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Effect of High Peroxide Value Fats on Performance of Broilers in a Normal Immune State

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Abstract: A floor pen trial was conducted to determine the effect of high peroxide value fats on performance of broilers in a normal immune state. Ross 708 Broilers were randomly assigned to 48 floor pens with each pen contained 30 birds. Dietary treatments were developed in a 3 x 2 factorial using three levels of fat rancidity, Peroxide Value (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). The trial measured the performance of the broilers based on the parameters of Feed Intake (FI), Weight Gain (WG) and feed conversion (F:G). An initial pen weight was taken on day 0 for each of the 48 pens and birds were weighed at 3, 5 and 7 weeks of age to calculate FE. At week 7, four birds per pen (32 birds/treatment) were sacrificed and processed in order to obtain a fat pad weight, carcass weight and percent meat yield. The results indicated that diets with a peroxide value of 75 or greater result in poorer feed conversion than the treatment with a peroxide value of 0. Furthermore, the addition of an antioxidant to the diets with a peroxide value of 75 or greater yielded a numerically improved feed conversion over the diets with the same peroxide value but no antioxidant.

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

INTRODUCTION

The use of fats and fat-containing animal by-products is well established in the United States. Fat addition to poultry rations provides a concentrated energy source that is capable of increasing growth rates, decreasing feed intake and increasing feed efficiency (Firman, 1995; Sell *et al*., 1986; Pesti *et al*., 2002). It has been estimated that use of rendered fat products may save up to $10 per ton of feed produced (Firman, 2006). Potential cost savings may be even greater in international markets in which poultry production of low- and middle-income countries continues to rise (Economic Research Service, USDA website, 2001). However, internationally there is a trend toward an underutilization of fats and animal meals containing fat compared to more traditional and more expensive ingredients like soybean meal and vegetable oil. One of the biggest problems with marketing and selling products such as tallow is the perception that rendered fats and fat-containing meals, are of poor quality due to oxidative rancidity.

Relatively little research has been done relating Peroxide Value (PV) of fats to broiler performance. The objective of this study was to look at how PV affected the performance of broilers in a normal immune state grown to market age based on Feed Intake (FI), Weight Gain (WG) and feed conversion (F:G).

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 (eight replications per treatment). Birds were housed in floor pens, (30 birds/pen) in an environmentally controlled, curtain-sided house with thermostatically controlled gas heat and 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 nipple drinkers and a hanging feeder. 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, as was measurement of peroxide value prior to feed mixing.

Fat preparation:

- A 55-gallon metal barrel/drum was filled approximately half full of animal/vegetable blended fat.

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Table 1: Composition of experimental basal diets for fat rancidity trial

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[1]	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
Coban	0.075	0.075	0.075
Vitamin premix[2]	0.075	0.075	0.075
Lysine HCl	0.053	0	0
Calcium trace mineral[3]	0.05	0.05	0.1
Choline chloride	0.044	0.019	0
Selenium premix[3]	0.03	0.03	0.03
Copper sulfate	0.013	0.013	0.013
Potassium chloride	0	1.699	0
Ethoxyquin (ppm)[1]	0	0	0
Calculated to contain			
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

[1]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. The combination of these two factors set up a 2 x 3 factorial to produce 6 treatments; PV0-, PV75-, PV150-, PV0+, PV75+ and PV150+.

[2]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.

[3]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

- The other half of the barrel was filled to approximately 80% full with soy oil so that the space required to aerate the fat was available.
- A barrel heat band was placed on the barrel approximately three inches from the bottom and set to warm the fat to approximately 135-140°F.
- Four ¼ inch aquarium air lines were connected by using three ¼ inch air line “t” type connectors. Air lines were attached to an air compressor and lines were weighted with metal washers before they were inserted into the barrel.
- The air compressor was turned on and adjusted to 3-5 psi.
- Peroxide value was tested twice daily until the value neared the desired level and then every 2-3 h until the desired level was achieved.

Peroxide value was tested using the American Oil Chemist Society (AOCS) official method (AOAC, 1993). Each peroxide value treatment (0, 75 and 150) was then divided into two, with one receiving an antioxidant at 125 ppm (Ethoxyquin, Novus Intl., St. Louis, MO). 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 body 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. 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 hot carcass weight, weight of the fat pad, chilled carcass weight, weights 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® 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

In this study, body weight gain, feed intake, feed conversion (F:G) and processing yields were measured in order to determine if different levels of fat rancidity, with and without the addition of an antioxidant, exerted an effect on broiler performance. Mixed results were observed.

Results for Weight Gain (BWG) are presented in Table 2. Significant differences ($p<0.05$) in BWG occurred only in the 21-35 day period. There were no differences ($p>0.05$) in BWG among the treatments during the 0-21 day and 35-49 day periods. The 0-49 day period also had no significant difference among the treatments for body weight gain. Within the 21-35 day period there was a significant difference ($p<0.05$) between the two treatments with the low peroxide values, PV0- and PV0+ and the treatments with higher peroxide values, PV75-, PV150- and PV75+. The treatment PV150+ was not significantly different when compared to all other treatments within the 21-35 day period ($p<0.05$). PV seemed to be the main effect, with the PVO treatments resulting in significantly improved performance except in the case of the PV150+ treatment. It would appear that

Table 2: Effects of fat rancidity level when an antioxidant was added or excluded on body weight gain on day 21, 35, 49 and 0-49

PV[1]	A[2]	0-21 days	21-35 days	35-49 days	0-49 days
		(kg)			
0	-	0.63[a]	1.19[a]	1.27[a]	3.14[a]
0	+	0.62[a]	1.19[a]	1.28[a]	3.14[a]
75	-	0.61[a]	1.13[b]	1.26[a]	3.03[a]
75	+	0.61[a]	1.13[b]	1.29[a]	3.05[a]
150	-	0.61[a]	1.13[b]	1.25[a]	3.06[a]
150	+	0.62[a]	1.16[ab]	1.28[a]	3.08[a]
Pooled SEM		0.007	0.009	0.022	0.028
Source of variation		p-value			
PV		0.1808	<0.0001	0.8613	0.0030
A		0.5249	0.3194	0.2028	0.4210
PV x A		0.8212	0.2196	0.8288	0.9619
Main effect mean					
PV	0	0.62	1.19[a]	1.28	3.14[a]
	75	0.61	1.13[b]	1.27	3.04[b]
	150	0.62	1.15[b]	1.27	3.07[b]
A	-	0.62	1.15	1.26	3.07
	+	0.62	1.16	1.28	3.09

[1]Peroxide Value (PV).
[2]Antioxidant (A) was either added (+) at 125 ppm or withheld (-).
[ab]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 day 21, 35, 49 and 0-49

PV[1]	A[2]	0-21 days	21-35 days	35-49 days	0-49 days
		(kg)			
0	-	1.02[a]	1.99[a]	2.68[a]	5.78[a]
0	+	1.02[a]	1.96[a]	2.73[a]	5.78[a]
75	-	1.02[a]	2.00[a]	2.69[a]	5.72[a]
75	+	1.01[a]	1.95[a]	2.68[a]	5.68[a]
150	-	1.02[a]	1.96[a]	2.68[a]	5.77[a]
150	+	1.04[a]	1.98[a]	2.67[a]	5.71[a]
Pooled SEM		0.009	0.021	0.032	0.068
Source of variation		p-value			
PV		0.2769	0.8069	0.6196	0.7513
A		0.9562	0.1404	0.8359	0.3707
PV x A		0.3991	0.1700	0.5439	0.7214
Main effect mean					
PV	0	1.02	1.98	2.71	5.78
	75	1.01	1.97	2.69	5.73
	150	1.03	1.97	2.68	5.74
A	-	1.02	1.98	2.69	5.77
	+	1.02	1.96	2.69	5.73

[1]Peroxide Value (PV).
[2]Antioxidant (A) was either added (+) at 125 ppm or withheld (-).
[a]Values within a column with no common superscript are significantly different (p<0.05)

the ethoxyquin supplementation may have exerted a positive effect, except that it is unclear why the PV75+ treatment did not also result in improved body weight gain over the other diets containing elevated levels of PV with no ethoxyquin.

Feed Intake (FI) data are summarized in Table 3. When looking at the FI among treatments there was not a significant difference (p>0.05) among the treatments for any of the three time frames, 0-21 days, 21-35 days, or 35-49 days. There also was no significant difference when the treatments were compared for the total feed intake, from 0-49 days.

The data for feed conversion (F:G) are presented on Table 4. The F:G for the 0-21 day period demonstrates that there was a significant difference (p<0.05) among treatments. The two treatments with the low peroxide values, PV0- and PV0+, had a significantly improved F:G when compared to the treatments with higher peroxide values, PV75-, PV150- and PV150+. The treatment PV75+ was not significantly different when compared to all other treatments, again indicating that the antioxidant may have had a beneficial effect at lower PV levels during the starter period. During the 21-35 day period the high rancidity diets PV150- and PV150+ were not significantly different from any of the other treatments. The PV0- diet resulted in significantly improved feed conversion over the diets containing the middle PV level of fat, PV75- and PV75+. There was also a significant

Table 4: Effects of fat rancidity level when an antioxidant was added or excluded on feed conversions on day 21, 35, 49 and 0-49

PV[1]	A[2]	0-21 days	21-35 days	35-49 days	0-49 days
		(kg:kg)			
0	-	1.62[b]	1.64[c]	2.05[a]	1.80[c]
0	+	1.62[b]	1.65[bc]	2.05[a]	1.81[bc]
75	-	1.67[a]	1.76[a]	2.07[a]	1.85[a]
75	+	1.64[ab]	1.72[ab]	2.05[a]	1.84[abc]
150	-	1.66[a]	1.70[abc]	2.05[a]	1.84[abc]
150	+	1.66[a]	1.69[abc]	2.05[a]	1.84[abc]
Pooled SEM		0.013	0.018	0.018	0.009
Source of variation		p-value			
PV		0.0091	<0.0001	0.8561	0.0008
A		0.4077	0.4104	0.5497	0.4871
PV x A		0.3230	0.5381	0.7110	0.2893
Main effect mean					
PV	0	1.62[b]	1.64[c]	2.05	1.81[b]
	75	1.65[a]	1.74[a]	2.06	1.85[a]
	150	1.66[a]	1.69[b]	2.05	1.84[a]
A	-	1.65	1.15	2.06	1.84
	+	1.64	1.16	2.05	1.83

[1]Peroxide Value (PV).

[2]Antioxidant (A) was either added (+) at 125 ppm or withheld (-).

[abc]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 bases on the percentage of chilled carcass weight

Treatment	Yield	Breast	Major	Minor	Fat pad	Leg	Thigh	Wing
	(%)							
PV0-	73.26[a]	15.46[a]	12.69[a]	2.76[a]	2.46[a]	6.12[a]	8.12[a]	5.34[a]
PV75-	72.17[a]	15.78[a]	12.92[a]	2.86[a]	2.83[a]	6.21[a]	8.14[a]	5.29[a]
PV150-	72.79[a]	15.73[a]	12.87[a]	2.86[a]	2.74[a]	6.12[a]	8.12[a]	5.12[a]
PV0+	72.68[a]	15.72[a]	12.93[a]	2.79[a]	2.73[a]	6.11[a]	8.26[a]	5.16[a]
PV75+	72.82[a]	15.61[a]	12.84[a]	2.77[a]	2.62[a]	6.25[a]	7.75[a]	5.30[a]
PV150+	72.31[a]	15.62[a]	12.80[a]	2.81[a]	2.57[a]	6.27[a]	7.99[a]	5.36[a]
Pooled SEM	0.321	0.179	0.158	0.059	0.14	0.089	0.143	0.065

[a]Values with differing letters are significantly ($p<0.05$) different

difference between the PV75- and the PV0+ treatments, with the PV0+ treatment resulting in improved F:G (1.65 versus 1.76 for the PV75-).

For the period of 35-49 day there was no difference among the treatments ($p>0.05$). However, the 0-49 day results were similar to those for the 21-35 day period with the high rancidity diets PV150- and PV150+ showing no significant differences ($p>0.05$) from any of the other treatments. The PV0- diet was significantly different ($p<0.05$) from the diets containing the middle PV level of fat, PV75- and PV75+. There was also a significant difference between the PV75- and the PV0+ treatments.

Mortality occurred randomly throughout treatments at a consistently low level. Therefore, statistical analysis was not run on the mortality data.

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

It is unclear why variable results in body weight gain and feed conversion occurred, especially within the 21-35 day period and it is difficult to conclude that either peroxide value or the addition of an antioxidant caused conclusive effects in this period. Research conducted by Cabel and coworkers (1988), in which a 4 x 3 factorial arrangement of diets containing 0, 50, 100, or 175 meq/kg peroxide and either 0, 63, or 125 ppm ethoxyquin was utilized, displayed results that were somewhat similar. Birds consuming treatments that contained fat with PVs of 100 or 175 had decreased weight gain at 21 and 42 days. At 49 days, only feed containing fat with a PV of 175 meq/kg resulted in significantly decreased body weights and the addition of an antioxidant to diets containing fat with a PV of 175 relieved the depression in gain. Feed efficiency in the same trial mirrored the body weight gain data, with a significant decrease in body weight occurring only at the highest peroxide level and the addition of ethoxyquin failing to correct the depression. Another trial conducted by Enberg and others (1996) utilized diets containing either fresh fat with a PV of 1 meq/kg or oxidized fat (PV = 156 meq/kg) fed to broilers from 0-35 days of age. Body weight gain was significantly decreased in birds that consumed the high PV treatment. The authors of these two trials came to the conclusion that fat containing elevated peroxide levels can result in decreased performance of broilers and Cabel and coworkers (1988) also concluded that the addition of ethoxyquin alleviated these negative effects. These conclusions have been somewhat inconsistently demonstrated in the literature. Similar finding have been found (Waldroup *et al.*, 1960; Inoue *et al.*, 1984; Shermer and Calabotta, 1985), although the level of rancidity needed to cause deleterious effects has not been agreed upon. Conversely, earlier research performed by other groups has been unable to report differences in performance of turkeys or broilers fed fat that was oxidized (Lea *et al.*, 1966; Carpenter and L'Estrange, 1966; L'Estrange *et al.*, 1966; Oertel and Hartfiel, 1982). However, in each of these experiments the authors included an antioxidant in the diets in an effort to keep a steady peroxide level and it is possible that the harmful effect of the peroxide level was negated by the antioxidant.

The results of the current study indicate that an elevated peroxide level may cause a depression in body weight gain, especially when an antioxidant is not utilized, in later phases of growth but that when looking at the overall growth period (0-49 days) no significant differences were seen. Only in the 21-35 day period was a difference in growth observed between the PV0 treatments and the PV75-, PV150- and PV75+ treatments, with a 5.04% decrease in growth occurring. Peroxide level, antioxidant inclusion, or period of growth did not seem to affect feed intake. Feed conversion varied across growth periods, peroxide values and antioxidant addition; however, during the overall 0-49 day growth period, F:G was significantly improved ($p<0.05$) in the non-antioxidant treatments for birds consuming PV0 fat over those consuming PV75- treatments (1.80 versus 1.85, respectively) and the improvement in F:G of the PV0- groups over the PV150- (1.80 versus 1.84, respectively) was approaching statistical significance, indicating that PV did have an overall negative effect on feed conversion. A study conducted by Dibner and coworkers (1996) that investigated the effects of feeding oxidized fats to broilers concluded that oxidant stress resulted in increased cell turnover in the gastrointestinal epithelium, increased hepatic cell proliferation and lower concentration of immunoglobulin in the intestinal tissue, as well as increased numbers of *E. coli* and decreased Lactobacilli populations in the small intestine. These indicators of oxidative stress and reduced gut health may help explain the poor F:G observed in treatments with elevated PV in the current trial. It appears from this experiment that peroxide level had no effect on processing yields regardless of antioxidant addition, indicating that it may be possible to feed rations with some level of rancidity. It is unknown at this time why live performance seemed to be depressed more in the 21-35 day period and why antioxidant addition seemed to ameliorate some of the negative performance at certain PV levels and not at others. Based on results from this study and those from the literature, it appears that the addition of an antioxidant, especially to feeds containing fat with a PV below 150 meq/kg, can be useful in alleviating some of the negative effects caused by oxidative rancidity.

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