Kafkas Univ Vet Fak Derg
16 (4): 537-546, 2010
DOI:10.9775/kvfd.2009.1094

RESEARCH ARTICLE

Effects of Nonylphenol on Growth Parameters and Antioxidant Defense System in Japanese Quails *(Coturnix japonica)* [1]

Gülcan AVCI * Cevdet UĞUZ ** İsmail BAYRAM *** Metin ERDOĞAN **
İsmail KÜÇÜKKURT * Mine DOSAY AKBULUT ** Mehmet ÖZDEMİR ****
Ömer Faruk LENGER ** Mesude İŞCAN ***** İnci TOGAN *****

[1] *Research was supported with a project numbered VHAG-1926 by the Scientific and Technological Research Council of Turkey in 2001-2003*

* Department of Biochemistry, Faculty of Veterinary Medicine, Afyon Kocatepe Üniversity, TR-03200 Afyonkarahisar - TÜRKİYE
** Department of Medical Biology and Genetics, Faculty of Veterinary Medicine, Afyon Kocatepe Üniversity, TR-03200 Afyonkarahisar - TÜRKİYE
*** Department of Animal Feeding, Faculty of Veterinary Medicine, Afyon Kocatepe Üniversity, TR-03200 Afyonkarahisar - TÜRKİYE
**** Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Afyon Kocatepe Üniversity, TR-03200 Afyonkarahisar - TÜRKİYE
***** Department of Biological Sciences, Middle East Technical University, TR-06531 Ankara - TÜRKİYE

Makale Kodu (Article Code): KVFD-2009-1094

Summary

Alkylpehnol polyethoxylates (APEOs) and the derivatives of APEOs such as nonylphenol (NP) are generally used as anti-oxidants in many organic compounds including detergents, herbicides and pesticides. In this study, NP effects on growth, egg production and hatching in Japanese quails were investigated. Also, NP effects on plasma vitamin levels and malondialdehyde (MDA) were determined. NP does not affect body weight gain (BWG), feed consumption (FC) and feed conversion ratio for growth (FCRG) in all experimental groups in growing chicks, while it cause a significant decrease in egg production (EP) ($P<0.01$). Also, the present study showed that NP concentrations, 10, 100 and 500 µg NP in ethanol (ETOH) /kg-feed, exposure cause an increase in β-Carotene, Vitamin A and MDA levels ($P<0.01$). There is also significant rise in Vitamin C level induced by NP in all experimental groups ($P<0.01$). In conclusion, NP has dramatic effects on EP rate and anti-oxidant system in a concentration dependent manner in Japanese quails.

Keywords: *Nonylphenol, Egg production, Antioxidant, Vitamin, Lipid peroxidation*

Japon Bıldırcınlarında *(Coturnix japonica)* Büyüme Parametreleri ve Antioksidan Savunma Sistemine Nonilfenolün Etkileri

Özet

Alkilfenol polietoksilatlar (AFEO) bileşikleri ve nonilfenol (NF) gibi AFEO türevleri, deterjanlar, ot ve böcek ilaçlarını da içeren pek çok organik bileşikte, genellikle antioksidan olarak kullanılmaktadır. Bu çalışmada, Japon Bıldırcınlarında büyüme, yumurta üretimi ve kuluçka randımanı üzerine nonilfenol etkileri araştırıldı. Ayrıca, nonilfenolün plazma vitamin düzeyleri ve malondialdehit (MDA) üzerine etkileri belirlendi. NF büyüme dönemindeki tüm deney guruplarında canlı ağırlık artışı (BWG) ve yemden yararlanma oranını (FCRG) etkilemezken, yumurta üretiminde önemli bir azalmaya neden olmaktadır ($P<0.01$). Ayrıca, bu çalışmada etanolde çözünen nonilfenolün 10, 100 ve 500 µg NP/kg-yem konsantrasyonları β-Karoten, Vitamin A ve MDA düzeylerinde bir artışa sebep olduğu ($P<0.01$) gözlenmiştir. Tüm deney gruplarındaki NF, Vitamin C düzeyinde önemli bir artışa neden olmaktadır. Sonuç olarak, konsantrasyona bağlı olarak NF Japon bıldırcınlarında EP oranını ve antioksidan sistemi dramatik bir şekilde etkilemektedir.

Anahtar sözcükler: *Nonilfenol, Yumurta üretimi, Antioksidan, Vitamin, Lipid peroksidasyon*

İletişim (Correspondence)
☎ +90 272 2281312/133
✉ cuguz@aku.edu.tr

INTRODUCTION

Alkylphenolpolyethoxylates (APEs), commonly called estrogenic environmental endocrine disrupters, are widely used as non-ionic surfactants and anti-oxidants in detergents, herbicides, pesticides, paints, plasticware, emulsifiers and intra vaginal spermicides [1,2]. APEs are produced about 500.000 metric tons in a year around the world and it has been shown that 60% of this produced amount accumulated in the streams, rivers, lakes and seas around the world [3,4].

APEs undergo biodegradation process to give short side chain derivatives such as nonylphenol (NP), octylphenol (OP) and butylphenol (BP) in anaerobic conditions in water [5]. APEs have been shown to have estrogenic [6], carcinogenic [7,8] and toxic effects [9] in both aquatic and terrestrial organisms. Nonylphenol (NP), one of the most abundant derivatives of APEs, has been demonstrated to stay biologically active state for a longer period of time in the body than that of natural estrogen [10].

A decade ago, it was believed that NP had been posing a direct threat to the health and fertility in aquatic organism, since the waste majority of NP is accumulated in the bodies of water around the world [4]. NP accumulation in aquatic environment through the sewage and household effluent poses indirect threat to the terrestrial organism through the consumption of fish or by the addition of fish meal to the animal feed ration. It has been reported that dietary fish meal are used as substitute in poultry rations [11-13].

Furthermore, recent findings have indicated that APEs especially NP is ubiquitously found in terrestrial food including fresh fruits and vegetables [14,15], human milk [16,17], in aquatic as well as livestock products and in rice [18]. The pathway for nonylphenol contamination of food was occur due to the use of cleaning agents in the food processing industries as well as application of pesticides [19]. It has also been reported by Kawaguchi et al.[20] that 200 µg NP/kg-feed was extracted from feed samples of animals including poultry. These reports strongly suggest that APEs particularly NP poses serious direct threats on the health, reproduction and fertility of the terrestrial organisms through direct intake of NP along with fruits, vegetables, and rice and livestock products.

Japanese quails were chosen as experimental animals in this study since they are fairly inexpensive and readily available in the market. Furthermore, they are good research model since they grow fast, reach sexual maturation very early and their eggs require very short incubation time for hatching, and finally they have small size for convenient handling and breeding. Japanese quails have been used in many NP related toxicological studies. For example, Razia et al.[21] reported that NP has suppressive effects on immune system and has estrogenic effects similar to 17β-estradiol on endocrine organs in Japanese quail embryo. Yoshimura and Fujita [22] also demonstrated that only a minor disorder occurs in the female reproductive tract of the F1 generation in Japanese quails treated with NP by intramuscular injection for 5 days.

Although APEs or the derivatives of APEs such as NP are generally used as anti-oxidant in detergents or in other organic compounds outlined above, it has been reported by Chitra et al.[23] that NP causes a decrease antioxidant enzymes activities including superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase, while causing an increase in the generation of H_2O_2 and lipid peroxidation. Uguz et al.[24,25] demonstrated that NP bioaccumulates and alters the activity of phase II enzyme glutathione-S-transferase [GST] in a dose and time dependent manner in the liver of rainbow trout. It has also been shown that man made estrogen-mimicking compounds commonly called xenobiotics and/or xenoestrogen such as NP could reduce the activity of antioxidant defense system against reactive oxygen species (ROS) [26,27].

Livingstone [28] reported that ROS could be generated in endogenous xenobiotic metabolism. Indeed, paranonylphenol and bisphenol A have been shown to stimulate the generation of ROS in striatum [29]. Therefore, living organisms have evolved to develop a defense systems against oxidative damage including antioxidant scavengers and specific antioxidant enzymes namely catalase and glutathione peroxidase [30].

This study was designed to determine the effects of nonylphenol, given orally by feed, on growth, egg production and hatching and its adverse effects on lipid peroxidation and vitamin levels in Japanese quails.

MATERIAL and METHODS

Chemicals

4-nonylphenol was purchased from Aldrich, Germany. Sodium tungustate, disodium hydrogen phosphate, sulfuric acid, oxalic acid, ethanol, hexane, butylhydroxy-toluene, retinol acetate, β-carotene sodium benzoate, sodium phosphate, hydrogen peroxide, uric acid, trichloroacetic acid, 2-thiobarbituric acid (Sigma-Aldrich, Germany). Ammonium iron (II) sulfate hexahydrate, EDTA, NaOH, L-ascorbic acid (Merck), acetic acid (Merck, Darmstadt, Germany).

Quails

Japanese quail layers were purchased from Quails Research Unit from the Directorate of Agricultural Research Ministry of Agriculture, Konya and transferred to the Quail Research Unit, Afyon Kocatepe University, Afyonkarahisar, Turkey. Quails were then bred and maintained in the cages at the Research Unit in Afyonkarahisar Feed Factory.

Nonylphenol Containing Feed Ration Preparation

Nonylphenol (NP) stock solution was prepared (1 gr NP/L ethanol) in absolute ethanol (ETOH). The stock solution was then added in 10 ml ethanol to attain final concentrations outlined below. For example, in order to attain 10 µg NP concentrations aliquots of 10 µl from 1 gr NP/L stock solution was added in 10 ml ethanol. The 10 ml volume of ethanol containing 0 (ethanol alone) 10, 100, 500, 1000 and 5000 µg NP were pulverized in 1 kg quails-feed spread on a nylon sheet and mixed thoroughly. Neither ethanol nor NP containing feed was fed to control group.

Feeding

A basal diet feed containing ingredients shown in *Table 1* containing different concentrations of NP was given ad libitum to growing quails, whereas another basal diet feed shown in *Table 2* containing NP concentrations was given ad libitum to quail layers.

Table 1. *Basal diet for quails growth*
Tablo 1. *Bıldırcınlar için temel büyüme rasyonu*

Feedstuffs	**%**
Corn	51.4
Plant oil	1.6
Soybean meal	28.2
Full fat soybean	5.1
Sunflower meal	7.0
Limestone	1.0
Calcium phosphate	0.5
Fishmeal	4.5
Salt	0.4
Vitamin *	0.1
Mineral **	0.2
Calculated values	
Crude protein (%)	24.0
Metabolisable energy (kcal/kg)	2900
Calcium (%)	0.80
Available phosphorus (%)	0.30

** Provided by per kg of diet: Vitamin A, 10 000 IU; Vitamin D_3, 1.000 IU; Vitamin E, 25 mg; Vitamin K_3, 3 mg; Vitamin B_1, 2 mg; Vitamin B_2, 6 mg; Niacin 20 mg; Vitamin B_6, 4 mg; Vitamin B_{12}, 15 mg, Folic acid, 0.8 mg; Choline chloride,*
*** Provided by per kg of diet: 300 mg; Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg, I, 1 mg; Co, 0.2 mg; Se*

Table 2. *Basal diet for quails layer*
Tablo 2. *Yumurtacı bıldırcınların temel rasyon*

Feedstuffs	**%**
Corn	51.4
Plant oil	2.7
Soybean meal	27.0
Barley	7.7
Limestone	5.5
Calcium phosphate	0.5
Fishmeal	4.5
Salt	0.4
Vitamin *	0.1
Mineral **	0.2
Calculated values	
Crude protein (%)	20.0
Metabolisable energy (kcal/kg)	2900
Calcium (%)	2.5
Available phosphorus (%)	0.35

** Provided by per kg of diet: Vitamin A, 10 000 IU; Vitamin D_3, 1.000 IU; Vitamin E, 25 mg; Vitamin K_3, 3 mg; Vitamin B_1, 2 mg; Vitamin B_2, 6 mg; Niacin 20 mg; Vitamin B_6, 4 mg; Vitamin B_{12}, 15 mg, Folic acid, 0.8 mg; Choline chloride,*
*** Provided by per kg of diet: 300 mg; Mn, 80 mg; Fe, 60 mg; Zn, 60 mg; Cu, 5 mg, I, 1 mg; Co, 0.2 mg; Se*

Assessing Growth and Feed Conversion Ratio

The total of 468 healthy chicks was used in this trial. Seven-day old chicks, 1:1 ratio of male and female, were exposed to 0 (ethanol alone for solvent control) 10, 100, 500, 1000 and 5000 µg NP/kg-feed. Control group was fed with neither ethanol nor NP containing feed. Each treatment as well as control group had 78 animals. Experimentation was continued for 120 days.

Feed consumption (FC) was determined daily by the subtraction of feed given from feed remained. Live weight gain was determined or assess growth (G) by weighing quails and recording at the end of every week during experimentation. Feed conversion ratio for growth (FCRG) was determined by dividing weekly consumed feed (gr) to weekly live-weight-gain (gr), while feed conversion ration for egg production (FCRE) was determined by dividing weekly consumed feed (kg) to weekly egg production (dozen) after feeding. In another word: FCRG = feed given (gr) - feed remain (gr)/live weight gain (gr); FCRE = feed given (kg) - feed remain (kg)/dozen egg.

Egg Production and Hatching Trial

Eight females and two males were randomly selected among six-week-old non-treated (42-day-old) quails and were put in the layer's cages. Each treatment group had 30 animals. The total of 210 quails including males and females were employed in this trial. Animals were fed

ad-libitum. Photoperiod was adjusted to 8:16 hr dark and light. This trial was continued for 8 weeks. During experimentation feed consumption was determined in every week and egg production was recorded daily.

Quails eggs were collected daily and stored in egg cone. Eggs collected within one week were put in incubator at 38ºC±0.5 with 65% humidity for 17 days. Eggs were hatched at day 17. The hatchability rate was 93%. To indicate the age of quails, the eighteenth day was named Day 1 post hatching (ph). Quails chicks were then moved from incubator to a warm room with a temperature at 30ºC on Day 2 ph. Chicks were fed with the chick growth ration in this room until Day 7 ph. The survival rate until Day 7 ph was 98%.

Blood Sampling

Blood samples were collected into sterilized tubes containing lithium heparin from the heart of 10 quails on Day 120 post hatching. Plasma was separated by using centrifuging the samples at 3000 rpm for 10 min at +4°C). Plasma vitamin C, A and malondialdehyde (MDA) levels were measured immediately.

Determination of Vitamin, MDA and AOA Levels

- Vitamin C Levels

Plasma vitamin C level was measured as described by Kway [31]. Briefly, after precipitation of proteins by phosphotungistic acid spectrophotometric measurement was employed to determine vitamin C level on the basis of the color formation in response to reaction between protein precipitate and ascorbic acid.

- Vitamin A Levels

The plasma vitamin A and β-carotene concentrations were measured as previously described by Suziki and Katoh [32]. Retinol peaks were detected at 325 nm using 1:1 ratio of ethanol and hexane, whereas β-carotene peaks were detected at 453 nm using 1:3 ratio of ethanol and hexane.

- MDA Levels

The MDA, a biomarker for oxidative stress was determined by using a method developed by Draper and Hardley [33]. Briefly, this method is based on MDA coupling with thiobarbituric acid.

- Antioxidant Activity (AOA)

Plasma AOA was assayed by colorimetric method as described by Koracevic et al.[34]. A standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction leading to the formation of hydroxyl radicals (•OH). These radical oxygen species (ROS) induced benzoate degradation causes the release of thiobarbituric acid reactive substances (TBARS) and the addition of antioxidants suppresses TBARS production. This reaction was then measured spectrophotometrically and the inhibition of color development defined as the AOA.

Statistical Analysis

Data were analyzed by one-way ANOVA [35] using SPSS progamme (SPSS Inc. 2001) and Tukey's test was used to compare means using pair-wise comparison [36].

RESULTS

Effects of NP on FC, FCR and EP

Results are shown through *Tables 3-7*. Survival rate for both quail layers and chicks throughout the study in all experimental groups were very high (*Tables 3* and *6*). NP exposure did not have significant effects on body weight gain (BWG), feed consumption (FC), and feed conversion ratio (FCR) (P>0.05) in comparison to control, while ETOH alone had significant effect on FC (P<0.05) *(Table 4)*. However, ETOH alone did not have any effects on any other parameters investigated (P>0.05) *(Table 4)*.

Table 3. *Effects of NP on mean survival rate of Quails in growth trials*
Tablo 3. *Büyüme döneminde Bıldırcınların ortalama yaşama gücüne NP'nin etkileri*

Parameter	Experimental Groups						
	Control	ETOH	10 µg NP/kg-Feed	100 µg NP/kg-Feed	500 µg NP/kg-Feed	1000 µg NP/kg-Feed	5000 µg NP/kg-Feed
Survival Rate (%)	88	89	88	90	88	89	89

NP had dramatic effects on egg production (EP), initial body weight (IBW) and final body weight (FBW) in Japanese quails layers and these effects were statistically significant and the levels of significances are shown in *Table 5*.

As shown in *Table 5* that NP did not have any effect on egg weight (EW), hatching rate (HR), egg fertility (EF), and Japanese quails chick weight (CW).

Table 4. *Effects of NP on BWG, FC, and FCR of Quails*
Tablo 4. *NP nin Bıldırcınların BWG, FC ve FCR üzerine etkileri*

1-6 Weeks of Age	Experimental Groups						
	Control Mean±SE	ETOH Mean±SE	10 µg NP/kg-Feed Mean±SE	100 µg NP/kg-Feed Mean±SE	500 µg NP/kg-Feed Mean±SE	1000 µg NP/kg-Feed Mean±SE	5000 µg NP/kg-Feed Mean±SE
BWG	172±1	174±2	168±3	174±4	169±4	174±4	171±2
FC (gr/35 day)	766±21	812±15*	769±37	774±16	732±27	808±27	767±23
FCR	4±0.13	5±1	5±0.2	4±0.1	4±0.18	5±0.1	4±0.1

** Significant at P<0.05,* ***BWG:*** *Body Weight Gain*

Table 5. *Effects of NP on egg production parameters*
Tablo 5. *Yumurta verim parametreleri üzerine NP nin etkileri*

Parameters	Experimental Groups							P
	Control Mean±SE	ETOH Mean±SE	10 µg NP/kg-Feed Mean±SE	100 µg NP/kg-Feed Mean±SE	500 µg NP/kg-Feed Mean±SE	1000 µg NP/kg-Feed Mean±SE	5000 µg NP/kg-Feed Mean±SE	
EP (yield/day)	78±2 ^{a}	77±2 ^{a}	81±2 ^{b}	81±1 ^{b}	77±2 ^{a}	77±2 ^{a}	73±2 ^{c}	**
FC (gr/day)	36±1	33.45±1	36±1	35±1	36±1	32±1	36±1	NS
FCRE (gr/kg)	1±0.01	1±0.1b	1±0.01	1±0.01	1±0.01	1±0.01	1±0.01	NS
EW (gr)	12±0.01	12±0.1	12±0.01	12±0.01	12±0.01	12±0.01	12±0.01	NS
IBW (gr)	201±5 ^{a}	206±6 ^{a}	216±4 ^{b}	209±4 ^{c}	209±3 ^{c}	214±4 ^{bc}	204±3 ^{a}	*
FBW (gr)	220±4 ^{a}	221±3 ^{a}	242±5 ^{b}	234±3 ^{c}	231±4 ^{c}	231±5 ^{c}	218±3 ^{a}	***

EP: *Egg production;* ***FC:*** *Feed Consumption;* ***FCR:*** *Feed Conversion Ratio;* ***EW:*** *Egg Weight;* ***IBW:*** *Initial Body Weight;* ***FBW:*** *Final Body Weight*
Mean values with different superscripts within a row differ significantly
NS: *Non significant;* ******* *Significant at P<0.05,* ******** *Significant at P<0.01,* ********* *Significant at P<0.001*

Table 6. *Effects of NP egg weight, hatching, egg fertility, chick weight and survival*
Tablo 6. *Yumurta ağırlığı, kuluçka randımanı, döllü yumurta, civciv ağırlığı ve yaşama gücü üzerine NP etkileri*

Parameter	Experimental Groups						
	Control Mean±SE	ETOH Mean±SE	10 µg NP/kg-Feed Mean±SE	100 µg NP/kg-Feed Mean±SE	500 µg NP/kg-Feed Mean±SE	1000 µg NP/kg-Feed Mean±SE	5000 µg NP/kg-Feed Mean±SE
EW (gr)	12±0.1	12±0.1	12±0.1	12±1	12±0.1	11±1	12±0.1
HR (%)	74±5	74±3	80±3	73±5	75±4	81±3	81±3
FE (%)	5±1	8±1	4±1	8±2	6±2	7±1	5±1
CW (gr)	8±0.1	8±0.1	8±0.1	8±0.1	8±0.1	8±0.1	8±0.1
Survival rate (%)	96	96	96	84	96	94	94

EW: *Egg weight;* ***HR:*** *Hatching Rate;* ***FE:*** *Fertile Eggs;* ***CW:*** *Chick Weight*

Effects of NP on Lipid Peroxidation and Vitamins

Results are shown in *Tables 7-10*. NP stimulated a significant increase in plasma vitamin C level in comparison to control (P<0.01). However, NP-induced increase in vitamin C level appeared to be not dose dependent since this increase was not significantly different among NP treated groups (P>0.01). Plasma vitamin C level in ethanol alone treated group was not affected significantly (P>0.01).

Effects of NP on AOA and MDA in Quails

As shown in *Table 8*, NP did not have any effects on plasma AOA level. The rise in plasma MDA levels was significant in NP groups in comparison to control group. However, the plasma level of MDA dropped back to control level in 1000 and 5000 µg NP in ETOH/kg-feed groups *(Table 9)*.

As shown in *Table 10*, vitamin A was not dramatically

Table 7. *Effects of NP on vitamin C level in quail*
Tablo 7. *Bıldırcınlarda vitamin C düzeyine NP nin etkileri*

Treatment Groups	N	Vitamin C (mg/dl±SE)
Control	10	2±0.1 [a]
ETOH alone	10	2±0.2 [ab]
10 µg NP in ETOH/kg-feed	10	3±0.2 [bc]
100 µg NP in ETOH/kg-feed	10	3±0.2 [bc]
500 µg NP in ETOH/kg-feed	10	3±0.1 [c]
1000 µg in ETOH NP/kg-feed	10	3±0.3 [c]
5000 µg NP in ETOH/kg-feed	10	4±0.4 [c]

[abc] *Different letters indicates significance at P<0.01*

Table 8. *Effects of NP on AOA level in quail*
Tablo 8. *Bıldırcınlarda AOA düzeyine NP nin etkileri*

Treatment Groups	N	AOA (mmol/L±SE)
Control	10	2±0.1
ETOH alone	10	2±0.1
10 µg NP in ETOH/kg-feed	10	2±0.2
100 µg NP in ETOH/kg-feed	10	2±0.1
500 µg NP in ETOH/kg-feed	10	2±0.2
1000 µg in ETOH NP/kg-feed	10	2±0. 2
5000 µg NP in ETOH/kg-feed	10	2±0.2

Table 9. *Effects of NP on MDA level in quail*
Tablo 9. *Bıldırcınlarda MDA düzeyine NP nin etkileri*

Treatment Groups	N	MDA (nmol/L±SE)
Control	10	3±0.3 [a]
ETOH alone	10	4±0 [b]
10 µg NP in ETOH/kg-feed	10	4±0.1 [b]
100 µg NP in ETOH/kg-feed	10	4±0.4 [b]
500 µg NP in ETOH/kg-feed	10	4±0.4 [b]
1000 µg in ETOH NP/kg-feed	10	3±0.3 [a]
5000 µg NP in ETOH/kg-feed	10	3±0.2 [a]

[abc] *Different letters indicates significance at P<0.01*

Table 10. *Effects of NP on vitamin A and β-carotene*
Tablo 10. *Vitamin A ve β-karoten üzerine NP'nin etkileri*

Treatment Groups	N	Vitamin A (µg/dl±SE)	β-Carotene (µg/dl±SE)
Control	10	94±13	77±12 [a]
ETOH alone	10	80±8	153±12 [b]
10 µg NP in ETOH/kg-feed	10	87±23	146±36 [b]
100 µg NP in ETOH/kg-feed	10	112±26	145±33 [b]
500 µg NP in ETOH/kg-feed	10	113±30	103±10 [ab]
1000 µg in ETOH NP/kg-feed	10	72±7	78±4 [a]
5000 µg NP in ETOH/kg-feed	10	87±4	73±8 [a]

[abc] *Different letters indicates significance at P<0.01*

changed in all experimental groups. There was a significant increase in the level of β-Carotene in ETOH alone group as well as in 10 and 100 µg NP in ETOH/kg-feed groups in comparison to control group (P<0.01). However, β-Carotene level was begun to decrease at 500 µg NP in ETOH/kg-feed groups, and the decrease was more dramatic in 1000, and 5000 µg NP in ETOH/kg-feed groups (*Table 10*).

DISCUSSION

Environmental endocrine disrupter namely NP is present in the environment and is contaminating food and bioaccumulating in living organisms. It appears that the contamination of NP in aquatic food primarily occur through the massive use of detergents, while the contamination of NP in terrestrial food mainly occurs through the extensive use of pesticides and herbicides. However, the contamination of NP to aquatic organism may occur through the use of pesticides since nonylphenol and the related nonyl phenol ethoxylates are used in pesticide products as "inert" ingredients and added as adjuvants with a 1.5 millions pound by pesticide users in a year [36]. Furthermore, the use of aquatic products such as fish meal in animal ration is posing a potential regarding to NP bioaccumulation in terrestrial organisms including human. Therefore, it has been highly intriguing for many researchers to determine the adverse effects of NP on living organism. In this study, we were interested in determining the effects of NP on growth parameters of Japanese quails such as BWG, FC, FCR, EP and the parameters of antioxidant defense system including vitamin A and C levels along with lipid peroxidation.

Effects of NP on FC, FCR and EP

As shown in *Table 3* and *6*, within the range of environmentally relevant or above concentrations of NP do not have dramatic effects on the survival of Japanese quails in our system of experimentation. Mortality rate are minimum in all experimental groups. This suggests that even the highest concentration of NP (5000 µg NP/kg-feed) used in this study is not lethal to growing chicks and layers of quails. However, all of these sub-lethal concentrations cause abnormalities in some of the parameters investigated in this study.

Cunny et al.[37] reported that 90 day feeding study with 40 mg NP/kg-feed caused reduction in daily body weight gain, while Chitra et al.[23] reported that growth and daily body weight gain were not affected by the exposure of rats with 1, 10 and 100 µg NP/kg-feed for 45 days. Different NP concentrations used in this study did not have any effects on BWG, FC and feed conversion

ratio for growth (FCRG) (P>0.05). Our finding confirms that environmentally relevant concentrations of NP exposed to quails for 120 days did not have any effect on daily body weight gain. On the other hand, environmentally relevant concentration may have adverse effects on pup's weight when NP orally administered to pregnant rats [39]. This suggests that NP did not have any effects on growth but it may have inhibitory effects on the growth of fetus. The finding of this study is supported by a finding reported by Chen et al.[40] that prenatal exposure of NP is transferred to fetus. However, it is not clear how NP inhibits the maternal growth of fetus.

In the present study, there was an interesting finding that the ethanol alone appeared to cause significant increase in FC (P<0.05) but it did not have any other effect on any other parameters including BWG, EP or FCR. It was not clear why ethanol induced FC in quails.

As it seen in *Table 5*, the most dramatic adverse effect of NP occurred on FCRE in laying quails older than 6 weeks of age in comparison to control group. Orally given NP within feed does not affect BWG in growing quails less than 6 weeks old, but it has significant detrimental effect on EP without having any effect on feed consumption in laying quails older than 6 weeks of age (P<0.001). This suggests that NP may have an effect on endocrine control of ovarian function in Japanese quails. NP is an estrogenic environmental disrupter, but it may not exert its estrogenic effects on chicks prior to sexual maturity since estrogen receptors are not expressed before sexual maturation. When quails reach sexual maturity at the six weeks of age, endocrine disrupting effect of NP may have become evident and follicular development could be affected. It has been reported that NP cause a change in the expression of mRNA for gonadotropin-releasing hormone (sGnRH), and estrogen receptor (ER) isoforms in the brain [41]. Since NP could change sGnRH, it may have disrupting effect on the pituitary-gonadal axis. NP may therefore have inhibitory effect on egg production by disrupting FSH secretion during follicular development or disrupting LH surge required for ovulation.

Effects of NP on Lipid Peroxidation and Vitamins

It has been shown that nonylphenol (NP) inhibits cellular respiration and growth in yeast by inducing the generation of ROS [42]. Joreno [43] reported that oxidative damage is generally determined by measuring anti-oxidant levels in the blood and tissue since it is very difficult to determine the activity of ROS or the level of ROS which has a very short life but has a very high detrimental effect.

The main target of ROS is membrane phoshpholipids. Therefore, the determination of lipid peroxidation is a first step toward assessing the oxidative damages in a cell [44]. The common indicators of lipid peroxidation and oxidative stress in tissues are the generation of thio-barbituric acid reactants such as malondialdehyde (MDA), lipid peroxides and hydroperoxides [45,46]. MDA is considered as an important indicator for ROS-induced oxidative damage since it is the end product of either oxidative breakdown of multiple unsaturated fatty acids or the oxygenation of arahydonic acid [44].

In this study, it is shown that NP causes ROS induced oxidative damage. As shown in *Table 9*, MDA level is significantly elevated in 10, 100, and 500 µg NP in ETOH/kg-feed groups in comparison to control (P<0.001). Elevation of MDA means NP induces the generation of ROS that cause oxidative damage in Japanese quails. The possible reason for not seeing a dramatic increase in MDA level in 1000 and 5000 µg NP in ETOH/kg-feed groups, high concentrations of NP including 1000 and 5000 µg NP in ETOH/kg-feed may have adverse effects on the integrity of cells or tissues that fails to exert their normal functions. For instance, Uguz et al.[24] reported that 220 µg NP/L exposure severe structural degenerations in the liver tissues of rainbow trout that glutathione-S-transferase activities dramatically depleted in liver cells while 22 and 66 µg NP/L stimulate GST activities. This suggests that higher concentrations of NP induce severe histopathological disorders in tissues of living organisms that it is not possible to measure or evaluate normal biochemical parameters.

The level of AOA is not a simple sum of the activities of antioxidative agents but it is the dynamic equilibrium of interaction among each antioxidant constituents with each other [34]. The present study showed that there was an increase in AOA level in comparison to control but this increase is not significantly different from control *(Table 8)*. As shown in Table 8 and 9, there is an increase in the levels of AOA and MDA in 100 µg NP in ETOH/kg-feed group. However, this increase is alleviated when NP concentrations are increased. It seems that AOA and MDA level is inversely related to NP concentrations. This may indicate that the high concentration of NP consumes the activity of antioxidants very quickly in quails. These findings are in accordance with the findings of Chitra et al.[23] that NP is consuming the antioxidants in a dose dependent manner in testicular cells of rats.

It is widely known that β-Carotene is a precursor for the synthesis vitamin A. It is also known that β-Carotene and vitamin A are very important for antioxidant system and β-Carotene has been shown to exert its antioxidant activity independently from vitamin A [47]. This study shows that, β-Carotene level is significantly elevated in

10 and 100 µg NP in ETOH/kg-feed groups in comparison to control (P<0.01), whereas its level drops back to control level as the concentration of NP increases *(Table 10)*. There is a similar pattern for rise and fall on vitamin A level as well. However, this fluctuation is not significant (P>0.01). Our results is confirming the findings reported by Gong and Han [48] that high NP concentrations induce the generation of ROS without causing any morphological changes in Sertoli cells within 2 h but the exposure of Sertoli cells to NP for 12 to 24 h induces the lipid peroxidation and the loss of mitochondrial membrane potential. These findings strongly suggest that the activity of antioxidant system and vitamins including vitamin A and β-Carotene is dramatically consumed when quails exposed to the high concentrations of NP.

Vitamin C is known as a cellular antioxidant due to its powerful reducing activity in the cell [49]. Besides, being a primary antioxidant like α-tochoferol and β-Carotene, ascorbic acid also serves as a secondary antioxidant due to its ability for reducing α-tochoferoksil [50,51]. Antioxidants including vitamin C prevent the hazardous effects of ROS. For example, vitamin C scavenges hydroxyl radicals and prevents lipid peroxidation in the cell membrane of the stomach [52]. However, it has been reported that vitamin C does not prevents but aggravates BPA, NP and OP induced brain damages [53]. This suggests that the interaction of vitamin C and NP is somewhat peculiar since NP does not consume vitamin C, it may rather induce the synthesis of vitamin C in a dose dependent manner. The present study showed that unlike MDA, AOA, and β-Carotene, there is a rise in vitamin C level when quails are exposed to high NP concentrations. This finding confirms the peculiarity of vitamin C and NP interaction as Aydogan et al.[52] reported. This may also suggest that vitamin C aggravates the adverse effects on Japanese quails and other vertebrates and mammals.

Rucker and Morris [49] reported that vitamin C is endogenously synthesized in birds and the present study shows that NP induces vitamin C synthesis in Japanese quails. Thus, the vitamin C may severely aggravates the adverse effects of NP in living organisms.

In conclusion, although different concentrations of NP used in this study are not lethal and did not have any significant effect on growth of chicks, it has dramatic effect on growth parameters such as BW and EP as well as on the parameters of antioxidant system including lipid peroxidation and the levels of vitamins. The most interesting finding of this study is that NP induces the synthesis of vitamin C in Japanese quails. Since NP induces vitamin C synthesis and vitamin C aggravates the oxidative effects of NP, the exposure of NP is highly detrimental to birds.

ACKNOWLEDGEMENT

Authors would like to thank to the Director of Afyon Feed Factory for providing space to conduct this study.

REFERENCES

1. Nimrod AC, Benson WH: Environmental estrogenic effects of akylphenol etoxylates. *Crit Rev Toxicol,* 26, 335-364, 1996.

2. Bolt HM, Janning P, Michna H, Degen GH: Comparative assessment of endocrine modulators with oestrogenic activity. I. Definition of a hygiene-based margin of safety (HBMOS) for xenooestrogens against the background of European developments. *Arch Toxicol,* 74, 649-662, 2001.

3. Renner R: European bans on surfactant trigger transatlantic debate. *Environ Sci Tech,* 31, 316-320, 1997.

4. Blankenship AL, Coady K: Nonylphenol. In, Wexler P (Ed.): Encyclopedia of Toxicology. 2nd ed., pp. 260-263, Elsevier Academic Press, Oxford, 2005.

5. Montogomery-Brown J, Reinhard M: Occurrence and behavior of alkylphenol polyethoxylates in the environment. *Environ Eng Sci,* 20, 471-486, 2003.

6. Jobling S, Sumpter JP: Detergent components in sewage are weakly oestrogenic to fish, An *in vitro* study using rainbow trout *(Onchorynchus mykiss)* hepatocytes. *Aquat Toxicol,* 27, 361-372, 1993.

7. Skakkebaek NE, Meyts ERD, Jorgensen N, Carlsen E, Petersen PM, Giwercman A, Andersen AG, Jensen TK, Andersson AM, Muller J: Germ cell cancer and disorders of spermatogenesis: An environmental connection? *APMIS,* 106, 3-11, 1998.

8. Veeramachaneni DNR: Deteriorating trends in male reproduction, Idiopathic or Environmental? *Anim Reprod Sci,* 60-61, 121-130, 2000.

9. Hughes PJ, McLellan H, Lowes DA, Khan SZ, Bilmen JG, Tovey SC, Godfrey RE, Michell RH, Kirk CJ, Michelangeli F: Estrogenic alkylphenols induce cell death by inhibiting testis endoplasmic reticulum Ca^{2+} pumps. *Biochem Biophysic Res Com,* 277, 568-574, 2000.

10. Tapiero H, Nguyen Ba G, Tew KD: Estrogens and environmental estrogens. *Biomed Pharmacother,* 56, 36-44, 2002.

11. Salami RI: Replacement of poultry visceral offal meal for fish meal in layers' diets. *Niger J Anim Product,* 24, 37-42, 1997.

12. Okon BI, Ogunmodede BK: Effects of replacing dietary fishmeal with periwinkle flesh on the performance of broiler chickens. *Niger J Anim Product,* 24, 37-42, 1995.

13. Abiola SS, Onunkwor EK: Replacement value of hatchery waste meal for fish meal in layer diets. *Bioresour Technol,* 95, 103-106, 2004.

14. Guenther K, Heinke V, Thiele B, Kleist E, Prast H, Raecker T: Endocrine disrupting nonylphenols are ubiquitous in food. *Environ Sci Tech,* 36, 1676-1680, 2002.

15. Yang DK, Ding WH: Determination of alkylphenolic residues in fresh fruits and vegetables by extractive steam distillation and gas chromatography-mass spectrometry. *J Chromat A,* 1088, 200-204, 2005.

16. Ye X, Kuklenyik ZL, Needham LL, Calafat AM: Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching-high performance liquid chromatography-isotope dilution tandem mass spectrometry. *J Chromat B,* 831, 110-115, 2006.

17. Ademollo N, Ferrara F, Delise M, Fabietti F, Funari E: Nonylphenol and octylphenol in human breast milk. *Environ Int,* 34, 984-987, 2008.

18. Lu CY, Ma YC: Oxidative stress associated with indoor air pollution and sick building syndrome-related symptoms among office workers in Taiwan. *Inhal Toxicol,* 19, 57-65, 2007.

19. Soares A, Guieysse B, Jefferson B, Cartmell E, Lester JN: Nonylphenol in the environment: A critical review on occurrence, fate, toxicity and treatment in wastewaters. *Environ Int,* 34, 1033-1049, 2008.

20. Kawaguchi M, Takahashia S, Seshimoa F, Sakui N, Okanouchia N, Itoa R, Inouea K, Yoshimuraa Y, Izumic SI, Makinoc T, Nakazawaa H: Determination of 4-tert.-octylphenol and 4-nonylphenol in laboratory animal feed sample by stir bar sorptive extraction followed by liquid desorption and column-switching liquid chromatography-mass spectrometry with solid-phase extraction. *J Chromat A,* 1046, 83-88, 2004.

21. Razia S, Maegawab Y, Tamotsua S, Oishia T: Histological changes in immune and endocrine organs of quail embryos: Exposure to estrogen and nonylphenol. *Ecotoxicol Environ Safety,* 65, 364-371, 2006.

22. Yoshimura Y, Fujita M: Endocrine disruption in avian reproduction: The histological analysis. *Av Poult Biol Rev,* 16, 29-40, 2005.

23. Chitra KC, Latchoumycandane C, Mathur PP: Effect of nonylphenol on the antioxidant system in epididymal sperm of rats. *Arch Toxicol,* l76, 545-551, 2002.

24. Uguz C, Iscan M, Ergüven A, Isgor B, Togan I: The bioaccumulation of nonylphenol and its adverse effects on the liver of rainbow trout *(Onchorynchus mykiss). Environ Res,* 92, 262-270, 2003.

25. Uguz C, Togan I, Eroglu Y, Tabak I, Zengin M, Iscan M: Alkylphenol concentrations in two rivers of Turkey. *Environ Toxicol Pharm,* 14, 87-88, 2003.

26. Solé M, de Alda MJL, Castillo M, Porte C, Pedersen KL, Barcelo´ D: Estrogenicity determination in sewage treatment plants and surface waters from the Catalonian area (NE Spain). *Environ Sci Tech,* 34, 5076-5083, 2000.

27. Vaccaro E, Meucci V, Intorre L, Soldani G, Di Bello D, Longo V, Gervasi P G, Pretti C: Effects of 17-beta-estradiol 4-nonylphenol and PCB 126 on the estrogenic activity and phase 1 and 2 biotransformation enzymes in male sea bass *(Dicentrarchus labrax). Aquat Toxicol,* 75, 293-305, 2005.

28. Livingstone DR: Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar Pollut Bull,* 42, 656-666, 2001.

29. Obata T: Environmental estrogen-like chemicals and hydroxyl radicals induced by MPTP in the striatum: A review. *Neurochem Res,* 27, 423-431, 2002.

30. Carrera EP, García-López A, del Pilar M, del Río M, Martínez-Rodríguez G, Solé M, Mancera JM: Effects of 17β-estradiol and 4-nonylphenol on osmoregulation and hepatic enzymes in gilthead sea bream (Sparus auratus). *Comp Biochem Physiol - Part C,* 145, 210-217, 2007.

31. Kway A: A simple colorimetric method for ascorbic acid determination in blood plasma. *Clin Chim Acta,* 86, 153-157, 1978.

32. Suzuki J, Katoh NA: Simple and cheap methods for measuring serum vitamin-A in cattle using only a spectrophotometer. *J Nip Juigaku Zasshi,* 52, 1281-1283, 1990.

33. Draper HH, Hardley M: Malondialdehyde determination as index of lipid peroxidation. *Meth Enzymol,* 186, 421-431, 1990.

34. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V: Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol,* 54, 356-361, 2001.

35. Ergün G, Aktaş S: ANOVA modellerinde kareler toplamı yöntemlerinin karşılaştırılması. *Kafkas Univ Vet Fak Derg,* 15 (3): 481-484, 2009.

36. Snedecor GW, Cochran WG: Statistical Methods. Ames, A: Iowa State University Pres. 1980

37. Cox C: Nonylphenol and related chemicals. *J Pest Reform,* 16(1): 15-20, 2003.

38. Cunny HC, Mayes BA, Rosica KA, Trutter JA, Van Miller JP: Subchronic toxicity (90-day) study with para-nonylphenol in rats. *Regul Toxicol Pharmacol,* 26, 172-178, 1997.

39. Latendresse JR, Newbold RR, Weis CC, Delclos KB: Polycystic kidney induced in F1 sprague-Dawley rats fed para-nonylphenol in a soy-free casein-containing diet. *Toxicol Sci,* 62, 140-147, 2001.

40. Chen ML, Chang CC, Shen YJ, Hung JH, Guo BR, Chuang HY, Mao IF: Quantification of prenatal exposure and maternal-fetal transfer of nonylphenol. *Chemos,* 73, 239-245, 2008.

41. Vetillard A, Bailhache T: Effects of 4-n-nonylphenol and tamoxifen on sGnRH, estrogen receptor and vitellogenin gene expression in juvenile rainbow trout. *Toxicol Sci,* 1-28, 2004.

42. Okai Y, Sato EF, Higashi-Okai K, Inoue M: Enhancing effect of the endocrine disruptor para-nonylphenol on the generation of reactive oxygen species in human blood neutrophils. *Environ Health Perspect,* 112, 553-556, 2004.

43. Joreno DR: Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic Biol Med,* 9 (6): 515-540, 1990.

44. Katz D, Mazor D, Dviansky A, Meyerstein N: Effect of radiation on red cell membrane and intra cellular oxidative defense system. Free Radic Res, 24 (3): 199-204, 1996.

45. Carr TP, Andersen CJ, Rudel LL: Enzymatic determination of triglyceride free cholesterol and total cholesterol in tissue lipid extracts. *Clin Biochem,* 26, 39-42, 1993.

46. Holley AE, Cheeseman KH: Measuring free radical reactions in vivo. *Br Med Bull,* 49, 494-505, 1993.

47. Moscio DP, Murphy EM, Sies H: Antioxidant defense systems: the role of carotenoids tocopherols and thiols. *Am J Clin Nut,* 53, 194-200, 1991.

48. Gong Y, Han XD: Nonylphenol-induced oxidative stress and cytotoxicity in testicular Sertoli cells. *Reprod Toxicol,* 22, 623-630, 2006.

49. Rucker BR, Morris JG: The vitamins. **In,** Kaneco JJ, Harvey JW, Bruss ML (Ed.): Clinical Biochemistry of Domestic

Animals. 5th ed. Chapter 24, pp. 703-739, Academic Press Inc, California, 1997.

50. Gey KF: On the antioxidant hypothesis with regard to arteriosclerosis. *Bibl Nutr Dieta,* 37, 53 91, 1986.

51. Wefers H, Sies H: The protection of ascorbate and glutathione against microsomal lipid peroxidation is dependent on vitamin E. *Eur J Biochem,* 174, 353-357, 1988.

52. Gartner LP, Hiatt LJ: Color Textbook of Histology, 2nd ed., Saunders, Pennsylvania, 2001.

53. Aydogan M, Korkmaz A, Barlas N, Kolankaya D: The effect of vitamin C on bisphenol A, nonylphenol and octylphenol induced brain damages of male rats. *Toxicol,* 249, 35-39, 2008.