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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Effect of High Peroxide Value Fats on Performance of Broilers in an Immune Challenged State

J. McGill, E. McGill, A. Kamyab and J.D. Firman  
Division of Animal Sciences, University of Missouri-Columbia, Columbia, Missouri 65211, USA

**Abstract:** A floor pen trial was conducted to determine the effect of high peroxide value fats on the performance of broilers in an immune challenged state. Ross 708 broilers were randomly assigned to 48 floor pens with each pen containing 30 birds. Dietary treatments were developed as a 3 x 2 factorial using three levels of fat rancidity, with Peroxide Values (PV) of 0, 75 and 150. One half of each peroxide value diet also received an antioxidant at 125 ppm. Six dietary treatments with eight replicates were fed to broilers from hatch to day 49. Diets were formulated based on standard industry diets with the exception of fat being forced into the diet at 3% for the starter ration (0-3 wks), 6% in the grower ration (3-5 wks) and 6% in the finisher ration (5-7 wks). At 4 weeks of age the broilers underwent a coccidial challenge. The trial measured the performance of the immune challenged broilers based on the parameters of Feed Intake (FI), Body Weight Gain (BWG) and feed conversion (F:G). An initial pen weight was taken on day 0 for each of the 48 pens. Birds were weighed at 3, 5 and 7 weeks of age to calculate F:G. At week 7, four birds per pen (32 birds/treatment) were sacrificed and processed in order to obtain a fat pad weight, carcass weight, percent meat yield and cecal scoring. The results indicated that birds consuming diets with a peroxide value of 75 or greater exhibited poorer feed conversion than the treatment with an acceptable peroxide value. Furthermore, diets with the added antioxidant demonstrated no statistical difference in feed conversion due to peroxide value. There were also no significant effects of the immune challenge in combination with peroxide levels on bird performance.

**Key words:** Peroxide value, fat, broiler, immunity

### INTRODUCTION

The benefits of added fat to poultry rations are well documented (Firman, 1995; Sell *et al.*, 1986; Pesti *et al.*, 2002). Significant cost savings may be achieved with fat addition (Firman, 2006). These cost savings may be especially relevant in international markets where there is a trend towards underutilization of animal fat and animal meals containing fat compared to usage of traditional, more expensive ingredients such as soybean meal and vegetable oil due to the perception that rendered fats may be of poor quality due to oxidative rancidity (McGill *et al.*, 2011).

One of the concerns with using oxidized fats is the potential for negative health effects. To date, there is very little research investigating the effects of autoxidized fats on the immune function of broilers. Despite this lack of research, it is known that the free-radical mechanism of autoxidation leads to the formation of several products that are known to be toxic (Sanders, 1994) and may compromise immune function and cell wall integrity (Sevanian and Peterson, 1986). It is apparent that additional investigation is needed in this area. The objective of this study was to look at how PV affected the growth of broilers based on Feed Intake (FI), Weight Gain (WG) and feed conversion (F:G), with and without an immune challenge.

### MATERIALS AND METHODS

**Animals and diets:** 1440 day-old, straight-run, Ross 708 broilers were obtained from a commercial hatchery and randomly assigned to one of six dietary treatments with eight replicates per treatment. Birds were housed in floor pens (30 birds/pen) in an environmentally controlled curtain-sided house with thermostatically controlled gas heat. The birds were exposed to 24 h of fluorescent lighting. Temperature and mortality were recorded daily. Access to experimental diets and water was provided *ad libitum* for the duration of the trial and each pen contained a hanging feeder and nipple drinkers. The birds were cared for using standard husbandry guidelines derived from standard operating procedures.

Six dietary treatments were replicated eight times for a total of 48 pens. Birds were fed diets formulated to resemble standard industry diets that met all of the NRC requirements (NRC, 1994) from hatch to 49 days of age. A 3 x 2 factorial was the model used for this trial with three levels of fat rancidity: Peroxide Value (PV) of 0, 75 and 150. Fat preparation (rancidity level) was done on site according to methods previously discussed (McGill *et al.*, 2011), as was measurement of peroxide value prior to feed mixing. Peroxide value was tested using the American Oil Chemist Society official method (AOAC,

Table 1: Composition of experimental basal diets for fat rancidity trial with the addition of an immune challenge

Treatment	Starter 0-3 weeks	Grower 3-5 weeks	Finisher 5-7 weeks
Corn	60.147	57.731	60.652
Soybean meal	29.45	30.95	26.958
Porkmeal	4.707	0	0
Animal/Vegetable blend <sup>1</sup>	3.0	6.0	6.0
Limestone	0.839	1.5	1.5
Dicalcium Phosphate	0.804	1.274	4.026
Salt	0.30	0.30	0.30
DL Methionine	0.214	0.084	0.072
Sodium Bicarbonate	0.2	0.2	0.2
Vitamin Premix <sup>2</sup>	0.075	0.075	0.075
Lysine HCl	0.053	0	0
Calcium Trace Mineral <sup>3</sup>	0.05	0.05	0.1
Choline Chloride	0.044	0.019	0
Selenium Premix <sup>3</sup>	0.03	0.03	0.03
Copper Sulfate	0.013	0.013	0.013
Potassium Chloride	0	1.699	0
Ethoxyquin, ppm <sup>1</sup>	0	0	0
<b>Calculated to contain</b>			
Crude protein, %	22	20	18.3
ME, kcal/kg	3075	3150	3150
Calcium, %	1.0	0.9	0.8
Available Phosphorus, %	0.45	0.35	0.3

<sup>1</sup>Animal/Vegetable blend was different in peroxide value (0, 75, or 150). The Ethoxyquin was either added (+) at 125 ppm or withheld (-) depending on treatment. All treatments were challenged with a coccidial challenge (C). The combination of these two factors set up a 2x3 factorial to produce 6 treatments; PV0-C, PV75-C, PV150-C, PV0+C, PV75+C and PV150+C (Table 1).

<sup>2</sup>Vitamin premix provided the following amounts per kilogram of diet: vitamin D3, 200 IU; vitamin A, 1,500 IU; vitamin E, 101 IU; niacin, 35 mg; D-Pantothenic acid, 14 mg; riboflavin, 4.5 mg; pyridoxine, 3.5 mg; menadione, 2 mg; folic acid, 0.55 mg; thiamine, 1.8 mg.

<sup>3</sup>Mineral premix provided the following amounts per pound of premix per ton of feed: Mn, 11.0%; Zn, 11.0%; Fe, 6.0%; I, 2,000 ppm; Mg, 2.68%; Se, 600 ppm

1993). Each peroxide value treatment (0, 75 and 150) was then divided so that one of the treatments for each level of rancidity contained the addition of an antioxidant (+A) at 125 ppm (Ethoxyquin, Novus Int., St. Louis, MO), while the remaining treatment for each level of rancidity had the antioxidant withheld (-A). Fat was set to a level of 3% within the starter diet (0-3 weeks) and 6% within the grower (3-5 weeks) and finisher diet (5-7 weeks) (Table 1). Diets were formulated using least-cost formulation software. Experimental diets were in mash form from 0-3 weeks of age and in pellet form thereafter.

**Measurements:** Birds and feed were weighed on a pen basis on days 0, 21, 35 and 49 to determine weight gain, feed intake and feed conversion and mortality was recorded daily. Feed:Gain was adjusted for mortality; weight of bird (mortality) was added to the pen weight gain, then feed consumed was divided by pen weight gain. An immune challenge was presented to the birds

by way of coccidiosis on day 28. The coccidial challenge was administered to the birds by using a live vaccination of Cocci-vac at four times the treatment dosage. On day 49, four birds from each pen (two males and two females) were wing-banded, individually weighed and removed from feed. On day 50 the 192 individually weighed birds were processed to determine the cecal score, hot carcass weight, weight of the fat pad, chilled carcass weight, weight of the major cuts including leg, thigh, wing, pectoralis major and pectoralis minor and percent yield.

**Statistical analysis:** Analysis of data was performed using pen as the experimental unit. The JMP<sup>®</sup> statistical analysis software package (SAS Institute; Cary, NC) was used to perform Analysis of Variance (ANOVA) with a factorial design using the general linear model. The level of significance was established at  $p < 0.05$ . Mean comparisons for all pairs were conducted using the Least Significant Difference test.

## RESULTS AND DISCUSSION

Body weight gain, feed intake, feed conversion and processing yields were measured to determine if different levels of fat rancidity, with or without the addition of an antioxidant, exerted an effect on the performance of broilers in an immune challenged state.

Results for Weight Gain (BWG) are demonstrated in Table 2. In the trial there were no differences ( $p > 0.05$ ) in BWG among the treatments during the 0-21 day, 21-35 day and 35-49 day periods. There were also no significant differences overall for the 0-49 day period.

Feed Intake (FI) data are listed in Table 3. When looking at the FI among treatments there were no significant differences ( $p > 0.05$ ) among the treatments for 0-21 days and 35-49 days periods. There was also no significant difference for the 0-49 day period among the treatments. There was a significant difference ( $p < 0.05$ ) among the treatments for the 21-35 day period. The two high rancidity levels, PV150-A and PV150+A and the control group, PV0-A, did not differ from the other three treatments, PV75-A, PV0+A and PV75+A. The PV75-A and PV0+A treatments did not differ from each other, but both were significantly different from the PV75+A treatment (PV75-A = 1.89 kg and PV0+A = 1.89 kg versus PV75+A = 1.82 kg).

The data for feed conversion (F:G) are provided in Table 4. The F:G for the 0-21 day period demonstrates that there was no significant difference ( $p > 0.05$ ) found among treatments. During the 21-35 day period the high rancidity diet without the antioxidant, PV150-A, was significantly different from the low rancidity diet containing the antioxidant, PV0+A. The four other diets (PV0-A, PV75-A, PV75+A and PV150+A) were not significantly different from each other within the 21-35 day period. For the period of 35-49 day there was no

Table 2: Effects of fat rancidity level when an antioxidant was added or excluded on body weight gain on days 21, 35, 49 and 0-49 in the presence of an immune challenge

PV <sup>1</sup>	A <sup>2</sup>	0-21 days (kg)	21-35 days (kg)	35-49 days (kg)	0-49 days (kg)
0	-	0.71 <sup>a</sup>	1.10 <sup>a</sup>	0.97 <sup>a</sup>	2.86 <sup>a</sup>
0	+	0.75 <sup>a</sup>	1.07 <sup>a</sup>	0.95 <sup>a</sup>	2.84 <sup>a</sup>
75	-	0.73 <sup>a</sup>	1.07 <sup>a</sup>	0.97 <sup>a</sup>	2.84 <sup>a</sup>
75	+	0.72 <sup>a</sup>	1.05 <sup>a</sup>	0.99 <sup>a</sup>	2.79 <sup>a</sup>
150	-	0.73 <sup>a</sup>	1.14 <sup>a</sup>	0.97 <sup>a</sup>	2.81 <sup>a</sup>
150	+	0.74 <sup>a</sup>	1.08 <sup>a</sup>	1.01 <sup>a</sup>	2.84 <sup>a</sup>
Pooled SEM		0.008	0.029	0.041	0.032
<b>Source of variation</b>		p-value			
PV		0.4885	0.2482	0.8276	0.6903
A		0.0927	0.0811	0.6630	0.6628
PV x A		0.0795	0.7375	0.7199	0.4927
<b>Main effect mean</b>					
<b>PV</b>	0	0.73	1.09	0.96	2.84
	75	0.72	1.06	0.98	2.82
	150	0.73	1.11	0.99	2.82
<b>A</b>	-	0.72	1.11	0.97	2.83
	+	0.73	1.06	0.98	2.82

<sup>1</sup>Peroxide Value (PV).

<sup>2</sup>Antioxidant (A) was either added (+) at 125 ppm or withheld (-).

<sup>a</sup>Values within a column with no common superscript are significantly different (p<0.05)

Table 3: Effects of fat rancidity level when an antioxidant was added or excluded on feed intake on days 21, 35, 49 and 0-49 in the presence of an immune challenge

PV <sup>1</sup>	A <sup>2</sup>	0-21 days (kg)	21-35 days (kg)	35-49 days (kg)	0-49 days (kg)
0	-	0.91 <sup>a</sup>	1.87 <sup>ab</sup>	2.48 <sup>a</sup>	5.36 <sup>a</sup>
0	+	0.98 <sup>a</sup>	1.89 <sup>a</sup>	2.48 <sup>a</sup>	5.45 <sup>a</sup>
75	-	0.98 <sup>a</sup>	1.89 <sup>a</sup>	2.52 <sup>a</sup>	5.49 <sup>a</sup>
75	+	0.95 <sup>a</sup>	1.82 <sup>b</sup>	2.49 <sup>a</sup>	5.32 <sup>a</sup>
150	-	0.96 <sup>a</sup>	1.87 <sup>ab</sup>	2.52 <sup>a</sup>	5.42 <sup>a</sup>
150	+	0.99 <sup>a</sup>	1.86 <sup>ab</sup>	2.43 <sup>a</sup>	5.30 <sup>a</sup>
Pooled SEM		0.021	0.016	0.038	0.079
<b>Source of variation</b>		p-value			
PV		0.4153	0.2772	0.6766	0.8142
A		0.2593	0.1284	0.1624	0.2965
PV x A		0.0860	0.0129	0.5038	0.2271
<b>Main effect mean</b>					
<b>PV</b>	0	0.95	1.88	2.48	5.40
	75	0.96	1.86	2.50	5.40
	150	0.97	1.87	2.47	5.36
<b>A</b>	-	0.95	1.88	2.50	5.42
	+	0.97	1.86	2.46	5.35

<sup>1</sup>Peroxide Value (PV).

<sup>2</sup>Antioxidant (A) was either added (+) at 125 ppm or withheld (-).

<sup>ab</sup>Values within a column with no common superscript are significantly different (p<0.05)

difference among the PV75-A, PV0+A and PV150+A treatments. The treatments PV0-A and PV75+A were significantly different from the PV150-A treatment. For the 0-49 day period the low rancidity level without the antioxidant, PV0-A, was statistically different than the PV75-A, PV150-A and PV0+A treatments. The treatments with increased rancidity and the addition of an antioxidant, PV75+A and PV150+A, were statistically similar to the four other treatments.

Mortality occurred randomly throughout treatments at a consistently low level. There were no significant differences among treatments even when exposed to an immune challenge.

Processing attributes are summarized in Table 5. All of the processing data were calculated as a percentage of chilled carcass weight. There were no significant differences (p>0.05) among treatments when comparing percent yield, breast, major, minor, fat pad, leg, thigh and wing.

Signs of coccidiosis such as bloody droppings and ruffled feathers were seen in the birds near the end of the trial, indicating a successful impairment of the immune system via coccidial challenge. During cecal examination, ceca were visually scored using criteria presented by Conway and McKenzie (1991) in which a scale from 1-4 is utilized based on occurrence and

Table 4: Effects of fat rancidity level when an antioxidant was added or excluded on feed conversion on days 21, 35, 49 and 0-49 in the presence of an immune challenge

PV <sup>1</sup>	A <sup>2</sup>	0-21 days (kg:kg)	21-35 days (kg:kg)	35-49 days (kg:kg)	0-49 days (kg:kg)
0	-	1.27 <sup>a</sup>	1.68 <sup>ab</sup>	2.37 <sup>b</sup>	1.82 <sup>b</sup>
0	+	1.31 <sup>a</sup>	1.77 <sup>a</sup>	2.41 <sup>ab</sup>	1.87 <sup>a</sup>
75	-	1.34 <sup>a</sup>	1.75 <sup>ab</sup>	2.41 <sup>ab</sup>	1.87 <sup>a</sup>
75	+	1.32 <sup>a</sup>	1.73 <sup>ab</sup>	2.38 <sup>b</sup>	1.86 <sup>ab</sup>
150	-	1.32 <sup>a</sup>	1.67 <sup>b</sup>	2.55 <sup>a</sup>	1.89 <sup>a</sup>
150	+	1.33 <sup>a</sup>	1.72 <sup>ab</sup>	2.41 <sup>ab</sup>	1.85 <sup>ab</sup>
Pooled SEM		0.020	0.032	0.046	0.012
<b>Source of variation</b>		p-value			
PV		0.1423	0.3733	0.1095	0.0322
A		0.4821	0.1116	0.2891	0.9319
PV x A		0.2569	0.0294	0.0419	0.0018
<b>Main effect mean</b>					
<b>PV</b>	0	1.29	1.73	2.39	1.85 <sup>b</sup>
	75	1.33	1.74	2.39	1.87 <sup>a</sup>
	150	1.32	1.69	2.47	1.88 <sup>a</sup>
<b>A</b>	-	1.31	1.70	2.44	1.86
	+	1.32	1.74	2.40	1.86

<sup>1</sup>Peroxide Value (PV).

<sup>2</sup>Antioxidant (A) was either added (+) at 125 ppm or withheld (-).

<sup>ab</sup>Values within a column with no common superscript are significantly different (p<0.05)

Table 5: Effects of fat rancidity level when an antioxidant was added (+) or excluded (-) on 0-49 day broiler carcass traits based on the percentage of chilled carcass weight in the presence of an immune challenge

Treatment	Yield	Breast	Major	Minor	Fat pad	Leg	Thigh	Wing
PV0-	75.59 <sup>a</sup>	15.07 <sup>a</sup>	12.22 <sup>a</sup>	2.84 <sup>a</sup>	2.42 <sup>a</sup>	6.87 <sup>a</sup>	8.14 <sup>a</sup>	5.58 <sup>a</sup>
PV75-	76.24 <sup>a</sup>	15.29 <sup>a</sup>	12.29 <sup>a</sup>	2.99 <sup>a</sup>	2.63 <sup>a</sup>	6.82 <sup>a</sup>	8.27 <sup>a</sup>	5.71 <sup>a</sup>
PV150-	74.22 <sup>a</sup>	15.08 <sup>a</sup>	12.16 <sup>a</sup>	2.92 <sup>a</sup>	2.18 <sup>a</sup>	7.03 <sup>a</sup>	8.07 <sup>a</sup>	5.65 <sup>a</sup>
PV0+	75.05 <sup>a</sup>	15.09 <sup>a</sup>	12.09 <sup>a</sup>	2.99 <sup>a</sup>	2.48 <sup>a</sup>	6.92 <sup>a</sup>	8.34 <sup>a</sup>	5.51 <sup>a</sup>
PV75+	75.48 <sup>a</sup>	15.32 <sup>a</sup>	12.26 <sup>a</sup>	3.06 <sup>a</sup>	2.65 <sup>a</sup>	6.97 <sup>a</sup>	8.20 <sup>a</sup>	5.54 <sup>a</sup>
PV150+	76.09 <sup>a</sup>	14.84 <sup>a</sup>	11.84 <sup>a</sup>	2.99 <sup>a</sup>	2.61 <sup>a</sup>	6.82 <sup>a</sup>	8.27 <sup>a</sup>	5.74 <sup>a</sup>
Pooled SEM	0.641	0.232	0.182	0.09	0.129	0.111	0.129	0.119

<sup>a</sup>Values with differing letters are significantly (p<0.05) different

severity of ulcers, lesions, hemorrhage and lining integrity, with a score of 1 denoting little or no presence of clear indicators of coccidiosis and 4 denoting severe indication of coccidiosis. Occasional, random occurrences of mild lesions in the cecal lining were observed across treatments, but none so severe as to receive a score above 1. Scores of 1 were assigned across all treatment groups.

The current trial revealed no negative effects on weight gain caused by either immune challenge or elevated peroxide values. Feed intake was only depressed during the 21-35 day period with the birds in the PV75+A treatment consuming the least amount of feed (1.82 kg). No significant differences (p>0.05) were seen for the overall 0-49 day period. Feed conversion varied somewhat across periods of growth, immune status and peroxide values, although F:G for the 0-49 day period was significantly improved (p<0.05) in the non-antioxidant treatments for birds consuming PV0 fat (PV0-A = 1.82 versus 1.87 and 1.89 for PV75-A and PV150-A, respectively), indicating that PV did have an overall negative effect on feed conversion and that antioxidant addition corrected that negative effect. No differences were seen in processing data (p>0.05).

Cecal examination did not reveal severe signs of coccidiosis even though mild signs of the challenge were observed in the live birds, so it is difficult from these data to draw conclusions on the effects of fat rancidity on birds with an immune challenge. It does not appear that diets containing oxidized fat worsened immune function in birds challenged with coccidiosis. However, a study in which oxidized fat (4 meq kg<sup>-1</sup> diet) without the addition of an antioxidant was fed to broilers found that oxidant stress resulted in increased cell turnover in the gastrointestinal epithelium, increased hepatic proliferation, reduced concentrations of immunoglobulin in intestinal tissue and increased numbers of *E. coli* and decreased *Lactobacilli* populations in the small intestine (Dibner *et al.*, 1996), suggesting that oxidized fat may affect the gut associated immune system. It is known that the free-radical mechanism of autoxidation leads to the formation of several products that are known to be toxic (Sanders, 1994) and may compromise immune function and cell wall integrity (Sevanian and Peterson, 1986). These include lipid free radicals, peroxy radicals and hydroperoxides that are very unstable and break down to a number of secondary products such as aldehydes and

alcohols, which contribute to the unpleasant flavors associated with rancid fats (Hamilton, 1994), or other polymers which are unavailable and therefore lower the energy content of the fat (Shermer and Giesen, 1997) and are capable of affecting the absorption of, or even destroying, fat-soluble vitamins (Sanders, 1994). A study conducted by Enberg and coworkers (1996) in which broiler hens were fed diets with 11% oxidized vegetable oil (156 meq O<sub>2</sub>/kg oil) and evaluated for nutrient balance and antioxidant status found decreased levels of liver vitamins A and E and that plasma concentrations of Thiobarbituric Acid-Reactive Substances (TBARS) were significantly higher than those in birds fed control rations. TBARS may be used as an indication of the presence of oxidation products and vitamins A and E act as part of the body's defense mechanism against lipid peroxidation by stabilizing free radicals while being consumed in the process (Tavarez *et al.*, 2011). The general lack of research in this area makes it apparent that additional investigation is needed.

The benefits of added fat in poultry diets are well established and the use of rendered fats in the United States is a common practice proven to be safe and cost effective. In many other countries, there is a significant potential market for rendered fats and fat-containing animal by-products, especially as world population increases and demand rises for poultry meat and eggs (Economic Research Service/USDA website, 2001). However, fear of decreased quality due to oxidative rancidity and the subsequent effects on performance and immunity may prevent utilization of these fat sources.

Currently, very little research has been conducted on the effect of feeding oxidized fats on immunity and bird health. While it seems that excessive peroxide values of individual fats (greater than 100 meq/kg) may cause performance problems, little evidence exists that fats with lower PVs should be of concern (McGill *et al.*, 2011). However, concern remains over the issue of oxidative rancidity, the toxic secondary products of oxidative decomposition and the potential for compromised immune function that might result. These results indicate that the inclusion of high peroxide value fats in broiler diets can cause a depression in overall live performance parameters, especially feed conversion, but that the addition of an antioxidant can improve performance. Birds also seemed to overcome the immune challenge of the coccidiostat administered at a higher than recommended dose. Continued research is imperative in order to define an acceptable level of rancidity and to determine if high levels of peroxide values affect immune function.

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