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EFFECT OF FEEDING DIFFERENT OILS ON PLASMA CORTICOSTERONE IN BROILER CHICKENS

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A study was conducted to examine the effects of different oils on the plasma corticosterone concentrations of broiler chickens fed *ad libitum* or deprived of feed for 24 hours. A total of 36 Ross broilers were randomly assigned to one of three dietary treatments at 10 days of age and fed a grower diet supplemented with 60 g/kg soybean oil (rich in linoleic acid, C18:2n-6), linseed oil (rich in α-linolenic acid, C18:3n-3) or fish oil (rich in C14:0, C16:0, C16:1n-7, C20:1n-9; eicosapentaenoic acid and docosahexaenoic acid, EPA, C20:5n-3 and DHA, C22:6n-3), respectively, for 18 days. Dietary supplementation of fish oil resulted in lower ($P < 0.05$) baseline plasma corticosterone levels of chickens fed *ad libitum* for 18 days compared to soybean and linseed oil supplementations. Feed deprivation for 24 h induced a significant ($P < 0.05$) increase in corticosterone concentration in every treatment group compared to the *ad libitum*-fed birds. The hormone levels of feed-deprived birds did not differ significantly among groups fed diets supplemented with different oils.

Key words: Polyunsaturated fatty acids, fish oil, plasma corticosterone, broiler chickens

Polyunsaturated fatty acids (PUFA) influence normal growth and development, gene expression, metabolic pathways, as well as central nervous, endocrine and immune functions (Simopoulos, 2003). Dietary n-3 and n-6 subtypes of PUFA have been shown to fulfil different physiological roles in metabolism and hormonal regulation. In mammals, diets containing high levels of n-6 PUFA may lead to insulin resistance while the incorporation of long-chain n-3 PUFA in phospholipids of skeletal muscle cells improves glucose uptake and insulin action (Storlien et al., 1991). The n-6 fatty acid arachidonic acid (AA; C20:4n-6) can be converted to eicosanoids of high pro-inflammatory potential (Lewis et al., 1990). By contrast, the n-3 fatty acids eicosapentaenoic acid (EPA; C20:5n-3)

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and docosahexaenoic acid (DHA; C22:6n-3) give rise to mediators that have been demonstrated to be anti-inflammatory and inflammation-resolving (Serhan et al., 2000). The metabolic effects of dietary PUFA have also been investigated in broiler chickens. In the fasted state, broilers fed diets enriched with n-6 or n-3 fatty acids (sunflower or linseed oil, respectively) developed lower plasma levels of insulin and cholesterol compared to those fed diets rich in saturated fatty acids (tallow) or monounsaturated fatty acids (different kinds of olive oils; Crespo and Esteve-Garcia, 2003). The protective effects of EPA and DHA from marine sources are well documented in chronic human and animal diseases including cancer, insulin resistance and cardiovascular disease (Calder, 2012). However, the specific biological effects of plant-derived α-linolenic acid (ALA; C18:3n-3) are largely unknown or conflicting (Anderson and Ma, 2009).

Recent studies in mammals have demonstrated that EPA and DHA may affect the function of the hypothalamic–pituitary–adrenal (HPA) axis and inhibit corticosterone secretion (Carsia et al., 2008; Liu et al., 2013). However, little information is available in birds whether ALA and its longer chain metabolites, EPA and DHA show similar or different metabolic effects on the adrenocortical function to those found in mammalian species. Thus, the aim of the present study was to examine the effects of different dietary sources of PUFA – soybean oil (rich in LA), linseed oil (rich in ALA) and fish oil (rich in EPA + DHA) – on plasma corticosterone concentrations.

Materials and methods

Animals, diets and experimental design

The experimental procedures, animal facilities and sample collection methods were approved by the National Ethics Committee for Animal Experiments of the Advisory Council on Animal Experiments (permit number: GK-2676/2012). One-day-old male broiler chickens of the Ross 308 strain were obtained from a commercial hatchery and assigned randomly to three floor pens, with 12 chickens per pen in an environmentally controlled room under standard conditions of temperature, humidity and ventilation (Aviagen Broiler Breeders, 2009).

Broilers were fed a commercial broiler starter diet until 10 days of age. At 10 days of age, the chickens were randomly assigned to one of three dietary treatments (n = 12 per treatment) and fed the experimental grower diets for 18 days on an *ad libitum* basis. Individual body weights and feed intake per group were measured at 10 and 28 days of age, and body weight gain and feed conversion ratio was calculated. The experimental diets were isonitrogenous and isoenergetic, and differed only in fatty acid composition. The diets were prepared weekly and stored below 20 °C. The composition and nutrient content of the experimental diets are presented in Table 1. The experimental diets were supple-

mented with 60 g/kg edible-grade soybean, linseed or fish oil, respectively. The fatty acid composition of the diets is shown in Table 2.

Table 1

Composition and nutrient content of the experimental diets

Ingredient	g/kg
Maize	422
Wheat	150
Extracted soybean meal	275
Maize gluten meal	50
Oil*	60
L-Lysine	3
DL-Methionine	1
Monocalcium phosphate	15
Limestone	16
Salt	3
Vitamin-trace mineral premix†	5
Calculated nutrient content‡	
Metabolisable energy (AMEn, MJ/kg)	13.2
Crude protein	210.0
Ether extract	82.8
Crude fibre	35.0
Calcium	9.0
Phosphorus (available)	4.5
Lysine	12.5
Methionine	4.6
Methionine + cystine	8.8

*Soybean, linseed or fish oil, respectively. The oils contained 1 mg/g added antioxidant (butylated hydroxytoluene); †Supplied the following per kg of diet: trans-retinol 3.3 mg, cholecalciferol 125 μg, DL-α-tocopheryl acetate 50 mg, menadione 3 mg, thiamine 2 mg, riboflavin 6 mg, pyridoxine 3 mg, cyanocobalamin 16 μg, D-calcium panthotenate 20 mg, niacin 20 mg, folic acid 1.75 mg, choline chloride 600 mg, butylated hydroxytoluene 80 mg, Zn 80 mg, Fe 80 mg, Mn 100 mg, Cu 8 mg, I 1 mg, Se 150 μg; ‡Based on analyses of ingredients

Sample collection and chemical analyses

Blood samples were collected from the wing vein of *ad libitum*-fed birds in heparinised tubes containing sodium fluoride at day 28 between 08:00 and 09:00 a.m. After blood sampling, chickens in each group were deprived of feed for 24 h. Blood sampling was repeated the next day between 08:00 and 09:00 a.m. Care was taken to ensure that the time that elapsed between catching a bird

and collecting the blood sample did not exceed 45 sec to minimise the effects of sampling on hormone and metabolite values. Plasma was obtained after centrifugation at 4,000 rpm for 20 min within 1 h of collection and stored at –20 °C.

Table 2

Fatty acid composition of the experimental diets (g/100 g total fatty acids extracted)

Fatty acids	Dietary treatments*		
	Soybean oil	Linseed oil	Fish oil
14:0	0.32	0.11	6.48
16:0	10.04	7.95	17.28
18:0	3.24	3.92	2.28
16:1n-7	0.11	0.11	4.68
18:1n-9	24.62	22.05	18.72
18:1n-7	0.76	0.42	1.32
20:1n-9	0.22	0.21	7.32
18:2n-6	54.00	25.55	20.28
18:3n-3	5.51	38.90	2.64
20:5n-3	0.32	0.21	8.40
22:5n-3	ND	0.21	0.84
22:6n-3	ND	ND	9.72
Total saturated	13.61	11.98	26.04
Total monounsaturated	25.70	22.79	32.04
Total polyunsaturated	59.83	64.87	41.88
n-6	54.00	25.55	20.28
n-3	5.83	39.32	21.60
n-6: n-3	9.26	0.65	0.94

ND = not detected; *Diets supplemented with 60 g/kg soybean oil, linseed oil or fish oil, respectively

Plasma corticosterone levels were measured by a high-sensitivity competitive enzyme-linked immunoassay method (Code AC-15F1, IDS Ltd, UK) using an Anthos Zenyth 3100 microplate reader (Biochrom Co., UK). In this analysis the intra-assay coefficient of variation (CV) was < 10%.

The total fat content of the diets was extracted and lipid extracts were converted to fatty acid methyl esters by using BF_3-methanol (Association of Official Analytical Chemists, 1990*a*, 1990*b*). The fatty acid methyl esters were separated and analysed by gas chromatography in a TRACE 2000 chromatograph (Thermo Finnigan Italia, S.p.A., Rodano, Milan, Italy) equipped with an Omegavax 320 capillary column (30 m length × 0.32 mm I.D., 0.25 µm film; Supelco, Bellefonte, USA). The temperature of oven and flame ionisation detector were set at 200 and 260 °C, respectively. Helium was used as a carrier gas (25 cm/sec, set at 200 °C) and the split ratio was 100:1. Individual fatty acids were identified by comparison to a known standard mixture of fatty acid methyl esters (PUFA-2, Supelco catalogue number 4-7015-U; Supelco, Bellefonte, PA, USA).

Statistical analysis

Statistical analysis of plasma corticosterone concentrations was carried out by two-way analysis of variance (ANOVA) using dietary fatty acid profile and feeding regimen (*ad libitum* feeding vs. feed deprivation) as main effects. Differences between means were determined by the Duncan multiple range test and considered significant when $P < 0.05$. Data for corticosterone concentrations were transformed logarithmically before ANOVA to achieve homogeneity of variances. The significance level of correlation coefficients between dietary fatty acids and baseline corticosterone levels was evaluated by *t*-test. All statistical analyses were conducted using the Statistica 5.0 statistical software (StatSoft, Tulsa, OK, USA). The results are presented as mean values with their standard errors.

Results

The body weight of birds fed different diets did not differ ($P > 0.05$) at 28 days of age (Table 3). The body weight gain and feed conversion ratio of chickens during the experimental period (from day 10 to day 28) were not affected by dietary treatments ($P > 0.05$; Table 3).

Table 3

Body weight (g), body weight gain (g/day) and feed conversion ratio (kg/kg) of birds fed diets supplemented with different oils

	Dietary treatments*			P value
	Soybean oil	Linseed oil	Fish oil	
Body weight (day 28)	1437.4 ± 43.9	1367.6 ± 43.3	1329.4 ± 30.8	NS
Body weight gain (days 10–28)	51.9 ± 2.0	50.9 ± 1.2	49.8 ± 1.3	NS
Feed conversion ratio (days 10–28)	1.56	1.56	1.59	–

Body weight and body weight gain values are means ± SE of 12 birds. Feed conversion ratio values are means calculated from the feed intake data measured for the whole treatment groups. NS = not significant, $P > 0.05$; *Diets supplemented with 60 g/kg soybean oil, linseed oil or fish oil, respectively

The plasma corticosterone results of chickens fed *ad libitum* and deprived of feed for 24 h are shown in Fig. 1. Dietary supplementation of soybean and linseed oil resulted in higher basal plasma corticosterone levels compared to fish oil supplementation ($P < 0.05$). Feed deprivation for 24 h induced an increase ($P < 0.05$) in corticosterone concentration in all treatment groups compared to the baseline values of *ad libitum*-fed birds. The hormone levels of feed-deprived birds did not differ significantly between groups fed different diets. The correlation coefficients between the proportion of fatty acids in diets and basal plasma corticosterone levels are presented in Table 4. The proportion of dietary fatty ac-

ids C14:0, C16:1n-7, C20:1n-9, C20:5n-3 and total saturated fatty acids showed a negative correlation with the plasma corticosterone values observed in the three dietary treatment groups ($P < 0.05$).

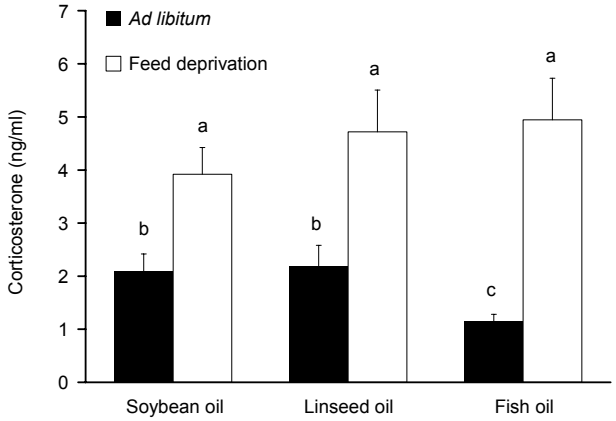

Fig. 1. Effect of dietary treatments on plasma corticosterone levels of broilers fed *ad libitum* or deprived of feed for 24 h (n = 12 per dietary treatment group). Chickens were fed diets supplemented with 60 g/kg soybean oil, linseed oil or fish oil, respectively. Values are means with their standard errors. Values not sharing common superscript letters are significantly different ($P < 0.05$)

Table 4

Correlation coefficients (r) between dietary fatty acids and baseline plasma corticosterone concentrations

Fatty acids	r	P value
14:0	–0.9988	< 0.05
16:0	–0.9908	NS
18:0	0,9406	NS
16:1n-7	–0.9969	< 0.05
18:1n-9	0.8637	NS
18:1n-7	–0.9540	NS
20:1n-9	–0.9970	< 0.05
18:2n-6	0.5567	NS
18:3n-3	0.6240	NS
20:5n-3	–0.9977	< 0.05
Total saturated	–0.9996	< 0.05
Total monounsaturated	–0.9728	NS
Total polyunsaturated	0.9914	NS
n-6	0.5567	NS
n-3	0.1123	NS
n-6:n-3	0.4032	NS

NS = not significant, $P > 0.05$

Discussion

This experiment focused on studying the effect of dietary fatty acids on plasma corticosterone concentrations in broilers. Plasma corticosterone level has been widely used as the most reliable indicator of acute stress in chickens (Thaxton and Puvadolpirod, 2000). Considering the age of the broilers, in this study the mean basal levels of this hormone in birds fed *ad libitum* were in the physiological range (Gonzales et al., 2003). Peak corticosterone concentrations after acute handling stress can reach a value ranging from 4 to 16 ng/ml (Beuving and Vonder, 1978). In conformity with the results of Scott et al. (1983), our study clearly showed that a 24-h feed deprivation significantly increased plasma corticosterone concentration in broilers irrespective of dietary treatments, and such feed deprivation led to the activation of the HPA axis.

To the best of our knowledge, this paper presents the first experimental demonstration of the effects of dietary fatty acids on plasma corticosterone concentrations in poultry. The *ad libitum* feeding of diets containing fish oil led to significantly lower baseline plasma corticosterone concentrations than the feeding of diets supplemented with either soybean or linseed oil. In contrast, we did not find significant dietary fatty acid effects on plasma corticosterone concentrations in response to feed deprivation.

In this experiment, correlations were found between several dietary fatty acids (saturated: C14:0; monounsaturated: C16:1n-7, C20:1n-9; and polyunsaturated: EPA, respectively) and baseline plasma corticosterone levels. These findings may contribute to the significant differences observed in our study. Circulating specific free fatty acids in the plasma are involved in the regulation of HPA axis activity. A fall of plasma palmitate, but not of oleate and linoleate, level can increase plasma corticosterone in rats (Oh et al., 2014). Thus, a higher intake of dietary palmitate may decrease plasma corticosterone. In our experiment, broilers fed diets containing fish oil had a higher intake of palmitate than chickens in other groups, and thus the effect of palmitate cannot be excluded. The correlation coefficient for palmitate was quite high but not significant. Based on the literature published on this topic, the possible role of the long-chain n-3 fatty acids EPA and DHA in the inhibition of corticosterone synthesis and/or secretion in broilers fed a diet containing fish oil can be supposed. Like in our study, however, most of the experimental setups did not allow the precise investigation of the effects of individual fatty acids. According to a single study published in birds up to now, a diet containing higher amounts of n-3 type fatty acids of marine origin resulted in lower levels of plasma corticosterone. In that experiment of Kitaysky et al. (2001) with feed-restricted red-legged kittiwake chicks at 65% of the *ad libitum* energy intake, a high-lipid diet rich in long-chain n-3 PUFA resulted in lower baseline and acute stress-induced levels of corticosterone compared to a low-lipid diet of the same fatty acid composition. Similarly, several

studies in mammals investigating the effects of dietary fatty acids also demonstrated the suppressive effect of long-chain n-3 fatty acids on the adrenocortical function both at adrenal and hypothalamic–pituitary level. Compared to the diet supplemented with butterfat rich in saturated fatty acids, feeding a diet supplemented with an n-3 fatty acid mixture containing EPA and DHA decreased cellular ACTH-induced corticosteroid production of rats by 67% (Carsia et al., 2008). In a recent study in piglets, a diet supplemented with fish oil resulted in the enrichment of EPA and DHA contents, respectively, in the hypothalamus and pituitary, and decreased plasma ACTH and cortisol levels (Liu et al., 2013). Mice fed diets supplemented with DHA showed significantly reduced hypothalamic corticotropin-releasing hormone (CRH) and pituitary ACTH levels, as well as reduced plasma ACTH and corticosterone in response to physical stress (Jiang et al., 2012).

Despite the experiments demonstrating the effect of long-chain n-3 PUFA on the HPA axis, the precise mechanism of that effect is still not clear. Different modes of n-3 PUFA action were observed, including effects on plasma membrane structure in model systems (Shaikh, 2012), and as ligands for peroxisome proliferator-activated receptors of rat cardiomyocytes (Di Nunzio et al., 2009) and specific G-protein-coupled receptors of humans and mice (Oh and Olefsky, 2012). In addition, EPA and DHA may impair prostaglandin synthesis in a state of inflammation and serve as a substrate for recently discovered lipid mediators which in turn affect the HPA function of mammalian species (Peterson et al., 1998; Shewchuk, 2014). Prostaglandins can stimulate ACTH secretion, and previous *in vitro* studies with chicken and turkey adrenal cells showed that prostaglandins can directly increase avian adrenocortical steroidogenesis (Kocsis et al., 1999).

In our experiment feeding a fish oil diet rich in long-chain marine-type EPA plus DHA and a diet containing high amounts of ALA of linseed oil origin resulted in significantly different baseline plasma corticosterone levels of *ad libitum*-fed chickens. Supposing the role of EPA and DHA in the regulation of plasma baseline corticosterone, the n-3 fatty acid ALA did not seem to have similar effects. The enzymatic conversion of ALA to EPA and DHA is possible in chickens (Gregory et al., 2013), but its efficiency does not seem to be sufficient to have effects similar to those of the long-chain n-3 PUFA in fish oil diets. Furthermore, the biological role of ALA in mammalian stress response is also unknown (Anderson and Ma, 2009).

Despite the numerous examples in mammals mentioned above, dietary fatty acids did not affect the plasma corticosterone response of chickens to feed deprivation. The reason for this is not clear and could be related to differences between the mammalian species investigated and chickens. However, in another experiment conducted by us a three-day feed restriction led to a significantly lower increase of corticosterone metabolite concentration in the faeces of hens

fed a diet containing fish oil compared to a diet supplemented with sunflower oil (unpublished results).

In conclusion, the present study indicates that feeding diets supplemented with fish oil can decrease the baseline plasma corticosterone concentration of chickens fed *ad libitum* compared to those receiving diets containing soybean or linseed oils, respectively. These effects observed in broilers may be related to several dietary fatty acids present in fish oil, and further studies on the effect exerted by individual fatty acids on the HPA axis and corticosterone level of birds need to be conducted.

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