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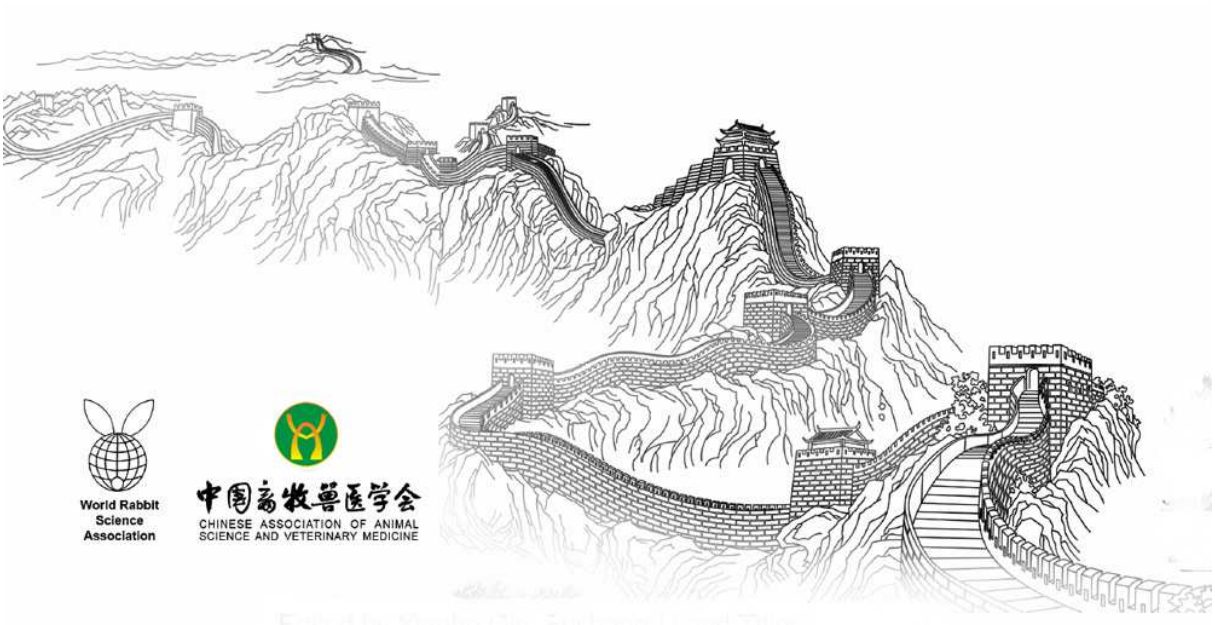

ORAL ADMINISTRATION OF n-3 POLYUNSATURATED FATTY ACIDS AND RABBIT REPRODUCTIVE PARAMETERS

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ABSTRACT

Multiple effects of n-3 polyunsaturated fatty acids (PUFAs) have been reported at plasma membrane level of different cell tissues and animal species, however, little has been done on rabbit gametes. This study was conducted to evaluate the effect of n-3 PUFAs oral administration on standard semen parameters and reproductive performance of rabbits. Ten sexually active bucks and twenty multiparous does were randomly assigned to either control or treatment groups, and received an oral dose of either 40mg/kg of body weight of n-3 PUFAs (from salmon oil, containing eicosapentanoic EPA and docosahexanoic acid DHA), or an equivalent volume of distilled water, once daily during 50 days. A total number of one hundred ejaculates were evaluated, before and after treatment; also, four groups of does were randomly assigned to see the n-3 PUFAs treated and non-treated male and female interactions on reproductive performance: group 1: (-)(-) does without treatment mated with bucks without treatment; group 2: (-)(n-3) does without treatment mated with bucks with n-3 PUFAs treatment; group 3: (n-3)(-) does with n-3 PUFAs treatment mated with bucks without treatment; and group 4: (n-3)(n-3) does with n-3 PUFAs treatment mated with bucks with n-3 PUFAs treatment. Sperm viability percentage was significantly increased after n-3 PUFAs oral administration (78.88±8.04 and 83.56±7.6%; P=0.01), compared to the control group (80.24±5.63 and 80.64±8.94 %; P>0.05), however, statistical differences on semen volume (0.65±0.27 and 0.64±0.29 ml) and percentage of morphoanomalies (5.42±1.84 and 5.58±3.35%) before and after treatment, respectively, were insignificant (P>0.05). Interactions between n-3 PUFAs treated and non-treated does and bucks had no significant effect on the reproductive performance evaluated, including conception rate, litter size and gestation length (P>0.05). No nest mortality was observed in the treated does. In conclusion, n-3 PUFAs oral administration to adult rabbits had a positive effect on sperm quality, but farther research needs to be performed to find defined effects of PUFAs on rabbit gametes and its impact on reproductive performance.

Key words: n-3 PUFAs, sperm quality, reproductive performance.

INTRODUCTION

The multiple effects of n-3 polyunsaturated fatty acids (n-3 PUFAs) can be reflected in plasma membrane, when n-3 PUFAs are introduced, the lipid composition of the microdomains gets altered, reducing the sphingomyelin and cholesterol content in lipid rafts (Raza *et al.*, 2003). Ma *et al*. (2004a) concluded that n-3 PUFAs incorporation alters membrane properties, such as protein functionality and microdomain localization of signaling proteins, resulting in modulation of downstream cellular signaling pathways. There is considerable evidence that dietary PUFAs supplementation can influence biosyntetic pathways involved in metabolism of important reproductive hormones such as prostaglandin, progesterone and oestradiol synthesis that have multiple roles in female regulation of reproductive function (Wathes *et al.*, 2007). Many reports have been made to see the effect of n-3 PUFAs administration on tissues of different species; however, less has been reported on rabbits and its effects on reproduction. Therefore, the aim of the present study was to evaluate the effects of n-3 PUFAs oral administration to adult rabbits, on their reproductive performance, including semen quality, conception rate, litter size, gestation length and nest mortality before weaning.

MATERIALS AND METHODS

Animals and experimental design

The present research was done in the Rabbit Production Area of the Faculty of Veterinary Medicine from the Universidad Autónoma del Estado de México, located in the center Valley of México, 2632 m.a.s.l., with cool weather most of the year. Animals were housed individually in wire cages (80 x 60 x 40 cm) provided with galvanized feeders and nipple drinkers. Water and commercial feed for breeders (containing about 17% crude protein and 2700 kcal ME/kg DM) was given *ad libitum*. All rabbits were maintained under the same environmental and management conditions. A total number of 10 Californian x New Zealand bucks and 20 New Zealand does of 21 months of age, weighing an average of 3845±560.11 and 4150±577.76 g, respectively, were used. All does had 3 previous parities and males were trained to the semen collection with artificial vagina having similar seminal parameters at the time the experiment begin. Treatments for n-3 PUFAs (n-3) or control group (-) given in distilled water were randomly assigned, therefore, half of the animals received either treatment during 50 days. The experiment started in April and ended in June, 2014 (spring and early summer) and the light regime received was about 15 to 16 h/day (natural light).

Treatment dose

The n-3 PUFAs were obtained from a commercial brand (Prime ™ Omega-3; Natrillium Inc, CA, USA) containing 360 mg/capsule of EPA (eicosapentanoic) and 240mg/capsule of DHA (docosahexaenoic)) for human use, which was extracted from fish oil. Dose was calculated for each individual, adjusting it to 40 mg/kg of body weight, and an equivalent volume of distilled water was given to the control group. Therefore the average dose received by each animal contained 3.15 kcal, 90 mg EPA and 60 mg DHA. Oral administration of either treatment was performed daily and individually with a disposable 1ml syringe per animal during 50 days. Does received the same treatment 20 days before mating and during the entire pregnancy period, therefore resulting a total of 50 days treatment.

Mating

Natural mating was allowed when does presented lordosis reflect and red and edematous vulvas, taking does into the cages of bucks, allowing two services per doe. Mating was performed 50 days after treatment was given to males, however does were mated after day 20 of treatment. Four groups of 5 does were randomly assigned to compare conception rate (number of pregnant does at parturition/ number of mated does x100), total born, gestation length, and nest mortality of groups with or without n-3 PUFAs treatments. Group 1: (-)(-) does without treatment mated with bucks without treatment; group 2: (-)(n-3) does without treatment mated with bucks with n-3 PUFAs treatment; group 3: (n-3)(-) does with n-3 PUFAs treatment mated with bucks without treatment; and group 4: (n-3)(n-3) does with n-3 PUFAs treatment mated with bucks with n-3 PUFAs treatment.

Semen collection and evaluation

Considering that the n-3 PUFAs effect would be reflected after a whole spermatogenic cycle, a total of 100 semen samples were evaluated starting at day 43 of treatment (10 samples we taken weekly during 10 weeks). Volume, motility, viability, concentration and morphoanomalies of each sample were evaluated and recorded immediately, after collection with an artificial vagina. Semen volume was measured using a graduated tube and progressive motility was observed under a light microscope placing a 10 µl drop of semen mixed with the same volume of tempered PBS under a tempered slide cover, ranging movement from 0 to 100% scale. Viability and morphoanomalies were determined by eosin-nigrosine staining, counting 100 cells twice in at least 8 different fields per duplicate and visualizing the sample under a 400x magnification. Sperm concentration was determined by Neubauer chamber.

Statistical Analysis

Statistical analysis of conception rate and litter size was performed by Chi-square (α=0.05), while variables of semen quality were analyzed by the repeated measures analysis (Friedman repeated measures analysis of variance on ranks) and pairwise multiple comparison procedures were performed by Tuckey´s test, using Sigma Plot, Version 12.5 (Systat Software, Inc.).

RESULTS AND DISCUSSION

Semen Parameters.
Differences in semen volume and motility were not observed (Table 1).

Table 1: Weakly evaluation of volume and motility of buck semen (n=10) in response to a treatment with n-3 PUFA´s, lasting 50 days. Week 1 corresponds to day 43 of treatment. .

Weeks	Volume (ml)		Motility (%)	
	control	omega-3	control	omega-3
1	0.74 ± 0.31	0.72 ± 0.33	72.00 ± 23.61	69.00 ± 19.49
2	1.00 ± 0.612	0.58 ±0.31	68.00 ± 27.97	75.00 ± 10.00
3	0.94 ± 0.60	0.64 ±0.22	72.00 ± 13.04	84.00 ± 5.47
4	0.94 ± 0.60	0.52 ± 0.38	87.00 ± 4.47	77 ± 12.04
5	0.84 ± 0.39	0.88 ± 0.27	73 ± 14.83	82.00 ± 8.37
6	1.16 ± 0.80	0.82 ± 0.42	88.00 ± 2.74	83.00 ± 4.47
7	1.06 ± 0.71	0.54 ± 0.18	70.00 ± 23.45	58.20 ± 19.47
8	0.54 ± 0.27	0.74 ± 0.38	75.00 ± 10.61	71.20 ± 19.48
9	1.04 ± 0.32	0.54 ± 0.25	62.00 ± 25.88	85.00 ± 5.00
10	0.96 ± 0.36	0.60 ± 0.24	75.00 ± 12.75	80.00 ± 14.58

Means and standard error are shown, the interaction time x treatment was not statistically different (P>0.05).

Along the entire experiment, a high range of variability was observed in sperm viability (70.2-90.8%) and concentration (110.5-528.3 x 10^6 sperm/ml) (Table 2). In contrast, the percentage of morphoanomalies did not differed in either group, ranging from 3 to 10 percent in both treatments (P>0.05). Therefore, a clear effect of time and the given treatment on semen parameters cannot be explain.

Table 2: Weakly evaluation of sperm viability and concentration of buck semen (n=10) in response to a treatment with n-3 PUFA´s, lasting50 days. Week 1 corresponds to day 43 of treatment

Weeks	Viability (%)		Sperm concentration (x10^6/ml)	
	control	omega-3	control	omega-3
1	79.6 ± 6.27^{b}	78.4 ± 8.96^{b}	528.3 ± 182.76^{a}	351.8 ± 229.44
2	70.2 ± 4.76$^{b, 1}$	76.0 ± 3.08^{b}	222.7 ± 119.05$^{a, b}$	238.6 ± 149.12
3	78.2 ± 4.09$^{b, 1}$	84.2 ± 4.76$^{a, b}$	212.2 ± 129.03$^{a, b}$	340.8 ± 83.71
4	87.2 ± 3.35^{a}	85.2 ± 4.97$^{a, b}$	478.5 ± 81.59^{a}	253.3 ± 161.79
5	77.6 ± 8.20^{b}	83.0 ± 6.86$^{a, b}$	354.7 ± 103.74$^{a, b}$	282.5 ± 66.33
6	92.6 ± 4.28$^{a, 2}$	92.8 ± 2.49$^{a, 2}$	317.8± 96.59$^{a, b}$	270.3 ± 115.55
7	82.2 ± 3.77$^{a, b}$	74.2 ± 5.31$^{b, 3}$	209.2 ± 107.89$^{a, b}$	249.6 ± 68.76
8	75.0 ± 3.54$^{b, 1}$	78.0 ± 5.09^{a,}	556.7 ± 64.98^{a}	488.3 ± 148.12^{1}
9	84.4 ± 5.18$^{a, b}$	87.8 ± 4.44$^{a, b, 2}$	209.9 ± 176.42$^{a, b}$	213.3 ± 118.38
10	88.8 ± 8.26^{a}	92.6 ± 1.67$^{a, 2}$	110.5 ± 52.23$^{b, 2}$	256.6 ± 207.06

Means and standard errors are shown. Lower-case superscripts indicate statistical differences among weeks within the same treatments (P<0.05). Different number superscripts indicate statistical differences between treatments (P<0.05).

Our results did not showed a significant increase on semen quality of bucks from the two groups along the experimental period. In contrast, Dolatpanah et al (2008), found an increase in goat semen quality, after supplementation of fish oil (2.5% dry matter), and observed a significant increase in concentration, viability and motility, but no differences in volume were observed, and reported that the best values were obtained when combining fish oil and vitamin E in goats feed. On the other hand, Al-Daraji et al (2010), reported semen improvement in Japanese quails given fish oil at 3% in the birds´ diets. Therefore, in our study, the amount of omega-3 from fish oil given, could have been too low to show an increase in bucks´ semen quality, since it was calculated according to bucks body weight. Previous studies demonstrated that modifications in the n-6/n-3 ratio in sperm and seminal plasma affect the sperm viability, inducing modifications of fertility rates (Blesbois et al., 1997).

Reproductive Performance.

Results of the n-3 PUFAs effect on reproductive performance of multiparous New Zealand does is set out on Table 3. No statistical differences in conception rate, litter size and gestation length were found ($P<0.05$), Others reported an improvement in conception rate of 8.3% and litter size of 0.84, and a decrease in nest mortality, after 4 months of supplementation with salmon fish oil and E-vitamin in a low fertility farm (Lleonart, 2005). Most of our results differ, probably due to differences in the length of PUFAs treatment, sample size, the amount of PUFAs given by other authors. Gestation length was not affected by the treatment, it contrasts with some reports were PUFAs supplementation, especially DHA, enhanced gestation length (Larqué *et al.*, 2012). Due to the limited size of the animal samples available to develop the present study, the fact that no statistical differences were found in the parameters analyzed, must be taken cautiously before denying the positive or negative effects of n-3 PUFAs on rabbit reproductive parameters.

Table 3 Reproductive performance of does under oral n-3 PUFAs treatment

	Group 1 (-) (-)	Group 2 (-) (n-3)	Group 3 (n-3) (-)	Group 4 (n-3) (n-3)
Conception rate (%)	100	60	60	80
(number of pregnant does / number of mated does)	(5/5)	(3/5)	(3/5)	(4/5)
Litter size at birth (average number of total born)	5.8 ± 3.13	5.6 ± 3.01	4.6 ± 2.51	7.25 ± 0.95
Gestation Length (days)	31.4±0.89	32.6±2.08	30.8±0.44	31.3±0.57
Nest mortality before weaning (%)	0.33	0.16	0	0

The first symbol corresponds to the groups of does (-) without treatment and (n-3) with n-3 PUFAs treatment, the second symbol indicates the group of bucks mated with the does: without treatment (-) or with n-3 PUFAs treatment (n-3).

CONCLUSIONS

Oral administration of 40 mg/kg of n-3 PUFAs to bucks, did not increased sperm quality after a 50 day period treatment; moreover, reproductive performance of rabbit does were not improved. Therefore, given these results, the reproduction of this experiment with a larger sample of bucks and does is strongly suggested, in order to better establish the n-3 PUFAs role on rabbit gametes and reproductive performance.

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