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FISH OIL AND DISEASE RESISTANCE IN THE CHICK - INTERACTIONS BETWEEN OMEGA-3 AND OMEGA-6 FATTY ACIDS - TRIAL AT UNIVERSITY OF CALIFORNIA, DAVIS

RESEARCH REPORT NUMBER: 1993-11

October 1993

CONFIDENTIAL

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Summary and Conclusion

The disease resistance of chickens was investigated in two trials comparing diets containing fish oil (rich in omega-3 [n-3] fatty acids) versus corn oil (rich in omega-6 [n-6] fatty acids). In trial 1 chicks fed 2% fish oil mounted a more effective immune response following vaccination against infectious bronchitis and also following the injection of an antigen. *In vitro* production of interleukin-1 [IL-1], a fever causing cytokine, was reduced. In trial 2, chickens challenged with injections of bacteria or bacterial toxin suffered less when fed diets with 2% fish oil compared with diets with 2% corn oil. Lower levels of fish oil (0.5% and 1.0%) did not have any of the above effects.

These results indicate that if fish oil replaces corn oil, provided 2% fish oil is fed, it will have a beneficial effect on disease resistance with resultant improvements in performance. Additional work is needed to investigate whether lower levels of fish oil (1% and 1½%) become effective when the omega 6 : omega 3 ratio is decreased further.

Extended Executive Summary

Background

Preliminary results from research at the University of California, Davis have shown that by introducing omega-3 fatty acids from fish oil into the diet of the chick, deleterious effects of a disease challenge may be reduced. It is believed that the fish oil played a role in moderating the bird's immune response, reducing the growth depressing effect of this response. In addition, the fish oil may have improved the bird's resistance to disease.

Large amounts of fish oil were used in the trials (5% or more in the diet), giving low ratios of omega-6 (unsaturated fatty acids typically from vegetable oils) to omega-3 (unsaturated fatty acid from fish oil) fatty acids. Such high inclusions of fish oil in the diet would not be appropriate in commercial diets. There is evidence from human work that the modifying effect of omega-3 fatty acids on the immune system may be optimum when the ratio of omega-6 : omega-3 fatty acids is in the order of 5:1. This would be achieved with a lower inclusion of fish oil, e.g. 1% menhaden oil.

The Trial

The present trial, in two parts, was designed to study further the effect of lower levels of fish oil (menhaden oil rich in omega-3 fatty acids) compared with corn oil (rich in

omega-6 fatty acids and almost free of omega-3 fatty acids). Inclusion rates of the two oils were 0.5, 1 and 2% in each case, added to a corn : soyabean meal diet (i.e. six dietary treatments). Diets were balanced in other respects.

In the first part (i), chicks were challenged with a vaccination against infectious bronchitis virus and by injection of phytohaemagglutinin into the wattle[1]. The effectiveness of immune response was measured from antibody titre and wattle[1] swelling following injection. The extent of the increase in the former and the greater the swelling of the latter indicate a more effective immune response. Interleukin-1 production was also accessed as a measure of inflammatory response. It is involved in the inflammatory responses. Rising levels are associated with decreased appetite, decreased skeletal muscle protein synthesis and increased metabolic rate (fever).

In the second part (ii) chicks were challenged with repeated injections of Salmonella lipopolysaccharide (LPS) or heat killed staphylococcus aureus; a control group were untreated. Feed intake, weight gain and feed efficiency were measured. Body temperature was measured (in the cloaca), the rise indicating the extent of the fever. Circulating levels of so-called acute phase proteins (hemopexin and metallothionein) were measured as an indication of the immune response.

Results of the Trial and Its Interpretation

In part (i) chicks fed 2% fish oil compared with 2% corn oil mounted a more effective immune response, judged by greater antibody titre (0.77 v 0.68), following vaccination against infectious bronchitis. They also had an improved cell-mediated immune response following the injection of phytohaemagglutinin as an antigen seen in the increased wattle index (2.4 v 2.1). *In vitro* production of interleukin-1 was reduced (1.8 v 2.8). All these differences were significant.

In part (ii) the bacterial challenges (LPS and S. aureus) depressed feed intake and weight gain and decreased feed efficiency as expected. This detrimental effect on performance was less marked with the high level of fish oil (2%) compared with corn oil. For example, growth was depressed by LPS in chicks receiving 2% corn oil by 16% whereas the depression was only 10% with fish oil (2%) the difference being significant; put another way, the fish oil (2%) fed birds grew 6% faster than corn oil (2%) fed birds following the LPS challenge and this difference, as well as the feed efficiency, was significantly better but there was no difference in feed intake. With S. aureus, 2% fish oil gave significantly better growth and feed intake without significant improvement in feed efficiency compared with the 2% corn oil treatment.

Further evidence of a greater detrimental effect of the challenges with bacteria in part (ii) is seen from the lower rise in body temperature with fish oil (2%) compared with corn oil (2%). Both with LPS and S.aureus the temperature rises were significantly less (0.9 v 1.2°C and 0.6 v 0.1°C respectively) with fish oil.

These results show that fish oil provides beneficial effects at lower dietary levels (2%)

[1]hanging skin below the beak

than previously tested (5%). Failure of even lower levels to provide benefits may be a result of the omega-6 : omega-3 ratios not being optimal. It is recommended that lower levels should be tested again (1% and 1½%) using lower omega-6 : omega-3 ratios by replacing corn with wheat, the lipid in wheat being lower in omega-6 fatty acids. Lowering this fatty acid ratio compared with that in the present trial [(5:1) - see Appendix Table 1] with 1% fish oil treatment might make this level of fish oil effective in improving disease resistance. Details of the trial follow.

DETAILS OF THE EXPERIMENT

Methods

An experiment was conducted to determine the impact of various fatty acid sources on immunocompetence. Chicks were fed corn-soybean diets based on the NRC (1984) standard research reference diets to which either 0.5, 1.0 or 2.0% corn oil, or menhaden fish oil was added. Each of the six diets was fed to four pens of five chicks starting when chicks were three days of age (see **Table 1** for Experimental Design and **Table 2** for Experimental Diets). When chicks were 14 days of age, they were vaccinated against infectious bronchitis virus (IBV; Bron-Newcavac-M, 10-006). On day 28, antibody titres were determined by ELISA using the ProFlock test kit (Kirkegaard and Perry Laboratories, Gathersburg, MD). Cell mediated immunity was evaluated by the PHA induced wattle inflammation assay as described by Klasing (1985). Sephadex elicited peritoneal macrophages were stimulated *in vitro* with LPS to determine the capacity of these cells to produce interleukin-1.

A second group of chicks were fed the same six diets in 12 pens of five chicks starting when chicks were three days of age. When chicks were 10 days of age, four pens per diet were injected every other day with Salmonella lipopolysaccharide (LPS); four pens were injected with heat-killed Staphylococcus aureus; four pens were not injected (control). Injections were to be repeated every other day for seven days to simulate an authentic infectious challenge. Gain, feed intake and feed efficiency were determined throughout the experiment. Circulating levels of acute phase proteins (haptoglobin and metallothionein) were determined on the final day of the injection schedule to give an index of the state of activity of the immune response. Cloacal temperature was determined six hours following the first immunogen injection to provide an index of the responsiveness of the hypothalamus to cytokines released during the immune stress.

Discussion

The immune system can respond to immunogens (foreign macromolecules) presented by invading microorganisms in four ways. First the immune system may actively decide not to respond, giving tolerance. Second the immune system may mount a response that primarily involves macrophages and heterophils (the avian equivalent of neutrophils), resulting in an inflammatory response. Third, the immune system may direct its response by evoking cell mediated immunity which utilises T cells (T cytotoxic and killer T cells).

Finally, the B cell may be the primary responding cell type giving humoral or antibody mediated immunity. Although none of these responses are mutually exclusive, in most instances only one of the four responses is predominant. The regulatory cells of the immune system (monocytes and T helper cells) produce a series of cytokines that direct the immune response along one of the four paths. Interleukin-1 and tumour necrosis factor, for example, orchestrate the inflammatory response. Interleukin-4 and interleukin-10 inhibit interleukin-1 release and decrease the size of the inflammatory response, while at the same time augmenting humoral immunity by stimulating the proliferation of B cells recognizing immunogen. Interleukin-12 blocks interleukin-4 release, thereby reducing humoral immunity and at the same time induces cell mediated immunity through stimulation of T cytotoxic cells. Thus, the coordinated action of cytokines released by regulatory cells during the initiation of the immune response directs the type of response.

The inflammatory response is the major component of the immune response that disrupts growth related physiology resulting in slower growing birds. Interleukin-1 decreases appetite, decreases skeletal muscle protein synthesis and increases metabolic rate (fever). Dietary n-3 fatty acids have been shown to decrease interleukin-1 and tumour necrosis factor production (J Nutr 122: 1942-1951, 1992; New Eng J Med 320: 265-271, 1989).

Nutritional modulation of the immune system that **minimises inflammatory responses and concurrently augments cell mediated or humoral immunity theoretically will maximise growth of birds with no loss or even an augmentation of immunity against most practical avian pathogens and also the efficacy of vaccination programmes.**

The results of this trial indicate that n-3 fatty acids from fish oil at 2% of the diet decreased the release of interleukin-1 by stimulated macrophages especially compared to the n-6 fatty acids supplied by 2% corn oil **(Table 3)**. At the same time, feeding n-3 fatty acids resulted in tendency for increased cell mediated immunity as determined by the wattle index and augmented humoral immunity as indicated by antibodies against IBV. Interleukin-1 induces fever and the synthesis of acute phase proteins such as hemopexin and metallothionein. The levels of acute phase proteins and fever were blunted by feeding fish oil **(Table 4)**, indicating lower interleukin-1 levels and decreased inflammatory response, *in vivo*. **Together these results indicate that n-3 fatty acids modulate cytokine production** by decreasing IL-1 possibly by increasing IL-4. We cannot measure IL-4 in chicks.

In chicks consuming n-6, corn oil (2%) based diets, a bacterial challenge simulated by injecting LPS results in about a 16% decrease in the rate of gain. **This is blunted by feeding fish oil**, resulting in only a 10% decrease in the rate of weight gain **(Table 5)**. Presumably, the modulation in sensitivity to a bacterial challenge as measured by weight gain is due to a shift in the immune response away from the inflammatory response and towards humoral and/or cell mediated responses.

TABLE 1

EXPERIMENTAL DESIGN

2 FAT SOURCES	-	Corn Oil Fish Oil
3 FAT LEVELS	-	0.05, 1 or 2%
Part (i) IMMUNOGEN	-	infectious bronchitis virus (IBV)
Part (ii) 3 IMMUNOGENS	-	None S. typh LPS S. aureus, heat killed

4 PENS OF 5 CHICKS/PEN (Non-injected birds were replicated in an additional 4 pens for antibody and PHA challenge):

MALE HUBBARD*HUBBARD

EXPERIMENTAL DIET:

NRC Standard reference diet
3200 kcal/kg, oil sources substituted for an isocaloric amount of corn starch.

Ingredient	g/kg
Corn	580
Soy	350
Corn starch	
0.5 % diet	50
1.0% diet	40
2.0% diet	20
Cellulose	
0.5% diet	0
1.0% diet	5
2.0% diet	15

All other ingredients as per NRC

TABLE 2

DAVIS IMMUNITY STUDY - DIETS FOR TRIAL PARTS (i) & (ii)

Diet	**Corn Oil**			**Menhaden Oil**		
	0.5	**1.0**	**2.0**	**0.5**	**1.0**	**2.0**
Corn	58	58	58	58	58	58
Soya	35	35	35	35	35	35
Starch	5	4	2	5	4	2
Cellulose	0	0.5	1.5	0	0.5	1.5
Oil	0.5	1.0	2.0	0.5	1.0	2.0
Min + vits etc	1.5	1.5	1.5	1.5	1.5	1.5
	100	**100**	**100**	**100**	**100**	**100**
Calculated Composition (%)						
Energy (MJ/kg)	12.2	12.2	12.3	12.2	12.2	12.3
Protein	19.6	19.6	19.6	19.6	19.6	19.6
Lysine	1.10	1.10	1.10	1.10	1.10	1.10
M + C	0.64	0.64	0.64	0.64	0.64	0.64
n-3	0.08	0.08	0.08	0.19	0.31	0.55
n-6	1.60	1.82	2.27	1.39	1.41	1.45
$\frac{\text{n-6}}{\text{n-3}}$	20	23	28	7	5	2.6

TABLE 3

EFFECT OF OIL SOURCE ON THE IMMUNE RESPONSE OF BROILER CHICKS

OIL SOURCE	LEVEL	ANTI-IBV[1]	WATTLE INDEX[2]	IL-1[3]
CORN OIL	0.5%	0.72	2.0	2.4
	1.0%	0.75	2.0	2.3
	2.0%	0.68	2.1	2.8
FISH OIL	0.5%	0.75	2.2	2.1
	1.0%	0.72	2.2	2.1
	2.0%	0.77	2.4	1.8
	LSD	0.08	0.26	0.39
	pooled SEM	0.03	0.11	0.18
	source	0.18	0.04	0.02
	level	0.63	0.32	0.87
	interaction	0.09	0.08	0.06

[1]Absorbance reading at 405 nm using the proflock elisa kit

[2]Swelling index which is the width of control wattle divided by the width of the injected wattle.

[3]Stimulation index which is the rate of T cell mitogenesis in the presence of IL-1 source divided by the rate in the absence.

TABLE 4

Effect of dietary oil source on immunologic stress in broilers.

OIL SOURCE	LEVEL	IMMUNOGEN	Body Temp C[1]	Hemopexin mg/100 ml	Metallo-thionein µg/g liver
CORN OIL	0.5%	none	40.7	3	21
	1.0%	none	40.7	5	12
	2.0%	none	40.8	2	19
FISH OIL	0.5%	none	40.8	2	23
	1.0%	none	40.7	6	26
	2.0%	none	40.7	3	19
CORN OIL	0.5%	LPS	41.9	26	75
	1.0%	LPS	42.1	28	58
	2.0%	LPS	42.0	33	77
FISH OIL	0.5%	LPS	42.0	27	56
	1.0%	LPS	41.8	24	69
	2.0%	LPS	41.6	22	44
CORN OIL	0.5%	S.aureus	41.3	23	31
	1.0%	S.aureus	41.2	25	43
	2.0%	S.aureus	41.4	25	37
FISH OIL	0.5%	S.aureus	41.3	24	30
	1.0%	S.aureus	41.2	20	36
	2.0%	S.aureus	40.8	21	24
	LSD		0.027	5.5	14
	SEM		0.005	1.8	3.1
P Values	source		0.28	0.28	0.19
	level		0.36	0.47	0.27
	immuno gen		0.001	0.001	0.001
	source x imm		0.33	0.49	0.37
	level x imm		0.21	0.17	0.41
	level x src		0.06	0.03	0.07

[1]Cloacal temperature taken at 4 hrs after first injection of immunogen.

Effect of dietary oil source on immunologic stress in broilers.

OIL SOURCE	LEVEL	IMMUNOGEN	Gain g/c/d	Feed g/c/d	Efficiency
CORN OIL	0.5%	none	21.5	27.56	0.78
	1.0%	none	21.3	27.66	0.77
	2.0%	none	20.9	27.14	0.77
FISH OIL	0.5%	none	21.0	27.63	0.76
	1.0%	none	21.2	27.53	0.77
	2.0%	none	21.2	27.53	0.77
CORN OIL	0.5%	LPS	18.0	25.00	0.72
	1.0%	LPS	17.7	24.25	0.73
	2.0%	LPS	17.8	24.72	0.72
FISH OIL	0.5%	LPS	17.9	24.52	0.73
	1.0%	LPS	18.0	24.66	0.73
	2.0%	LPS	18.9	24.87	0.76
CORN OIL	0.5%	S.aureus	18.6	24.80	0.75
	1.0%	S.aureus	19.0	26.03	0.73
	2.0%	S.aureus	18.5	25.00	0.74
FISH OIL	0.5%	S.aureus	18.7	24.93	0.75
	1.0%	S.aureus	18.8	25.07	0.75
	2.0%	S.aureus	19.5	26.00	0.75
	LSD		0.8	1.0	0.02
	SEM		0.13	0.19	0.02
P Values	source		0.18	0.36	0.11
	level		0.33	0.41	0.17
	immunogen		0.001	0.001	0.007
	source x imm		0.04	0.22	0.03
	level x imm		0.31	0.41	0.26
	level x src		0.07	0.56	0.08