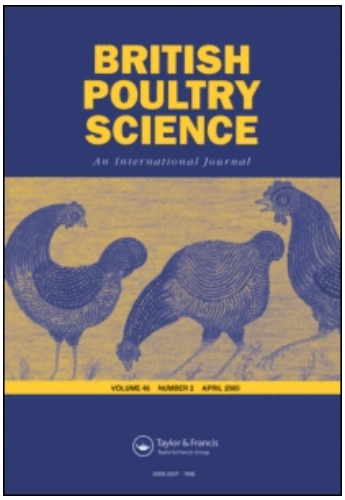

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SHORT COMMUNICATION

Learning ability of 1-d-old partridges (*Alectoris rufa*) from eggs laid by hens fed with different n-3 fatty acid concentrations

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Abstract 1. The diets of commercial strains of laying partridge are usually lower in polyunsaturated fatty acids (PUFA) and n-3 fatty acids than the diets of wild partridges. The aim of this experiment was to examine the effects of three different PUFA and n-3 concentrations in partridge laying diets.
2. Offspring learning ability (passive avoidance test of 1-d-old chicks) was used to assess the effect of three different maternal diets (144 chicks were tested for each diet). A negative experience, allowing the bird to peck a bead bathed in a bitter liquid (methyl anthranilate—MA), was used for this purpose. The adults had been fed one of three different diets with n-3 contents of 0·48, 4·04 or 7·60 g/kg.
3. There was better memory retention in the offspring of adults fed the intermediate n-3 content compared to those fed the lower content. Discrimination ratio (DR) of the latency time toward the wrong (red) bead was less for the lower n-3 content (0·48) than for the middle n-3 PUFA content (0·43). DR of the number of pecks toward the wrong beads was greater for the lower n-3 content (0·51) than for the middle n-3 PUFA content (0·71).
4. The partridges fed the diet containing the lowest concentration of n-3 and PUFA were unable to express the expected behavioural score (neural embryo development index) given the genetic characteristics of the animals.

INTRODUCTION

Essential fatty acids contribute to the structure of all tissues and are involved in cell membrane synthesis. Long-chain polyunsaturated fatty acids (l.c. PUFA) are particularly represented in the brain, retina and other neural tissues and they seem to be involved in the functional activity of the brain. Lipids are about 50 to 60% of human brain dry matter of which approximately 35% are l.c. PUFA. Arachidonic acid (AA) and docosahexaenoic acid (DHA) are the main constituents of the l.c. PUFA in the brain. These fatty acids have two different main origins: (a) through biosynthesis from their precursors, primarily linoleic acid and α-linolenic acid (LNA); (b) directly from dietary sources (Wainwright *et al.*, 1999).

DHA, a 22-carbon fatty acid with 6 double bonds, the last of which in the n-3 position, is considered essential for neuronal plasticity and development (Itokazu *et al.*, 2000). Docosapentaenoic acid (DPA), eicosapentaenoic acid (EPA) and LNA are n-3 fatty acids involved in the same processes and they can also be used as DHA precursors. An inadequate PUFA content in the diet, particularly of n-3 and n-6 fatty acids, may cause imperfect development of the tissues and their functionality might be impaired or reduced. Some kinds of algae and fish industry by-products (mainly oil) are foods particularly rich in PUFA. The algae are the primary producers and fish that eat the algae store these fatty acids in the liver. These two different sources have a different PUFA concentration and bio-availability in the digestive tract of different animal species. Other conventional foods such as soybean and other oil seeds, some species of fresh forages such as alfalfa, and unconventional food such as snails, worms and insects, also contain high concentrations of n-3 PUFA (Bagliacca *et al.*, 2000).

In bird species, the only source of PUFA for the embryo is the yolk. For this reason, the yolk

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fatty acid composition is essential for the optimal development of the neural embryo-tissues. Many studies have demonstrated the possibility of increasing the n-3 yolk content by dietary alteration (Cheriam and Sim, 1991). Learning ability and memory performance have been studied on several mammalian species, such as humans, pigs, mice and rats (Helland *et al*., 2003) but few studies have been carried out in avian species (Bagliacca *et al*., 2000). The ingredients of commercial laying diets greatly differ from the diet ingredients selected by partridge in their natural environment (Bagliacca *et al*., 2000).

The aim of the present study was to examine the effect of three different n-3 PUFA diet contents supplied to red partridge laying hens (*Alectoris rufa rufa*), on CNS functionality by means of the learning ability of their offspring (1-d-old chicks).

METHODS

Animals and diets

Eggs laid by three different parent groups (called 480, 4040 and 7600) were used for the experiment. Each group was composed of 48 red partridge pairs, 2 and 3 years old. Pairs were distributed at random in the groups and in the cages. The cages (45 cm × 80 cm × 35 cm: w × d × h, with a mesh floor of 1 cm × 1 cm, wire cross section 2·0 mm), each held one pair of birds, were equipped with one feeder (20 cm × 7 cm × 15 cm, with a capacity of 1·2 kg approximately) and were kept outdoors in a fenced area. An artificially extended photoperiod was used (natural + artificial 16L:8D; 35 lux light intensity), eggs were collected daily, stored (14°C, RH 70%) for one week and incubated each week.

Each parent group was fed with a different diet starting from 30 d before the laying period began. The only treatment difference was the diet of the breeding pairs.

The diets, formulated for partridge laying hens, contained two different commercial fat sources: diet 480, 30 g/kg palm oil (as fatty acid calcium salts); diet 4040, 15 g/kg palm oil (as fatty acid calcium salts), 15 g/kg oil mixture (linseed oil 600 g/kg and fish oil 400 g/kg, micro-encapsulated); diet 7600, 30 g/kg oil mixture (composed as described above). Diet ingredients and their fatty acid compositions are shown in Table 1.

The third, 7th and 11th hatches were used to test learning ability and memory retention and a total of 144 chicks (48 chicks for each group) were chosen at random from each hatch for this purpose.

Passive avoidance test

A passive avoidance learning (PAL) task was used to assess brain development through the learning ability and memory retention of the 1-d-old chicks (Andrew, 1991). Sociable birds cannot be tested alone because they show signs of distress if placed alone in a strange environment and the presence of more than one bird in the same box seems not to affect the test results (Andrew, 1991). For this reason, three partridge chicks (just hatched) coming from three different parent groups were placed together in one shaving floor cardboard holding box (26 cm × 21 cm). The boxes were continuously warmed by 100 W red bulbs (30 cm high) and the room was kept at a constant temperature of 27°C. To recognise chicks from the different dietary treatments (480, 4040, 7600) they were marked on the head by different coloured pens. The chicks were kept in the experimental boxes for at least 30 min prior to beginning the trials. The test was based on the chick's natural

Table 1. *Ingredients and chemical composition of the diets*

Ingredient (g/kg 'as is basis')		
Soft wheat		220
Soybean meal solv. extr.		190
Barley		170
Sunflower seed meal solv. extr.		150
Maize		100
Sorghum		50
Fat supplement[1]		30
Calcium carbonate		46
Lignosulphonate		10
Vitamin and mineral premix[2]		4
Sodium bicarbonate		3
Sodium chloride		3
Dicalcium phosphate		14·85
L-Lysine HCl		1·65
DL-Methionine		7·50
Total		1000·00

Calculated analysis		Diet		
	Unit	480	4040	7600
Metabolisable energy	MJ/kg	11·1	11·2	11·3
Crude protein	g/kg	210	209	209
Fat	g/kg	50·3	50·4	50·6
NSP (Non-starch polysacc.)	g/kg	259	260	262
LA C18:2	g/kg	13·7	13·9	14·1
LNA C18:3 $\omega 3$	g/kg	0·48	1·43	2·80
EPA C20:5 $\omega 3$	g/kg	–	0·68	1·37
DPA C22:5 $\omega 3$	g/kg	–	0·33	0·67
DHA C22:6 $\omega 3$	g/kg	–	1·75	3·50
Total $\omega 3$	g/kg	0·48	4·04	7·60
Total $\omega 6$	g/kg	13·72	12·44	11·16
$\omega 6/\omega 3$		28·60	3·10	1·50

[1] Diet 480 30 g/kg saponified palm oil; diet 4040 15 g/kg saponified palm oil and 15 g/kg micro-encapsulated oil mixture; diet 7600 30 g/kg micro-encapsulated oil mixture.

[2] Supplied (mg/kg diet): retinol 4·5, cholecalciferol 0·075, DL-α-tocopherol 30, menadione 3, thiamine 2, riboflavin 8, pyridoxin 5, cyanocobalamin 0·03, D-biotin 0·1, nicotinic acid 40, pantothenic acid 15, folic acid 1·25, choline chloride 600, Mn 150, Zn 60, Fe 35, Co 0·5, Cu 10, J 0·5, Se 0·1, ethoxyquin 2·5.

behaviour to peck at small moving objects. The protocol originally described by Andrew (1991), was partially modified by introducing a pre-training treatment before the passive training, to increase retention and reduce variability (Burne and Rogers, 1997).

The test was composed of 4 different stages. In the pre-training stage chicks were trained to peck a white bead (2 mm diameter). In the training stage chicks were presented (1 cm in front of its beak) with a red bead and than a blue bead (both 4 mm diameter). In the testing stage the red bead bathed in a bitter liquid (methyl anthranilate—MA) was presented. Finally, in the post-testing stage the red bead and the blue bead were presented again, as described for the training stage.

Two testers worked in pairs with the same testing group. The first tester operated as follows: he presented to each chick the bead fixed at the end of a wire 20 cm long (1 mm cross section), announced the first peck and counted the number of pecks given by the chick. The second tester recorded the time elapsed (latency) between the bead presentation and the first peck given, announced the elapsed time and recorded the number of pecks given (in 20 s in the pre-training stage and in 10 s in the training, testing and post-testing stages). Between each stage and single activity, pauses of different duration were observed. The whole procedure and pause durations are shown in Table 2.

In the post-testing stage the modification of the behaviour by learning is usually observed as much more marked avoidance in pecking the red bead than the blue bead. The intensity of this behavioural modification in each group is used as a neural development index.

Statistical analysis

All the observed times were converted into their reciprocal value because the original data were not normally distributed. Latencies longer than 20 s during the pre-training or longer than 10 s during training, testing or post-testing were considered infinite (equal to zero its reciprocal value). Latencies were analysed by ANOVA (by considering the effects of the n-3 content in the hen diet as a categorical variable). Number of pecks were analysed by the Wilcoxon/Kruskal–Wallis non-parametric test (Rank Sums) in relationship to the categorical effect and by logistic fit (ordinal values) in relationship to the n-3 content. The differences between the post-testing stage and the training stage were analysed on post-testing crude values covariated per the training stage latencies. Discrimination ratios (DR), calculated as blue colour latency (or number of pecks) divided per red colour plus blue colour latencies (or number of pecks) (Burne and Rogers, 1997), were first checked for the normality of their distribution and then analysed by ANOVA (SAS Institute, 2002).

RESULT

One-trial passive avoidance learning task

The results of this task have been used to study temporal structure and memory formation in chicks (Andrew, 1991). In our study, 1-d-old partridge chicks were very active when they were just hatched and each chick pecked the bead at least once in two different stages of the entire trials (pre-training, training, testing or post-testing). Table 3 reports the number of observations, latency time (mean values), number of pecks (mean values) and standard error of mean (pooled) and referred only to the training stage and post-testing stage. There were no treatment differences ($P > 0{\cdot}05$) in the pre-training stage or testing stage.

A difference ($P < 0{\cdot}05$) in latency times for pecking the red bead between the experimental group 7600 and the control group 480 (Table 3) was observed in the training stage. No difference ($P > 0{\cdot}05$) was observed either for the blue bead or for the DR. In the post-testing stage (after experiencing MA) all the groups showed longer latency times. Group 4040 showed a longer latency to peck the red bead than groups 480 and 7600 ($P < 0{\cdot}05$). The ability to remember the unpleasant taste of the red bead and the

Table 2. *Outline of the one-trial passive avoidance task*

Stage	Activity	Stimulus	Interval between trials (min)
1	Three chicks placed in each box	Rest	>30
2	Pre-training treatment 1	Chrome bead (2 mm Ø) + water	20
	Pre-training treatment 2	Chrome bead (2 mm Ø) + water	30
3	Training treatment 1	Red bead (4 mm Ø) + water	6
	Training treatment 2	Blue bead (4 mm Ø) + water	30
4	Testing treatment	Red bead (4 mm Ø) + MA	30
5	Post-testing—responding 1	Dry red bead (4 mm Ø)	6
	Post-testing—responding 2	Dry blue bead (4 mm Ø)	

Table 3. *Influence of n-3 content of the hen diet on the latency and number of pecks of the partridge chicks*

Group		Training stage			Post-testing stage			Variation		
		Red bead	Blue bead	DR[1]	Red bead	Blue bead	DR[1]	Diff. red	Diff. blue	Diff. DR[1]
Latency time										
480	*n*	149	149	149	149	149	149	149	149	149
	Means	6·72^{b}	6·74	0·50	14·00^{a}	13·00	0·48^{b}	7·24^{b}	6·31	−0·02
4040	*n*	140	140	140	140	140	140	140	140	140
	Means	5·23^{ab}	5·09	0·50	16·40^{b}	13·30	0·43^{a}	11·20^{a}	8·23	−0·07
7600	*n*	143	143	143	143	143	143	143	143	143
	Means	4·28^{a}	4·87	0·51	14·00^{a}	12·00	0·46^{ab}	9·68^{ab}	7·17	−0·05
Pooled SEM		0·33	0·33	0·01	0·38	0·4	0·01	0·47	0·48	0·02
Number of pecks										
480	*n*	149	149	133	149	149	84	149	149	78
	Mean	3·33	3·10^{B}	0·49	1·08^{a}	0·90	0·51^{b}	−2·25	−2·20	0·01^{b}
4040	*n*	140	140	134	140	140	70	140	140	68
	Mean	3·41	3·50^{AB}	0·49	0·53^{b}	1·19	0·71^{a}	−2·89	−2·31	0·26^{a}
7600	*n*	143	143	138	143	143	88	143	143	86
	Mean	3·78	4·13^{A}	0·50	1·08^{a}	1·31	0·60^{ab}	−2·71	−2·83	0·12^{ab}
Pooled SEM[2]		0·15	0·16	0·02	0·09	0·09	0·03	0·16	0·17	0·03

Note: Means with different letters differ ($P<0·05$).
[1]DR = blue bead value/(red bead value + blue bead value).
[2]Calculated by analysis of variance.

consequently longer latency time showed by group 4040 chicks, was confirmed by the DR values, which were different ($P<0·05$) between group 4040 and group 480.

The poor retention memory shown by group 480 was confirmed by the differences between post- and pre-testing stages for the red bead. In this case, the latency time between group 4040 and group 480 was significant ($P<0·05$). The number of pecks in the training stage tended to increase in the chicks whose parents had been fed with higher n-3 content diets (Table 3). In particular group 7600 pecked the blue bead more than group 480 ($P<0·05$).

After the testing stage (after experiencing MA), all chicks markedly reduced their number of pecks toward all beads (red and blue). Most of them pecked the red bead only once and only pecked the blue bead slightly more (Table 3). Group 480 and group 7600 pecked the red bead more than group 4040 ($P<0·05$). This evidence has been partially confirmed by DR values in the post-testing stage and by different DR values (post-testing–training stage), which were both significantly different, between the 4040 and 480 groups ($P<0·05$).

DISCUSSION

The dietary n-3 content and/or the n-6/n-3 ratio can change the behaviour of animals like mice and chicks (Raygada *et al.*, 1998; Bagliacca *et al.*, 2000). Our study suggests that the behaviour of partridge chicks can be affected by the maternal partridge diet. Diets with a low PUFA content and/or an incorrect ratio of n-3 and n-6 fatty acids affected behaviour. A diet lower in PUFA and n-3 fatty acids concentration than the one commonly eaten by game bird hens under natural conditions (Bagliacca *et al.*, 2000) worsened the chicks' memory retention, when it was measured by means of the one-trial passive avoidance task (Andrew, 1991). When maternal dietary PUFA and n-3 fatty acid concentration was similar to that used in commercial diets the latency times, number of pecks toward the previously tested bitter bead and discrimination ability of 1-d-old chicks worsened compared to higher n-3 diet concentrations (4·04 g/kg). At the same time, the lack of statistically significant difference between the intermediate and the highest n-3 content diets, seems to demonstrate that n-6/n-3 ratio affected the partridge chicks' behaviour more than n-3 diet content per se. As in mice (Wainwright, 2002), the superior behavioural score was reached at the n-6/n-3 ratio of 4, which is close to that of our diet 4040 (3·1).

In captive birds reared for release into the wild, the nutritional requirements derived by studying Galliformes reared in captivity may be inadequate, so that using diets, apparently equal in chemical composition but differing in their ingredients, may impose a handicap when captive-reared birds are released. The n-3 content of the diet, together with other unconventional nutrient characteristics (Bagliacca *et al.*, 1998), must always be evaluated so that birds are fully fit to survive after their release.

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