International Fishmeal & Oil Manufacturers Association

STUDIES ON DISEASE RESISTANCE OF BROILERS FED FISH OIL AND FISH MEAL - University of Davis, California, USA

by D Korver and K Klasing

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CONTENTS

EXECUTIVE SUMMARY

STUDIES ON DISEASE RESISTANCE OF BROILERS FED FISH OIL AND FISH MEAL - EFFECT ON IMMUNE RESPONSE

At the University of California, Davis, Professor Kirk Klasing - a leading poultry immunologist - has established a procedure to challenge broilers with immunogens (disease related compounds) and monitor the animals reaction to the 'disease'. The techniques enable different dietary treatments to be screened rapidly Several dietary oils including fish oil, and fish meal as a source of fish lipids, have now been tested in a series of trials.

The immunogens used included a lipoprotein polysaccharide extract from *Salmonella typhimurium* and heat killed *Staphylococcus aureus* and infectious bronchitis (IBV). Both are from potentially pathogenic organisms but are non-replicating. They were injected into the birds to give fever symptoms shortly afterwards.

Parameters measured include body weight gain, feed intake, feed efficiency, antibody titres to infectious bronchitis following vaccination, wattle inflammation, acute phase proteins (hemopexin and metallothionein), body temperature and interleukin 1 production from isolated macrophages *in vitro*. Further details of these measurements and their significance are given in Appendix 1.

Two trials (1 and 2) financed by the Association are summarised. They have also been submitted for publication - the paper follows. This provides a detailed account of these trials. In addition, a further two trials have been undertaken (3 and 4).

The trials cover a range of dietary oil treatments including fish oil, corn oil, linseed oil and fish meal as a source of fish lipids. The levels of fish oil in the diet included 0.5, 1.0, 1.5, 2 and 6.6%. Fish meal was included at 10%, 10.6%, 15.9% and 21.2%. Maize or wheat were the main starch sources. Diets were equated for nitrogen and energy.

Generally fish oil improved most of the immune reactions provided between 1% and 2% was used; 2% gave the best responses. In particular the reduction in growth rate, feed intake and efficiency during disease challenge were not as great with fish oil as with corn oil. Levels of fever inducing interleukin 1 were reduced, and in some trials rise in body temperature was not as great with fish oil (2%) compared with corn oil (2%) in the diet.

Antibody titres to infectious bronchitis and wattle index were increased with 2% fish oil indicating better humoral and cell mediated immune responses.

A wide range of $\omega 3$:$\omega 6$ ratios were tested. Generally beneficial growth and immune responses, etc., were greater with increasing ratios. However, the one treatment

with more than 2% fish oil (6.6% was tested in trial 2), showed no benefits. The results do not indicate an optimum ω3:ω6 ratio, but in the case of the 6.6% fish oil treatment, the ratio (1.32:1) may have been excessively high. Fish oil appeared to give better responses with wheat rather than maize, possibly because of the higher ratio of ω3:ω6 achieved (0.98:1).

Linseed oil which is rich in ω3 fatty acids of short chain length (C_{18}) and has a high ω3:ω6 ratio was also included as a dietary treatment in experiment 2. The inclusion was 2%. Whilst growth and immune response of challenged birds was improved compared with corn oil (2%), results generally were intermediate to the fish oil, taking corn oil as the basis for the comparison.

A summary of the results of the trials indicating if responses were beneficial (+), detrimental (-), variable +/- or not different (NS) are indicated for each parameter (Table 1). In addition, a legend, (Appendix 1) follows along with an explanation of the parameters measured. As the highest levels of fish oils (excluding a 6.6% treatment) gave the most promising results these are the values to which the summary table refers. An outline of the dietary treatments used in each trial, the results and a brief summary appear in Appendix 2. To simplify the presentation of the results the parameters measured have been presented graphically for each challenge.

A further trial has been undertaken at the University of California, Davis where birds were challenged with coccidiosis. This was not funded by the Association and only a summary is available (see Appendix 3). This showed 4% fish oil prevented weight loss in challenged birds, whereas birds with 4% corn oil in the diet had 6% lower weight gains.

GENERAL CONCLUSION

The results of these experiments show the potential for the use of practical amounts of fish oil in poultry diets as a means of ameliorating the negative effects on performance, and in particular lower growth, associated with an inflammatory response. The levels of fish oil in the diet need not be very high; for example, these experiments showed beneficial effects with as little as 1% dietary fish oil. At low levels of fish oil, the most important factor seems to be not the absolute level, but the ratio of n-3:n-6 PUFA. Typical corn-soy-corn oil diets have a ratio of about 0.07. Increasing the ratio to approximately 0.2 improved performance as well as responses of birds to an inflammatory challenge. It is interesting to note that feeding a high (6.6%) level of fish oil did not confer extra advantage, if anything, the beneficial effects were lost in spite of this diet having the highest n-3:n-6 ratio of any of the diets tested. Thus, the effect of ratio may be lost if the level of fish oil in the diet is too high.

Interleukin-1 activity is extremely important in mediating an inflammatory response, and improvements in performance of fish oil-fed chicks after immune challenge seem to be associated with decreased IL-1 activity. Of all the indices of the inflammatory response, the measurement was the most predictive of performance.

TABLE 1: SUMMARY OF RESULTS FROM DAVIS TRIALS

	gain	feed	FCE	IBV	wattle	IL-1 plas	IL-1 leuk	HP	MT	Temp	Organs	Minerals
Experiment 1	**IFOMA 1**											
Immunogen†	-	-	-					-	-	-		
Corn Oil 2%	-	NS	-	NS	-	-		-	-	-		
Fish Oil 2%	+	NS	+	NS	+	+		+	+	+		
Experiment 2	**IFOMA 2**											
Immunogen	-	-	-					-	-	-		
Corn Oil 2%	-	+/-	+/-	-	-	-		+/-	-	NS		
Fish Oil 2%	+	+/-	+/-	+	+	+		+/-	+	NS		
Experiment 3												
Immunogen	-	-	-							-	-	-
Corn Oil 6.6%	NS	+	NS							NS	NS	NS
Fish Oil 6.6%	NS	-	NS							NS	NS	NS
Experiment 4												
Immunogen	-	-	-						-		-	NS
Corn Oil 2%	-	NS	+/-						NS		+/-	NS
Fish Oil 2%	+	NS	+						NS		+/-	NS
Fish Meal 20%	+/-	NS	+/-						NS		+/-	NS

†immunogen - effect of immune challenge where given when feeding control diet.

APPENDIX 1

LEGEND:

- \+ indicates changes which would result in improved performance and/or health of the bird. May include non-significant trends
- \- indicates changes which would tend to decrease performance or increase susceptibility to disease
- +/- indicates that there may be effects of level of oil or interactions
- NS no significant differences or apparent trends
- shaded measurement not taken for a given experiment

- Most of the corn oil/menhaden oil comparisons in the table are for injected birds. In most cases, differences among diets for non-immunologically challenged birds were not different.

Gain	Improved body weight gain is obviously desirable
Feed	Increased feed consumption is usually closely related to body weight gain; during an inflammatory response, feed consumption typically drops. However, decreased feed consumption without a concurrent decrease in body weight gain would result in improved efficiency (FCE).
FCE	Feed conversion efficiency. Improvements increase profitability
IBV	Infectious bronchitis virus antibody titers. An indication of specific (humoral) immunity, not necessarily related to the inflammatory response and the depressions in growth rate associated with that type of response. The inflammatory response does have a protective function, but when activated inappropriately, there is no benefit to such a response, and nutrients are diverted away from growth. Ideally, this type of immune response would become more active to compensate for decreased inflammatory responses. However, unaltered IBV titers vs corn oil diets is not necessarily bad.
wattle	Wattle swelling index. Indication of specific (cell-mediated) immune response. Phytohemagglutinin injected into the wattle of the chicken recruits basophils and T cells, this influx results in an increase in the thickness of the wattle. NOT an inflammatory response, does not involve phagocytic cells, and mediators of the inflammatory response are not released in a large amount. See also information for IBV titers.
IL-1 plas	Levels of plasma interleukin-1. Indicates IL-1 circulating in the bloodstream. We feel this is the most important indicator of the inflammatory response. It is directly responsible for increased metabolic rate (fever), loss of appetite, and via the induction of other mediators of inflammation, the loss of skeletal muscle (meat) and the induction of acute phase protein synthesis. An increase in circulating IL-1 tends to be closely associated with many of the symptoms of an inflammatory response, including decreased performance.
IL-1 leuk	IL-1 released from leukocytes (eg. macrophages, splenocytes) in tissue culture in response to a stimulus which stresses the cells. An indication of the levels of IL-1 which the cells are capable of releasing. Decreases in this measurement indicate that, to a given stimulus, the cells will

	respond to a lesser degree.
HP	Hemopexin. One of about 20 acute phase proteins (APP) which the liver produces during the acute phase of an inflammatory response. As it is one of many APP, a lack of change in HP levels may not mean that an acute phase is not underway, but increases in HP would tend to indicate such a situation.
MT	Metallothionein. See above (i.e. HP).
Temp	Body temperature. Increases (fever) are associated with the inflammatory response, and are due to increases in metabolic rate, which is maintained at the expense of body weight gain. Also, since the birds tend not to eat when they have a fever, often body tissue is catabolized to meet the energetic needs of the fever.
Organ	Organ indices (liver, spleen, intestines, depending on experiment). Internal organs such as these tend to increase as a proportion of body weight during an inflammatory response. Thus, not only are immunogen treated birds not gaining weight as fast, but the weight they gain may be mainly as internal organs, and not as lean tissue (meat).
Minerals	Plasma minerals (copper, zinc, iron, depending on experiment). A response common to all inflammatory responses, regardless of the challenge, is the alteration of mineral availability and presence in the blood. Iron and zinc are removed to a great extent from the plasma and sequestered in organs such as the liver and spleen, while the copper content of plasma increases due to the increased production of ceruloplasmin, an acute phase protein involved in copper transport through the blood.

Many of the factors we measure (IL-1, hemopexin, metallothionein), as well as the proteins involved in mineral transport and metabolism have important functions beyond the inflammatory response. Therefore, the mere presence of these factors does not indicate an immune stress. However, it is the changes in these parameters which we feel are important. As the inflammatory and specific immune responses are extremely complex, and not fully understood, it is important not to look at any one specific parameter, but to look at everything in context. Non-significant effects for the various parameters may be misleading, as there is a progression in the appearance or alterations of the factors we measure. However, it becomes prohibitive to take measurements when the alterations in each factor is expected to be maximal. Also, the bottom line is the performance of the birds, especially in response to immunogen injection.

APPENDIX 2

EFFECT OF DIETARY FISH OIL AND FISH MEAL ON IMMUNE RESPONSES OF BROILERS
UNIVERSITY OF CALIFORNIA, DAVIS

Experiment 1

Parameters Measured

- Body Weight Gain (g/chick/d)
- Feed Consumption (g/chick/d)
- Feed Conversion Efficiency (g gain/g feed)
- Infectious bronchitis virus antibody titers
- Cell-mediated immunity (PHA-induced wattle inflammation)
- Peritoneal macrophage IL-1 production
- Circulating levels of hemopexin; liver cytosolic metallothionein
- Body Temperature 6 Hours Post-Injection

Materials and 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 (each having an n-3:n-6 PUFA ratio of 0.07), or menhaden fish oil (n-3:n-6 PUFA ratios of 0.18. 0.28 and 0.47, respectively) was added. Each of the 6 diets was fed to 4 pens of 5 chicks starting when chicks were 3 days of age. When chicks were 14 days of age, they were vaccinated against infectious bronchitis virus (IBV; Bron-Newcavac-M, 10-006). On day 28, antibody titers 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 6 diets in 12 pens of 5 chicks starting when chicks are 3 days of age. When chicks were 10 days of age, 4 pens per diet were injected every other day with *Salmonella* lipopolysaccharide (LPS); 4 pens were injected with heat-killed Staphylococcus aureus; 4 pens were not injected (control). Injections were repeated every other day for 7 days to simulate an authentic infectious challenge. Gain, feed intake and feed conversion efficiency (FCE) were determined throughout the experiment. Circulating levels of hemopexin, and liver cytosolic metallothionein (both acute phase proteins) 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 6 hrs following the first immunogen injection to provide an index of the responsiveness of the hypothalamus to cytokines released during the immune stress.

Statistical analysis

Data were analyzed by analysis of variance using the general linear models procedure of SAS. The experimental unit in this study was the pen.

Results

Performance characteristics were not significantly different among the various dietary treatments (Table 1.1). However, immunogen injection significantly decreased body weight gain ($P<.0001$), feed consumption ($P<.0001$) and feed conversion efficiency ($P<.0007$). There was an

interaction between oil source and immunogen treatment for body weight gain (P<.04) and feed conversion efficiency (P<.03). Although immunogen treatment decreased gain and FCE for all dietary treatments, chicks were more affected by immunogen when fed corn oil than when fed fish oil. There was also a trend towards a level by oil source interaction for gain (P<.07) and FCE (P<.08). As the level of fish oil in the diet increased, the effect of immunogen treatment tended to decrease. However, increasing the level of corn oil in the diet did not appear to have the same effect.

Body temperature of chicks was not affected by either source of dietary oil or by the level of its inclusion in the diet (Table 1.2). Injection of immunogen, however, increased body temperature by 2% across dietary treatments (P<.0001). There was a trend (P<.06) towards a level by source interaction in which temperature decreased as fish oil in the diet increased, but remained fairly constant as corn oil in the diet increased. Hemopexin levels were increased 610% by immunogen injection (P<.0001). There was a level by source interaction; as fish oil increased in the diet, hemopexin levels decreased, while as the level of corn oil increased in the diet, hemopexin levels increased (P<.03). Metallothionein levels increased 125% in response to immunogen injection (P<.0001).

Antibody titers to infectious bronchitis virus were not altered by dietary treatment (Table 1.3). PHA-induced wattle inflammation was increased when chicks were fed fish oil (P<.04). Peritoneal macrophage IL-1 was decreased in chicks fed fish oil *vs* those fed corn oil (P<.02).

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 utilizes 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 tumor necrosis factor, for example, orchestrate the inflammatory response. Interleukin 4 and interleukin 10 inhibit Interleukin-1 release and decreases 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 tumor necrosis factor production (J Nutr 122:1942-1951, 1992; New Eng J Med 320: 265-271, 1989)

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

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. 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, 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 can not 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. 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 toward humoral and/or cell mediated responses.

Summary

Even moderate levels of fish oil in the diet can ameliorate the negative effect of an immune challenge as compared to diets containing fish oil. Both performance characteristics and indices of immune status can be improved by feeding fish oil. The ratios of n-3:n-6 PUFA used in this experiment were relatively low (e.g. 0.18, 0.28 and 0.47), and yet the beneficial effects were still observed.

Table 1.1 - Experiment 1

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

Table 1.2 - Experiment 1

Effect of dietary oil source on immunologic stress in broilers.

OIL SOURCE	LEVEL	IMMUNOGEN	Body Temp C[1]	Hemopexin mg/100 ml	MT[2] 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
	immunogen		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.
[2]Metallothionein

Table 1.3 - Experiment 1

EFFECT OF OIL SOURCE ON THE IMMUNE RESPONSE OF BROILER CHICKS

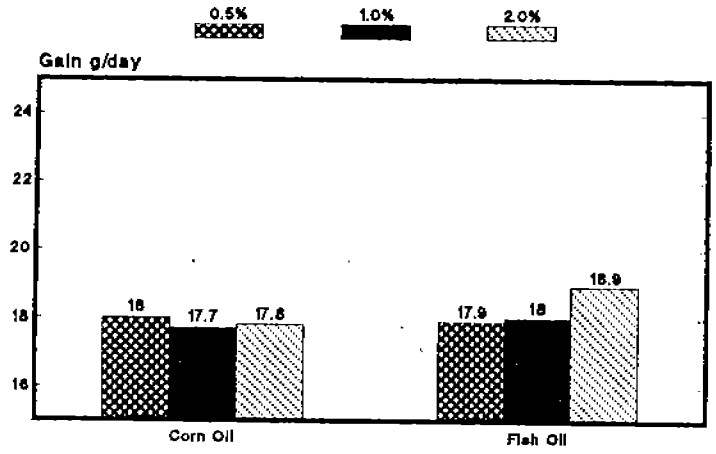
OIL SOURCE	LEVEL	ANTI-IBV[1]	WATTLE INDEX[2]	IL-1[3]
CORN OIL	0.5%	0.72	2.0	2.4
	1.0%	0.75	2.0	2.3
	2.0%	0.68	2.1	2.8
FISH OIL	0.5%	0.75	2.2	2.1
	1.0%	0.72	2.2	2.1
	2.0%	0.77	2.4	1.8
	LSD	0.08	0.26	0.39
	Pooled SEM	0.03	0.11	0.18
P values	source	0.18	0.04	0.02
	level	0.63	0.32	0.87
	interaction	0.09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

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

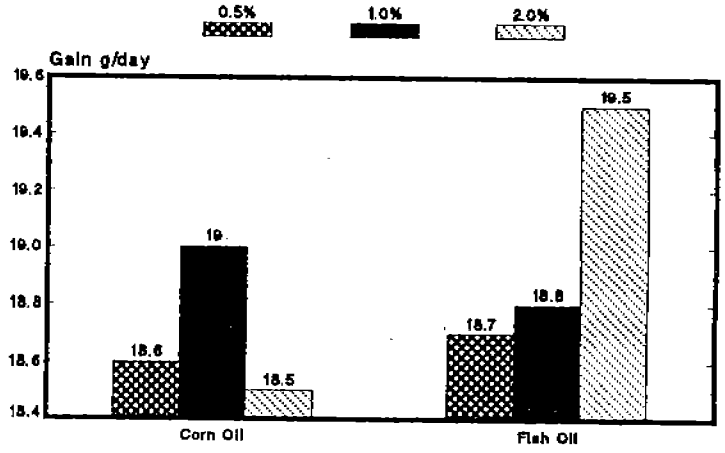
[2]Swelling index which is the width of control wattle divided by the width of the injected wattle.

[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.

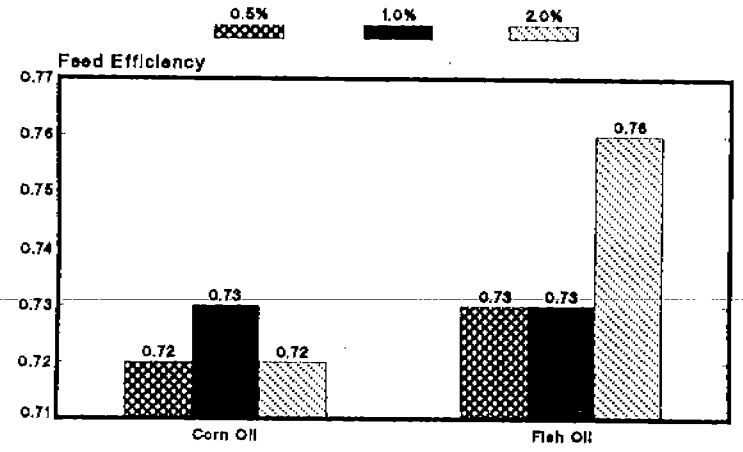
EXPT.1 – WEIGHT GAIN OF BROILERS – LPS CHALLENGE



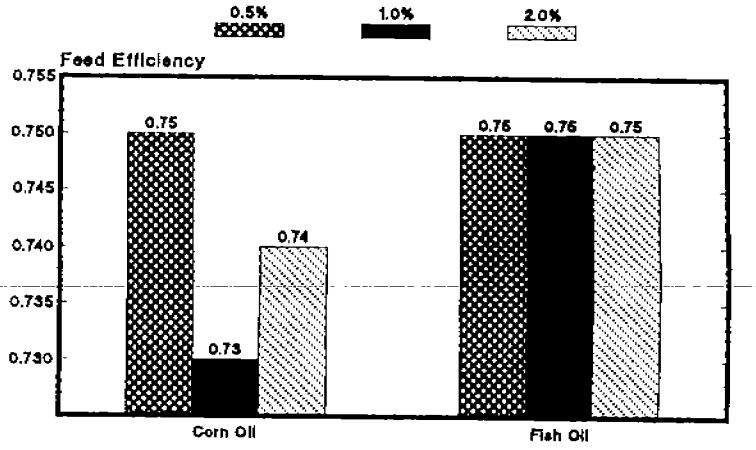
EXPT.1 – WEIGHT GAIN OF BROILERS – STAPH. AUREUS CHALLENGE



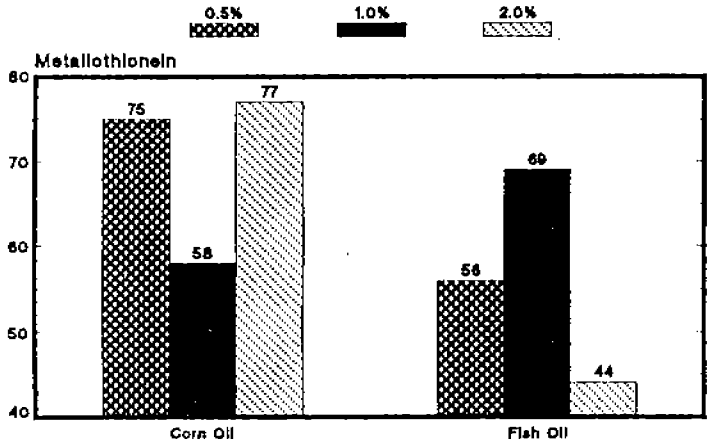
EXPT.1 – FEED EFFICIENCY OF BROILERS – LPS CHALLENGE



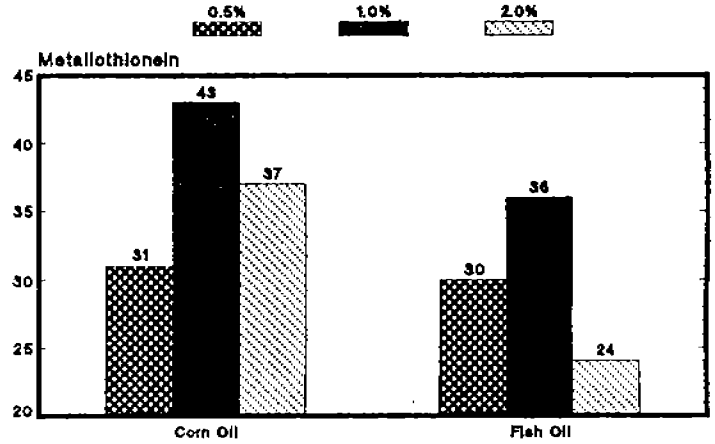
EXPT.1 – FEED EFFICIENCY OF BROILERS – STAPH. AUREUS CHALLENGE



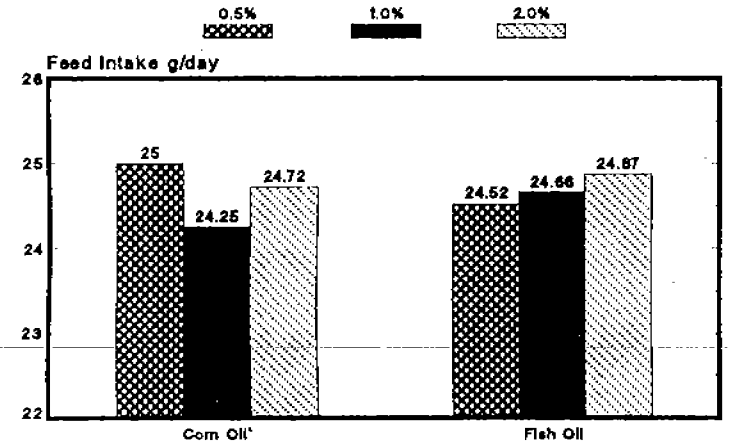
EXPT.1 – METALLOTHIONEIN IN BROILERS – LPS CHALLENGE



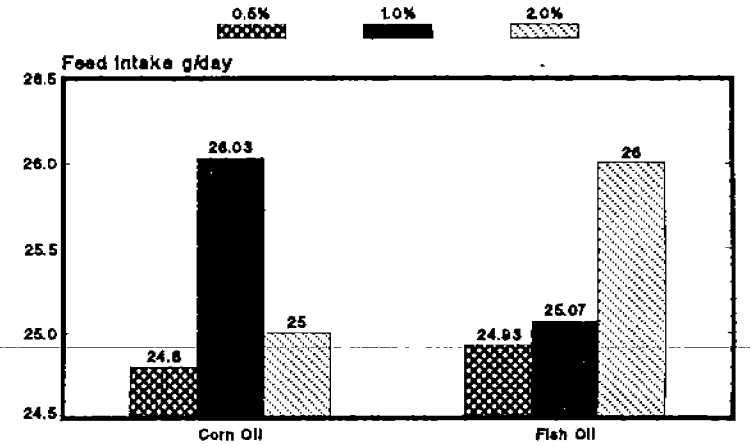
EXPT.1 – METALLOTHIONEIN IN BROILERS – STAPH. AUREUS CHALLENGE



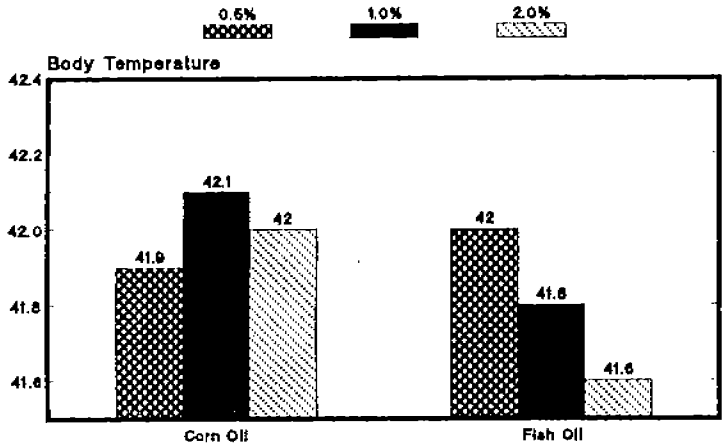
EXPT.1 – FEED INTAKE OF BROILERS – LPS CHALLENGE



EXPT.1 – FEED INTAKE OF BROILERS – STAPH. AUREUS CHALLENGE



EXPT.1 – BODY TEMPERATURE OF BROILERS – LPS CHALLENGE



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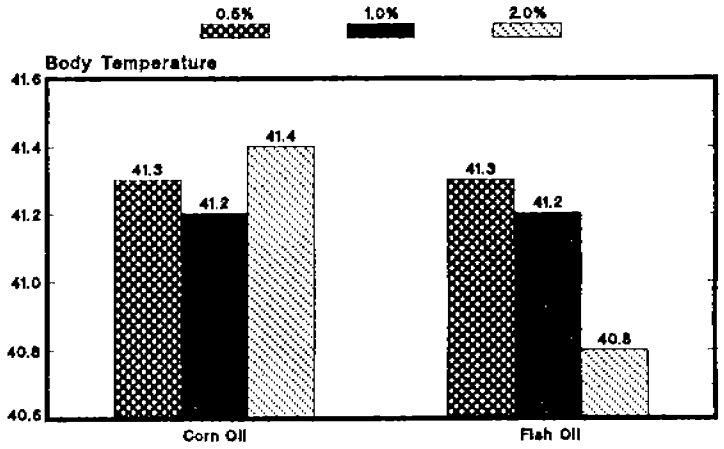
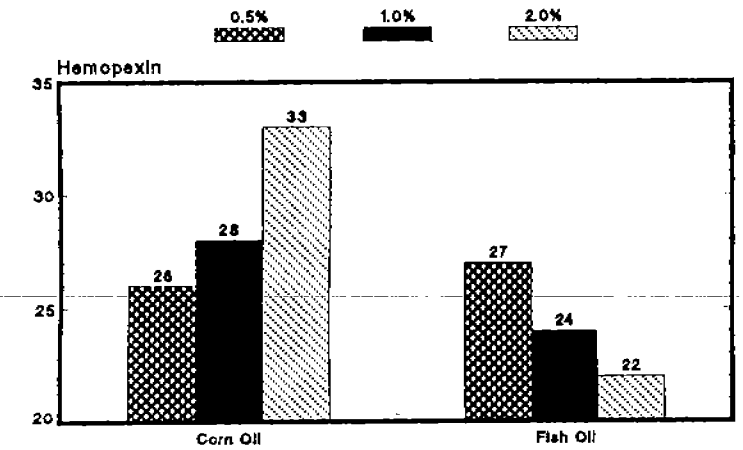
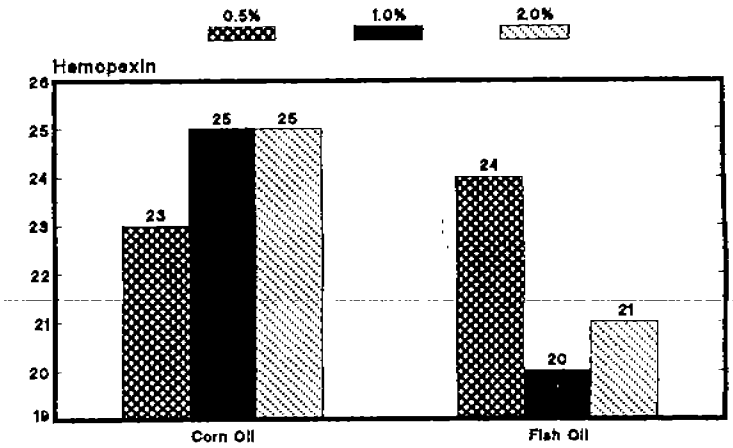


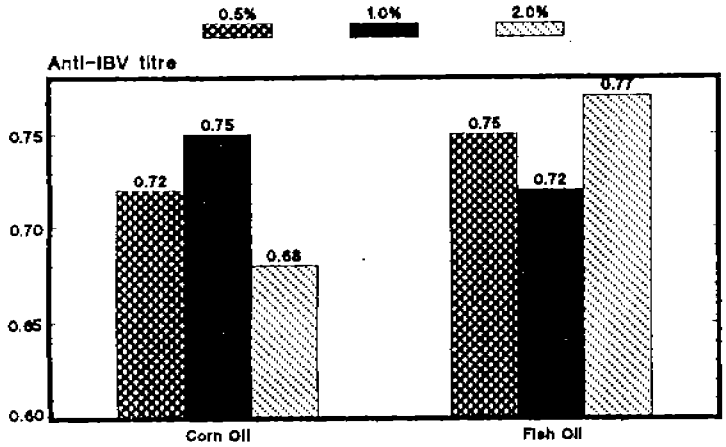
EXPT.1 – HEMOPEXIN IN BROILERS – LPS CHALLENGE



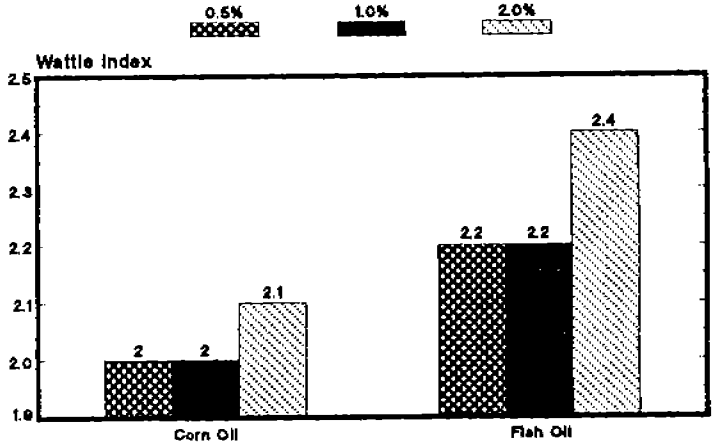
EXPT.1 – HEMOPEXIN IN BROILERS STAPH. AUREUS CHALLENGE



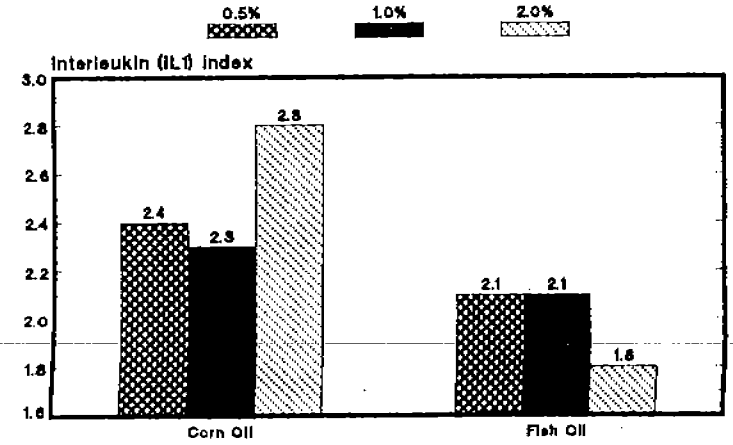
EXPT.1 - ANTI-IBV IN BROILERS



EXPT.1 - WATTLE INDEX IN BROILERS



EXPT.1 - INTERLEUKIN (IL1) IN BROILERS



Experiment 2

Parameters Measured

As in Experiment 1.

Materials and Methods

This experiment was a repeat of experiment 1 with different dietary treatments. Nine diets were used with 0, 1.0, 1.5 or 2.0% menhaden oil, 0 or 2.0% corn oil, 0 or 2.0% linseed oil and a combined fish oil (0.5%) fish meal (0.1%) treatment (Table 1a.1).

With these combinations with corn or wheat as the main cereal, ω3:ω6 ratios ranged from 0.07 to 0.98. Diets were also iso caloric and iso nitrogenous.

Experiment 2 Summary

Broilers received diets equated for nitrogen and energy content with different sources of oil - fish oil 1%, 1.5% and 2%, corn oil 2%, linseed oil 2% and fish oil 0.5% plus 10% fish meal. No effects of diet were seen in growth, feed conversion and a number of other parameters (as measured in experiment 1). The ω3:ω6 ratio ranged from 0.07 to 0.98. Immunogen injection (LPS or Staphylococcus aureus) depressed growth rate (Table 1a.2). The depression was not as great with fish oil, particularly with 2% fish oil with wheat (2% fish oil v 2% corn oil - gain 25.2g/day v 23.7 and 25.3 v 24.8 for LPS and S.aureus respectively). Feed intake and feed efficiency did not differ.

There was a tendency for a lower rise in body temperature (Table 1a.3), lower acute phase proteins and lower interleukin 1 levels (Table 1a.4) with fish oil v corn oil. Generally linseed oil was intermediate. This suggests the fever was less pronounced with fish oil, inflammatory response, and cytokine production being decreased.

Table 2a.1 - Experiment 2

Dietary composition (g/kg) and calculated (n-3):(n-6) polyunsaturated fatty acid ratio[7].

Ingredient	1% FO[8], cereal	1.5% FO, cereal	2% FO, cereal	1.5% FO, corn	2% FO, corn	2% CO[9], corn	2% CO, cereal	2% LO[10], corn	.5% FO, 10% FM[11], corn
Corn	80	80	80	515	515	515	80	515	560
Soy	350	350	350	385	385	385	350	385	225
Wheat	330	330	330	0	0	0	330	0	0
Barley	150	150	150	0	0	0	150	0	0
Fish meal	0	0	0	0	0	0	0	0	100
Corn Starch	40	30	20	10	0	0	20	0	10
Fish oil	10	15	20	20	20	0	0	0	5
Corn oil	0	0	0	0	0	20	20	0	0
Linseed oil	0	0	0	0	0	0	0	20	0
Cellulose	----------all diets adjusted to 100% with cellulose----------								
(n-3):(n-6) PUFA ratio	0.57	0.78	0.98	0.4	0.5	0.07	0.08	0.73	0.33

[7]Vitamins, mineals and amino acids supplemented to meet NRC (1984) reccomendations for practical broiler diets

[8]Fish oil (menhaden)

[9]Corn oil

[10]Linseed oil

[11]Fish meal

Table 1a.2 - Experiment 2

Effect of dietary oil source and immunologic stress on broiler performance.

DIET	IMMUNOGEN	Gain g/chick/d	Feed g/chick/d	Efficiency gain/feed
1% Fish oil, cereal	none	26.8	37.2	0.72
	LPS	23.8	35.1	0.68
	S. aureus	24.8	35.4	0.7
1.5% Fish oil, cereal	none	26.5	35.8	0.74
	LPS	24.4	34.9	0.7
	S. aureus	25.5	35.9	0.71
1.5% Fish oil, corn	none	27.2	36	0.75
	LPS	24.7	35.1	0.7
	S. aureus	25.5	37	0.69
2% Fish oil, corn	none	27.5	35.9	0.77
	LPS	25.1	34.9	0.72
	S. aureus	25.9	36	0.72
2% Corn oil, corn	none	27.7	36.7	0.75
	LPS	24	34.8	0.69
	S. aureus	25.5	35.9	0.71
2% Linseed oil, corn	none	27.4	37.5	0.73
	LPS	24.8	36.5	0.68
	S. aureus	25	35.7	0.7
Fish oil/meal, corn	none	27.9	36.5	0.76
	LPS	25.1	34.6	0.73
	S. aureus	25.7	35.2	0.73
2% Fish oil, cereal	none	26.7	36.1	0.74
	LPS	25.2	35.1	0.72
	S. aureus	25.3	36.7	0.69
2% Corn oil, cereal	none	26.6	36.6	0.73
	LPS	23.7	35.4	0.67
	S. aureus	24.8	35.5	0.7
	SEM	0.15	1.4	0.09
	LSD[1]	0.7	0.6	0.03
P VALUES[2]	Diet	0.09	0.36	0.17
	Immunogen	0.001	0.04	0.001
	Diet x Immunogen	0.03	0.09	0.05

[1]Least Significant Difference following one way analysis of variance.
[2]Probability values following 2 way analysis of variance

Table 1a.3 - Experiment 2

Effect of dietary oil source on indices of immunologic stress in broilers.

DIET	IMMUNOGEN	Body Temp ° C[1]	Hemopexin mg/100 ml	MT[2] µg/g liver
1% Fish oil, cereal	none	40.4	7	12
	LPS	41.8	41	89
	S. aureus	41	39	83
1.5% Fish oil, cereal	none	40.5	11	10
	LPS	41.3	43	69
	S. aureus	41.1	35	76
1.5% Fish oil, corn	none	40.7	8	9
	LPS	41.9	47	79
	S. aureus	41.5	40	89
2% Fish oil, corn	none	40.5	6	11
	LPS	41.4	36	77
	S. aureus	41.4	36	76
2% Corn oil, corn	none	40.6	9	8
	LPS	42	45	87
	S. aureus	41.7	43	89
2% Linseed oil, corn	none	40.6	8	9
	LPS	41.7	39	77
	S. aureus	41.3	38	75
Fish oil/meal, corn	none	40.6	10	15
	LPS	41.5	37	81
	S. aureus	41.4	33	77
2% Fish oil, cereal	none	40.5	7	12
	LPS	40.9	39	77
	S. aureus	41	31	72
2% Corn oil, cereal	none	40.5	7	14
	LPS	41.9	43	85
	S. aureus	41.4	40	81
	SEM			
	LSD[1]	0.04	4	9
P VALUES[3]	Diet	0.11	0.27	0.16
	Immunogen	0.001	0.04	0.001
	Diet x Immun	0.11	0.04	0.06

[1]Least Significant Difference following one way analysis of variance.
[2]Metallothionein
[3]Probability values following 2 way analysis of variance

Table 2a.4 - Experiment 2

Effect of dietary oil source on indices of specific and inflammatory responses of broiler chicks.

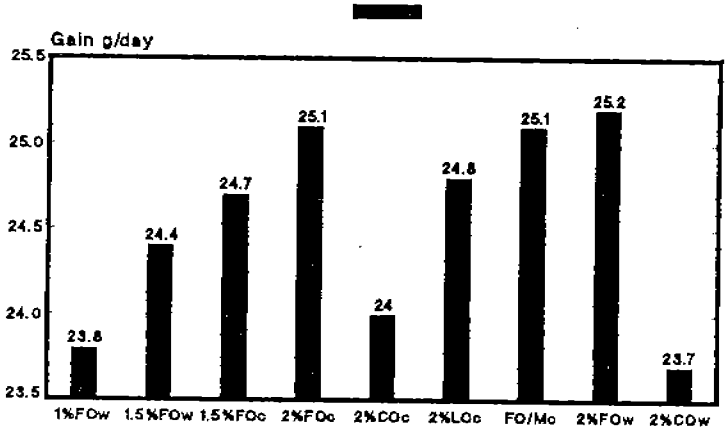
OIL SOURCE	ANTI-IBV[1]	WATTLE INDEX[2]	IL-1[3]
1% Fish oil, cereal	0.64	1.92	2.8
1.5% Fish oil, cereal	0.66	1.95	2.4
1.5% Fish oil, corn	0.65	1.77	2.8
2% Fish oil, corn	0.66	1.63	2.8
2% Corn oil, corn	0.62	1.44	3.2
2% Linseed oil, corn	0.67	1.73	2.8
Fish oil/meal, corn	0.64	1.77	2.6
2% Fish oil, cereal	0.67	2.09	2.5
2% Corn oil, cereal	0.63	1.53	2.9
LSD	0.07	0.37	0.36
Pooled SEM	0.02	0.18	0.22
P values			
diet	0.134	0.038	0.045

[1]Absorbance reading at 405 nm using the Proflock ELISA kit

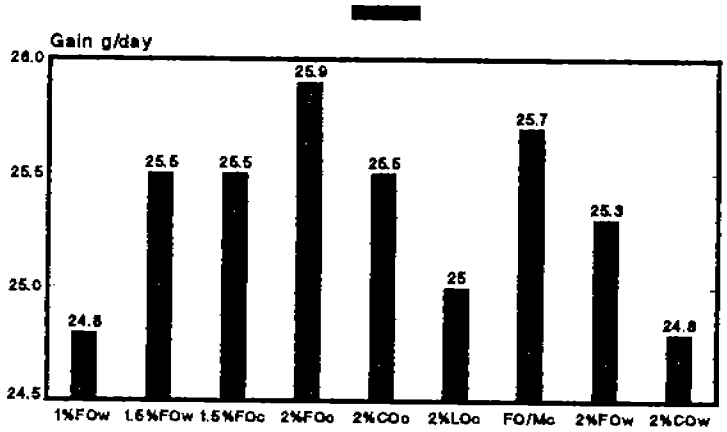
[2]Swelling index which is the width of control wattle divided by the width of the injected wattle.

[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.

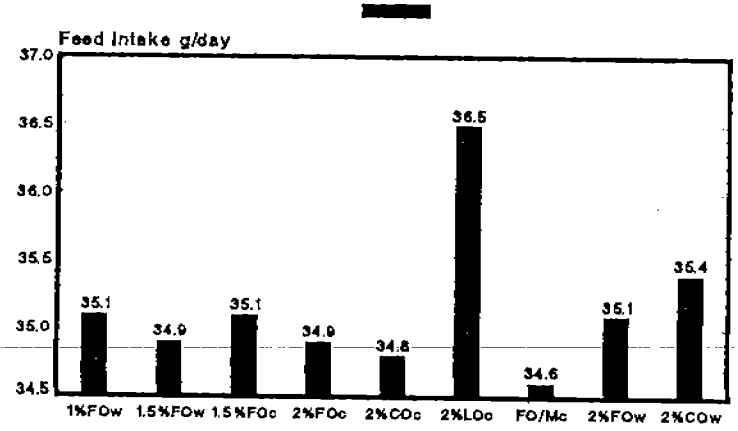
EXPT. 2 – BODY WEIGHT GAIN OF BROILERS – LPS CHALLENGE



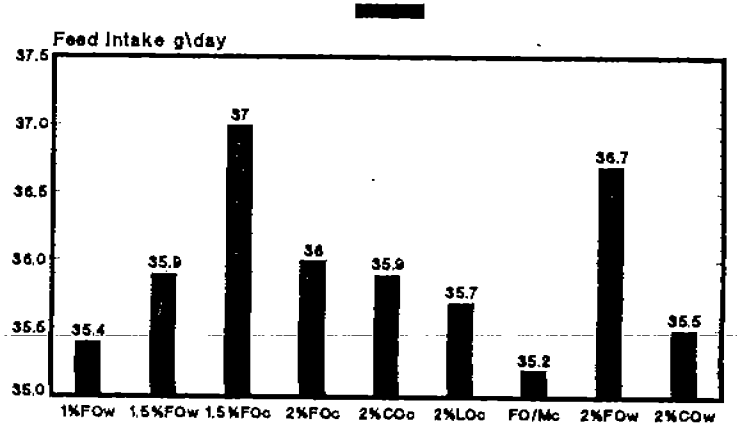
EXPT. 2 – BODY WEIGHT GAIN OF BROILERS – STAPH. AUREUS CHALLENGE



EXPT. 2 – FEED INTAKE OF BROILERS – LPS CHALLENGE

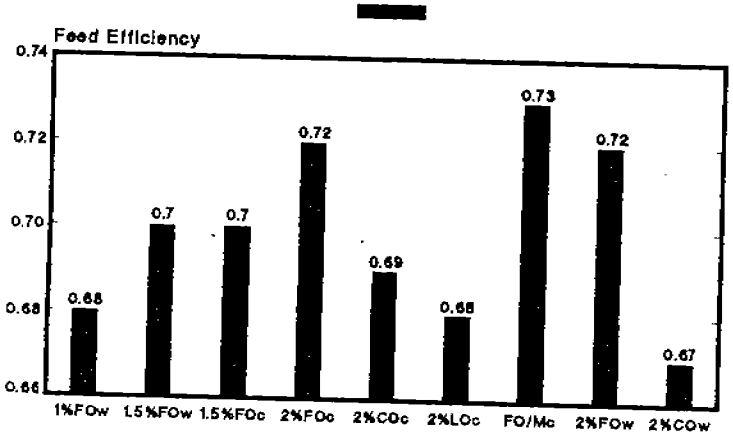


EXPT. 2 – FEED INTAKE OF BROILERS – STAPH. AUREUS CHALLENGE

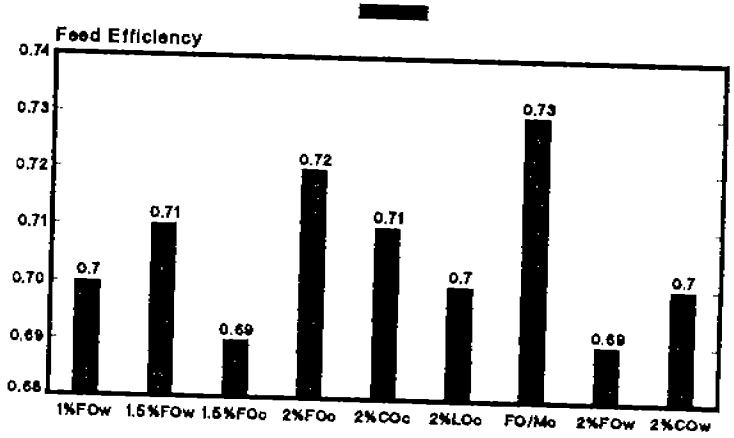


FO = Fish Oil; CO = Corn Oil; LO = Linseed Oil; FO/M = Fish Oil/Meal
c = cereal; w = wheat

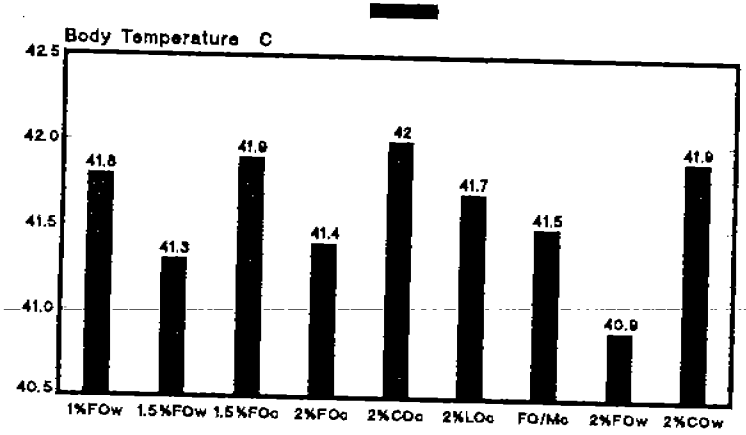
EXPT. 2 – FEED EFFICIENCY OF BROILERS – LPS CHALLENGE



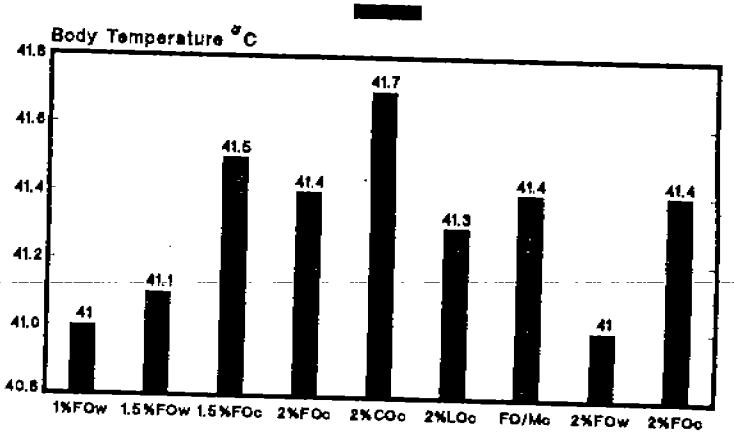
EXPT. 2 – FEED EFFICIENCY OF BROILERS – STAPH. AUREUS CHALLENGE



EXPT. 2 – BODY TEMPERATURE OF BROILERS LPS CHALLENGE

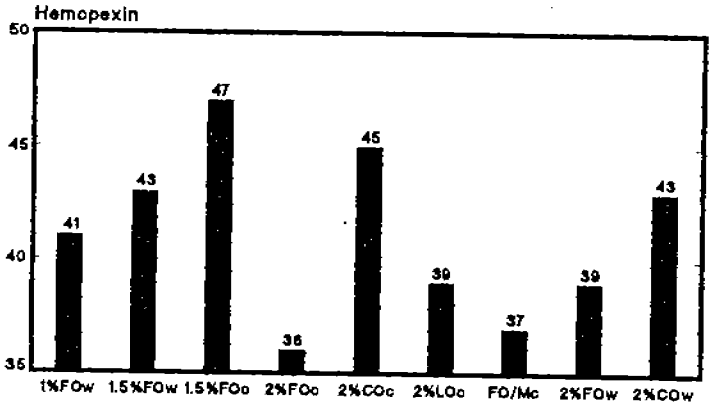


EXPT. 2 – BODY TEMPERATURE OF BROILERS – STAPH. AUREUS CHALLENGE

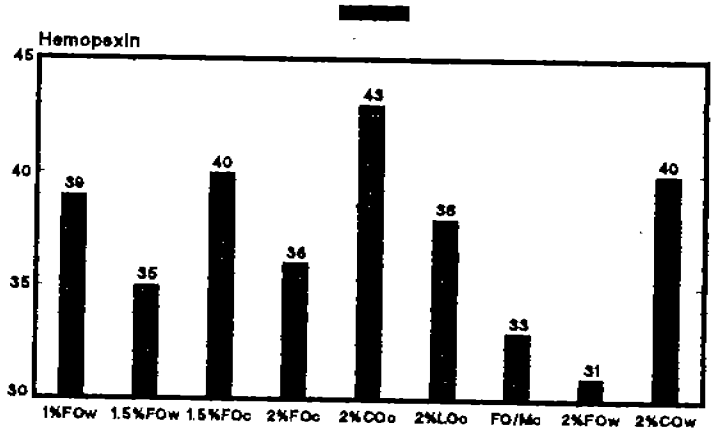


FO = Fish Oil; CO = Corn Oil; LO = Linseed Oil; FO/M = Fish Oil/Meal

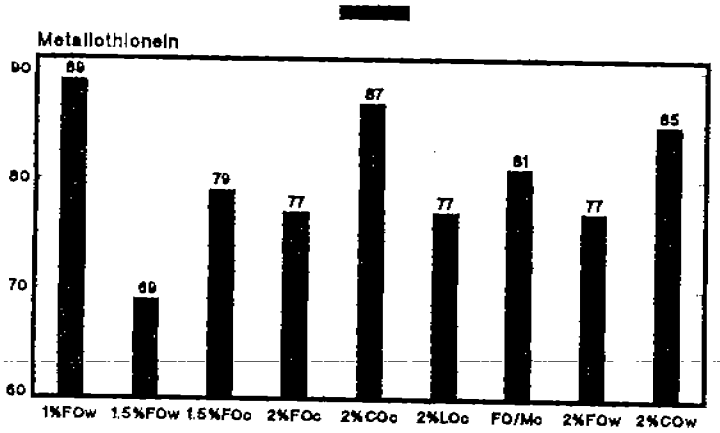
EXPT. 2 – HEMOPEXIN IN BROILERS –
LPS CHALLENGE



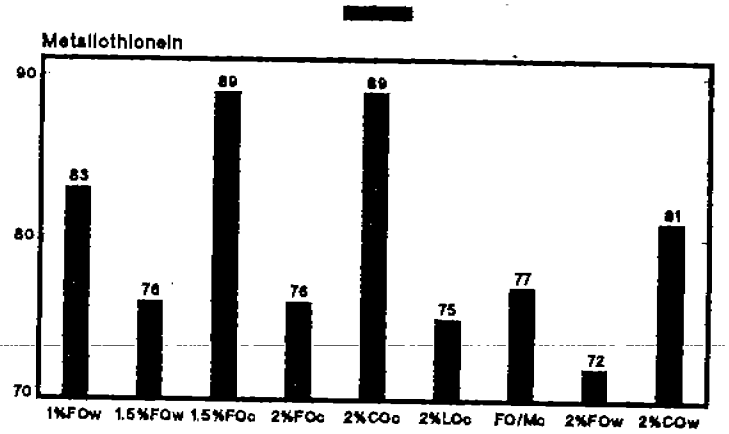
EXPT. 2 – HEMOPEXIN IN BROILERS –
STAPH. AUREUS CHALLENGE



EXPT. 2 – METALLOTHIONEIN IN BROILERS –
LPS CHALLENGE



EXPT. 2 – METALLOTHIONEIN IN BROILERS –
STAPH. AUREUS CHALLENGE



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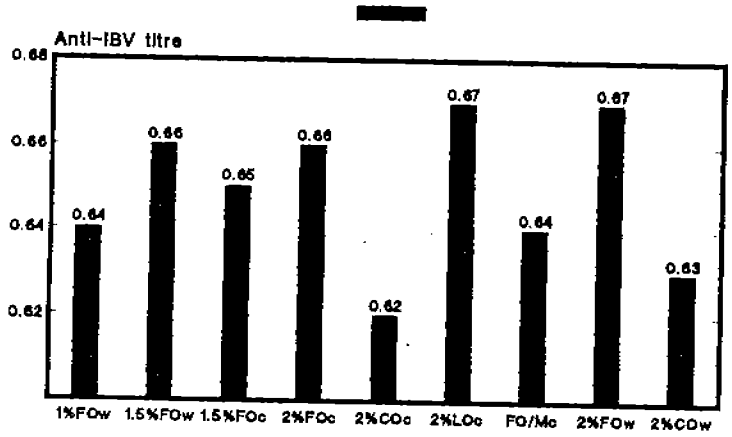
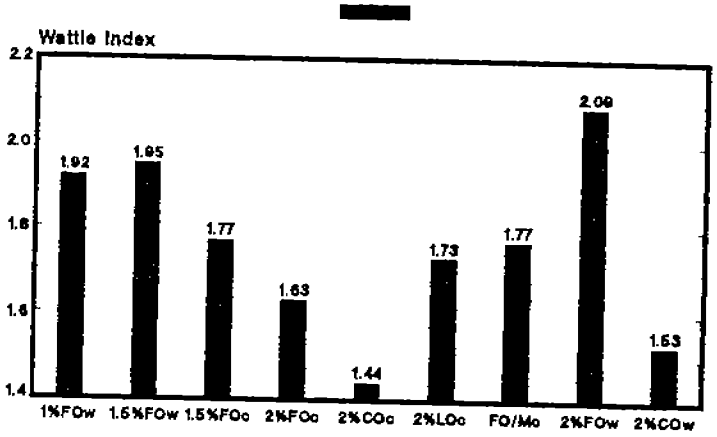
c = cereal; w = wheat

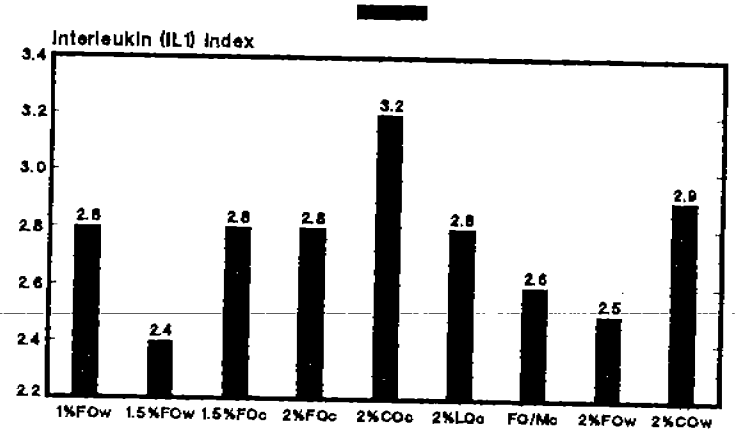
EXPT. 2 – ANTI-IBV IN BROILERS



EXPT. 2 – WATTLE INDEX IN BROILERS



EXPT. 2 – INTERLEUKIN (IL1) IN BROILERS



FO = Fish Oil; CO = Corn Oil: LO = Linseed Oil: FO/M = Fish Oil/Meal

Experiment 3 Effect of Energy Level and Fatty Acid Type on Performance and Immune Function of Broiler Chicks

Study ID 1S93

Parameters Measured

- Body Weight Gain (g/chick/d)
- Feed Consumption (g/chick/d)
- Feed Conversion Efficiency (g gain/g feed)
- Body Temperature 4 Hours Post-Injection
- Liver, Spleen, Bursa, Intestine Indices ((organ wt/body weight)*1000)
- Plasma Zinc, Copper

Materials and Methods

Experimental diets were formulated to meet NRC recommended levels of all nutrients. A low energy diet (Diet 1) contained 2700 kcal ME/kg. Four high energy diets contained 3300 kcal ME/kg and contained 6.6% of either corn oil, safflower oil, menhaden oil or tallow (Diets 2-5, respectively). Dietary composition, including n-3:n-6 PUFA ratio is shown in Table 3.1. Vitamin and mineral mixes were formulated according to the NRC Standard Reference Diets.

Chicks were fed a commercial broiler starter diet from 1 to 3 days of age. Starting on day 4, each of the experimental diets was fed to 60 chicks per treatment (n=300). At 11 days of age, chick and feed weights were measured, and the chicks injected with 3 ml of a 100 µg/ml solution of *Salmonella typhimurium* lipopolysaccharide (LPS; Sigma Chemical Co., St. Louis, MO). The chicks were injected with 3 ml of 5% sephadex on day 13, and 2 ml of 50% Freund's complete adjuvant on day 15. Cloacal temperatures were measured 4 hours after each of the first two injections. On day 16, body weights and feed consumption were recorded.

Statistical analysis

The experimental unit in this study was the pen. All data were analyzed by analysis of variance using the General Linear Models (GLM) procedure of SAS. Means separations were carried out using the Least Significant Difference (LSD) test. Results of statistical analyses in this section are given indicating mean, LSD (where possible), pooled standard error of the mean, and the P value as determined by GLM. Letter superscripts next to treatment means indicate differences among individual treatments. However, in the absence of a P value less than 0.05 as determined by GLM, significance of differences determined by LSD may not be statistically valid. In such cases, the letters are included only to assist in identifying trends towards significance. As there were differences in pen weights when the chicks were switched to the experimental diets, day 4 (initial) pen weight was used as a covariate to adjust body weight gain, feed consumption and feed conversion efficiency means.

Results and Discussion

Body weight gain (g/chick/d) during the pre-injection period (day 4-10) was greatest ($P<.0001$) in the corn oil, safflower oil and tallow treatments, and least in the cellulose and

menhaden oil diets (Table 3.2). In control birds, there was a trend (P<.07) towards the corn oil and safflower oil diets having higher rates of gain than the cellulose diet during the entire course of the experiment. Over the course of the experiment, immune challenged birds fed the low energy diet had the lowest rate of body weight gain (P<.04); those fed menhaden oil diet gained weight at a rate which was not significantly different than any of the other diets. Across dietary treatment, injection of immunogen resulted in a 18% decrease in gain during the injection period (P<.0001), and a 10% decrease overall (P<.0001).

The low energy and tallow diets were consumed by the birds at the greatest rate (P<.0001), and the corn-, safflower- and menhaden oil diets at the lowest rate from days 4 through ten (Table 3.3). After day 11, there were no differences in feed consumption due to diet. However, immunogen injection decreased feed consumption across dietary treatment by 16% during the injection period (P<.0001), and by 9% from day 4 to day 16 (P<.0001).

Feed conversion efficiency (g gain/g feed) during the pre-injection period were greater for the corn and safflower treatments than the menhaden and tallow treatments (Table 3.4). These diets were all greater than the cellulose diet (P<.0001). In control birds, the safflower diet again had the greatest, and the cellulose diet the poorest feed conversion efficiency both during the injection period (P<.03), and throughout the experiment (P<.0008). In immunogen-treated birds, all diets resulted in significantly greater FCE than the low energy cellulose diet (P<.004). Immunogen injection decreased FCE by 4% (P<.03) during the injection period, but this effect was not observed when the day 4 to day 16 data was analyzed.

The low energy diet tended to result in the poorest characteristics of any of the dietary treatments, both in control and immunologically challenged chicks. Chicks fed the corn oil diet tended to have better performance characteristics over the duration of the experiment than chicks fed other diets, although the differences were only significant in the case of the low energy diet. Contrary to expectations, feeding of the menhaden oil diet did not ameliorate the decrease in performance due to immunogen treatment with respect to the other diets. It is possible that the relatively high (6.6%) level of fish oil in the diet decreased palatability of the feed. Beneficial effects of fish oil have been observed in other experiments where the level of menhaden oil inclusion was much lower, and the ratio of n-3:n-6 PUFA was more moderate.

Body temperature (Table 3.5) of chicks after the first injection was 0.34% less in the control chicks than in the injected chicks (P<.006). Dietary treatment did not have an effect on body temperature at this time. After the injection on day 13, there was a trend toward higher temperature in chicks fed the cellulose diet, and lower in the safflower oil diet. (P<.06). Across dietary treatments, there were no differences in temperature between injected and non-injected chicks.

Liver indices (Table 3.6) of chicks fed the corn oil diet were 9% lower than both the cellulose and menhaden oil diets (P<.03). Liver indices were not different in immunogen treated birds. Immunogen treatment increased liver index by 17% *vs* non-injected controls (P<.0001). Spleen indices were not affected by dietary treatment in either the control or immunogen-challenged birds. Across dietary treatment, immunogen treatment increased spleen index by 23% (P<.0001). Bursa index of immune-challenged birds fed the cellulose and safflower oil diets were 26% and 16% greater than those fed the tallow diet, respectively (P<.05). Differences in bursa indices of control birds were not significant. Across dietary treatment, immunogen challenge

decreased bursa index by 9% (P<.03). Differences in intestine index were not affected by dietary treatment in either the control or the injected birds. Across dietary treatment, injection of immunogens increased intestine index by 10% (P<.0001).

Plasma zinc levels were unaffected by dietary treatment in both the immunogen and control birds (Table 3.7). Immunogen injection treatment decreased plasma zinc levels by 12% (P<.0005). Plasma copper levels were unaffected by both dietary and immunogen treatment.

Summary

This experiment demonstrates that simply increasing the amount of fish oil in the diet is not sufficient to improve performance. From the measurements taken and the results given, it is not possible to determine what is responsible for the lack of effect of fish oil relative to the other oils used. It may be a decrease in palatability, although consumption of the fish meal diet was not significantly different from the high n-6 PUFA (corn oil and safflower oil) diets during the injection period. Another possibility is that a certain level of n-6 PUFA are necessary for maintaining normal function and optimal response of the immune system to an inflammatory challenge. As well, others have observed a bell shaped dose-response curve to dietary fish oil, in which response increases until an optimal level is reached, and then response decreaxses again as this level is surpassed.

Table 3.1 - Experiment 3
Dietary Composition (g/kg) and calculated n-3:n-6 polyunsaturated fatty acid ratio

INGREDIENT	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
	Per cent of Diet				
Corn	57.11	57.11	57.11	57.11	57.11
Soybean Meal	32.06	32.06	32.06	32.06	32.06
Cellulose	6.97	0	0	0	0
Corn Oil	0	6.64	0	0	0
Safflower Oil	0	0	6.97	0	0
Menhaden Oil	0	0	0	6.97	0
Tallow	0	0	0	0	6.97
Dicalcium P	1.69	1.69	1.69	1.69	1.69
Limestone	1.39	1.39	1.39	1.39	1.39
Salt	0.22	0.22	0.22	0.22	0.22
D,L-Met	0.22	0.22	0.22	0.22	0.22
Vit/Min Premix	0.25	0.25	0.25	0.25	0.25
Ethoxyquin	0.08	0.08	0.08	0.08	0.08
Total	100	100	100	100	100
n-3:n-6 Ratio	0.07	0.07	0.03	1.32	0.06

Table 3.2 - Experiment 3

Body Weight Gain (g/chick/d)								
		All Birds			Control Birds		Injected Birds	
Diet	Treatment	D 4-10	D 11-16	D 4-16	D 11-16	D 4-16	D 11-16	D 4-16
Diet 1	7% Cellulose (Low E)	20.66^{b}	30.02^{b}	24.56^{b}	33.74^{b}	26.43^{b}	26.30^{b}	22.68^{b}
Diet 2	7% Corn Oil	22.19^{a}	33.23^{a}	26.84^{a}	36.80^{a}	28.26^{a}	29.65^{ab}	25.43^{a}
Diet 3	7% Saff. Oil	22.04^{a}	32.39^{a}	26.35^{a}	35.75^{ab}	27.74^{a}	29.03^{ab}	24.96^{a}
Diet 4	7% Menhaden Oil	20.73^{b}	33.07^{a}	25.87^{a}	35.60^{ab}	27.22^{ab}	30.53^{a}	24.53^{ab}
Diet 5	7% Tallow	22.36^{a}	32.05^{ab}	26.39^{a}	35.10^{ab}	27.53^{ab}	29.00^{ab}	25.26^{a}
LSD		0.62	2.36	1.17	2.85	1.19	3.68	1.85
Pooled SEM		0.75	2.88	1.42	2.39	1.00	3.09	1.55
P values								
Diet		0.0001	0.0636	0.0032	0.2970	0.0529	0.2134	0.0350
Injection			0.0001	0.0001				
Diet X Injection			0.8620	0.7827				
Initial BW (@ day 4)			0.0315	0.0001	0.0010	0.0001	0.7143	0.0384

^{abc}Means within a column having different superscripts and P<.05 are significantly different

Table 3.3 - Experiment 3

Feed Consumption (g/chick/d)								
		All Birds			Control Birds		Injected Birds	
Diet	Treatment	D 4-10	D 11-16	D 4-16	D 11-16	D 4-16	D 11-16	D 4-16
Diet 1	7% Cellulose (Low E)	32.55^{a}	48.28	39.11^{a}	53.41	41.61^{a}	43.14	36.60^{ab}
Diet 2	7% Corn Oil	31.20^{b}	49.36	38.70^{ab}	53.59	40.64^{ab}	45.13	36.76^{ab}
Diet 3	7% Saff. Oil	30.12^{b}	47.16	37.21^{b}	51.44	38.83^{b}	43.59	35.86^{b}
Diet 4	7% Menhaden Oil	30.45^{b}	49.48	38.38^{ab}	53.91	40.64^{ab}	45.05	36.12^{b}
Diet 5	7% Tallow	32.76^{a}	48.82	39.35^{a}	51.87	40.58^{ab}	45.96	38.12^{a}
LSD							4.15	1.93
Pooled SEM		1.41	3.87	1.89	4.18	2.11	3.48	1.62
P values								
Diet		0.0001	0.6047	0.0811	0.8117	0.3342	0.6129	0.1688
Injection			0.0001	0.0001				
Diet X Injection			0.7314	0.4581				
Initial BW (@ day 4)			0.1742	0.0025	0.0904	0.0133	0.7531	0.0543

^{abc}Means within a column having different superscripts and P<.05 are significantly different

Table 3.4 - Experiment 3

Feed Conversion Efficiency (g gain/g feed)								
		All Birds			Control Birds		Injected Birds	
Diet	Treatment	D 4-10	D 11-16	D 4-16	D 11-16	D 4-16	D 11-16	D 4-16
Diet 1	7% Cellulose (Low E)	0.64^{c}	0.62^{b}	0.63^{c}	0.63^{c}	0.64^{c}	0.61^{b}	0.62^{b}
Diet 2	7% Corn Oil	0.72^{a}	0.68^{a}	0.69^{ab}	0.69^{ab}	0.70^{ab}	0.66^{ab}	0.69^{a}
Diet 3	7% Saff. Oil	0.74^{a}	0.68^{a}	0.71^{a}	0.71^{a}	0.73^{a}	0.66^{a}	0.70^{a}
Diet 4	7% Menhaden Oil	0.68^{b}	0.67^{a}	0.68^{b}	0.66^{bc}	0.67^{b}	0.68^{a}	0.68^{a}
Diet 5	7% Tallow	0.68^{b}	0.66^{ab}	0.67^{b}	0.68^{ab}	0.68^{b}	0.63^{ab}	0.66^{a}
LSD							0.05	0.04
Pooled SEM		0.04	0.004	0.03	0.04	0.03	0.04	0.03
P values								
Diet		0.0001	0.0041	0.0001	0.0214	0.0008	0.0860	0.0031
Injection			0.0202	0.1809				
Diet X Injection			0.3861	0.6779				
Initial BW (@ day 4)			0.2744	0.0152	0.0942	0.0055	0.8476	0.3438

^{abc}Means within a column having different superscripts and P<.05 are significantly different

Table 3.5 - Experiment 3

Diet	Treatment	Temperature Day 11 (°C)			Temperature Day 13 (°C)		
		All Birds	Control	Injected	All Birds	Control	Injected
Diet 1	7% Cellulose (Low E)	41.40	41.19	41.61	41.30	41.13^{b}	41.47^{a}
Diet 2	7% Corn Oil	41.24	41.22	41.27	41.32	41.31^{a}	41.33^{ab}
Diet 3	7% Saff. Oil	41.25	41.21	41.29	41.20	41.24^{ab}	41.15^{b}
Diet 4	7% Menhaden Oil	41.29	41.27	41.31	41.32	41.27^{ab}	41.37^{a}
Diet 5	7% Tallow	41.37	41.16	41.59	41.33	41.32^{a}	41.33^{ab}
LSD			0.31		0.24	0.31	0.38
Pooled SEM		0.70	0.38	0.91	0.42	0.38	0.46
P values							
Diet		0.5482	0.7786	0.2677	0.2665	0.1953	0.0547
Injection		0.0058			0.0824		
Diet X Injection		0.1720			0.0255		

^{abc}Means within a column having different superscripts and P<.05 are significantly different

Table 3.6 - Experiment 3

Diet	Treatment	Liver Index			Spleen Index			Bursa Index			Intestine Index		
		All Birds	Control	Injected	All Birds	Control	Injected	All Birds	Control	Injected	All Birds	Control	Injected
1	7% Cellulose (Low E)	3.62	3.39^{a}	3.84	0.11	0.10^{b}	0.12	0.24^{a}	0.24	0.24^{a}	19.52^{a}	18.12	20.91
2	7% Corn Oil	3.47	3.10^{b}	3.83	0.11	0.10^{ab}	0.13	0.21^{bc}	0.21	0.21^{ab}	18.44^{b}	17.75	19.17
3	7% Safflower Oil	3.52	3.20^{ab}	3.84	0.12	0.10^{ab}	0.14	0.23^{ab}	0.24	0.22^{a}	18.50^{b}	17.50	19.50
4	7% Menhaden Oil	3.67	3.39^{a}	3.93	0.12	0.10^{ab}	0.14	0.23^{ab}	0.25	0.21^{ab}	18.85^{ab}	18.16	19.51
5	7% Tallow	3.47	3.25^{ab}	3.70	0.12	0.12^{a}	0.12	0.20^{c}	0.22	0.19^{b}	18.63^{ab}	17.85	19.40
LSD													
Pooled SEM		0.59	0.35	0.75	0.04	0.03	0.04	0.06	0.06	0.06	2.48	1.58	3.13
P values													
Diet		0.3901	0.0231	0.8975	0.7467	0.3093	0.5074	0.0190	0.2681	0.0454	0.2062	0.5901	0.3238
Injection		0.0001			0.0001			0.0206			0.0001		
Diet X Injection		0.7164			0.2125			0.4620			0.5892		

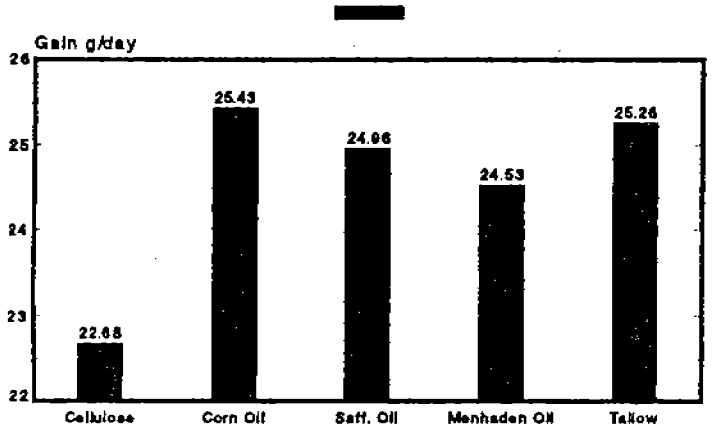
^{abc}Means within a column having different superscripts and P<.05 are significantly different

Table 3.7 - Experiment 3

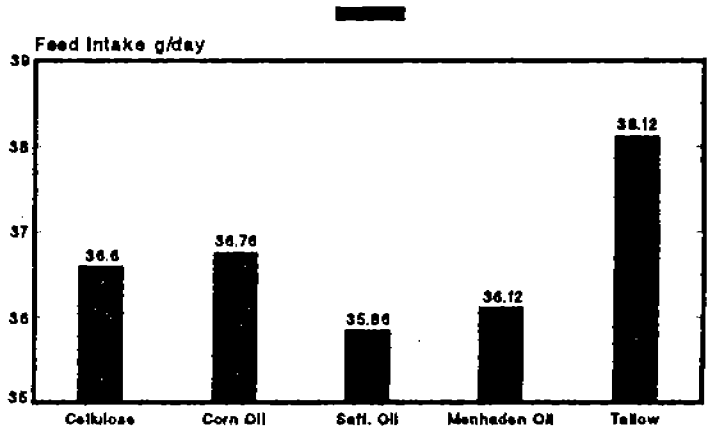
Diet	Treatment	Plasma Zinc (µg/ml or ppm)			Plasma Copper (µg/l or ppb)		
		All Birds	Control	Injected	All Birds	Control	Injected
Diet 1	7% Cellulose (Low E)	1.37	1.47	1.28^{ab}	3.99	4.63^{a}	3.14
Diet 2	7% Corn Oil	1.29	1.49	1.08^{b}	3.83	3.28^{b}	4.38
Diet 3	7% Saff. Oil	1.33	1.39	1.27^{ab}	3.68	3.94^{ab}	3.43
Diet 4	7% Menhaden Oil	1.29	1.38	1.19^{ab}	3.73	3.92^{ab}	3.54
Diet 5	7% Tallow	1.35	1.32	1.38^{a}	3.59	3.52^{b}	3.68
LSD							
Pooled SEM		0.32	0.31	0.34	1.58	1.57	1.58
P values							
Diet		0.7236	0.5216	0.1108	0.8768	0.1609	0.3595
Injection		0.0005			0.4664		
Diet X Injection		0.0613			0.0463		

^{abc}Means within a column having different superscripts and P<.05 are significantly different

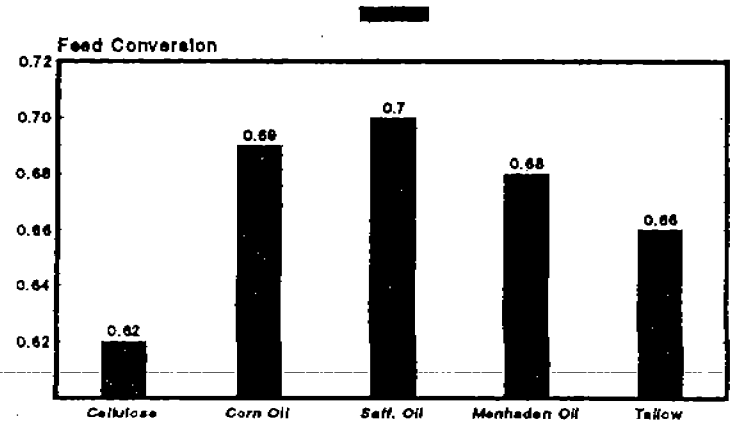
EXPT.3 - BODY WEIGHT GAIN OF BROILERS - LPS CHALLENGE



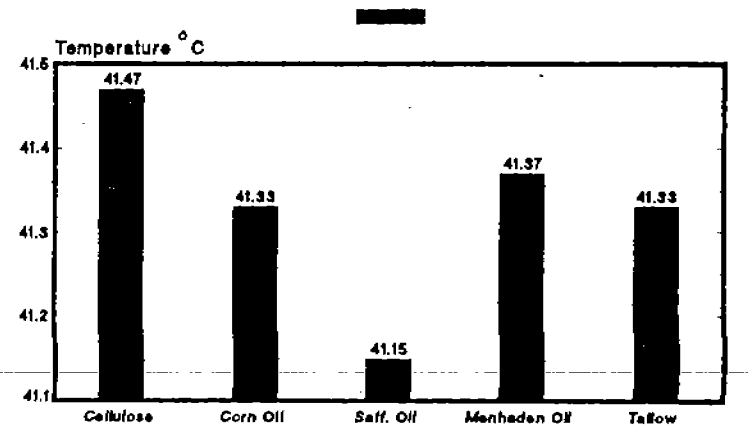
EXPT.3 - FEED INTAKE OF BROILERS - LPS CHALLENGE



EXPT. 3 - FEED EFFICIENCY OF BROILERS - LPS CHALLENGE



EXPT.3 - BODY TEMPERATURE OF BROILERS - LPS CHALLENGE



Experiment 4 Effect of Fish Oil and Fish Meal on Broiler Performance and Immune Function

Study ID 9401

Parameters Measured

- Body Weight Gain (g/chick/d)
- Feed Consumption (g/chick/d)
- Feed Conversion Efficiency (g gain/g feed)
- Liver Index ((liver wt/body wt)*1000)
- Liver Cytosolic Metallothionein (μg MT/g protein)
- Plasma Zinc, Iron

Materials and Methods

This study examined the effects of dietary fat type (tallow, corn oil, fish oil, and oil provided by fish meal) on broiler performance and immune function. From one to three days of age, the chicks received a commercial broiler starter diet. At that point, chicks were fed one of eight diets formulated to provide NRC recommended levels of all nutrients. Diet composition including ratio of n-3:n-6 PUFA is shown in Table 4.1. Diets were isocaloric and isonitrogenous. Three diets contained fish oil (1, 1.5 or 2%); three diets contained fish meal (10.6, 15.9 or 21.2%) and were formulated to provide 1, 1.5 or 2% fish oil from the fish meal.

Chicks were housed in Petersime battery brooders and fed *ad libitum*. Each dietary treatment was assigned to 8 pens of 5 chicks per pen. At 10 days of age, pen and feeder weights were measured. The chicks in four pens per dietary treatment were then injected with 3 ml of a 100 μg/ml solution of *Salmonella typhimurium* LPS. The remaining 4 pens per diet served as a non-injected control. The measurements and injection treatments were repeated at 12 and 14 days of age. Body weight gain, feed consumption, and feed conversion efficiency (FCE) were calculated. At 15 days of age, blood was sampled from 3 chicks per pen; the samples were then pooled within a pen, centrifuged and the plasma removed and frozen at -20°C. Livers were removed, immediately freeze-clamped and weighed. The livers were stored at -70°C until liver cytosolic metallothionein (MT) was assayed.

Statistical analysis

The experimental unit in this study was the pen. All data were analyzed by analysis of variance using the General Linear Models (GLM) procedure of SAS. Means separations were carried out using the Least Significant Difference (LSD) test. Results of statistical analyses in this section are given indicating mean, LSD (where possible), pooled standard error of the mean, and the P value as determined by GLM. Letter superscripts next to treatment means indicate differences among individual treatments. However, in the absence of a P value less than 0.05 as determined by GLM, significance of differences determined by LSD may not be valid. In such cases, the letters are included only to assist in identifying trends towards significance. As dietary treatment resulted in differing pen weights at the start of the injection period, pen weight at the initiation of injection treatment was used as a covariate to adjust body weight gain, feed

consumption and feed conversion efficiency means.

Results and Discussion

Body weight gains of LPS-challenged birds during the injection period were 19% greater (P<.004) for chicks fed the 2% fish oil diet than chicks fed the corn oil diet (Table 4.2). Gain of control birds were not affected by dietary treatment. LPS injection decreased body weight gain by 21% across dietary treatments (P<.0001). Orthogonal contrast analysis (Table 4.3) showed that in control birds, the fish oil diets resulted in body weight gains 10% greater than the tallow diet (P<.05), and 11% greater than the corn oil diet (P<.05). In the LPS-injected birds, the fish oil diets improved gain by 11% *vs* the fish meal diets (P<.02) and by 14% *vs* the corn oil diet (P<.03).

Feed consumption data by diet is shown in Table 4.4. Differences due to individual diets were not significant; across dietary treatments, LPS challenge resulted in a 12% decrease in feed consumption (P<.0001). Immune-challenged chicks fed the fish oil diets consumed 8% less (P<.03), and those fed fish meal 9% less (P<.005) feed than those chicks fed the tallow diet (Table 4.5).

Table 3.6 shows feed conversion efficiencies by diet. Control birds fed the 10.6% fish meal diet converted feed to body weight gain least efficiently (P<.004). LPS-injected chicks fed the 2% fish oil diet converted feed to body weight 32% more efficiently than those fed the highest level of fish meal (P<.04). As shown in Table 3.7, control birds fed fish oil converted feed into tissue mass 2% more efficiently than those fed fish meal (P<.006), 8% more efficiently than those fed the tallow diet (P<.004), and 3 % more efficiently than those fed the corn oil diet (P<.002). LPS-challenged chicks fed fish oil had FCE 12% greater than fish meal (P<.02) and 16% greater than the tallow diet (P<.03). The fish oil diet resulted in a nearly significant (P<.08) 12% improvement in FCE *vs* corn oil. Across dietary treatments, immune challenge caused a 9% decrease in FCE (P<.0001).

Body weight gains of chicks fed fish oil were consistently greater than the gains of the chicks fed corn oil, for both injected and non-injected groups. Feeding of fish meal decreased gain compared to the fish oil diets when the chicks were challenged with LPS. However, the fish meal diets did not decrease gain when compared with either tallow or corn oil, two common commercial oil sources.

Decreased feed consumption is commonly associated with an inflammatory response. Economically, this decrease will only be important if there is a concomitant decrease in growth rate. However, despite fish oil diets resulting in a greater decrease in feed consumption than the tallow diet, chicks fed the former diets gained more body mass. The fish oil diet also resulted in improved FCE *vs* the corn oil diet in control birds, and a nearly significant (P<.08) improvement in LPS-injected chicks.

Differences among liver index and liver cytosolic MT were not significant when analyzed by diet (Table 4.8), although LPS-injected birds fed the 21.2% fish meal diet had liver indices which were nearly different than the 1.5 and 2% fish oil, and the 15.9% fish meal diets (P<.08). When analyzed by dietary oil type (Table 4.9), the fish oil diets resulted in a nearly significant (P<.06) 15% increase in liver index *vs* the corn oil diet. Liver cytosolic MT of fish meal-fed controls was 20% greater than fish oil-fed birds (P<.009) and 37% greater than corn oil-fed

controls (P<.003). Differences were not significant when the chicks were immunologically stressed. MT levels of tallow- and fish meal-fed chicks were not significantly increased by LPS injection (data not shown), but chicks fed the corn oil and fish oil diets had MT levels which were 50 % (P<.001) and 29% (P<.04) greater, respectively than non-injected chicks fed the corresponding diets. LPS challenge decreased liver index by 30% (P<.0001) and increased by 16% liver cytosolic MT (P<.0001). The decrease in liver index is surprising, as an inflammatory response involves a production by the liver of a large amount of acute phase proteins, and usually, an increase of liver index.

Although non-injected, corn oil-fed birds had the lowest level of liver cytosolic MT, this diet resulted in the highest levels among immune challenged chicks. These birds had the greatest increase in MT due to injection treatment of any group. Surprisingly, the levels of this acute phase protein also increased significantly in fish oil-fed birds. Differences between this experiment and Experiment 1 may be due to differences in personnel responsible for carrying out assays.

Changes in plasma zinc and iron were affected by neither diet (Table 4.10), dietary oil type nor LPS injection (Table 4.11).

Summary

The most striking observation of this experiment is the disparity in response to immune challenge of chicks fed fish oil *vs* those fed fish meal. Long chain n-3 PUFA seem to be associated with, and may be responsible for improvements in performance seen in several other experiments using fish oil. As the fish meal composition tends to be enriched in these PUFA relative to the oil, one would expect even greater improvements in performance in chicks fed these diets. However, this was not the case. A possible explanation is that the fish meal and oil undergo different processing conditions. The processing of the meal may adversely affect the PUFA, which are highly susceptible to oxidation. Another possibility is that the processing may have a negative impact on the protein or other non-oil components of the meal. These components may then cause deleterious effects which might mask the beneficial effects of the oil. As well, the fish oil and the oil from fish meal contain different concentrations of various n-3 PUFA, and these differences may have an impact on the effect of the oil. It may be interesting to conduct a feeding trial in which chicks are fed either fish oil, or oil which has been extracted from fish meal.

Clearly, inclusion of even relatively low levels of fish oil in the diet have beneficial effects on the performance of chicks. The effects are especially pronounced when compared with diets containing corn oil, which is high in n-6 PUFA.

Table 4.1 - Experiment 4

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
	Per cent of Diet							
Corn	56.76	56.76	56.27	56.27	56.27	65.2	67.55	69.12
Soybean Meal	33.43	33.43	33.43	33.43	33.43	19.31	13.53	7.94
Tallow	3.73	5.69	4.71	4.22	3.73	2.84	1.86	1.09
Corn Oil	2	0	0	0	0	0	0	0
Fish Oil	0	0	1	1.5	2	0	0	0
Fish Meal	0	0	0	0	0	10.59	15.88	21.18
Limestone	1.47	1.47	1.47	1.47	1.47	0.94	0.36	0
Vit/Min Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Dicalcium P	1.67	1.67	1.67	1.67	1.67	0.18	0	0
Salt	0.25	0.25	0.25	0.25	0.25	0.18	0.14	0.1
D,L-Met	0.25	0.25	0.25	0.25	0.25	0.18	0.13	0.08
Choline Chloride	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100	100	100	100
n-3:n-6 Ratio	0.07	0.06	0.25	0.34	0.43	0.22	0.30	0.38

Table 4.2 - Experiment 4

Body Weight Gain (g/chick/d)										
		All Birds			Control Birds			LPS-Injected Birds		
Diet	Treatment	D 10-12	D 12-14	D 10-14	D 10-12	D 12-14	D 10-14	D 10-12	D 12-14	D 10-14
Diet 1	5.7% Tallow	26.65^{abc}	36.13^{bc}	31.41^{bc}	31.20	36.00^{c}	33.60^{b}	22.10^{ab}	36.33^{ab}	29.21^{bc}
Diet 2	Corn Oil (2%)	25.44^{c}	35.20^{c}	30.32^{c}	29.43	37.23^{abc}	33.33^{b}	21.45^{ab}	33.18^{b}	27.31^{cd}
Diet 3	Fish Oil (1%)	28.08^{abc}	39.11^{ab}	33.59^{ab}	33.95	39.23^{abc}	36.59^{ab}	22.20^{ab}	39.00^{a}	30.60^{ab}
Diet 4	Fish Oil (1.5%)	29.61^{ab}	38.54^{ab}	34.08^{a}	35.23	40.35^{ab}	37.79^{a}	24.00^{a}	36.73^{ab}	30.62^{ab}
Diet 5	Fish Oil (2%)	29.14^{abc}	39.70^{a}	34.42^{a}	32.18	40.50^{a}	36.34^{ab}	26.10^{a}	38.90^{a}	32.50^{a}
Diet 6	Fish Meal (10.6%)	27.21^{abc}	34.81^{c}	31.01^{c}	31.45	35.63^{c}	33.54^{b}	22.98^{ab}	34.00^{b}	28.49^{bcd}
Diet 7	Fish Meal (15.9%)	30.26^{a}	34.79^{c}	32.53^{abc}	34.45	36.13^{bc}	35.29^{ab}	26.08^{a}	33.45^{b}	29.76^{abc}
Diet 8	Fish Meal (21.2%)	26.31^{bc}	37.19^{abc}	31.09^{c}	34.20	38.58^{abc}	36.39^{ab}	18.43^{b}	35.79^{a}	25.78^{d}
	LSD	3.88	3.10	2.37	6.16	4.23	3.92	5.14	4.86	2.92
	Pooled SEM	3.86	3.08	2.35	4.21	2.89	2.68	3.52	3.33	2.00
	P values									
	Diet	0.1638	0.0058	0.0038	0.5213	0.1217	0.1894	0.0890	0.1184	0.0035
	Injection	0.0001	0.0113	0.0001						
	Diet X Injection	0.3178	0.8270	0.1506						
	Day 10 BW	0.7373	0.9049	0.8591	0.5359	0.9527	0.6490	0.6229	0.9007	0.5630

^{abc}Means within a column having different superscripts and P<.05 are significantly different

Table 4.3 - Experiment 4

Body Weight Gain (g/chick/d)

Contrast	Contrast P Values			Treatment Means			
All Birds	D 10-12	D 12-14	D 10-14	Treatment	D 10-12	D 12-14	D 10-14
Fish Meal vs Fish Oil	0.3425	0.0013	0.0018	Tallow	26.65	36.16	31.41
Fish Meal vs Tallow	0.4790	0.6823	0.9240	Corn Oil	25.44	35.20	30.32
Fish Meal vs Corn Oil	0.1145	0.7939	0.2115	Fish Meal	27.93	35.60	31.54
Fish Oil vs Tallow	0.1435	0.0258	0.0090	Fish Oil	28.94	39.12	34.02
Fish Oil vs Corn Oil	0.0499	0.0169	0.0021				
Control Birds	D 10-12	D 12-14	D 10-14		D 10-12	D 12-14	D 10-14
Fish Meal vs Fish Oil	0.6376	0.0204	0.0994	Tallow	31.20	36.00	33.60
Fish Meal vs Tallow	0.4857	0.6465	0.4280	Corn Oil	29.43	37.23	33.33
Fish Meal vs Corn Oil	0.0956	0.7877	0.2355	Fish Meal	33.37	36.77	35.07
Fish Oil vs Tallow	0.2833	0.0247	0.0416	Fish Oil	33.79	40.03	36.90
Fish Oil vs Corn Oil	0.0815	0.1975	0.0427				
LPS-Injected Birds	D 10-12	D 12-14	D 10-14		D 10-12	D 12-14	D 10-14
Fish Meal vs Fish Oil	0.5497	0.0401	0.0113	Tallow	22.10	36.33	29.21
Fish Meal vs Tallow	0.7720	0.3539	0.3802	Corn Oil	21.45	33.18	27.31
Fish Meal vs Corn Oil	0.6900	0.5492	0.6382	Fish Meal	22.49	34.42	28.01
Fish Oil vs Tallow	0.4338	0.3768	0.1640	Fish Oil	24.10	38.21	31.15
Fish Oil vs Corn Oil	0.4515	0.0518	0.0275				

[abc]Means within a column having different superscripts and P<.05 are significantly different

Table 4.4 - Experiment 4

Feed Consumption (g/chick/d)

	Treatment	All Birds			Control Birds			LPS-Injected Birds		
		D 10-12	D 12-14	D 10-14	D 10-12	D 12-14	D 10-14	D 10-12	D 12-14	D 10-14
Diet 1	5.7% Tallow	56.86^{a}	50.98^{ab}	53.92^{a}	59.75^{a}	50.28	55.01	53.98^{a}	51.68^{a}	52.83^{a}
Diet 2	Corn Oil (2%)	49.43^{b}	50.60^{ab}	50.01^{b}	51.48^{c}	53.38	52.43	47.38^{ab}	47.83^{ab}	47.60^{b}
Diet 3	Fish Oil (1%)	53.00^{ab}	52.20^{ab}	52.60^{ab}	58.00^{ab}	54.28	56.14	48.00^{ab}	50.13^{ab}	49.06^{ab}
Diet 4	Fish Oil (1.5%)	49.13^{b}	52.41^{ab}	50.77^{b}	54.20^{abc}	54.95	54.58	44.05^{b}	49.88^{ab}	46.96^{b}
Diet 5	Fish Oil (2%)	50.39^{b}	54.65^{a}	52.52^{ab}	54.18^{abc}	57.20	55.69	46.60^{ab}	52.10^{a}	49.35^{ab}
Diet 6	Fish Meal (10.6%)	51.98^{b}	52.66^{ab}	52.32^{ab}	55.55^{abc}	56.13	55.84	48.40^{ab}	49.20^{ab}	48.80^{ab}
Diet 7	Fish Meal (15.9%)	50.90^{b}	50.99^{ab}	50.94^{ab}	54.10^{abc}	53.78	53.94	47.70^{ab}	48.20^{ab}	47.95^{b}
Diet 8	Fish Meal (21.2%)	51.40^{b}	49.10^{b}	50.71^{b}	52.10^{bc}	54.48	53.29	50.70^{ab}	43.68^{b}	48.13^{b}
	LSD	4.81	4.89	3.01	6.37	6.97	4.74	7.68	7.37	4.09
	Pooled SEM	4.78	4.86	3.00	4.35	4.76	3.24	5.25	5.04	2.80
	P values									
	Diet	0.0578	0.4819	0.1792	0.1708	0.6380	0.7034	0.3174	0.3925	0.1724
	Injection	0.0001	0.0001	0.0001						
	Diet X Injection	0.6360	0.4715	0.6928						
	Day 10 BW	0.0008	0.7625	0.0032	0.0016	0.9667	0.0234	0.1629	0.6315	0.0904

^{abc}Means within a column having different superscripts and P<.05 are significantly different

Table 4.5 - Experiment 4

Feed Consumption (g/chick/d)

Contrast	Contrast P Values			Treatment Means			
All Birds	D 10-12	D 12-14	D 10-14	Treatment	D 10-12	D 12-14	D 10-14
Fish Meal vs Fish Oil	0.0416	0.2290	0.4075	Tallow	56.86	50.98	53.92
Fish Meal vs Tallow	0.0637	0.9708	0.1798	Corn Oil	49.43	50.60	50.01
Fish Meal vs Corn Oil	0.9115	0.9550	0.9346	Fish Meal	51.43	50.91	51.32
Fish Oil vs Tallow	0.0008	0.3188	0.0462	Fish Oil	50.84	53.09	51.96
Fish Oil vs Corn Oil	0.1467	0.3987	0.6336				
Control Birds	D 10-12	D 12-14	D 10-14		D 10-12	D 12-14	D 10-14
Fish Meal vs Fish Oil	0.5135	0.7640	0.8261	Tallow	59.75	50.28	55.01
Fish Meal vs Tallow	0.1839	0.1227	0.7992	Corn Oil	51.48	53.38	52.43
Fish Meal vs Corn Oil	0.6673	0.6484	0.9628	Fish Meal	53.92	54.79	54.35
Fish Oil vs Tallow	0.0668	0.0721	0.9265	Fish Oil	55.46	55.48	55.47
Fish Oil vs Corn Oil	0.4328	0.5573	0.9225				
LPS-Injected Birds	D 10-12	D 12-14	D 10-14		D 10-12	D 12-14	D 10-14
Fish Meal vs Fish Oil	0.0734	0.2615	0.3613	Tallow	53.98	51.68	52.83
Fish Meal vs Tallow	0.1983	0.1576	0.0264	Corn Oil	47.38	47.83	47.60
Fish Meal vs Corn Oil	0.8294	0.7182	0.9445	Fish Meal	48.93	47.03	48.29
Fish Oil vs Tallow	0.0086	0.6480	0.0050	Fish Oil	46.22	50.70	48.46
Fish Oil vs Corn Oil	0.2603	0.6103	0.5515				

[abc]Means within a column having different superscripts and P<.05 are significantly different

Table 4.6 - Experiment 4

Feed Conversion Efficiency (g gain/g feed)

	Treatment	All Birds			Control Birds			LPS-Injected Birds		
		D 10-12	D 12-14	D 10-14	D 10-12	D 12-14	D 10-14	D 10-12	D 12-14	D 10-14
Diet 1	5.7% Tallow	0.47^{d}	0.72^{abc}	0.58^{d}	0.52^{d}	0.73^{a}	0.61^{bc}	0.41^{ab}	0.70^{ab}	0.55^{bc}
Diet 2	Corn Oil (2%)	0.52^{cd}	0.70^{abc}	0.61^{bcd}	0.59^{bc}	0.70^{ab}	0.64^{bc}	0.46^{ab}	0.69^{b}	0.57^{abc}
Diet 3	Fish Oil (1%)	0.52^{bcd}	0.75^{ab}	0.64^{abc}	0.59^{bc}	0.72^{a}	0.65^{ab}	0.46^{ab}	0.78^{ab}	0.62^{ab}
Diet 4	Fish Oil (1.5%)	0.60^{a}	0.74^{ab}	0.67^{a}	0.65^{a}	0.73^{a}	0.69^{a}	0.55^{a}	0.76^{ab}	0.65^{ab}
Diet 5	Fish Oil (2%)	0.57^{abc}	0.73^{ab}	0.65^{ab}	0.58^{bcd}	0.71^{ab}	0.65^{ab}	0.56^{a}	0.75^{ab}	0.66^{a}
Diet 6	Fish Meal (10.6%)	0.52^{cd}	0.66^{bc}	0.59^{cd}	0.57^{cd}	0.64^{b}	0.60^{c}	0.47^{a}	0.69^{b}	0.58^{abc}
Diet 7	Fish Meal (15.9%)	0.59^{ab}	0.60^{c}	0.64^{abc}	0.64^{ab}	0.67^{ab}	0.65^{ab}	0.55^{a}	0.70^{b}	0.62^{ab}
Diet 8	Fish Meal (21.2%)	0.47^{cd}	0.80^{a}	0.59^{cd}	0.66^{a}	0.71^{ab}	0.68^{a}	0.28^{b}	0.89^{a}	0.50^{c}
	LSD	0.07	0.13	0.05	0.06	0.08	0.04	0.12	0.19	0.10
	Pooled SEM	0.07	0.13	0.05	0.04	0.05	0.03	0.08	0.13	0.07
	P values									
	Diet	0.0045	0.0969	0.0055	0.0019	0.1503	0.0033	0.0614	0.4338	0.0338
	Injection	0.0001	0.5680	0.0001						
	Diet X Injection	0.0619	0.3696	0.0196						
	Day 10 BW	0.0008	0.9774	0.0152	0.0001	0.9204	0.0004	0.4859	0.8039	0.5313

^{abc}Means within a column having different superscripts and $P<.05$ are significantly different

Table 4.7 - Experiment 4 Feed Conversion Efficiency (g gain/g feed)

Contrast	Contrast P Values			Treatment Means			
All Birds	D 10-12	D 12-14	D 10-14	Treatment	D 10-12	D 12-14	D 10-14
Fish Meal vs Fish Oil	0.0120	0.4631	0.0002	Tallow	0.47	0.72	0.58
Fish Meal vs Tallow	0.0002	0.9085	0.5902	Corn Oil	0.52	0.70	0..61
Fish Meal vs Corn Oil	0.0015	0.6106	0.4440	Fish Meal	0.53	0.72	0.61
Fish Oil vs Tallow	0.0611	0.6394	0.0005	Fish Oil	0.56	0.74	0.65
Fish Oil vs Corn Oil	0.0616	0.3584	0.0017				
Control Birds	D 10-12	D 12-14	D 10-14		D 10-12	D 12-14	D 10-14
Fish Meal vs Fish Oil	0.1114	0.0453	0.0052	Tallow	0.52	0.73	0.61
Fish Meal vs Tallow	0.0223	0.0538	0.4137	Corn Oil	0.59	0.70	0.64
Fish Meal vs Corn Oil	0.0022	0.4163	0.0647	Fish Meal	0.62	0.67	0.65
Fish Oil vs Tallow	0.0009	0.6513	0.0036	Fish Oil	0.61	0.72	0.66
Fish Oil vs Corn Oil	0.0005	0.5505	0.0014				
LPS-Injected Birds	D 10-12	D 12-14	D 10-14		D 10-12	D 12-14	D 10-14
Fish Meal vs Fish Oil	0.1093	0.9480	0.0143	Tallow	0.41	0.70	0.55
Fish Meal vs Tallow	0.3448	0.5125	0.8176	Corn Oil	0.46	0.69	0.57
Fish Meal vs Corn Oil	0.6734	0.3821	0.9635	Fish Meal	0.47	0.76	0.57
Fish Oil vs Tallow	0.0282	0.4872	0.0221	Fish Oil	0.52	0.75	0.64
Fish Oil vs Corn Oil	0.1379	0.4411	0.0775				

[abc]Means within a column having different superscripts and P<.05 are significantly different

Table 4.8 - Experiment 4

Diet	Treatment	Liver Index (liver wt./body wt.)*1000			Liver Cytosolic MT (µg MT/g protein)		
		All Birds	Control	Injected	All Birds	Control	Injected
Diet 1	5.7% Tallow	26.65^{abc}	31.20	22.10^{ab}	547.32	534.68^{abc}	559.96
Diet 2	Corn Oil (2%)	25.44^{c}	29.43	21.45^{ab}	511.56	409.61^{c}	613.51
Diet 3	Fish Oil (1%)	28.08^{abc}	33.95	22.20^{ab}	550.37	496.29^{abc}	604.45
Diet 4	Fish Oil (1.5%)	29.61^{ab}	35.23	24.00^{a}	522.26	442.37^{bc}	602.15
Diet 5	Fish Oil (2%)	29.14^{abc}	32.18	26.10^{a}	541.35	469.34^{bc}	613.36
Diet 6	Fish Meal (10.6%)	27.21^{abc}	31.45	22.98^{ab}	583.55	549.82^{ab}	617.28
Diet 7	Fish Meal (15.9%)	30.26^{a}	34.45	26.08^{a}	593.60	608.39^{a}	578.81
Diet 8	Fish Meal (21.2%)	26.31^{bc}	34.2	18.43^{b}	568.98	526.03^{abc}	611.93
	LSD	3.85	6.07	5.05	82.32	128.81	109.41
	Pooled SEM	3.83	4.16	3.46	81.89	88.26	74.97
	P values						
	Diet	0.1545	0.5044	0.0784	0.4661	0.0854	0.9502
	Injection	0.0001			0.0001		
	Diet X Injection	0.3055			0.1320		

^{abc}Means within a column having different superscripts and P<.05 are significantly different

Table 4.9 - Experiment 4

Contrast	Liver Index ((liver wt/body wt)*1000)			Liver Cytosolic MT(µg MT/g protein)		
All Birds	Contrast P Values	Treatment	Mean	Contrast P Values	Treatment	Mean
Fish Meal vs Fish Oil	0.3639	Tallow	26.65	0.0685	Tallow	547.32
Fish Meal vs Tallow	0.4169	Corn Oil	25.44	0.3041	Corn Oil	511.56
Fish Meal vs Corn Oil	0.1172	Fish Meal	27.93	0.0402	Fish Meal	582.04
Fish Oil vs Tallow	0.1489	Fish Oil	28.94	0.7815	Fish Oil	537.99
Fish Oil vs Corn Oil	0.0295			0.4330		
Control Birds						
Fish Meal vs Fish Oil	0.7908	Tallow	31.20	0.0083	Tallow	534.68
Fish Meal vs Tallow	0.3316	Corn Oil	29.43	0.5744	Corn Oil	409.61
Fish Meal vs Corn Oil	0.0807	Fish Meal	33.37	0.0024	Fish Meal	561.41
Fish Oil vs Tallow	0.2480	Fish Oil	33.78	0.1733	Fish Oil	469.33
Fish Oil vs Corn Oil	0.0543			0.2126		
LPS-Injected Birds						
Fish Meal vs Fish Oil	0.3083	Tallow	22.10	0.9057	Tallow	559.96
Fish Meal vs Tallow	0.8600	Corn Oil	21.45	0.3707	Corn Oil	613.51
Fish Meal vs Corn Oil	0.6394	Fish Meal	22.49	0.8197	Fish Meal	602.67
Fish Oil vs Tallow	0.3698	Fish Oil	24.10	0.3282	Fish Oil	606.65
Fish Oil vs Corn Oil	0.2362			0.8845		

[abc]Means within a column having different superscripts and P<.05 are significantly different

Table 4.10 - Experiment 4

Diet	Treatment	Plasma Zinc(µg/ml)			Plasma Iron (µg/ml)		
		All Birds	Control	Injected	All Birds	Control	Injected
Diet 1	5.7% Tallow	1.82	1.77	1.86	0.81	0.71	0.91^{ab}
Diet 2	Corn Oil (2%)	1.68	1.62	1.74	1.02	1.11	0.93^{ab}
Diet 3	Fish Oil (1%)	2.53	1.63	3.44	0.91	0.79	1.02^{ab}
Diet 4	Fish Oil (1.5%)	1.98	2.42	1.53	0.83	0.75	0.90^{ab}
Diet 5	Fish Oil (2%)	2.94	3.02	2.85	0.95	0.66	1.25^{a}
Diet 6	Fish Meal (10.6%)	2.46	1.59	3.32	1.11	1.09	1.12^{ab}
Diet 7	Fish Meal (15.9%)	3.42	3.13	3.71	0.80	0.81	0.78^{b}
Diet 8	Fish Meal (21.2%)	1.42	1.31	1.52	0.95	0.91	0.98^{ab}
	LSD						
	Pooled SEM	1.88	1.69	2.09	0.44	0.54	0.24
	P values						
	Diet	0.6148	0.7631	0.8041	0.9115	0.9574	0.3801
	Injection	0.2966			0.1606		
	Diet X Injection	0.9262			0.8936		

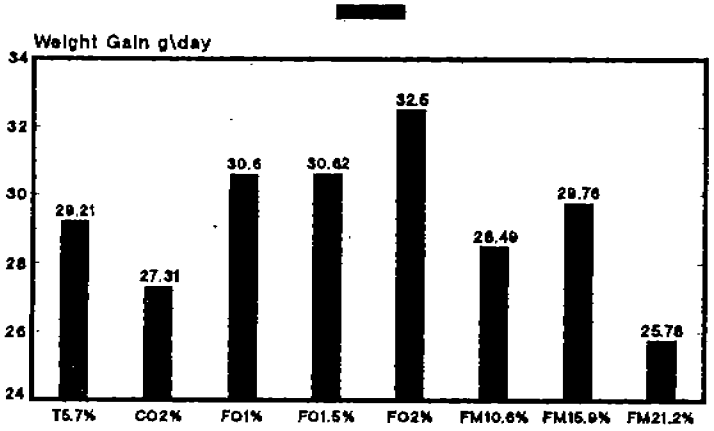
^{abc}Means within a column having different superscripts and P<.05 are significantly different

Table 4.11 - Experiment 4

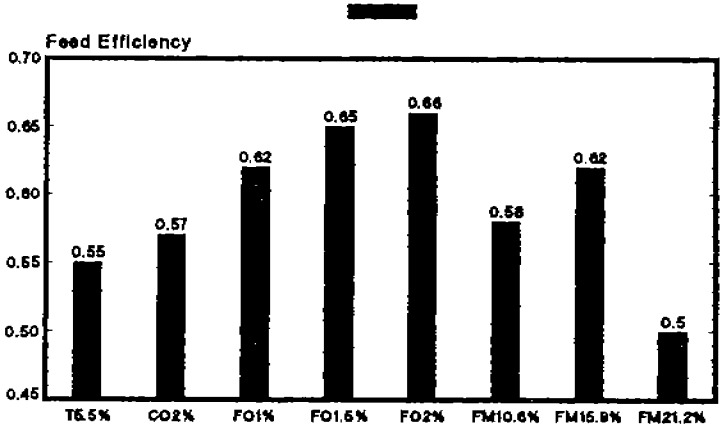
Contrast	Plasma Zinc (µg/ml)			Plasma Iron (µg/ml)		
All Birds	Contrast P Values	Treatment	Plasma Zn	Contrast P Values	Treatment	Plasma Fe
Fish Meal vs Fish Oil	0.9398	Tallow	1.82	0.7172	Tallow	0.81
Fish Meal vs Tallow	0.4692	Corn Oil	1.68	0.4667	Corn Oil	1.02
Fish Meal vs Corn Oil	0.4710	Fish Meal	2.43	0.7713	Fish Meal	0.95
Fish Oil vs Tallow	0.4475	Fish Oil	2.49	0.6611	Fish Oil	0.90
Fish Oil vs Corn Oil	0.4513			0.6125		
Control Birds						
Fish Meal vs Fish Oil	0.6811	Tallow	1.77	0.2965	Tallow	0.71
Fish Meal vs Tallow	0.8310	Corn Oil	1.62	0.3758	Corn Oil	1.11
Fish Meal vs Corn Oil	0.7894	Fish Meal	2.01	0.6149	Fish Meal	0.94
Fish Oil vs Tallow	0.5994	Fish Oil	2.36	0.9125	Fish Oil	0.73
Fish Oil vs Corn Oil	0.6143			0.2710		
LPS-Injected Birds						
Fish Meal vs Fish Oil	0.8059	Tallow	1.86	0.6836	Tallow	0.91
Fish Meal vs Tallow	0.4359	Corn Oil	1.74	0.8529	Corn Oil	0.93
Fish Meal vs Corn Oil	0.4526	Fish Meal	2.85	0.9280	Fish Meal	0.96
Fish Oil vs Tallow	0.5800	Fish Oil	2.61	0.6326	Fish Oil	1.06
Fish Oil vs Corn Oil	0.5740			0.7267		

[abc]Means within a column having different superscripts and P<.05 are significantly different

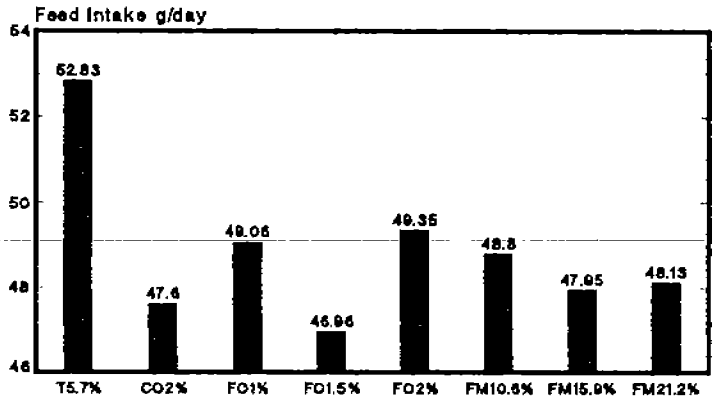
EXPT.4– WEIGHT GAIN OF BROILERS –
LPS CHALLENGE



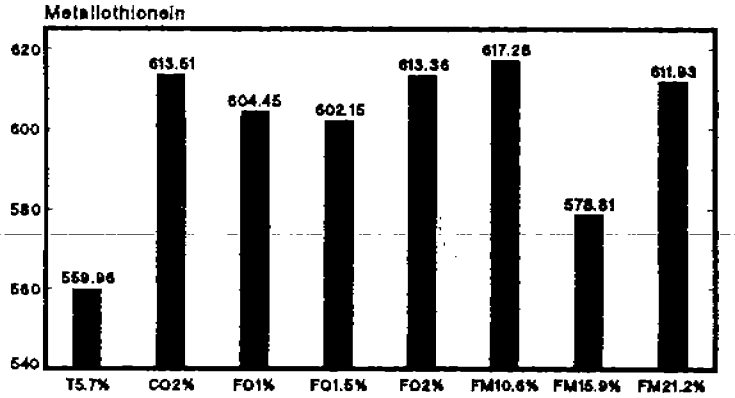
EXPT4 – FEED EFFICIENCY OF BROILERS –
LPS CHALLENGE



EXPT.4– FEED INTAKE OF BROILERS –
LPS CHALLENGE



EXPT4 – METALLOTHIONEIN IN BROILERS –



APPENDIX 3

EXPERIMENT 5

The following is an abstract which will be presented at the 1995 Poultry Science Association Annual Meeting in Edmonton, Alberta, Canada in August.

FISH OIL AND FENLEUTON, A LIPOXYGENASE INHIBITOR, IMPROVE GROWTH OF BROILER CHICKS CHALLENGED WITH COCCIDIA. D. R. Korver* and K. C. Klasing Department of Avian Sciences, University of California, Davis, Davis, CA 95616.

Day-old broiler chicks were fed diets containing 4% of either corn oil (high in n-6 PUFA) or menhaden fish oil (high in n-3 PUFA). On day 14, half of the birds on each of the dietary treatments received 33 mg fenleuton (a lipoxygenase (LO) inhibitor)/kg of diet (2 X 2 factorial). On day 23 half of the birds on each diet/drug treatment were dosed with 50, 000 coccidia oocysts per bird (*Eimeria tenella*) (2 X 2 X 2 factorial). Birds were killed on day 27 and liver samples taken. Fish oil addition to the diet resulted in a nearly significant (P<.06) 8% increase in weight gain and increased feed consumption by 4% (P<.004) *vs* corn oil diets. Fenleuton resulted in weight gain 4% greater than the control diets (P<.04), and a 3% improvement in feed conversion efficiency (P<.04). Corn oil-fed birds challenged with coccidia had 6% lower body weight gains than unchallenged corn oil-fed birds (P<.05). Coccidial infection had no effect on body weight gains of chicks fed fish oil. The addition of fenleuton to corn oil-containing diets tended (P<.06) to abrogate an 11% depression of body weight gain due to coccidial infection. Liver indices (liver weight*1000/body weight) increased by 10% (P<.0002) in the fish oil *vs* corn oil diets and by 6% in the fenleuton-containing *vs* the diets containing no drug (P<.02). Inhibition of LO may affect the response of broiler chicks to an infectious challenge.

APPENDIX 4

ALTERATIONS IN IMMUNE RESPONSE BY DIETARY FISH OIL*

BY DOUGLAS R. KORVER AND KIRK C. KLASING

ABSTRACT

(Key Words: broiler, fish oil, inflammatory response, immune (n-3) polyunsaturated fatty acid)

*Paper submitted for publication

An inflammatory response can increase organ mass relative to body mass (Roura et al., 1992), metabolic rate, synthesis of acute phase proteins, and decrease feed consumption and muscle protein accretion (Klasing and Johnstone, 1991). One strategy to achieve faster growth rate and improve meat yield in broilers is to minimize factors which divert nutrients away from growth.

In mammals, the fatty acid composition of phospholipid membranes in immune cells and target cells can affect the degree of inflammatory response to a given immunological challenge *in vitro* (Prescott, 1984; Billiar et al., 1988) and *in vivo* (German et al., 1987). Membranes enriched in (n-3) polyunsaturated fatty acids (PUFA) at the expense of (n-6) PUFA release a lower level as well as less potent mediators of inflammation (Prescott, 1984; Billiar et al., 1988). These mediators, the eicosanoids, are involved in the release and function of pro-inflammatory cytokines such as tumor necrosis factor (TNF) (Scales et al., 1989), interleukin-1 (IL-1), and IL-6 (Navarra et al., 1992). Two eicosanoids important in the inflammatory response are prostaglandins of the E series (PGE) and leukotrienes of the B series (LTB).

Although most research of dietary oils has utilized high levels (<5%) of inclusion in the diet, in mice the ratio of (n-3):(n-6) PUFA appears to be more important in modulating eicosanoid biosynthesis than the absolute level of (n-3) PUFA in the diet (Boudreau et al., 1991, Broughton *et al.*, 1991). German et al. (1988) demonstrated that at high levels of dietary linoleic acid, fish oil supplementation had a minimal effect on leukotriene production.

Inclusion of fish oil in the diet has been shown to increase the proportion of (n-3) PUFA relative to (n-6) PUFA in the tissues of humans (Schmidt et al., 1991), rats (Billiar et al., 1988), mice (German et al., 1987; Whelan et al., 1991), and poultry (Fritsche et al., 1991b; Chanmugam

et al., 1992). The enrichment of (n-3) PUFA is associated with decreases in the inflammatory response, improvements in growth rate, and either increased or no change in specific immunity. The inclusion of fish oil in the diet of mammals appears to improve both humoral immunity and ameliorate the suppression of the cellular immune response caused by PGE_2. Dietary fish oil supplementation in humans decreases neutrophil chemotaxis in vitro (Schmidt et al., 1991). Administration of exogenous PGE_2 to the central nervous system of rats decreased in vitro cellular immune responses (Rassnick et al., 1995). Decreasing the production of PGE_2 by feeding fish oil should therefore result in increased cellular responses. Mice fed diets containing 17% fish oil had antibody responses to SRBC which were significantly higher than mice fed 20% corn oil diets (Fritsche et al., 1992).

Fritsche et al. (1991a,) reported that chicks fed a diet containing 7% menhaden oil had higher antibody responses to SRBC than chicks fed 7% of either lard, corn oil or canola oil. Cellular immune response as measured by antibody-dependent cell cytotoxicity of splenocytes was decreased in broilers fed 7% fish oil vs those fed 7% corn oil, although cytotoxicity of peripheral blood leukocytes was not affected by dietary treatment (Fritsche and Cassity, 1992).

The endogenous mediators of inflammation can themselves be involved in the pathogenesis of several diseases, including rheumatoid arthritis (Ridderstad et al., 1991), systemic lupus erythematosus (Das, 1994) and atherosclerosis (Makheja, 1992). Mice suffering from murine lupus nephritis and fed diets containing fish oil have decreased renal expression of IL-1, IL-6 and TNF mRNA expression *vs.* those fed diets containing corn oil (Chandresekar and Fernandes, 1994). The use of fish oil not only affects the release of immune system mediators from various immune tissues, but affects target tissue response to those mediators. Rats fed diets

containing fish oil have decreased pyrogenic responses to endogenous IL-1 (Cooper and Rothwell, 1993) and the anorexic responses to endogenous TNF (Mulrooney and Grimble, 1993).

The experiments presented here were conducted to determine if the inclusion of low levels of fish oil in broiler diets could improve performance during an inflammatory response. Further, the experiments were designed to relate any changes in performance to indices of inflammatory and specific immune responses.

MATERIALS AND METHODS

Birds and Management

Two experiments were conducted to determine the impact of various fatty acid sources on immunocompetence. Male commercial Hubbard x Hubbard broiler chicks (A & M Hatchery, Santa Rosa, CA) were raised in Petersime brooder batteries[2] with raised floors and provided a nutritionally complete corn and soybean meal based starter diet with 13.4 kJ/g (Klasing and Barnes,1988) prior to the experiment. When chicks were 3 days of age, experimental chicks were selected from a two-fold larger population to obtain uniform body weights and randomly assigned to dietary treatments. All experiments were approved by the University of California, Davis animal use committee.

The corn-soy experimental diets used in experiment 1 were based on the NRC (1984) standard research reference diet for chicks to which 0.5, 1.0 or 2% of either corn oil or menhaden oil was added. Table 1 shows composition and calculated (n-3):(n-6) PUFA ratio of each diet. Nine diets were used in Experiment 2 (Table 2), with menhaden, linseed or corn oil as the source of dietary fat, and either corn or mixed cereal based diets. Diets were kept isocaloric and

[2]Petersime Incubator Co., Gettysburg, OH 45238

isonitrogenous by adding appropriate amounts of corn starch and cellulose. Each of the six (Experiment 1) or nine (Experiment 2) experimental diets was fed to four pens of five chicks each. When the chicks were 14 days of age, they were vaccinated, i. m., with 5 mg/kg body weight infectious bronchitis virus (IBV; Bron-Newcavac-M, 10-006[3]). On day 28, venous blood was taken and IBV antibody titers were determined by ELISA.[3] Delayed-type hypersensitivity, a measure of cell-mediated immunity was evaluated by the Phytohemagglutinin (PHA)-P-induced wattle inflammation assay as described by Klasing (1988). On day 29, sephadex-elicited peritoneal macrophages were purified as described by Klasing and Peng (1987) and stimulated in vitro with *Salmonella typhimurium* lipopolysaccharide (LPS) to determine the capacity of these cells to produce interleukin 1 (IL-1). IL-1 activity was measured by PHA-P-induced comitogenesis of thymocytes (Klasing and Peng, 1987). Briefly, thymocytes were isolated from several thymic lobes of a 6-week-old broiler chick and minced in ice-cold RPMI 1640 medium.[4] The cells and medium were transferred to a 50 mL conical centrifuge tube and put on ice. After allowing debris to settle for ten minutes, cells remaining in suspension were removed and washed 3 times by centrifugation at 600 x g for 10 min. Centrifugation concentrated red blood cells at the bottom of the tube; these cells were discarded. Thymocytes (2×10^6 cells/well) were plated in 96-well plates[5] with 1 µg PHA-P/well and 50 µl of a **1:10 dilution** of the macrophage-conditioned medium. After incubation at 42°C in a 5% CO_2 humidified atmosphere for 48 h, each well was pulsed with 1µCi [^{3}H]thymidine and incubated for an additional 16 h. At that time, cells were

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[3]Kirkegaard and Perry Laboratories, Gathersburg, MD **ZIP CODE**

[4]Sigma Chemical Co., St. Louis, MO 63178

[5]Corning, Corning NY 14831

harvested using a Skatron cell harvester, and activity of incorporated thymidine was determined by scintillation counting. All solutions and suspensions used in thymocyte culture were made up in RPMI 1640 supplemented with 5% fetal bovine serum, 100 U/mL penicillin and 100 µg/ mL streptomycin. IL-1 activity is given as the stimulation index, which is the ratio of cpm [^{3}H]thymidine incorporated into DNA of thymocytes incubated with macrophage-conditioned medium plus PHA-P to cpm in thymocytes incubated in medium plus PHA-P.

A second group of chicks were fed the same 6 (experiment 1) or 9 (experiment 2) diets in 12 pens of 5 chicks starting when chicks are 3 days of age. When chicks were 10 days of age, 4 pens per diet were injected, i. p., with 3 mL of a 100 µg/ml solution of *S. typhimurium* (LPS); 4 pens were injected with 3 x 10^9 heat-killed *Staphylococcus aureus*/kg body weight; 4 pens were not injected and served as controls. Injections were repeated every other day 3 times to simulate an authentic infectious challenge. *Salmonella typhimurium* lipopolysaccharide was purchased from Sigma Chemical (St. Louis, MO), reconstituted in saline (9 g/L) to 100 mg/L and sterilized by passing through a 0.45 µm filter. *S. aureus* were grown in nutrient broth, washed in saline, heat-killed at 85°C for 10 min and suspended in 9 g/L saline at 10^{12} cells/L.

Gain, feed intake and feed conversion efficiency (FCE) were determined throughout the experiment. Circulating levels of the acute phase protein, hemopexin was determined on the final day of the injection schedule to give an index of the acute phase response. Hemopexin concentrations were determined by rocket gel electrophoresis using a rabbit anti-chick hemopexin antibody. Cloacal temperature was determined 6 hrs following the first immunogen injection to provide an index of the responsiveness of the hypothalamus to cytokines released during the immune stress. Livers were removed on the last day of the experiment, freeze-clamped in liquid

nitrogen, and kept frozen until analysis. The concentration of the acute phase protein metallothionein (MT) in the liver was assessed by the cadmium-109 affinity assay described by Eaton and Toal (1982).

Statistical analysis

For experiment 1, data were analyzed by a three-way analysis of variance with oil source, oil level, and immunogen as main effects and for their interactions using the general linear model procedure of the Statistical Analysis System (SAS) computer program (SAS Institute, 1985). For experiment 2, data were analyzed by a two-way analysis of variance with dietary treatment and immunogen as main effects and for interactions using the general linear model procedure of SAS. When main effects due to dietary treatment, level, or immunogen were significant ($P<0.05$), significant differences between main effect means were determined by the method of Tukey using SAS. All dependent variables were also analyzed by a One Way ANOVA with 18 unrelated treatments (experiment 1) or 27 unrelated treatments (experiment 2) and the SEM and LSD are reported.

RESULTS

Performance characteristics were not significantly different among the various dietary treatments in experiment 1 (Table 3). Immunogen injection significantly decreased body weight gain ($P<.0001$), feed consumption ($P<.0001$) and feed conversion efficiency ($P<.0007$). There was an interaction between oil source and immunogen treatment ($P<.04$) for body weight gain where chicks fed corn oil and injected with either LPS or *S. aureus* gained less body weight than those fed fish oil and treated with the corresponding immunogen. Immunogen challenged chicks fed corn oil gained body weight less efficiently than those challenged chicks fed fish oil, but this

difference was not seen in control chicks. There was also a non-significant trend towards a level by oil source interaction for gain (P<.07) and FCE (P<.08). As the level of fish oil in the diet increased, the negative effect of immunogen treatment tended to decrease. However, increasing the level of corn oil in the diet did not appear to have the same effect.

Performance data from experiment 2 is shown in Table 4. Immunogen injection significantly decreased body weight gain (P<.0001), feed consumption (P<.04) and feed conversion efficiency (P<.0001). The decrease in rate of gain due to LPS injection was 13.4% for the 2% corn oil/corn treatment and only 5.6% for the 2% menhaden oil/cereal treatment. There was a diet by immunogen interaction (P<.05) for feed conversion efficiency and a non-significant trend for feed consumption (P<.09).

Body temperature of chicks was not affected by either source of dietary oil or by the level of its inclusion in the diet in experiment 1 (Table 5). Injection of immunogen, however, increased body temperature by 2% across dietary treatments (P<.0001). There was a non-significant trend (P<.06) towards a level by source interaction in which the febrile response to immunogen decreased as fish oil in the diet increased, but remained fairly constant as corn oil in the diet increased. Hemopexin levels were increased 610% (P<.0001) in experiment 1 (Table 5). There was a level by source interaction; as fish oil increased in the diet, hemopexin levels decreased, while as the level of corn oil increased in the diet, hemopexin levels increased (P<.03). Metallothionein levels increased 125% (P<.0001) (Table 5) in response to immunogen injection. There was a nearly significant level by source interaction for MT response (P<.07). Levels in immunogen-treated chicks fed corn oil tended to increase as corn oil increased, while increasing fish oil in the diet from 0.5% to 2% tended to decrease MT levels in response to immunogen.

Dietary treatment did not affect body temperature of chicks in experiment 2 (Table 6), although immunogen treatment increased temperature across all dietary treatments ($P<.0001$). Hemopexin levels were elevated 383% ($P<.04$) (Table 6) by immunogen injection. There was a diet by immunogen interaction ($P<.04$) in which diets providing a greater level of corn oil (either as oil or as grain) tended to have greater increases in hemopexin production in response to immunogen treatment. As the (n-3):(n-6) PUFA ratio increased, hemopexin production was stimulated to a lesser extent by immunogen treatment.

Metallothionein levels increased by 619% ($P<.0001$) (Table 6) in response to immunogen injection. There was a nearly significant diet by immunogen interaction ($P<.06$) for MT, in which corn oil-containing diets had higher MT levels than the high (n-3) PUFA diets.

Antibody titers to infectious bronchitis virus were not altered by dietary treatment in experiment 1 (Table 7). There was a non-significant trend towards a source by level interaction ($P<.09$), in which as corn oil increased from 0.5% to 2% of the diet, antibody titers decreased, while increasing dietary fish oil from 0.5% to 2% increased antibody titers. PHA-induced wattle inflammation was increased ($P<.04$) when chicks were fed fish oil *vs.* corn oil-fed chicks (Table 7). Peritoneal macrophage IL-1 was decreased ($P<.02$) in chicks fed fish oil *vs* those fed corn oil (Table 7). There was a non-significant trend towards a level by source interaction ($P<.06$) for IL-1. Increasing corn oil in the diet from 0.5% to 2% tended to increase released IL-1 activity, while increasing fish oil from 0.5% to 2% tended to decrease IL-1 activity.

Antibody titers to infectious bronchitis virus were not altered by dietary treatment in experiment 2 (Table 8). PHA-induced wattle swelling was increased ($P<.038$) when chicks were fed fish oil *vs.* corn oil-fed chicks (Table 8). Peritoneal macrophage IL-1 was decreased ($P<.045$)

in chicks fed fish oil *vs* those fed corn oil (Table 8).

DISCUSSION

Performance of broiler chicks which were not challenged with inflammatory agents was not significantly affected by dietary oil treatment. Others have reported a similar lack of effect of dietary fish oil in unchallenged birds. For example, fish oil supplemented diets result in broiler weight gains which are either not different from diets containing corn oil and linseed oil (Chanmugam *et al.*, 1992), or greater than diets containing lard, corn oil, canola oil, or linseed oil (Fritsche *et al.*, 1991a,b). Similar results have been reported in experiments using mice (Hardardottir and Kinsella, 1992). Although little data is available on the effect of dietary fish oil on body weight gain in mammals, injection of juvenile rats with LPS has been shown to result in significant growth depression (Peisen *et al.*, 1995), as was the case in these experiments.

When the birds were challenged with either LPS or *S. aureus*, inclusion of fish oil in the diet mitigated the decrease in body weight gain and feed conversion efficiency. In experiment 1, chicks consuming (n-3):(n-6) (2% corn oil) diets, a bacterial challenge simulated by injecting LPS resulted in about a 16% decrease in the rate of gain. This was abrogated by feeding fish oil, resulting in only a 10% decrease in the rate of weight gain. In the second experiment, the efficacy of fish oil was examined with two different dietary backgrounds, either cereal or corn. For rate of gain, a significant source by immunogen interaction indicates that the magnitude of growth depression was dependent on the diet. Menhaden oil tended to be more efficacious in ameliorating LPS induced growth depression in the cereal diets than the corn diets, but the cereal diets supported slightly slower growth rates in the absence of a challenge. In environments which are extremely clean or where birds are not exposed to infectious challenges, there may be no

benefit or detriment to feeding fish oil. When the birds are challenged, however, the fish oil diets resulted in greater growth rates than did the corn oil diets. Thus, fish oil may be most effective when the birds are challenged by pathogens. When birds are reared in a dirty environment with the build up of dust, dander and feces, constant stimulation of the inflammatory response by environmental immunogens increases the level of the catabolic cytokine, IL-1, altering the birds' metabolism and redirecting nutrients away from growth and toward an inflammatory response (Roura *et al.*, 1992). In these situations fish oil may minimize the catabolic effect of pathogens or environmental immunogens by decreasing production of pro-inflammatory cytokines and acute phase proteins which would decrease growth rate.

Injection of immunogen in the first experiment caused significant increases in body temperature and the levels of the acute phase proteins hemopexin and metallothionein in all dietary treatments. Hemopexin levels in injected chicks fed fish oil were lower than those in injected chicks fed corn oil. Although not significant, body temperature and MT levels tended to be higher in corn oil-fed birds than in fish oil-fed birds. In the second experiment, a significant source by immunogen interaction for hemopexin and metallothionein indicates that the magnitude of acute phase protein induction was also dependent on the diet. Chicks fed diets containing menhaden oil at 1.5% or 2% had lower acute phase protein levels following LPS or *S. aureus* than those fed corn oil. A similar trend also followed for body temperature. Together the weight gain and acute phase protein data of the second trial support the results of the first experiment in that fish oil (and possibly meal) is immunomodulatory and blunts the detrimental impact of an inflammatory response on gain and feed conversion. Clearly, immunogens alter the metabolism of broiler chickens. The inclusion of fish oil in the diet appears to minimize the decrease in growth

rate associated acute phase response, which is consistent with a reduced release of catabolic cytokines following a challenge with immunogen.

The (n-3) fatty acids from fish oil at 2% of the diet decreased the release of IL-1 by stimulated macrophages especially compared to the (n-6) fatty acids supplied by 2% corn oil. In chicks fed the either the cereal or corn based diets, serum IL-1 levels were significantly depressed by substituting menhaden oil for corn oil at either the 1.5% or 2 % level. Fish meal had a similar effect on IL-1 levels as the oil. IL-1 levels in chicks fed cereal based diets tended to be lower than levels in chicks fed corn based diets.

Interleukin 1 induces fever (Dinarello, 1988), and along with IL-6 and TNF, the synthesis of acute phase proteins such as hemopexin (Klasing, 1984; Baumann and Gauldie, 1994) and metallothionein (Bremner and Beattie, 1990). The levels of acute phase proteins and fever were blunted by feeding fish oil, 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. The mechanism by which (n-3) fatty acids specifically decrease the inflammatory response was not investigated in these experiments. Increasing the (n-3):(n-6) concentration would increase the (n-3) content of membrane phspholipids of both target and effector cells. This in turn may result in decrease pro-inflammatory signals released by effector cells, and decrease responsiveness of target cells to pro-inflammmatory signals.

The inflammatory response is thought to be the major component of the immune response that disrupts growth related physiology resulting in slower growing birds. Mammalian interleukin 1 decreases appetite, decreases skeletal muscle protein accretion, stimulates T cell proliferation and increases metabolic rate, resulting in fever (Dinarello, 1988). Klasing *et al.* (1987) reported

that injections of chicken IL-1 preparations resulted in increased body temperature and decreased body weight gain of White Leghorn chicks. Dietary (n-3) fatty acids have been shown to decrease interleukin-1 and tumor necrosis factor production by cultured human mononuclear cells (Endres *et al.*, 1989).

Increasing dietary (n-3) fatty acids in experiment 1 resulted in increased cell-mediated immunity as determined by the wattle delayed-type hypersensitivity assay. In trial 2, cell mediated immunity was stimulated by the substitution of menhaden oil for corn oil in chicks fed the cereal based diet. Although a similar trend was seen in chicks fed the corn based diet, this was not significant at $p<0.05$. Fish oil diets have been reported to improve, decrease, or not affect indices of specific immunity depending on the index of immune function, the level of fish oil inclusion in the diet, and the level of dietary fat. SRBC antibody responses of chicks fed either 20% corn oil or 17% fish oil and either 300 or 900 mg vitamin E/kg diet were significantly higher than corn oil-fed birds fed the same levels of vitamin E (Fritsche *et al.*, 1992). In non-infected mice, feeding of a high (n-3) PUFA diet (20% fish oil) resulted in the greatest percentage and number of T cells, but in *Listeria*-infected mice, this diet resulted in the lowest percentage of T cells in the peritoneum when compared to mice fed 20% sunflower oil and coconut oils. B cell populations were not affected by dietary fat in non-infected mice, but the fish oil diet resulted in the highest percentage of B cells in infected mice (Huang *et al.*, 1992). Splenocyte natural killer cell activity of mice fed 10% fish oil was decreased 25% compared to that of mice fed a the same level of corn oil, although cell-mediated cytotoxicity of cytotoxic T lymphocytes and peritoneal cells was not affected (Fritsche and Johnston, 1990). Level of inclusion appears to play a role in the effect of fish oil, since this oil was found to be immunosuppressive in the host vs. graft model in mice only

at high levels (10%) of dietary energy (Hinds and Sanders, 1993). As demonstrated in our studies, high levels of supplementation are not needed to show a beneficial effect on broiler performance. Providing a large amount of fish oil may prove to be detrimental to the specific immune system. In humans, inclusion of fish oil at .54% of calories in a low fat diet decreased T cell proliferation in response to Con A and PHA, while inclusion of only .13% of calories as fish oil in a similar diet resulted in an improvement in the same indices. Delayed type hypersensitivity was decreased vs. baseline in the higher fish oil dietary treatment group, but there was no change in the low-fish oil dietary treatment (Meydani *et al.*, 1993).

The modulation in sensitivity to a bacterial challenge as measured by weight gain in this experiment appears to be due to a shift in the immune response away from the inflammatory response and toward humoral and/or cell mediated responses. Indeed, indices of the inflammatory response were decreased in fish oil-fed birds, while indices of specific immunity were either unchanged or increased by fish oil treatment. The implications of such a shift in the resistance of chickens to commercially relevant infectious challenges needs to be investigated. The inflammatory response is the first line of defense to novel pathogens, but cells and mediators of the inflammatory response have been implicated in the pathology of many poultry diseases including coccidiosis (Trout and Lillehoj, 1993) and *S. enteritidis* (Tellez *et al.*, 1994; Kogut *et al.*, 1995).

Even moderate levels of fish oil in the diet appear to ameliorate the negative effect of an immune challenge as compared to diets containing low ratios of (n-3):(n-6) PUFA. Both performance characteristics and indices of immune status can be improved by feeding fish oil. The ratios of (n-3):(n-6) PUFA of the fish oil diets used in experiment 1 were relatively low (e.g. 0.18,

0.28 and 0.47), and yet the beneficial effects were still observed. In experiment 2, the beneficial effect of feeding fish oil was greater in the cereal diets than the corn oil diets, most likely due to the lower amounts of (n-6) PUFA provided by the cereal. Conversely, the corn diets provided more (n-6) PUFA, thereby decreasing the (n-3):(n-6) ratio of these diets.

The results of this experiment give insight into a possible method to decrease losses in performance which might occur due to low-level stimulation of the inflammatory response by environmental immunogens. However, more work is needed to determine if the shift away from an inflammatory response and towards a specific immune response will confer suitable protection from disease in commercial settings.

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TABLE 1. **Dietary composition (g/kg) and calculated (n-3):(n-6) polyunsaturated fatty acid ratio[6]. Experiment 1**

Ingredient	0.5% FO	1% FO	2% FO	0.5% C	1% CO	2% CO
Corn	580	580	580	580	580	580
Soy	350	350	350	350	350	350
Corn Starch	50	40	20	50	40	20
Cellulose	0	5	15	0	5	15
Fish Oil	5	10	20	0	0	0
Corn Oil	0	0	0	5	10	20
(n-3):(n-6) PUFA ratio	.18	.28	.47	.07	.07	.07

[6]Vitamins, mineals and amino acids supplemented to meet NRC (1984) reccomendations for practical broiler diets

TABLE 2. **Dietary composition (g/kg) and calculated (n-3):(n-6) polyunsaturated fatty acid ratio[7]. Experiment 2**

Ingredient	1% FO[8], cereal	1.5% FO, cereal	2% FO, cereal	1.5% FO, corn	2% FO, corn	2% CO[9], corn	2% CO, cereal	2% LO[10], corn	.5% FO, 10% FM[11], corn
Corn	80	80	80	515	515	515	80	515	560
Soy	350	350	350	385	385	385	350	385	225
Wheat	330	330	330	0	0	0	330	0	0
Barley	150	150	150	0	0	0	150	0	0
Fish meal	0	0	0	0	0	0	0	0	100
Corn Starch	40	30	20	10	0	0	20	0	10
Fish oil	10	15	20	20	20	0	0	0	5
Corn oil	0	0	0	0	0	20	20	0	0
Linseed oil	0	0	0	0	0	0	0	20	0
Cellulose	----------all diets adjusted to 100% with cellulose----------								
(n-3):(n-6) PUFA ratio	0.57	0.78	0.98	0.4	0.5	0.07	0.08	0.73	0.33

[7]Vitamins, mineals and amino acids supplemented to meet NRC (1984) reccomendations for practical broiler diets

[8]Fish oil (menhaden)

[9]Corn oil

[10]Linseed oil

[11]Fish meal

TABLE 3. Effect of dietary oil source and immunologic stress on broiler performance. Experiment 1

OIL SOURCE	LEVEL	IMMUNOGEN	Gain g/chick/d	Feed g/chick/d	Efficiency gain/feed
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

TABLE 4. **Effect of dietary oil source and immunologic stress on broiler performance. Experiment 2**

DIET	IMMUNOGEN	Gain g/chick/d	Feed g/chick/d	Efficiency gain/feed
1% Fish oil,	none	26.8	37.2	0.72
cereal	LPS	23.8	35.1	0.68
	S. aureus	24.8	35.4	0.7
1.5% Fish oil,	none	26.5	35.8	0.74
cereal	LPS	24.4	34.9	0.7
	S. aureus	25.5	35.9	0.71
1.5% Fish oil,	none	27.2	36	0.75
corn	LPS	24.7	35.1	0.7
	S. aureus	25.5	37	0.69
2% Fish oil,	none	27.5	35.9	0.77
corn	LPS	25.1	34.9	0.72
	S. aureus	25.9	36	0.72
2% Corn oil,	none	27.7	36.7	0.75
corn	LPS	24	34.8	0.69
	S. aureus	25.5	35.9	0.71
2% Linseed oil,	none	27.4	37.5	0.73
corn	LPS	24.8	36.5	0.68
	S. aureus	25	35.7	0.7
Fish oil/meal,	none	27.9	36.5	0.76
corn	LPS	25.1	34.6	0.73
	S. aureus	25.7	35.2	0.73
2% Fish oil,	none	26.7	36.1	0.74
cereal	LPS	25.2	35.1	0.72
	S. aureus	25.3	36.7	0.69
2% Corn oil,	none	26.6	36.6	0.73
cereal	LPS	23.7	35.4	0.67
	S. aureus	24.8	35.5	0.7
	SEM	0.15	1.4	0.09
	LSD[1]	0.7	0.6	0.03
P VALUES[2]	Diet	0.09	0.36	0.17
	Immunogen	0.001	0.04	0.001
	Diet x Immunogen	0.03	0.09	0.05

[1]Least Significant Difference following one way analysis of variance.
[2]Probability values following 2 way analysis of variance

TABLE 5. **Effect of dietary oil source on indices of immunologic stress in broilers. Experiment 1**

OIL SOURCE	LEVEL	IMMUNOGEN	Body Temp ° C[1]	Hemopexin mg/100 ml	MT[2] 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
	immunogen		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.
[2]Metallothionein

TABLE 6. **Effect of dietary oil source on indices of immunologic stress in broilers. Experiment 2**

DIET	IMMUNOGEN	Body Temp ° C[1]	Hemopexin mg/100 ml	MT[2] µg/g liver
1% Fish oil, cereal	none	40.4	7	12
	LPS	41.8	41	89
	S. aureus	41	39	83
1.5% Fish oil, cereal	none	40.5	11	10
	LPS	41.3	43	69
	S. aureus	41.1	35	76
1.5% Fish oil, corn	none	40.7	8	9
	LPS	41.9	47	79
	S. aureus	41.5	40	89
2% Fish oil, corn	none	40.5	6	11
	LPS	41.4	36	77
	S. aureus	41.4	36	76
2% Corn oil, corn	none	40.6	9	8
	LPS	42	45	87
	S. aureus	41.7	43	89
2% Linseed oil, corn	none	40.6	8	9
	LPS	41.7	39	77
	S. aureus	41.3	38	75
Fish oil/meal, corn	none	40.6	10	15
	LPS	41.5	37	81
	S. aureus	41.4	33	77
2% Fish oil, cereal	none	40.5	7	12
	LPS	40.9	39	77
	S. aureus	41	31	72
2% Corn oil, cereal	none	40.5	7	14
	LPS	41.9	43	85
	S. aureus	41.4	40	81
	SEM			
	LSD[1]	0.04	4	9
P VALUES[3]	Diet	0.11	0.27	0.16
	Immunogen	0.001	0.04	0.001
	Diet x Immun	0.11	0.04	0.06

[1]Least Significant Difference following one way analysis of variance.
[2]Metallothionein
[3]Probability values following 2 way analysis of variance

TABLE 7. **Effect of dietary oil source on indices of specific and inflammatory responses of broiler chicks. Experiment 1**

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
P values	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 8. **Effect of dietary oil source on indices of specific and inflammatory responses of broiler chicks. Experiment 2**

OIL SOURCE	ANTI-IBV[1]	WATTLE INDEX[2]	IL-1[3]
1% Fish oil, cereal	0.64	1.92	2.8
1.5% Fish oil, cereal	0.66	1.95	2.4
1.5% Fish oil, corn	0.65	1.77	2.8
2% Fish oil, corn	0.66	1.63	2.8
2% Corn oil, corn	0.62	1.44	3.2
2% Linseed oil, corn	0.67	1.73	2.8
Fish oil/meal, corn	0.64	1.77	2.6
2% Fish oil, cereal	0.67	2.09	2.5
2% Corn oil, cereal	0.63	1.53	2.9
LSD	0.07	0.37	0.36
Pooled SEM	0.02	0.18	0.22
P values			
diet	0.134	0.038	0.045

[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.