Scientific Research Publishing

Open Journal of Animal Sciences, 2021, 11, 197-207
https://www.scirp.org/journal/ojas
ISSN Online: 2161-7627
ISSN Print: 2161-7597

Effect of Stabilized Fish Oil Source on Sperm Quality and Production of Boars

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How to cite this paper: Parsley, M.A., Wilson, M.E., Gall, T.J. and Ballard, M.R.M. (2021) Effect of Stabilized Fish Oil Source on Sperm Quality and Production of Boars. *Open Journal of Animal Sciences*, **11**, 197-207.
https://doi.org/10.4236/ojas.2021.112015

Received: March 3, 2021
Accepted: April 12, 2021
Published: April 15, 2021

Abstract

Research findings for supplementing boar stud diets with fish oils are inconsistent. This study was designed to address three possible causes of performance variation of boars to fish oil supplementation: stability of the fatty acid source, level of inclusion and breed of boars tested. Three groups of 87 boars each, from two genetic lines (PIC 337 and PIC 800), were assigned to treatment based on age, mean sperm production (previous 12 weeks), and body condition score. All boars received a corn-soybean meal diet with a commercial fish oil supplement providing 1.83 g/boar/day of docosahexaenoic acid (DHA) as a preconditioning diet. On 10-Aug., 2020, the DHA source was changed to a stabilized starch imbedded source of refined fish oil (Salmate®), providing 1.83 g/b/d for the test diet. Two additional levels providing 2.38 and 2.94 g/b/d of DHA were fed for a 9 week pretreatment period and during the test period. Salmate® fed at 2.38 g/b/d of DHA resulted in a reduction in the number of rejected ejaculates ($P < 0.045$) by 7.5% and 6.4% compared to the lowest and highest inclusion rates, respectively. There were no treatments by genetic line interactions. A retrospective study of semen production and quality of 77 boars on the Salmate® diet containing 1.83 g/b/d DHA was done to compare to the original source of DHA at the same inclusion level. There were no differences in semen quality parameters between the 2 lipid sources. Ejaculate volume increased from 177.9 ml to 233.4 ml ($P < 0.001$) and total sperm cells per ejaculate increased from 69.7×10^9 to 82.0×10^9 ($P < 0.001$) due to substitution of Salmate®. Adding Salmate® at 2.38 g/b/d resulted in a lower number of rejected ejaculates per boar by 7.5% and 6.4% vs. 1.83 and 2.94 g/b/d, respectively, and boars fed Salmate® at 1.83 g/b/d produced 17% more doses than the competing product.

Keywords

Boars, Docosahexaenoic Acid (DHA), Salmate®, Total Sperm, Semen Doses

1. Introduction

Artificial insemination is a common practice in many parts of the world and has resulted in rapid genetic improvement and competitiveness in the swine industry. Boars are selected for breeding based on the traits that they possess, but genetic progress and efficient production can only be achieved if sperm quality is up to standards and is adequate to insure sow pregnancy.

While sperm quality and yield are influenced by many factors, a key component is an insurance that the diet boars receive is nutritionally adequate with requirements for minerals, vitamins and amino acids having been previously established by the National Research Council [1]. Requirements for essential fatty acids, however, have not been succinctly determined. The beneficial effects of essential fatty acids, in particular docosahexaenoic acid (DHA) on spermatogenesis, sperm maturation, sperm quality and male reproductive system maintenance are well known [2]. Importantly, the inclusion of DHA in the diet has been demonstrated to lengthen storage time and improve freezability of boar semen after dilution [3].

Other studies have demonstrated the beneficial effects of adding DHA to diets for boars. It has been demonstrated that tuna oil with added vitamin E improved sperm cell viability and progressive motility [4]. When the fish oil based supplement was added to boar diets it was found to increase sperm per ejaculate by 11% in pigs receiving the supplement over the experimental period compared with control pigs over the same time period [5].

The uptake of omega fatty acids by sperm cells has been found to be much greater when menhaden oil was added to the boars' diet, but this did not translate into improved sperm production or quality [6]. Exposure to stress was suggested as a mitigating factor in this trial [7]. It has been suggested that Duroc boars may be less responsive to added fatty acids than boars of the Large White or Pietrain breeds [8].

Sperm cells are rich in polyunsaturated fatty acids and are therefore susceptible to oxidation. When the pro-oxidant to antioxidant balance shifts to favor oxidation, sperm concentration and motility can become impaired [9]. The presence of reactive oxygen species, emanating from dietary lipids, has been identified as a cause of low sperm motility and testicular anomalies [2]. Hence, any beneficial effects of DHA can be neutralized or even become negative under such conditions. The purpose of this study was to evaluate the inclusion of a stabilized marine lipid (Salmate®) in diets provided to mature boars in a commercial stud at 3 levels compared to a traditional source of marine polyunsaturated fatty acids. In addition, 2 different genetic lines were evaluated, PIC 337 (Pietrain cross) and

PIC 800 (Duroc), to determine if the responses differed by breed.

2. Materials and Methods

2.1. Animals and Treatments

The experiment was conducted at the Geode Gene Transfer Center boar stud (TriOak Foods, Inc., IA, USA). Boars were housed in individual stalls on slatted floors over a deep pit in an air-conditioned barn with a positive pressure air filtration system. Fresh water, sourced from a public (rural) water supply, was provided by individual nipple waterers and feed was supplied individually each morning based on the body condition score (BCS) of each boar. Using a BCS ranking system of 1 - 5, a BCS of 3+ is considered ideal, and 3.2 kg of feed per boar per day was provided to maintain boars at this level. Boars with a lower BCS were fed additional feed and boars with a higher BCS were fed slightly less.

Boars were Pig Improvement Company (PIC, Hendersonville, TN, USA) terminal sires developed for optimum growth performance and carcass quality. PIC 337 (Pietrain cross) and PIC 800 (Duroc) lines were used in the study. Three groups of 87 boars per group, representing all of the mature boars available at the time the study was run, were assigned with each group made up of 54 PIC 337 and 33 PIC 800 boars. A power test was not run to determine the number of boars required to answer these questions prior to the initiation of the trial. Boars were assigned to treatment to equalize age, average sperm production in the previous 12 weeks and BCS.

Boars received a corn-soybean meal diet that was balanced for net energy and formulated to meet or exceed requirements for amino acids, minerals and vitamins as specified by NRS [1]. All boars were maintained on the control diet before the start of the study for a minimum of 12 weeks (**Table 1**). The control diet provided 1.83 g of DHA per boar per day from a commercially available marine omega-3 fatty acid supplement, fed according to the manufacturer's recommendations. The 3 test diets contained an omega-3 supplement composed of refined fish oils, vitamin E and hydrophobic starch (Salmate®, The Ballard Group Inc., Cincinnati, OH, USA) at an inclusion level calculated to provide 1.83 g, 2.38 g and 2.94 g per boar per day of DHA for test diets 1, 2 and 3 respectively.

2.2. Trial Part 1

For the first part of the experiment, test diets 1 through 3 were compared to determine if the level of inclusion of supplemental DHA via the Salmate® ingredient influenced semen volume, sperm concentration and sperm quality characteristics in the 2 boar lines. The source of the marine fatty acids in the boar stud diet was changed to Salmate® on 10-Aug., 2020 and all boars received the test diet from 10-Aug., 2020 until completion of the trial on 02-Nov., 2020. For test diets 2 and 3, boars received the additional Salmate® via top-dress at 8 g and 16 g per boar per day, respectively. Semen samples collected between 12-Oct., 2020 and 02-Nov., 2020 were included in the analysis, allowing a 9 week period of exposure to the product for sperm cellular growth and development.

Table 1. Composition of diets.

Ingredient Name	Diet (Kg)	
	Control	Test
Corn Grain	665.15	670.25
Soybean meal	162.75	162.75
Soybean Hulls	100	100
Monocalcium phosphate	19.2	19.2
Choice White Grease	9.7	9.7
Calcium carbonate	9.1	9.1
Omega 3 Supplement[a]	13.3	0
Salmate®-Omega 3 Supplement[a]	0	8.2
Salt	6.05	6.05
Vitamin -Mineral Premix[b]	14.75	14.75
KCal Net Energy	2,358	2,344

[a]Omega 3 sources supplied 1.83 g DHA per boar per day for each diet; [b]Vitamin-mineral premix, synthetic amino acids, choline chloride, and phytase were isogenous for both diets.

2.3. Trial Part 2

For the second part of the study, production data from 77 of the 87 boars on the control diet (previous DHA source) were compared to data from the same boars on test diet 1, with both diets providing 1.83 g DHA per boar per day. Boars were eligible for inclusion if they had received the control diet for a minimum of 9 weeks prior to collection of semen parameter data, and were in production through 02-Nov., 2020. Data collected from 14-June, 2020 through 05-July, 2020 were used to represent the control feeding period. Semen collected between 12-Oct., 2020 and 02-Nov., 2020 from the same boars was evaluated as the test feeding period.

2.4. Semen Collection

Semen was collected by the gloved hand technique from boars previously trained to mount dummy sows. Ejaculate volume was determined by weight. Within 2 minutes of completion of ejaculation, a sample of the raw semen was diluted at a 1:14 ratio with AndroStar Plus® semen extender (Minitube USA, Inc., Verona, WI, USA) and processed through an eFlow® chamber to determine sperm concentration and quality (Androvision® Computer Assisted Sperm Analysis System (CASA), Minitube USA, Inc., Verona, WI, USA). Once these measurements were completed, the data were automatically transferred into Prism® software (Minitube USA, Inc., Verona, WI, USA). The CASA analysis yielded data on sperm concentration, motility, both progressive and non-progressive, and gross morphology, which included percent proximal and distal cytoplasmic tail droplets as well as translocated tail abnormalities. Findings were automatically trans-

ferred into Prism® software and recorded. From these results, the program determined the number of semen doses to make at 2.25×10^9 total sperm per dose for ejaculates that met minimum requirements for customer use.

Ejaculates were required to meet the following criteria to be accepted for use:

1) Combined percent of morphological defects (proximal and distal cytoplasmic droplets and translocated tails) could not exceed 25%.

2) Total motility must be >80% and progressive motility must be >70%.

3) Minimum doses per ejaculate must be >5.

2.5. Chemical Analysis

Feed samples for each of the diets were analyzed by Eurofins, Des Moines, IA, USA to determine the level of fatty acids supplied by the diets. This analysis of the average value of DHA level in the feed samples was within 7% variance of calculated levels of DHA.

2.6. Statistical Analyses

For sperm quality parameters, statistical analysis was conducted using a general linear model determined through stepwise regression and included the fatty acid treatment, the 2 genetic lines of boars, the age of the boars and the number of days rest between the collection of semen. The analysis was carried out using Minitab 16 Statistical Software® (Minitab LLC, State College, PA, USA). Chi-square analysis was conducted to assess differences between ejaculate rejection rates. A two tailed test with Yates corrected was used.

3. Results

3.1. Results for Trial Part 1

Nine of the boars originally assigned to treatment were not available for the duration of the trial. Two boars in each of treatments 1 and 2 died, and 5 boars from treatment 1 were moved out of the program by facility management. The remaining 159 PIC 337 boars averaged 824 days of age, with a range from 332 to 1341 days of age. For the PIC 800 boars, 93 boars remained that averaged 538 days of age, with a range from 366 to 1192. The number of days between semen collections averaged 6.84 ± 3.01 for the PIC 337 line and 6.01 ± 2.86 days for the PIC 800 boars. In the final analysis, there were 51 PIC 337 and 29 PIC 800 boars in test diet 1, 54 PIC 337 and 31 PIC 800 boars in test diet 2, and 54 PIC 337 and 33 PIC 800 boars in test diet 3.

Results showing the effects of the 3 dietary levels of Salmate® are provided in (Table 2). There were noteworthy differences between the 2 genetic lines for this phase of the research. Sperm concentration was greater for the PIC 800 line ($P < 0.001$), while ejaculate volume was greater with the PIC 337 line ($P < 0.001$). Other differences were not significant ($P > 0.2$) between the lines. There were no differences in the individual sperm quality parameters measured that could be attributed to the dietary treatments.

Table 2. Results obtained from ANOVA General Linear Model (Part 1).

	Treatment			Line		SEM	P Value		
	Test 1	Test 2	Test 3	L337	L800		Treatment	Line	T x L
Motility, %	83.1	83.6	82.8	83.5	82.5	0.38	0.869	0.347	0.788
Progressive motility, %	76.1	76.2	76.2	77.2	75.6	0.49	0.951	0.318	0.898
Proximal droplets, %	7.0	8.6	8.1	8.1	7.6	0.24	0.345	0.259	0.062
Distal droplets, %	9.6	9.5	9.5	9.4	9.9	0.48	0.971	0.583	0.446
Translocated tails, %	0.3	0.7	0.3	0.5	0.3	0.11	0.156	0.274	0.450
Concentration $\times 10^9$	0.391	0.412	0.394	0.347	0.450	0.02	0.570	0.001	0.424
Doses possible[3]	34.2	34.6	33.7	34.0	34.3	0.58	0.872	0.824	0.168
Volume, ml	231.6	219.6	227.4	255.3	210.4	4.97	0.553	0.001	0.187
Total sperm, $\times 10^9$	81.8	82.8	80.3	80.4	82.7	1.40	0.765	0.486	0.194

Mean of 2.25×10^9 cells/dose.

There were no diets by genetic line interactions for any of the parameters measured.

Findings for semen rejection parameters are provided in (Table 3). The table shows the incidences of both the rejected as well as the retained occurrences for each measurement and when combined, they add up to the total number of samples within each treatment. The overall rejection rate was low with all treatments. While there were no differences in the individual semen quality parameters, the intermediate treatment level resulted in fewer ejaculates being rejected due to low motility ($P < 0.026$) and fewer total ejaculates being discarded ($P < 0.045$). This overall increase in the percent of ejaculates accepted for treatment 2 equates to 7.5% and 6.4% more ejaculates processed compared to the lowest and highest levels of DHA inclusion, respectively.

3.2. Results for Trial Part 2

Ten of the originally assigned boars were not available at the end of the trial and were not included in either data set. Two died, 3 were not available for the entire control period and the remaining 5 boars were transferred out of the program by facility management. The remaining were 49 PIC 337 and 28 PIC 800 boars. For this part of the trial, the PIC 337 boars averaged 762 days of age, with a range of 251 to 1340 days of age. The PIC 800 boars averaged 482 days of age, with a range of 247 to 1133 days of age. The PIC 337 boars averaged 6.76 ± 1.38 days rest between collections while the PIC 800 boars averaged 6.06 ± 1.49 days rest. Results (Table 4) showed that there were no differences in the extent of sperm abnormalities between the 2 dietary fatty acid sources [proximal cytoplasmic droplets ($P = 0.33$), distal cytoplasmic droplets ($P = 0.68$), translocated tail abnormalities ($P = 0.21$)]. However, the concentration of sperm was lower ($P < 0.023$) for the boars during the test period (Salmate®) compared to the control feeding period. This was offset by a greater volume of semen per ejaculate during

the test feeding period ($P < 0.001$), resulting in a greater number of total sperm cells ($P < 0.001$) and significantly more insemination doses being produced per ejaculate ($P < 0.001$).

Results in Part 2 of this study (Table 5) show that there were no statistical differences in the number of rejected ejaculates between the Salmate® treatment and the previous source of DHA with all P values greater than 0.17 for each of the parameters measured.

Table 3. Effects of Salmate® on factors resulting in ejaculate rejection (Trial Part 1).

	Treatment			P Value
	1	2	3	
Tail Abnormality	2	1	1	0.735
Normal tail morphology	246	261	269	
No Sperm	0	1	0	0.783
Normal ejaculate	248	261	270	
Training	3	0	1	0.326
Normal collection	245	262	269	
Under 5 doses	1	0	0	0.811
Normal production level	247	262	270	
Distal Droplets	15	15	16	0.989
Normal tail morphology	233	246	254	
Proximal droplets	1	1	2	0.833
Normal tail morphology	247	261	268	
Low motility	25a	12b	28a	0.0261
Normal motility	223	250	242	
Total discarded	47	30b	48a	0.0448
Total processed	201	231	221	

Table 4. Results obtained from ANOVA General Linear Model (Part 2)[1].

	Treatment		Line		SEM	P Value		
	Before	After	PIC 337	PIC 800		Treatment	Line	T*L[2]
Motility, %	84.5	83.1	85.2	82.4	0.55	0.137	0.003	0.965
Progressive motility, %	77.1	76	75.5	74.5	0.74	0.409	0.004	0.997
Proximal droplets	6.4	6.9	6.6	6.7	0.24	0.331	0.742	0.122
Distal droplets	9.4	9.8	8.6	10.5	0.68	0.678	0.145	0.884
Translocated tails	0.5	0.3	0.3	0.5	0.05	0.21	0.349	0.433
Concentration	0.428	0.387	0.369	0.455	0.01	0.023	0.001	0.855
Doses possible[3]	29.2	34.3	32	31.4	0.76	0.001	0.706	0.468
Volume, ml	172.8	234.8	228.9	178.9	6.35	0.001	0.001	0.617
Total sperm, $\times 10^9$	69.7	82	76	75.8	1.84	0.001	0.964	0.584

[1]GLM included treatment, boar line, and number of days of rest; [2]Treatment *Line interaction; [3]Mean of 2.25×10^9 cells/dose.

Table 5. Effects of Salmate on factors resulting in ejaculate rejection (Part 2).

	Treatment		P Value
	Control	Test	
Tail Abnormality	1	2	0.854
Normal tail morphology	294	237	
No Sperm	1	0	0.812
Normal ejaculate	294	239	
Training	0	3	0.176
Normal collection	295	235	
Under 5 doses	1	1	0.917
Normal production level	294	238	
Distal Droplets	19	15	0.930
Normal tail morphology	275	224	
Proximal droplets	2	1	0.687
Normal tail morphology	292	238	
Low motility	18	22	0.234
Normal motility	277	217	
Total discarded	42	44	0.236
Total processed	253	195	

4. Discussion

4.1. Trial Part 1

There were no effects of DHA level fed on the individual semen quality parameters measured ($P > 0.15$) in this trial (Table 2). However, (Table 3) clearly shows that the overall ejaculate rejection rate was significantly lower ($P < 0.045$) for the intermediate level of Salmate® compared to the lowest and highest levels of inclusion, resulting in 7.5% and 6.4% more ejaculates passing minimum standards for processing into semen doses. It has been found previously that the concentration of DHA was lower in low motility boar sperm [10], but these researchers accepted 60% motility as adequate, which is much lower than this trial. It has also been determined that DHA improved semen volume and total sperm numbers per ejaculate, much as occurred in Part 2 of this trial, but did not influence motility when 80% was used as the cut-off point for rejected ejaculates [11]. The fact that there was no additional response to the highest inclusion level of DHA warrants further evaluation of the 3 levels of DHA investigated in this study to determine whether higher levels of DHA coupled with adjustments to the diet could have a larger impact on semen quality parameters, ejaculate rejection rates, and/or total semen production than were observed in this trial.

When the trial was designed, it was not known if the PIC 800 (Durocs) would have a higher requirement for DHA, as suggested by previous work [8]. The lack

of an interaction between genetic lines and DHA feeding levels in this trial indicates that this is not the case. One limitation of this trial is there was no negative control group that did not receive supplemental DHA in the diet. Additional research is also warranted to further evaluate potential performance differences between the Duroc line tested in this study and other Duroc lines.

4.2. Trial Part 2

These results clearly demonstrated a significant advantage ($P < 0.001$) for the stabilized marine lipid supplement (Salmate®) which increased total sperm output per ejaculate over the control in Part 2 of this experiment. While the motility and progressive motility remained unchanged for these boars, the volume of semen and the total number of sperm cells per ejaculate increased with the treatment regimen, providing a clear economic advantage to this collection facility. It has been previously reported that boar diets containing supplemental algae rich in DHA increased semen volume without altering semen quality parameters [11].

All results presented are for the same boars and demonstrate an improvement in total sperm output when the stabilized fatty acid supplement (Salmate®) was substituted for the DHA product previously used by this facility. It can be argued that the time periods for Part 2 of the trial were different, potentially favoring the Salmate® product. However, this should have worked against the treatment feeding program. It was previously found that sperm concentration, volume and number of potential doses of semen declined with declining photoperiod [12]. Day length was reduced for the treatment group of boars compared to the controls in this trial.

Age was rejected as a factor in the regression model due to the short time between sampling periods. In an extensive meta-analysis of over 250,000 records, it was determined that age influences semen production, but only over the long term, with optimum age being 3.5 years for maximum sperm output for boars [13]. Due to housing conditions, heat stress would not have been a factor in this study. PIC 800 boars were included in the trial because a previous publication showed that omega-3 supplemented diets positively affected both sperm morphology in Large White and Pietrain breeds as well as the osmotic resistance of Pietrain spermatozoa [8]. No effects were seen in the Duroc boars in that study. However, in this trial, the PIC 800 (Duroc) line responded positively to the treatment program with an increase in total sperm output.

As with Part 1 of this trial, there is the limitation that no negative control was included that would demonstrate differences between the two DHA products added and no DHA in the diet.

4.3. Conclusions

Salmate® significantly reduced the percent of rejected ejaculates when fed at 2.38 g DHA per boar per day, compared to 1.83 g and 2.94 g, by 7.5% and 6.4%, re-

spectively, in mature boars in a commercial boar stud in two terminal genetic lines. Further investigation is warranted to determine if increasing levels of this protected DHA product (Salmate®) coupled with other dietary adjustments would result in further increases in sperm output, improved sperm quality and/or further reductions in the percent of rejected ejaculates. Salmate® also significantly increased total sperm output without altering sperm cell quality or the percent of rejected ejaculates when compared to another commercially available product when both provided 1.83 g DHA per boar per day. Although sperm concentration was reduced, total volume, and thus, the total number of sperm cells increased per ejaculate by 17%.

Acknowledgements

The authors wish to acknowledge Dr. Essi Evans, E + E Technical Advisory Services, for sorting and compiling the data and providing all statistical analyses.

Conflict of Interest

Mr. Michael Parsley markets Salmate®. Mr. Malcolm Ballard's company manufactures Salmate®. Both were involved in the designing of this experiment but were not involved in the collection or analysis of data or the interpretation of the results.

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