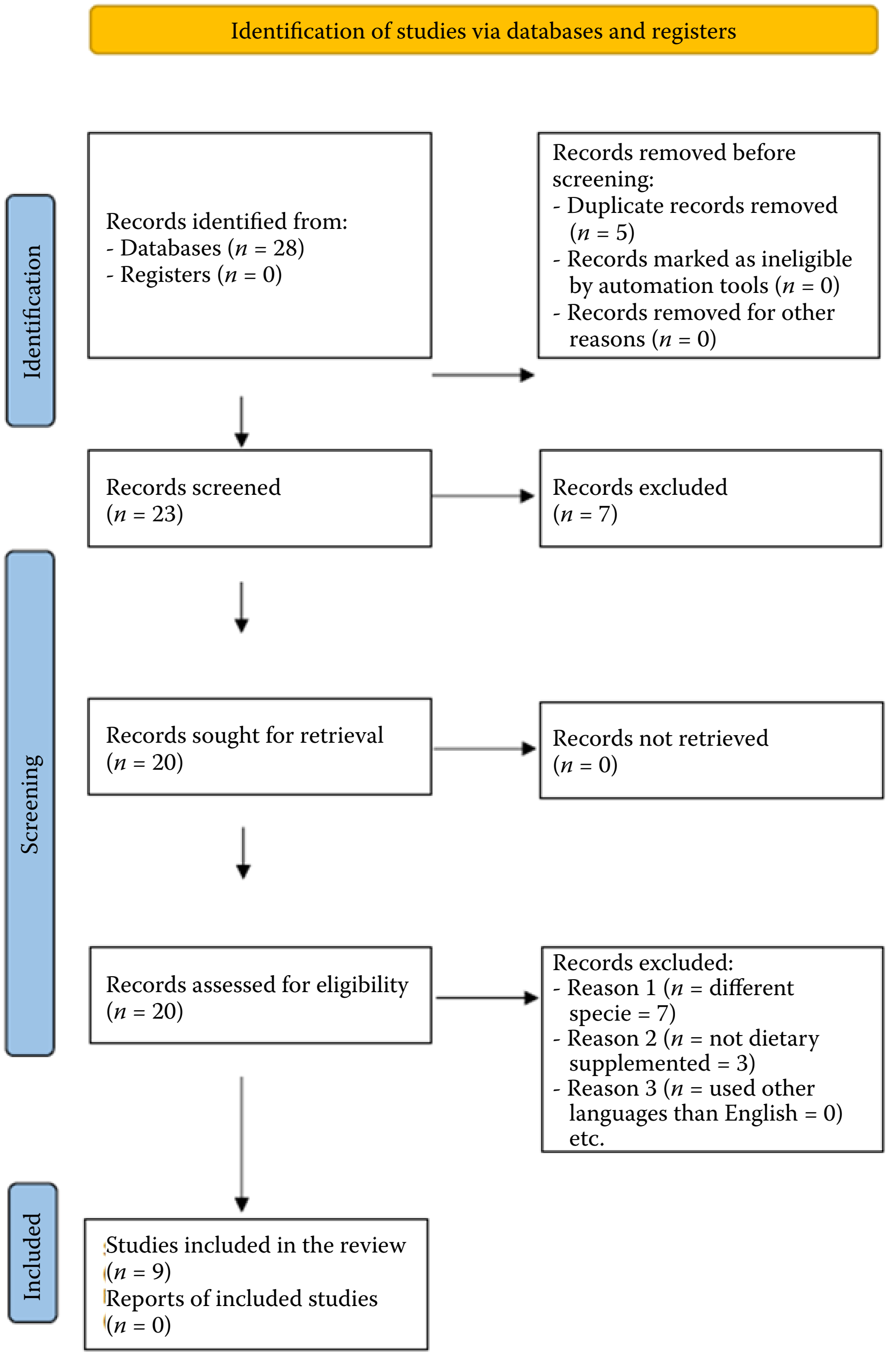

Figure 1. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)

Inclusion and exclusion criteria

To ensure inclusion, all research articles addressing the influence of FLAX, FO, OMFA, linseed oil, and alpha-linolenic acid on the semen quality of bulls were gathered from the search engines listed above. Moreover, only research articles written in English were considered.

For exclusion purposes, all research and review articles addressing FLAX, FO, OMFA, linseed oil, and alpha-linolenic acid on other species, such as boars, rams, goats, chickens, and rats, as well as equines, were excluded. Furthermore, all research articles that did not specify the type of oil used were also excluded from this meta-analysis review.

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Statistical analysis

Data were collected from the included studies, recorded in Microsoft Excel, and analysed using RStudio software v5.1 (Posit, PBC, Boston, MA, USA). The metafor package was downloaded and used to generate a summary of the data and funnel plots. The different effects were analysed using a random-effects model. The variations among the included studies were tested using heterogeneity (I^2).

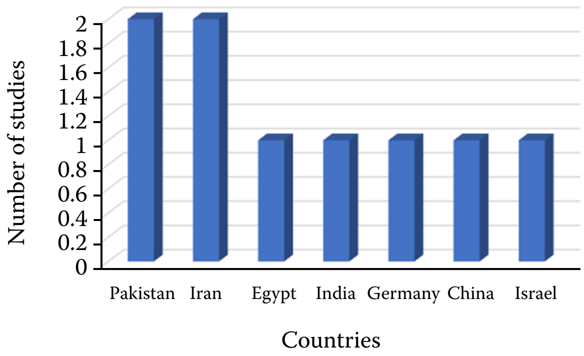

Figure 2. Publication by country

RESULTS

Study characterisation, publication by year, and publication by country

Figure 2 and Table 1 show the characterisation of included studies, indicating a total of 9 studies with 12 experiments.

Most of the studies were published in Pakistan (Shah et al. 2016; Ahmad et al. 2019), and Iran (Gholami et al. 2010; Khoshniat et al. 2020), with Egypt (El-Hamd et al. 2019), India (Perumal et al. 2019), Germany (Gurler et al. 2015), China (Yuan et al. 2023), and Israel (Moallem et al. 2015) contributing one study per country. One study was published in 2010 (Gholami et al. 2010), two in 2015 (Gurler et al. 2015; Moallem et al. 2015), one in 2016 (Shah et al. 2016), two in 2019 (El-Hamd et al. 2019; Perumal et al. 2019), one in 2020 (Khoshniat et al. 2020), and one in the year 2023 (Yuan et al. 2023).

Distribution of articles according to the source of OMFA

Figure 3 illustrates the distribution of studies according to the source of OMFA. The majority of included studies used flaxseed oil (El-Hamd et al. 2019; Moallem et al. 2015; Perumal et al. 2019; Shah et al. 2016; Yuan et al. 2023), linseed oil (Ahmad et al. 2019; Khoshniat et al. 2020), omega-3 fatty acids (Gholami et al. 2010), alpha-linolenic acid (Gurler et al. 2015), and fish oil (Moallem et al. 2015).

Table 1. Study characterisation

Study	Year	Country	Breed	Age	Type of OMFA	Dosage	Duration
Moallem et al.	2015(1)	Israel	Israel Holstein	6 years	flaxseed oil	450 g/bull	13 weeks
Moallem et al.	2015(2)	Israel	Israel Holstein	6 years	fish oil	450 g/bull	13 weeks
Ahmad et al.	2019	Pakistan	Nili Ravi	3.5–4 years	linseed oil	125 g/bull	12 weeks
Gholami et al.	2010	Iran	Holstein	2.5–6 years	omega-3	25 g/bull	12 weeks
El-Hamd et al.	2019(1)	Egypt	Holstein	14 months	flaxseed oil	200 g/kg/bull	28 weeks
El-Hamd et al.	2019(2)	Egypt	Holstein	14 months	flaxseed	400 g/kg/bull	28 weeks
Shah et al.	2016(1)	Pakistan	Nili Ravi	matured	flaxseed oil	125 g/bull	12 weeks
Shah et al.	2016(2)	Pakistan	Nili Ravi	matured	flaxseed oil	250 g/bull	12 weeks
Perumal et al.	2019	India	Mithun	4–6 years	flaxseed oil	150 g/bull	12 weeks
Khoshniat et al.	2020	Iran	Holstein	mature	linseed oil	300 g/bull	12 weeks
Gurler et al.	2015	Germany	Holstein	3 years	alpha-linolenic acid	800 g/bull	12 weeks
Yuan et al.	2023	China	Simental	2–3 years	flaxseed oil	24 g/bull	10 weeks

Only fresh semen quality parameters were considered; dosages from studies such as Gholami et al. (2010) and El-Hamd et al. (2019) were converted from % to g for uniformity; Dosages from studies such as Ahmad et al. (2019), Shah et al. (2016), Perumal et al. (2019) were all converted from ml to grams

OMFA = omega-3 fatty acids

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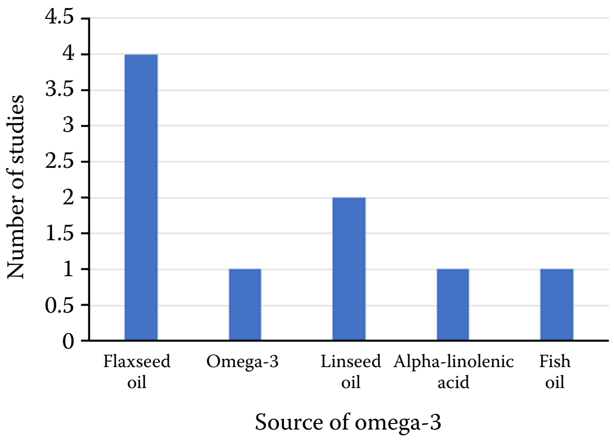

Figure 3. Distribution of studies according to the source of OMFA

OMFA = omega-3 fatty acids

Effect of OMFA on semen volume

The current meta-analysis, comprising 8 trials from 6 research articles, revealed a small but significant positive effect (MD = 0.28, 95% CI: 0.13–0.44) (Figure 4). Substantial heterogeneity (I^2 = 94.4%) was observed, indicating a high variability in the results across all 8 trials. Based on these results, semen volume increased by 0.28 ml when the diet of bulls was enriched with OMFA in comparison with the control group. Moreover, the confidence interval could not cross zero, implying a statistical difference between the treated group and the control group (P < 0.000 1). Notably, the heterogeneity (I^2) was very high (94.4%), which implies substantial differences between the included studies. However, when the weight of the included studies was evaluated, experiments (1 and 2) by El-Hamd et al. (2019) were dominant, ranging from 30.5% both. Therefore, these experiments were then excluded, and another analysis was conducted.

Figure 5 represents results from different studies without the dominant studies from Figure 4. The second analysis performed without dominant experiments (1 and 2) by El-Hamd et al. (2019) showed the negative effect when MD = –0.03, 95% CI: –0.88; 0.82 with the confidence interval

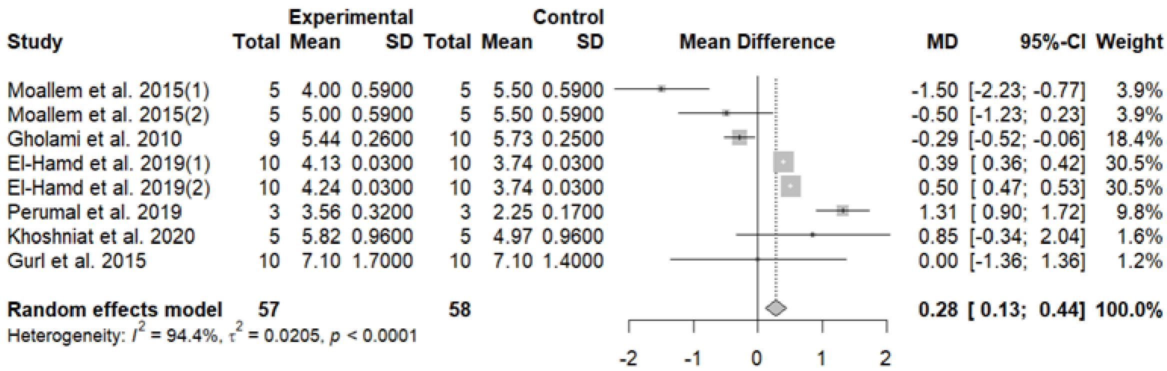

Figure 4. Effect of enriching bull's diets with OMFA on semen volume with dominant experiments (1 and 2) by El-Hamd et al. (2019)

OMFA = omega-3 fatty acids

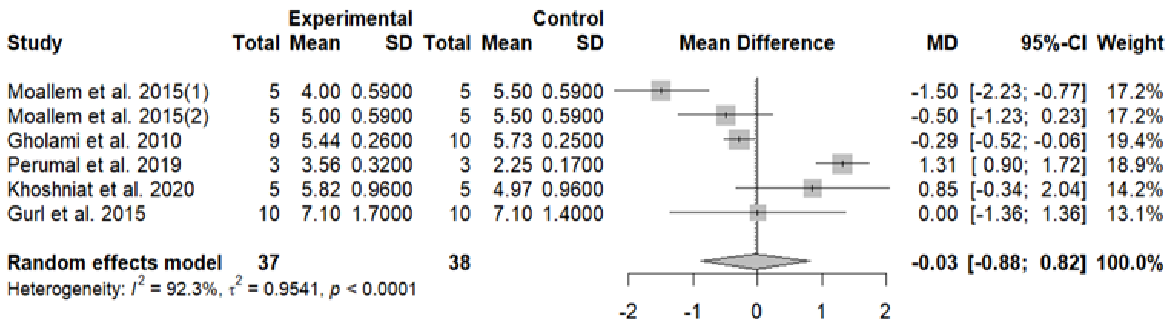

Figure 5. Effect of enriching bull's diets with OMFA on semen volume without experiments (experiment 1 and experiment 2) by El-Hamd et al. (2019)

OMFA = omega-3 fatty acids

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crossing zero, implying a non-significant difference. Nevertheless, the semen volume decreased by –0.03 ml when omega-3 fatty acid was supplemented in comparison with the control group. Notably, heterogeneity was very high (I^2 = 92.3%). The heterogeneity did not decrease significantly when dominant experiments by El-Hamd et al. (2019) were included (I^2 = 94.4%) and when these experiments were excluded (I^2 = 92.3%).

Omega-3 effect on sperm concentration

Sperm concentration was further evaluated in three studies involving five different trials, and the results are presented in Figure 6. According to these studies, supplementing omega-3 sources in bulls leads to an increase of sperm concentration by 71.12 × 10^6 cells/ml (MD = 71.12, 95% CI: 15.97–126.26). It was also observed that the confidence interval (CI) could not cross the zero border line (15.97; 126.26), confirming the differences (P < 0.000 2) between the treated bulls and the control. However, the substantially high heterogeneity (I^2 = 84.60%) confirmed that the included studies differed significantly. Notably, only one study (Perumal et al. 2019) had higher weights (36.6%).

Due to the study by Perumal et al. (2019) having the most significant influence (36.6%), another meta-analysis excluding this study was conducted, and the results are presented in Figures 7 and 8. In this analysis, it was found that the sperm concentration

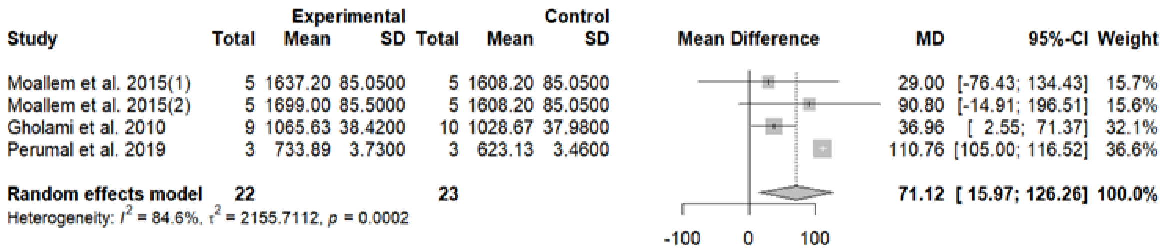

Figure 6. Effect of enriching bull's diet with OMFA on sperm concentration with Perumal et al. (2019)
OMFA = omega-3 fatty acids

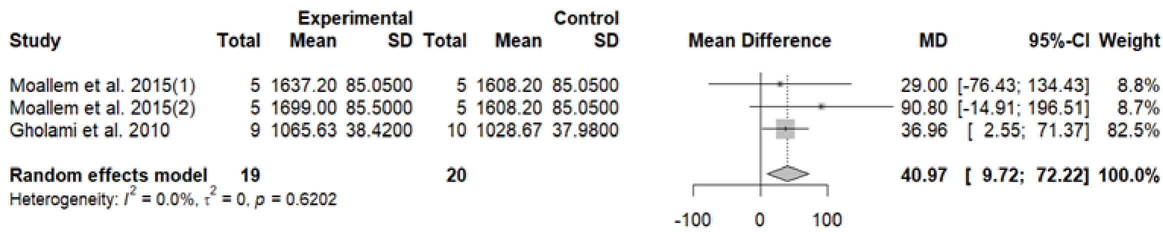

Figure 7. Effect of dietary OMFA on sperm concentration without dominant study by Perumal et al. (2019)
OMFA = omega-3 fatty acids

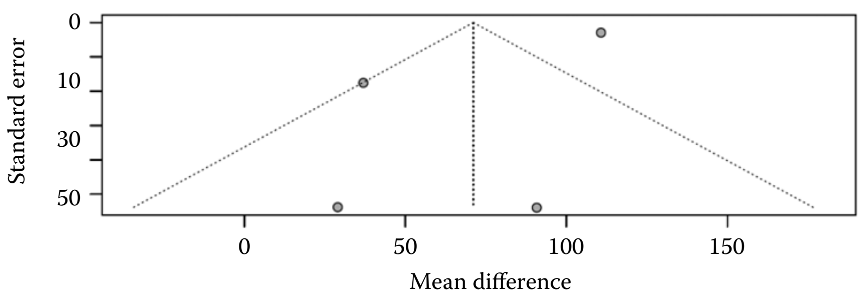

Figure 8. Funnel plot on the effect of enriching the bulls' diet with OMFA on sperm concentration with the dominant study by Perumal et al. (2019)
OMFA = omega-3 fatty acids

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increased by 40.97 × 10^6 cells/ml when dietary omega-3 was supplemented to bulls. There was no heterogeneity (I^2 = 0.00%) found in this meta-analysis for sperm concentration, implying that the studies were consistent and unbiased (Figure 9; funnel plot).

Effect of enriching the bulls' diet with OMFA on live sperm

A meta-analysis of live sperm following the enrichment of bull diets with omega-3 fatty acids from various studies was conducted, and the results are presented in Figure 10.

Four studies for the live sperm were included, consisting of five different trials. According to this meta-analysis, live sperm increased by 11.93% when bulls were supplemented with omega-3 fatty acids. Moreover, our meta-analysis found a statistical difference [MD = 11.93; CI (7.30; 16.55)] between the treated groups and the control. Nevertheless, the heterogeneity was substantially high (I^2 = 94.30%), implying significant differences between the included studies.

Notably, all included studies in this meta-analysis contributed almost similar weight to these results, ranging from 18.40% to 21.30%.

Effect of enriching the bulls' diet with OMFA on sperm motility

The meta-analysis for sperm motility following dietary supplementation of omega-3 sources and the results are presented in Figure 11. The current meta-analysis observed that sperm motility increased by 9.24% when omega-3 was used in bulls [MD = 9.24; CI (7.39; 11.09)]. The confidence interval (CI) ranged from 7.39 to 11.09, confirming a positive effect of enriching the diet of bulls with omega-3 on the effective population size and it did not cross the zero border. This CI observed for sperm motility further implied a substantial difference between the treated group and the control. However, there were substantial differences between the included studies [heterogeneity (I^2 = 100%)]. There was no dominant study in this analysis, as the study weight ranged between 4.70% and 15.30%.

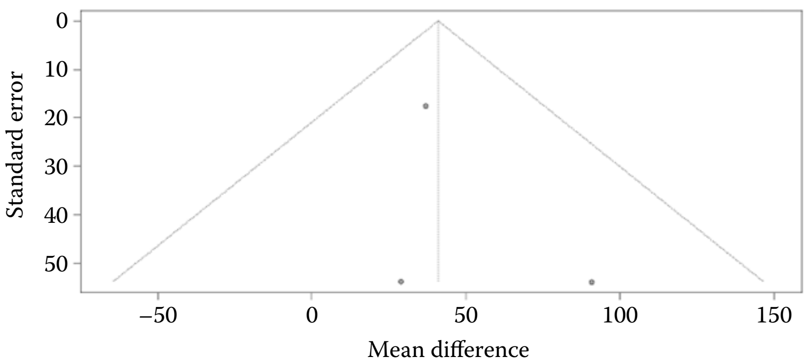

Figure 9. Funnel plot on the effect of enriching bulls' diet with OMFA on sperm concentration without the dominant study by Perumal et al. (2019)
OMFA = omega-3 fatty acids

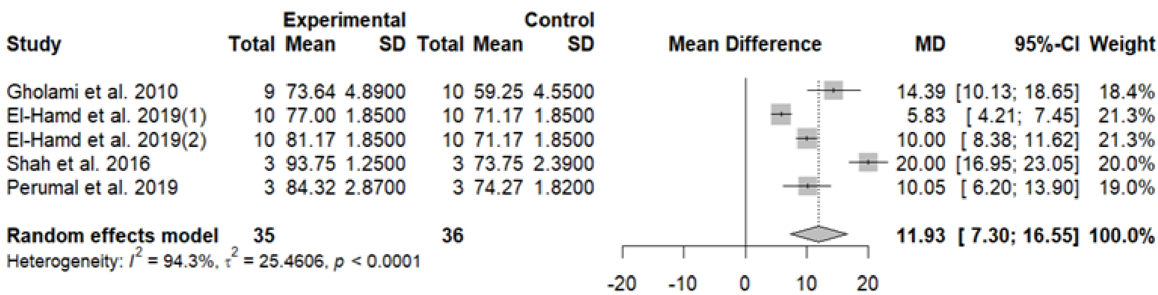

Figure 10. Effect of enriching the bulls' diet with OMFA on live sperm
OMFA = omega-3 fatty acids

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Effect of enriching the bulls' diet with OMFA on plasma membrane integrity

The plasma membrane integrity was also included and determined in the current meta-analysis, and the results are presented in Figure 12. In this meta-analysis, a total of five studies consisting of eight trials was used to analyse the effect of enriching the diet of bulls with omega-3 fatty acids on the plasma membrane integrity [MD = 11.60; CI (6.30; 16.89)]. Supplementing dietary OMFA sources in bulls led to an increase in the plasma membrane integrity by 11.60% in comparison with the control group. A positive effect of dietary OMFA on the plasma membrane integrity was observed, with the confidence interval ranging from 6.30 to 16.89 and not crossing the zero border. Heterogeneity (I^2 = 98.2%) continued to be substantially high for the plasma membrane integrity, as observed in the other semen parameters included in this study.

Effect of enriching the bulls' diet with OMFA on sperm progressive motility

Figure 13 represents the meta-analysis results following enriching the diet with OMFA from different studies in bulls. According to these results,

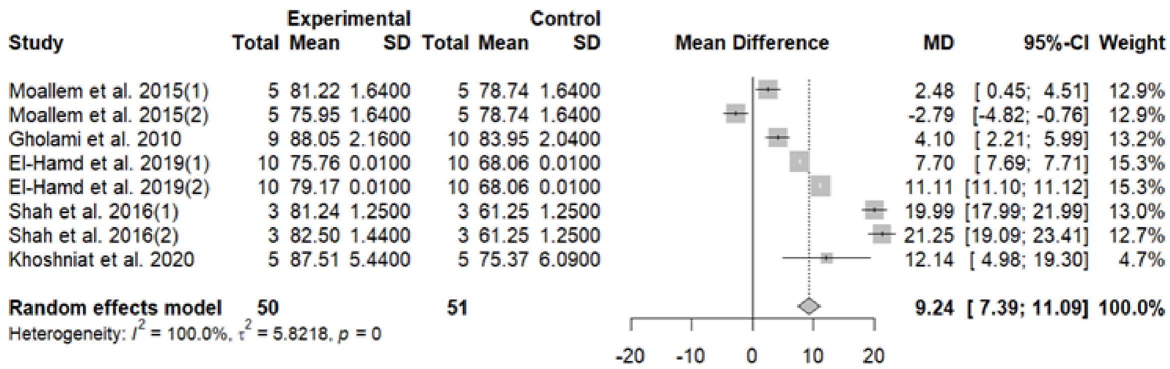

Figure 11. Effect of enriching the bulls' diet with OMFA on sperm motility
OMFA = omega-3 fatty acids

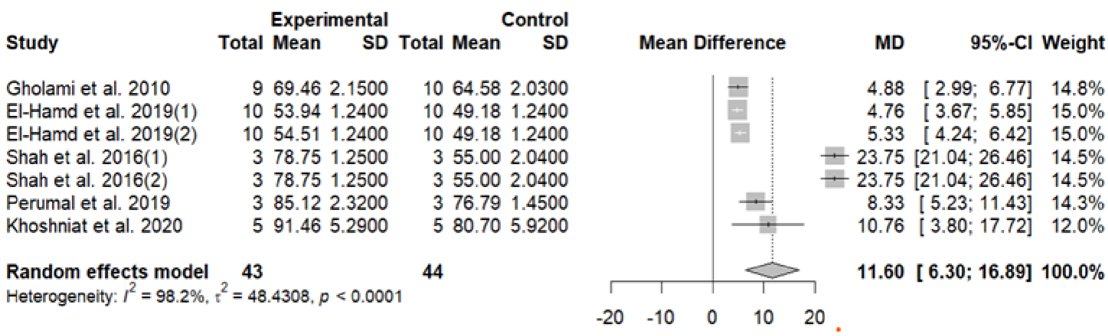

Figure 12. Effect of enriching the diets of bulls with OMFA on plasma membrane
OMFA = omega-3 fatty acids

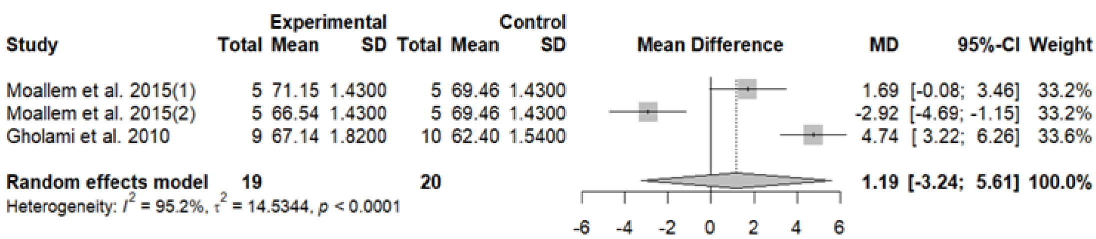

Figure 13. Effect of enriching the bulls' diet with OMFA on progressive sperm motility
OMFA = omega-3 fatty acids

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progressive motility increased by only 1.19% when omega-3 fatty acids were supplemented in bulls. Nevertheless, a negative effect was observed where the confidence interval crossed the zero border [CI (–3.24; 5.61)]. Furthermore, the substantially high heterogeneity (I^2 = 95.20%) shows that the included studies differed significantly in this parameter.

DISCUSSION

Effect of enriching the bulls' diet with OMFA on semen volume

Mammals are unable to synthesise LCPUFAs as they lack delta-12 and 15 desaturase enzymes (Ngcobo et al. 2021), hence the need for dietary supplementation (Perumal et al. 2019). Omega-3 supplementation in livestock animals was well-documented in rabbits (Castellini et al. 2019), sheep (Ngcobo et al. 2023), boars (Singh et al. 2020; Singh et al. 2023), and in bucks (Dolatpanah et al. 2008). However, some studies reported a significant effect of OMFA while others reported a non-significant difference in some parameters. Reproductive efficiency in mammals is mostly affected by the semen quantity and quality (Arrebola and Abecia 2017); hence, the interest in the role of OMFA in the improvement of semen quality (Ngcobo et al. 2023). This meta-analysis aimed to evaluate the effect of enriching the diet of bulls with omega-3 fatty acids on semen volume in bulls.

According to the results obtained from this study, supplementing OMFA sources results in an increase of semen volume by 0.34 ml. There are many other studies done previously on the omega-3 influence on semen volume in bulls (Khoshniat et al. 2020) and in rams (Ngcobo et al. 2023). Nevertheless, supplementation of OMFA (fish oil) in bulls could not improve semen volume (Castellini et al. 2019). In contrast, Khoshvaght et al. (2016) found that supplementing fish oil in Holstein bulls increased semen volume. Moreover, when flaxseed oil was used in Friesian bulls, there was a clear improvement in semen volume (El-Hamd et al. 2019). Moreover, different sources of OMFA were used in these studies, including flaxseed oil, linseed oil, fish oil, omega-3 oil, and alpha-linoleic acid. These differences might be due to the level of inclusion, and the current meta-analysis could not include meta-regression for sub-groupings due to the included studies being fewer than ten studies. Shah et al. (2016) found that supplementing flaxseed oil (125 and 250 ml/day) improved semen volume after 12 weeks.

Effect of enriching diet with OMFA on sperm concentration

The sperm concentration is the total number of spermatozoa per unit volume of semen (Brito et al. 2016). This sperm parameter can be measured by counting sperm in a haemocytometer or using a spectrophotometer (Boe-Hansen and Satake 2019). When a haemocytometer is used, sperm concentration can be calculated as the total sperm per ejaculate by multiplying the concentration by the ejaculate volume (Boe-Hansen and Satake 2019).

On the other hand, when a spectrophotometer is used, spermatozoa should be mixed with sodium citrate (Ngcobo et al. 2023). In the current meta-analysis, the included studies showed that supplementing OMFA improves sperm concentration (MD = 71.12, 95% CI: 15.97–126.26) significantly.

Previous studies confirmed that sperm concentration can be significantly improved when flaxseed oil (2% and 4%) is supplemented in Friesian bulls (El-Hamd et al. 2019). Furthermore, supplementing flaxseed oil (125 and 250 ml/day) led to higher sperm concentration in Nilli Ravi bulls (Shah et al. 2016). Notably, the level of flaxseed oil does not influence the sperm quality results (Khoshniat et al. 2020).

Effect of enriching the bulls' diet with OMFA on live sperm

Live sperm can be defined as viable spermatozoa and are crucial for fertility (Zoca et al. 2023). This meta-analysis evaluated the effectiveness of omega-3 fatty acids on live spermatozoa. It was found that supplementing OMFA can improve live sperm by 11.93% [MD = 11.93; CI (7.30; 16.55)]. Live sperm were improved in Friesian bulls following the flaxseed oil supplementation (El-Hamd et al. 2019). Live sperm were measured before the supplementation of different OMFA sources. For instance, Ahmad et al. (2019) found that linseed oil can improve live sperm. Moreover, similar results

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were obtained in Nilli Ravi bulls following the dietary flaxseed oil (125 and 250 ml/day) supplementation (Shah et al. 2016). Nevertheless, substantial heterogeneity suggested higher variability in the included studies.

Effect of enriching the bulls' diet with OMFA on sperm motility

Sperm motility refers to the motile spermatozoa. This parameter has been found to correlate with fertility in bulls (Rosyada et al. 2021), hence the interest in improving sperm motility using dietary omega-3 fatty acids. This meta-analysis confirmed that dietary omega-3 improves sperm motility by 9.24% [MD = 9.24; CI (7.39; 11.09)]. Previous studies observed an increase in sperm motility following dietary flaxseed oil supplementation in Friesian bulls (El-Hamd et al. 2019). Supplementing omega-3 fatty acids has been proven to increase sperm motility in bulls (Castellini et al. 2019). Moreover, supplementing linseed oil in Nili Ravi bulls improved sperm motility significantly (Ahmad et al. 2019). In Mithun bulls, flaxseed oil supplementation (150 ml) improved both fresh and frozen-thawed sperm motility (Perumal et al. 2019). In Nilli Ravi bulls, supplementing flaxseed oil (125 and 250 ml/day), sperm motility was improved after 8 weeks (Shah et al. 2016). Nevertheless, Yuan et al. (2023) found that flaxseed oil improves semen quality when supplemented with vitamin E. Similar results were observed in sheep when flaxseed oil was supplemented with ascorbic acid (Ngcobo et al. 2023).

Effect of enriching the bulls' diet with OMFA on plasma membrane integrity

The plasma membrane integrity increased in the flaxseed oil-supplemented group (El-Hamd et al. 2019). Plasma membranes were improved in the linseed-supplemented group (Ahmad et al. 2019). Moreover, flaxseed oil has been proven to improve even frozen-thawed sperm motility (Perumal et al. 2019). The plasma membrane integrity was improved in Nili Ravi bulls following the supplementation of flaxseed oil (125 and 250 ml/day) (Shah et al. 2016).

Effect of enriching the bulls' diet with omega-3 on sperm progressive motility

Progressive motility could be defined as the percentage of the spermatozoa actively moving forward (Peter et al. 2021). Progressive motility has been found to correlate with semen quality and *in vitro* fertility (Li et al. 2016). In this meta-analysis, there was no significant improvement in progressive motility following the OMFA-enriched bull diet. The major long-chain polyunsaturated fatty acids found in omega-3 fatty acids are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). They are both known for enhancing the sperm progressive motility (Lass and Belluzzi 2019; Ngcobo et al. 2021), when fed for long enough (Hosseini and Khalili 2017).

Nevertheless, previous studies reported that progressive motility was improved in Holstein bulls when fish oil was supplemented (Khoshniat et al. 2020). On the other hand, it appears that individual variability may affect the absorption of omega-3 fatty acids among different animals following dietary supplementation, such as metabolic responses and rumen biohydrogenation efficiency (Toral et al. 2021).

CONCLUSION

The meta-analysis demonstrated a positive effect of enriching the bull diet with omega-3 fatty acids on bull semen volume, live sperm, sperm motility, and plasma membrane integrity. This meta-analysis demonstrated that enriching the bull diet with OMFA improves the majority of semen parameters, except for sperm progressive motility, and this might be because LCPUFAs require a longer period of time to be incorporated into testicular and epididymal tissues to improve progressive sperm motility. Notably, some studies were weighted more heavily than others for sperm concentration, and a split analysis (excluding dominant studies) was conducted. Nevertheless, substantial heterogeneity was observed, indicating a high variability among the studies. The limited number of studies (<10 studies) led to an inability to conduct meta-regression to find the causes of high variability among the included studies. It was recommended that future studies should be conducted using a bigger sample size so that meta-regression and subgrouping can be conducted.

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Conflict of interest

The authors declare no conflict of interest.

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