

## **Effects of dietary supplementation of codfish liver oil on semen quality of boars during the hot season<sup>(1)</sup>**

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### **ABSTRACT**

The purpose of this study was to investigate the effect of codfish liver oil as dietary supplementation on the semen quality of boars during the hot season. A total of 18 TLRI Black Pig No. 1 (TBP) boars were selected and randomly divided into three groups. Boars in each group were fed either basic diet (CP 15%, ME 3,250 kcal/kg) (control, group A), or diets supplemented with 3% (group B) or 5% (group C) of codfish liver oil. Semen samples were collected once a week for a 3-month period starting from June 1, 2002. The semen characteristics, including semen volume (gel free), sperm concentration and total sperms per ejaculate, sperm motility, and percentage of abnormal sperm, were measured. The results indicated that diets supplemented with codfish liver oil, either 3% or 5%, significantly ( $P < 0.05$ ) increased sperm concentration and total sperm per ejaculate of the boars. The supplementation of codfish liver oil also reduced the percentage of abnormal sperm when compared with the control group ( $P < 0.05$ ). In conclusion, we found diets supplemented with codfish liver oil rich in decosahexaenoic acid (DHA) improved the semen quality of boars in the hot season.

(Key Words: Boar, Decosahexaenoic acid, Semen quality)

### **INTRODUCTION**

The semen quality is closely related to the reproductive efficiency of boars (Ciereszko *et al.*, 2000). The high ambient temperature seriously affects the reproductive performances of boars, which includes the damage to the spermatogenic process, and thus reduces the semen quality and reproduction efficiency of breeding boars (Hsu *et al.*, 1996; Cameron and Blackshaw, 1980). In Taiwan, the high temperature in

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summer was proven to be detrimental to the semen quality of boars (Liao *et al.*, 1996).

The plasma membrane of sperm contains abundant polyunsaturated fatty acid (PUFA), especially decosahexaenoic acid (DHA) (Lenzi *et al.*, 2000). DHA assure membrane integrity by maintaining fluidity of cell membrane (Yousdim and Deans, 2000). The high ambient temperature peroxidized the fatty acid on membrane and therefore, caused the PUFA and DHA loss on membrane (Tirosh *et al.*, 1997). Boars fed on diet with tuna oil could produce more motile sperms with normal acrosome (Rooke *et al.*, 2001). The purpose of this study was to evaluate the effect of dietary supplementation of codfish liver oil, containing high amount of DHA, on the improvement of boar semen quality in the hot season.

## MATERIALS AND METHODS

### 1. Animals and management

A total of 18 TLRI Black Pig No.1 (TBP) boars with 12-36 months of age were randomly allocated into three dietary treatments. The control group (A) was fed with basal diet containing 15.2% crude protein, digestible energy of 3,283 kcal/kg and 1.2% DHA. The boars in experimental groups were fed on basal diet supplemented with 3% (group B) or 5% (group C) (w/w) commercial codfish liver oil to make the DHA content was 6.4% and 8.0%, respectively. Each boar was confined in a galvanized pen (3 m × 1.25 m) and fed daily with 2.5 kg diet and water was provided *ad libitum*. Experimental diets were provided from May 6, 2002 and semen characteristics were evaluated at June 10. The daily ambient temperatures were recorded with a thermometer at the south and north parts of boar barn. The management and feeding of boars were made to comply with the Animal Welfare Requirements of LRI-COA.

### 2. Semen collection and evaluation of semen characteristics

Boar semen was collected by using the hand gloved method once a week. The pH value of semen was determined by using a pH meter while the volume (gel free) was measured by directly reading from the scales on semen collection bottle. The sperm concentration, motility, and percentage of progressive sperm were determined by using CASA (Version 10 HTM-IVOS, Hamilton-Thorne Research) as described by Yang *et al.* (2001). Total sperms per ejaculate was obtained by semen volume time sperm concentration. Sperm morphology and percentage of abnormal sperm was microscopically examined using the haemacytometer method under 200X magnification. Stained semen smears were prepared by using nigrosine-eosin dye.

### 3. Statistical analysis

Semen characteristics were subjected to analysis of variance for a repeated measurements design using the general linear model by SAS (SAS, 2002). Boar served as an experiment unit. The statistical model included treatment, and the treatment X time interactions. If a significant treatment X time

interaction was detected, then means among treatments within times were compared using the PDIFF option of the least square means statement in the GLM procedure.

## RESULTS AND DISCUSSION

### 1. Ambient temperature in boar barn

The temperature inside the boar barn during the experimental period was shown in Fig. 1. The high temperature ranged from 30.1°C to 35.9°C and low temperature was between 22.9°C and 26.1°C during the experiment period. Higher temperature was observed from mid June to mid July. Stone (1982) indicated that the sperm production was normal when boars were raised under 16 - 29°C. Liao *et al.* (1996) indicated that the high ambient temperature had a negative effect on the sperm production and reproductive efficiency of boars. Balta Moner (1996) suggested that the optimal temperature for boars was 16 - 28°C. Nevertheless, Corcuera *et al.* (2002) indicated that the optimal temperature was 20 - 24 °C for sperm production of boars. The ambient temperature in this study was higher than those temperatures reported in the previous literatures. The daily high temperature (29 - 34°C) in the present experiment was more severe than the optimal thermoneutral temperature for boars, which had caused a negative environmental effect on semen production.

### 2. Effect of dietary Codfish Liver Oil on the semen quality of boar

#### (1) Semen volume (gel – free)

The semen volume collected from boars of group B or C was significantly ( $P < 0.05$ ) less than that of the control group (Table 1). Significant ( $P < 0.05$ ) difference was also found on the percentage of abnormal sperm in semen among groups. During July and August, the percentage of abnormal sperm in semen from group A (control) was higher ( $P < 0.05$ ) than group B (3% codfish liver oil) and group C (5% codfish liver oil) (Fig. 2). This indicated that the dietary supplementation of codfish liver oil had beneficial effect on sperm morphology during the hot season.

#### (2) Sperm concentration and total sperms per ejaculate

The sperm concentration for boar semen in group B and C which supplemented with 3% or 5% codfish liver oil were higher than that in the control group during the period from mid July to the end of experiment (Fig. 3) (July, 29 to August, 26). At the 7<sup>th</sup> week after treatment, the sperm concentration of boars in group B was significantly ( $P < 0.05$ ) higher than the other groups.

The total sperms per ejaculate collected during July and August from boars of all groups decreased when compared with June. Nevertheless, the extent of decline on total sperms per ejaculate in group B and C were not so serious as the group A (Fig. 4). On June, 24 and July, 28, the total sperm from boars of group B and C (added 3% and 5% codfish oil) were significantly ( $P < 0.05$ ) higher than the control group. These results implied that the supplementation of 3% or 5% codfish liver oil might ameliorate the adverse effect of high temperature on total sperm production.

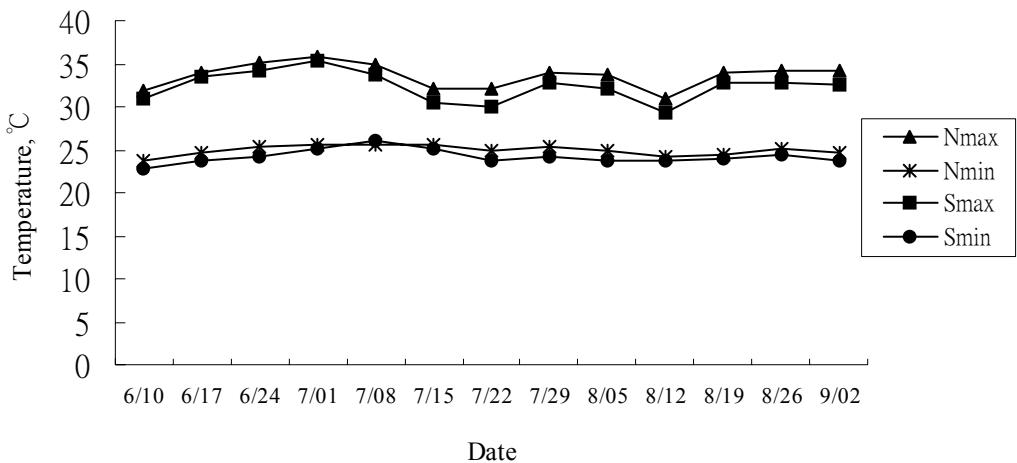


Figure 1 The temperatures recorded at different sites of boar barn during experimental period. Nmax and Nmin are the highest and the lowest temperature, respectively, at the north side of the boar barn; Smax and Smin are the counterpart at the south side of the boar barn.

Table 1 Effects of dietary supplementation of codfish liver oil on semen characteristics of TBP during July to August in 2002

Items	Group A	Group B	Group C	SEM
	Control	3% codfish liver oil	5% codfish liver oil	
Semen volume of ejaculate, mL	217.6 <sup>a</sup>	152.7 <sup>b</sup>	183.4 <sup>c</sup>	7.27
pH value	7.21	7.34	7.25	0.05
Sperm concentration, $\times 10^6/\text{mL}$	233.79	307.34	327.11	51.00
Total sperm, $\times 10^9/\text{ejaculate}$	50.21	44.37	59.40	13.45
Sperm motility, %	18.21	27.11	24.50	4.81
Abnormal sperm, %	13.35 <sup>a</sup>	6.19 <sup>b</sup>	4.54 <sup>b</sup>	2.23

<sup>a, b, c</sup>: Means in the same row without a common superscripts differ significantly ( $P < 0.05$ ).

The sperm concentration in group A and B reduced during hot season, which was consistent with the result of Liao *et al.* (1996). During high ambient temperature, the reduction in sperm concentration for group B was milder than that in group A, especially during July, 29 to August, 26. For group C, which was fortified with 5% codfish liver oil, sperm concentration was increased during hot season. The result showed that dietary supplementation of codfish liver oil increased the dietary DHA content and the sperm concentration during hot season. Noteworthy, at the 11<sup>th</sup> to 17<sup>th</sup> (July 22 to Sep. 2) week of the experiment, the sperm concentration in group B and C, which were supplemented with 3% or 5% codfish liver oil, were higher than those in the control group (Fig 3). This period located in the August which was about 6 weeks after the experimental animals experienced heat stress. Cameron and Blackshaw

(1980) indicated that the interval from boar subjected to heat stress and the depression on semen quality was 5 to 6 wks, which was consistent to the present experiment. Thus, the results of this study, suggested that supplementation of codfish liver oil is beneficial to the sperm production of boar under heat stress.

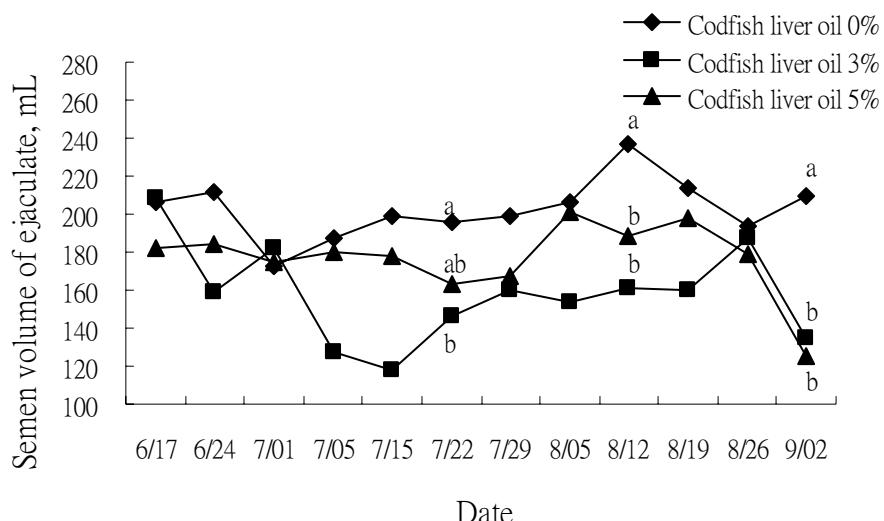


Figure 2 Effects of dietary supplementation of codfish liver oil on semen volume (mean, n=6) of ejaculate for TBP boars. <sup>a,b</sup>: Means within the same date without a common superscript differ significantly ( $P < 0.05$ ). Pooled SE = 12.

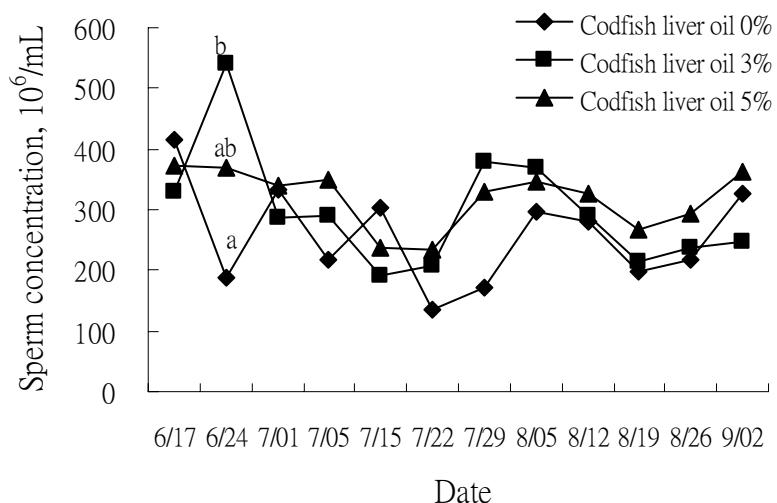


Figure 3 Effects of dietary supplementation of codfish liver oil on sperm concentrations of TBP boars. <sup>a,b</sup>: means at the same date without a common superscript differ significantly ( $P < 0.05$ ). Pooled SE = 63.

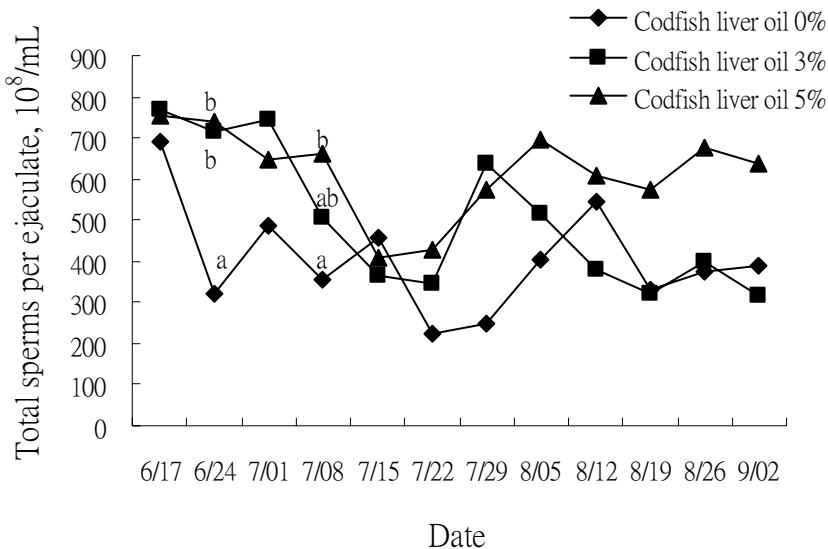


Figure 4 Effects of dietary supplementation of codfish liver oil on total sperm of TBP boars. <sup>a, b</sup>. means at the same date without a common superscript differ significantly ( $P < 0.05$ ). Pooled SE = 74.

### (3) Sperm motility and percentage of abnormal sperm

The sperm motility of boar semen in this experiment was measured according to the percentage between the linear progressive sperm, fast progressive sperm and immotile sperm. The sperm motility was numerically increased and percentage of abnormal sperm was decreased in group B and C when compared with the control group ( $P < 0.05$ ) (Table 1). The sperm motility in group B and C was increased during Mid July to August when compared with that of group A (Fig. 5).

The cell membrane of boar sperm contains large amount of PUFA and the high ambient temperature causes the peroxidative damage and destroys the sperm membrane (Tirosh *et al.*, 1997). Therefore, the dietary supplementation of codfish liver oil for boars during hot season may replenish the DHA content which can significantly decrease the percentage of abnormal sperm and improve the semen quality. At the 7<sup>th</sup> (June 24) and 9<sup>th</sup> (July 1) wk after providing the experimental diets, the percentage of abnormal sperm in group B and C were lower ( $P < 0.05$ ) than that of the group A (Fig. 6). These results demonstrated that the addition of DHA from codfish liver oil might alleviate the adverse effect of hot temperature on sperm motility and maintain morphologically normal sperm.

During hot season, the dietary supplementation of DHA from codfish liver oil increased the sperm concentration, sperm motility, and reduced percentage of abnormal sperm. The effect was observed when 3% or 5% codfish liver oil was added. In conclusion, the dietary supplementation of 3% or 5% codfish liver oil improves the boar semen quality during the hot season.

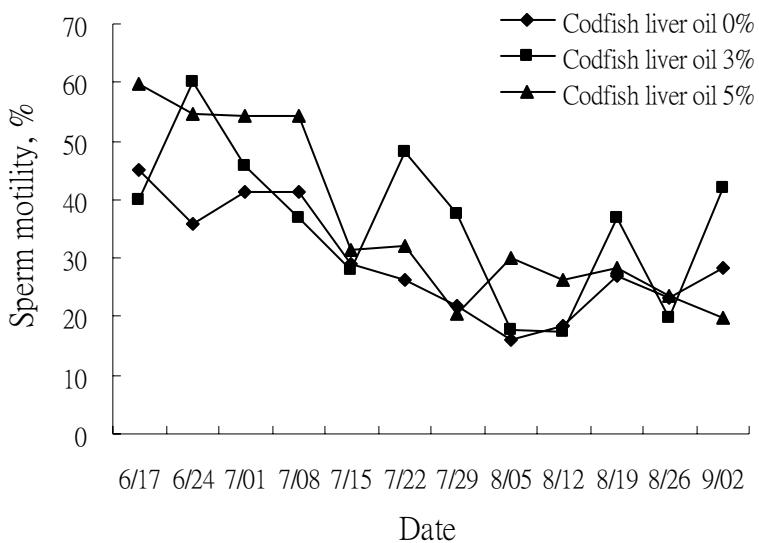


Figure 5 Effects of dietary supplementation of codfish liver oil on sperm motility of TBP boars.  
Pooled SE = 1.2.

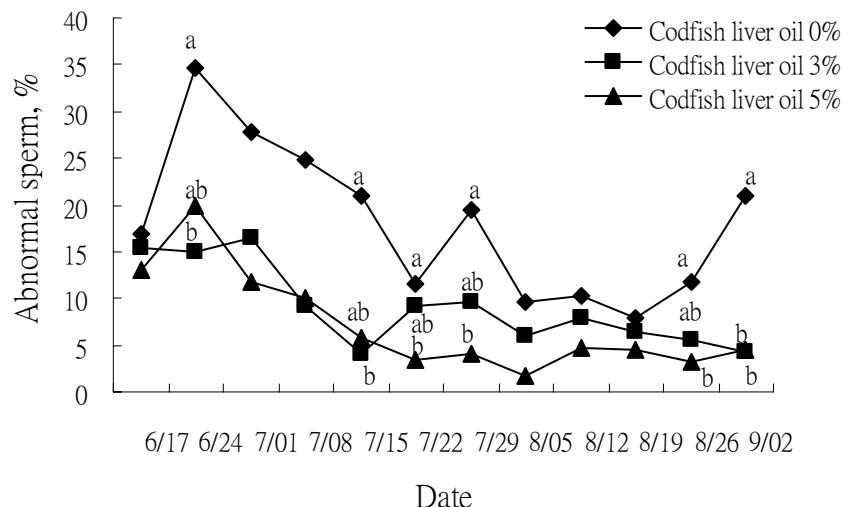


Figure 6 Effects of dietary supplementation of codfish liver oil on percentage of abnormal sperm of TBP boars. <sup>a,b</sup>: means at within the same date without a common superscript differ significantly ( $P < 0.05$ ). Pooled SE = 2.1.

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# 公豬飼糧中添加鱈魚肝油對熱季精液品質 之影響<sup>(1)</sup>

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**摘要：**本試驗嘗試在熱季時，於公豬飼糧中添加含高量二十二碳六烯酸（DHA）之鱈魚肝油，以補充 DHA 量，評估對改善熱季公豬精液品質之效果。試驗共採用 12 至 36 月齡之畜試黑豬一號公豬 18 頭，逢機分置於三種飼糧處理組，每處理組 6 頭，分別餵飼含粗蛋白質 15%、可消化能 3,250 kcal/kg 的基礎飼糧（group A）、基礎飼糧額外添加 3% 鱈魚肝油（group B）以及基礎飼糧額外添加 5% 鱈魚肝油（group C）等飼糧。試驗從 2002 年 6 月 1 日開始，共進行 3 個月，公豬每 7 天採精 1 次，測定包括精液量、精子濃度、精子活力及畸形率等精液性狀。結果顯示，在飼糧中添加含有 DHA 之鱈魚肝油 3% 或 5%（group B 與 group C），可顯著地 ( $P < 0.05$ ) 提高精液中精子濃度及總精子數，且精子活力較佳，畸形精子率顯著地 ( $P < 0.05$ ) 較對照組（group A）為低，顯示熱季中於飼糧中添加鱈魚肝油可改善公豬精液品質。

（關鍵語：公豬、二十二碳六烯酸、精液品質）

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