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Effect of Omega6:Omega3 Fatty Acid Ratios on Semen Quality of Malaysian Village Roosters

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Abstract: This experiment was conducted to study the effects of different dietary oil sources on qualitative and quantitative features of Malaysian village rooster's semen. Forty five Malaysian village roosters (BA Breed) at 30 weeks of age were randomly assigned to 1 of the 3 dietary treatments with 3 oil sources (fish oil, vegetable oil and cooked oil). Semen collected from roosters 2 times a week and analyzed by computer-assisted sperm analyzer (Hamilton Thorne Motility Analyzer; IVOS, Beverly, MA). The viability (live to dead spermatozoa ratio) was measured using eosin-nigrosin smears under light microscope and semen volume was determined by reading the scale on the tube. The results showed that in comparison to either vegetable or cooked oils, the addition of fish oil as a source of omega3 fatty acids to diets, significantly ($P < 0.05$) increased motile spermatozoa and average smoothed path velocity (VAP) but decreased static type of spermatozoa in village rooster's semen ($P < 0.05$). Conversely, the addition of cooked oil decreased motility and VAP of rooster's semen ($P < 0.05$). Dietary treatments with different oil sources had no significant effect on progressive traits such as semen volume, semen concentration and live and dead spermatozoa ($P > 0.05$). It was concluded that the addition of polyunsaturated fatty acids may improve semen quality in hot climates.

Key words: Village roosters · Oil sources · Semen quality · Motility

Abbreviation Key: VAP: Smoothed Path Velocity · VCL= Track Velocity (microns/sec) · VSL= Straight Line Velocity · ALH= Amplitude of Lateral Head Displacement · BCF= Beat Cross Frequency · LIN= Linearity (ratio of VSL/VCL) · STR= Straightness (ratio of VSL/VAP) · Elongation= head shape · DOS= superoxide dismutase activity · ROS= reactive oxygen species · TRP= turmeric rhizome powder

INTRODUCTION

Chicken spermatozoa are unique in their structure and chemical composition. The most important feature of lipid composition of the avian semen is the extremely high proportions of long chain polyunsaturated fatty acids (PUFAs) in the phospholipid fraction of spermatozoa. High PUFA proportion of the avian sperm is necessity antioxidant order to maintain specific membrane properties (fluidity, flexibility, etc).

The phospholipids of avian spermatozoa are characterized by very high proportions of C20-22n-6 polyunsaturated fatty acids (PUFAs), mainly docosatetraenoic (22:4n-6) and arachidonic (20:4n-6) acids [1-3]. It is known that the fatty acid composition of sperm membranes, especially their unsaturated components, determine their biophysical characteristics such as fluidity and flexibility as appropriate for their specific functions, including sperm motility and fertilizing capacity [4-5]. High levels of PUFAs may promote lipid peroxidation and limit the viability of chicken and turkey spermatozoa. Animal fat and oils from restaurant residuals have trans fatty acids. Trans fats interfere with enzymes needed to produce sex hormones; they decrease the levels of testosterone in male animals and increase the level of abnormal sperm [6]. Studying on women who consumed

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1-2.3% of their energy from trans fats showed that consumption of 2% of energy from trans fats rather than from n-6 PUFAs was associated with a significantly greater risk of ovulatory infertility. On the other hand, inclusion of calcium salt of trans fatty acid resulted in improvement in uterine health and increasing fertility in lactating dairy cow [7]. Furthermore, it is suggested that trans-octadecenoic fatty acids might influence uterine health and reproductive efficiency of early lactation dairy cows by altering prostaglandin release by the uterus [8].

In birds, fatty acids of the n-6 series (mainly 20:4n-6 and 22:4n-6) predominate. Therefore, the purposes of this research detailed herein were: 1) to test the efficacy of dietary long chain polyunsaturated fatty acids (PUFAs) on semen quality; 2) to compare the effects of cooked and fresh oil fatty acids on semen quality of village roosters.

MATERIALS AND METHODS

Birds and Housing: Village (also referred to as local or indigenous) chickens are the most common type of poultry raised in Malaysia. Forty five village chicken roosters, at 30 weeks of age were obtained from local breeder company in Putra Malaysia city (Serdang) and were assigned to 3 dietary treatments (Cod liver oil as a source of omega3 fatty acids, vegetable oil as a source of omega6 fatty acids and cooked oil containing trans fatty acids) with 15 roosters in each treatment. Roosters were housed in 3-floor cages (80×80×50 cm) with 3 roosters in each. Twelve hours light periods (12L: 12D), with incandescent light bulb was maintained. The average house temperature was 32°C and relative humidity was 80% saturation.

Dietary Treatments: Effects of dietary PUFAs were studied in a completely randomized design (CRD) using three sources of oil (Cod liver as fish oil, soybean oil as vegetable oil and cooked palm oil containing trans fatty acids as cooked oil) are shown in Table 1. Fatty acid profile of the oils were analyzed with gas chromatography (Table 2) then added to the diets to achieve the omega6:omega3 fatty acid ratio of 4 in fish and vegetable oil diets and 20 in cooked oil diet. All 3 corn-soybean mash diets were isocaloric and isonitrogenous. Feed and water were provided *ad-libitum* for 6 weeks of study. Roosters were adapted to the diets for the first 3 weeks of experiment and then the other 3 weeks dedicated for semen collection. All birds were in healthy conditions and no mortality and clinical sign of diseases were observed during the entire period of experiment.

Table 1: Experimental diets with different oil sources and omega6:omega3 ratios (%)

	Oil[2]				Oil		
Ingredients (%)	Fish[3]	Vegetable	Cooked	Composition	Fish	Vegetable	Cooked
Corn	37.88	34.34	35.12	CP (%)	12.9	12.9	13.5
Wheat	20.00	20.00	20.00	AME (Kcal/kg)	2740	2740	2740
Soybean Meal	9.91	9.64	12.74	T. P (%)	0.757	0.771	0.726
Barley	14.33	15.11	20.00	Av. P (%)	0.4	0.4	0.4
Wheat Bran	12.79	15.00	6.29	Ca (%)	1.2	1.2	1.2
Limestone	1.94	1.95	1.91	Na (%)	0.157	0.158	0.157
Di-calcium phosphate	1.61	1.59	1.67	Met (%)	0.259	0.259	0.249
Fish Oil	0.48	0.00	0.00	Met + Cys (%)	0.46	0.46	0.46
Vegetable Oil	0.00	1.22	0.00	Lys (%)	0.56	0.56	1.30
Cooked Oil	0.00	0.00	0.30	Fat (%)	2.99	3.71	2.59
Salt	0.33	0.33	0.33	Omega3 FAs	0.30	0.28	0.06
Vit & Min premix[1]	0.60	0.60	0.60	Omega6 FAs	1.21	1.13	1.21
Vitamin E	0.20	0.20	0.20	Omega6/omega3	4	4	20
DL-Met	0.04	0.04	0.02	Fiber	4.54	4.74	4.29
HCl-Lys	0.00	0.00	0.83	Choline	0.085	0.086	0.091

[1]Each kg of diet contains: Vitamin A, 36670 IU; vitamin D3, 3500 IU; vitamin K3, 5 mg; vitamin E, 340 IU; vitamin B1, 0.25 mg; vitamin B2, 12 mg; vitamin B5, 15 mg; vitamin B6, 4 mg; vitamin B9, 2 mg; vitamin B12, 0.03 mg; Choline chloride, 1000 mg; Iron, 50 mg; Zn, 100 mg; Mn, 120 mg; Cu, 10 mg; Se, 0.3 mg; Antioxidant, 1000 mg.

[2]Cod liver oil as fish oil; soybean oil as vegetable oil; cooked palm oil as cooked oil.

Table 2: Fatty acid profile of oils used in the experimental diets (%)

Fatty acids	Cooked oil[1]	Vegetable oil	fish oil
14:0	1.02	1.11	5.23
14:1	nd	nd	0.44
15:0	0.04	0.05	nd
16:0	34.66	36.77	15.08
16:1	0.22	0.42	7.59
18:0	3.78	4.12	2.99
18:1	45.98	44.58	20.31
18:1 Trans	1.36	nd	0.59
18:2 n-6	12.76	12.62	3.47
18:3 n-3	0.18	0.17	11.05
20:4 n-6	nd	nd	10.91
20:5 n-3	nd	0.05	9.5
22:5 n-3	nd	nd	1.65
22:6 n-3	nd	0.09	11.18
Total saturated fatty acids	39.5	42.05	23.3
Total unsaturated fatty acids	60.5	57.93	76.69
Total MUFA[2]	47.56	45	28.93
Total n-3 PUFAs	0.18	0.31	33.38
Total n-6 PUFAs	12.76	12.62	14.38
Total trans fatty acids	1.36	nd	0.59
n-6:n-3 ratio	70.9	40.71	0.431
Unsaturated to Saturated fatty acids ratio	1.53	1.38	3.29
PUFAs to Saturated fatty acids ratio	0.32	0.3	2.05

[1]cooked palm oil as cooked oil; soybean oil as vegetable oil; cod liver oil as fish oil.

[2]PUFAs, poly-unsaturated fatty acids; MUFA, mono-unsaturated fatty acids; n-3, omega3 fatty acids; n-6, omega6 fatty acids; nd, not detected.

Semen Collection: The roosters were adapted to the diets and were trained for semen collection within 3 weeks. The semen was collected twice a week as described by Burrows and Quinn method [9]. The semen volume was determined by reading the scale on the tube, which was started from 0 to 15 ml with a precision of 0.1 ml. Collected semen samples were diluted 1:1 (v/v) with TUR-2 diluent [10]. The viability (live to dead spermatozoa ratio) tests was performed using eosin-nigrosin smears [11] under light microscope. The experimental protocols were reviewed and approved by the Animal Care Committee of the University of Putra Malaysia.

Evaluation of Sperm Motility and Concentration: Sperm motility was determined using a computer-assisted sperm analyzer (Hamilton Thorne Motility Analyzer; IVOS, Beverly, MA). An aliquot of each sample (5 µL) was diluted with 100 µL of phosphate buffer saline (PBS). A subsample of this solution (5 µL) was placed on a Standard Count Analysis Chamber (Spectrum Technologies, Healdsburg, CA) and analyzed for motility. The following settings were used for computer-assisted sperm analyzer analysis: 30 frames acquired, frame rate of 60 Hz, minimum contrast of 25, minimum cell size of 4 pixels, path velocity (VAP) cutoff of 5 µm/s, progressive minimum VAP cutoff of 50 µm/s, progressive velocity cutoff of 10 µm/s, static head size of 0.27 to 4.24, magnification of 1.95 and a minimum of 500 sperm from at least 5 fields were observed for motility analysis.

Statistical Analysis: All measured criteria on the effect of dietary polyunsaturated fatty acids as n6:n3 of Malaysian chicken roosters on semen quality achieved by computer-assisted sperm analyzer were analyzed by one-way ANOVA using GLM of SAS [12] with oils as main effects. Semen qualitative and quantitative features data were analyzed by one-way ANOVA using SAS. Duncan's multiple range tests was used to compare the treatment means ($P < 0.05$).

RESULTS AND DISCUSSION

The effects of added dietary oils on semen quality of Malaysian roosters are shown in table 3. The results showed that semen parameters were significantly influenced by dietary added oils (fish, vegetable and

Table 3: Effects of dietary sources of oils on semen quality of village roosters

Parameters	Fish oil[1]	Vegetable oil	Cooked oil	±MSE	P-value
Motile spermatozoa%	68.9[a]	60.67[b]	62.75[b]	2.484	0.008
Progressive spermatozoa%	23.47	26.24	28.23	1.381	0.14
VAP (μm/s)	86.28[a]	79.36[b]	73.3[b]	3.749	0.002
VSL (μm/s)	71.48	68.2	66.94	1.362	0.198
VCL (μm/s)	134.13[a]	110.76[b]	99.99[c]	10.07	0.0001
ALH (μm/s)	6.01[a]	4.55[b]	4.48[c]	0.494	0.0001
BCF (μm/s)	17.143[b]	20.01[a]	20.75[a]	1.100	0.009
STR	76.23[c]	82.15[b]	87.46[a]	3.279	0.0001
LIN	56.31[c]	62.14[b]	69.31[a]	3.756	0.0001
Elongation	44.31[b]	47.76[b]	52.41[a]	2.345	0.0003
Spermatozoa type					
Rapid%	32.75[b]	39.51[a]	41.89[a]	2.736	0.0042
Medium%	35.86[a]	21.06[b]	22.51[b]	4.712	0.0001
Slow%	10.31[c]	14.98[b]	17.22[a]	2.034	0.0001
Static%	21.08[b]	25.87[a]	20.92[b]	1.616	0.0002

[a–d] Means within a row with no common superscript are significantly different ($P < 0.05$).

[1]Cod liver oil as fish oil; soybean oil as vegetable oil; cooked palm oil as cooked oil; VAP: Smoothed Path Velocity; VCL= Track Velocity (microns/sec); VSL= Straight Line Velocity; ALH= Amplitude of Lateral Head Displacement; BCF= Beat Cross Frequency; LIN= Linearity (ratio of VSL/VCL); STR= Straightness (ratio of VSL/VAP); Elongation= head shape.

Table 4: Effect of dietary oil sources on evaluation of semen parameters in village roosters

Parameters	Fish oil[1]	Vegetable oil	Cooked oil	±MSE	P-value
Volume (ml)	0.49	0.53	0.54	0.053	0.75
Live Sperm (%)	88.10	90.63	92.34	0.862	0.20
Death sperm (%)	11.90	9.37	7.66	0.862	0.21
Concentration (10^9)	2.32	2.26	2.35	0.190	0.22

[1]Cod liver oil as fish oil; soybean oil as vegetable oil; cooked palm oil as cooked oil

cooked). Addition of fish oil as a source of omega3 fatty acids significantly increased sperm motility, VAP, VCL, ALH and medium type spermatozoa but significantly decreased spermatozoa linear movement, STR, rapid, static and slow spermatozoa type ($P < 0.05$). On the other hand, the addition of vegetable oil as a source of omega6 fatty acids significantly decreased the VAP, motility, medium and slow spermatozoa type and simultaneously increased spermatozoa linearity movement, rapid and static spermatozoa type ($P < 0.05$). Addition of cooked oil as a source of trans fatty acids to the village rooster's diet significantly increased rapid motility of spermatozoa, STR, linearity movement and slow spermatozoa type, but decreased sperm motility, VAP and static type of spermatozoa ($P < 0.05$). The added oils had no significant effect on spermatozoa progressive motility, semen volume, semen concentration, live and dead spermatozoa during this experiment ($P > 0.05$) (Table 4).

The effects of dietary oils on the semen qualitative and quantitative features in village rooster were investigated. The reproductive performance of roosters like semen volume, semen concentration and spermatozoa livability did not influence by dietary oils. However, dietary treatments significantly influenced the motility of spermatozoa ($P < 0.05$). Several studies have shown that the proportion of motile spermatozoa in human and chicken semen is positively correlated with DHA [3, 13]. The results of this study are partially in agreement with those [14] who reported that using fish and corn oils did not influence the semen volume, sperm concentration and motility of turkey and the proportion of viable spermatozoa was significantly increased in the ejaculates collected from the birds fed fish oil as compared to the cooked oil fed birds. It is well known that an adequate lipid composition of sperm membranes is needed for sperm motility, maturation, the ability to undergo the acrosome reaction and sperm interaction with uterine components and oocyte [15]. Addition of fish oil to male broiler breeder diets after 33 weeks of age resulted in a 6.4% increase in their fertility at 49 weeks of age [15]. It is most likely that the biophysical properties of DHA contribute to the membrane fluidity and flexibility

demanded by the motility of the tail [16]. Other studies showed that the proportion of 22:4n-6 in total sperm phospholipids showed a significant positive correlation with sperm motility and fertilizing ability [17-18].

Sperm motility is a critical factor in maintenance of the fertility. In birds, the vaginal portion of the hen's oviduct regulates sperm entry and only motile sperm are able to traverse the vagina and enter into the hen's sperm storage tubules (SST) and one obvious factor critical to fertilization is sperm motility [19-20]. There is an interrelationship between sperm mobility, sperm storage in the hen and the fertility.

A positive correlation between VSL, LIN, BCF and sperm mobility of the whole population [21] has been found. The LIN parameter is a measure of linearity and the BCF motion parameter indicates the number of times the sperm track crosses the smoothed path, both of which indicate linear progression. Thus, high-mobility sperm swim faster and straighter than did low-mobility sperm. The computer-assisted sperm analyzer parameters such as VSL, LIN and BCF were all positively correlated with sperm mobility in the mobile subpopulations [21]. It is known that in turkeys, VSL is positively correlated with sperm mobility [21] which measures the ability of sperm to directionally move in a viscous media and is highly correlated with fertility [22]. These results are also confirmed by others [23-24].

Animal fat and oils from restaurant residuals have trans fatty acids. Trans fats interfere with enzymes needed to produce sex hormones; they decrease the levels of testosterone in male animals and increase the level of abnormal sperm. Chavarro and his colleagues [6] reported that each 2% increase in the intake of energy from trans unsaturated fats, as opposed to that of carbohydrates, was associated with a 73% greater risk of ovulatory infertility. Trans fatty acids were present in human sperm and were related inversely to sperm concentration ($r = -0.44$) [6]. A lower concentration of trans fatty acids not only improves the nutritional quality of the milk for human consumption, but also likely to improve the reproductive performance of the cows [25]. They showed that cows fed lower levels of trans fatty acids had a significantly better reproductive performance, whereas the other fatty acids had no influence on the animals' fertility. Cooking vegetable oils changes the fatty acid profile of vegetable oils and increase their trans fatty acids. The results of this study showed that cooking vegetable oil increased trans fatty acids like C18:1 trans and decreased total omega3 fatty acids of cooked oil. As omega3 fatty acids decreased in the diets, sperm motility and rapid type spermatozoa in village rooster's semen were also decreased. This might be related to the decreasing fluidity and flexibility of spermatozoa membrane caused by trans fatty acids.

Hydrogenated fats and specific fatty acid isomers can influence the activity of the desaturases, elongases, acyltransferases, oxygenases and prostaglandin synthetases [26]. Trans fatty acids, in particular trans-l8:2 fatty acids, have the ability to inhibit the elongation-desaturation reactions of linoleic acid [27]. It is obvious that giving hydrogenated fat containing a high concentration of trans-18:1 fatty acid may accentuate essential-fatty-acid deficiency in rats [28]. Impairment of reproductive functions is one of the earlier symptoms of the lack of essential fatty acids [29].

Sperm velocity and linearity appear to be important characteristics of bird sperm function. Sperm mobility is significantly correlated with adenosine triphosphate (ATP) content of sperm in roosters and that rates of mitochondrial ATP synthesis vary according to sperm mobility phenotype and this suggests that sperm with low mobility may not generate adequate ATP for efficient movement [22]. Sperm that were highly mobile also displayed increased velocity parameters and decreased deviation from linearity. This suggests that highly mobile sperm might be more efficient at traveling through the reproductive tract and reaching the site of sperm storage and/or fertilization.

CONCLUSIONS

From the results of this study it was concluded that the addition of oils containing trans fatty acids may decrease the omega3 fatty acid content of the semen and may decrease the qualitative features of the semen. Therefore, it is suggested that using fresh and high quality oils containing omega3 fatty acids may improve the semen quality of roosters.

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