EFFECT OF HIGH PEROXIDE VALUE FATS ON PERFORMANCE OF BROILERS IN NORMAL AND IMMUNE CHALLENGED STATES

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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

EFFECT OF HIGH PEROXIDE VALUE FATS ON PERFORMANCE OF BROILERS IN NORMAL AND IMMUNE CHALLENGED STATES

Presented by Jeremy P. McGill

A candidate for the degree MASTER OF SCIENCE,

And hereby certify that in our opinion it is worthy of acceptance

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CHAPTER 1

REVIEW OF LITERATURE

INTRODUCTION

The utilization of rendered fats, blended fats containing both vegetable oils and rendered fats, and animal by-products that contain fat has been well established in the United States for many years. The addition of high levels of fat is not uncommon due to the advanced rendering industry and, therefore, relatively inexpensive fat sources. In the United States, it is estimated that use of rendered fat products may save up to $10 per ton of feed produced (Firman, 2006). The use of these fats in poultry diets provides many additional benefits including, but not limited to, a cost effective concentrated energy source, a source of linoleic acid, increased growth rates, and increased feed efficiency.

While a variety of fats and animal by-products are used regularly in the United States, many other countries utilize primarily vegetable oils, the

use of which can be both sporadic and at considerably lower levels. One of the problems associated with using rendered fats is the perception that they might be of decreased quality due to oxidative rancidity, and so fats of vegetable origin are used as an alternative. These fats are often more expensive when compared to rendered products, which may contribute to the overall lower utilization of added fat. Additionally, feed ingredients such as corn are often less available in other countries and soybean meal is frequently more expensive, leading to the use of lower quality and lower energy ingredients. This, paired with the low utilization of fats, may lead to diets that are lower in metabolizable energy (ME) than those seen in the United States and therefore lower performance levels. However, as these trends have occurred, worldwide poultry meat output rose from 8.9 to 70.4 million tons between 1961 and 2001 with middle-income countries making the greatest overall increase. In terms of world chicken production alone, middle- and low-income countries achieved the greatest increases from 1961 to 2001, rising 1,139 percent and 898 percent, respectively (Economic Research Service, USDA website, 2003). Greater flexibility in the use of additional sources of fat could result in considerable cost savings worldwide.

STRUCTURE AND COMPOSITION OF FATS AND OILS

Natural fats and oils are triglycerides, consisting of three fatty acids linked to a glycerol molecule. The fatty acids may contain anywhere from 4 to 36 carbons, and are categorized as saturated or unsaturated. The chemical structure of saturated fatty acids does not contain double bonds, while unsaturated fatty acids contain at least one double bond (Nelson and Cox, 2008). Both the length of the fatty acid and the amount of double bonds determine the melting point and stability of each individual fat (Dozier, 2003). Typically the more unsaturated a lipid is, the more likely it will be in a liquid state at room temperature due to a lower melting point. Therefore, most vegetable oils such as canola oil or corn oil are more unsaturated, while animal fats such as lard or tallow tend to have a higher degree of saturation and tend to be solid at room temperature (Cheeke, 2005). The hardness or softness of a fat can be measured by either titer or iodine value. Titer is a measurement (in degrees) obtained by determining the solidification point of fatty acids in fats. A fat is classified as tallow if the titer value is equal to or greater than 40 or as grease if the value is less than 40. Iodine value (IV) defines the amount of iodine (in grams) that is

absorbed by 100 grams of fat. Unsaturated fats have greater IVs than saturated fats (Pearl, 2004; Shermer and Giesen, 1997).

FEED FAT QUALITY

Several factors can influence the quality of fat used in animal feed, including free fatty acid levels, moisture, insolubles, and unsaponifiables (MIU), and rancidity (Firman, 2006). Free fatty acids (FFA) are fatty acids that are not involved in ester linkage to glycerol. FFA are produced as a byproduct of hydrolysis in fat. It has been thought that high levels of FFA (> 20%) usually indicated a possible issue with rancidity (Pearl, 2004; Dozier, 2003; Zumbado, *et al.,* 1999; Gray and Robinson, 1941; Branion *et al.,* 1938) and could result in poor performance. Interestingly, other studies have shown that varying levels of FFA in poultry diets up to 50% do not negatively affect bird performance, nutritive value, or acceptability as long as the fats or fat blends have similar fatty acid profiles (level and saturated:unsaturated ratio) and do not have a high level of rancidity as indicated by peroxide value (PV) (Waldroup, *et al.,* 1995; De Groote, *et al.,* 1971; Lewis and Payne, 1963; Siedler, *et al.,* 1955, Treat, *et al.,* 1960). Only when accompanied by a high PV or a higher degree of saturation were

performance issues noted (Wiseman and Salvador, 1991). These studies suggest that although FFA levels may indicate the potential for problems with rancidity, the FFA themselves are not responsible for decreased performance.

MIU are the non-fat products that can decrease the energy content of a fat (Association of Official Analytical Chemists, 1982). Moisture is one of these factors influencing the quality of fat. A high level of moisture is one causative agent in hydrolytic rancidity in fat, which can decrease stability (Rossell, 1994). Insolubles make up another factor that influences fat quality. These may include traces of bone, hair, feathers, dirt, etc., and can create problems with clogging components of fat handling machinery (Pearl, 2004) while contributing little nutritive value. Unsaponifiable matter includes a variety of compounds, such as fatty alcohols, hydrocarbons, pigments, and sterols that are not hydrolyzed by the saponification process in which triglycerides are converted to glycerol and fatty acids (Pearl, 2004). Considered contaminants, these compounds have a low digestible energy content, thereby lowering metabolizable energy (Dozier, 2003), and some contain what is known as the chick edema factor which is highly toxic, producing edema, liver damage, kidney damage, and other performance

decreasing symptoms (Firestone, 1968). Acceptable levels of MIU vary depending on the source of the quality specifications, but typically the level of moisture is recommended to be below 1.0%, impurities less than 0.60%, and unsaponifiables less than 1.0%.

ADDED FAT IN POULTRY RATIONS

Adding fat to livestock and poultry rations provides many benefits. It is an excellent source of energy, providing 2.25 times the energy of starches or sugars (Church, 1991), making it an ideal method to raise the energy density of a diet. As a concentrated energy source it may be a more cost efficient option than alternative energy sources. Practical benefits of adding fat to rations include a reduction in dust and dust losses, decreased particle separation, a source of lubrication for feed mill machinery, and an increase in the palatability of the ration. It is a good source of linoleic acid (18:2), which is required by poultry (National Research Council, 1994). Fat will typically be added to most poultry rations at a minimum of 1-3%, an amount sufficient to provide the essential fatty acids required and lend beneficial physical improvements. Additionally, supplementing fat to poultry rations

can result in increased growth rates, decreased feed intake, and increased feed efficiency (Firman, 1995; Sell *et al.,* 1986; Pesti *et al.,* 2002).

The Effect of Age on Fat Utilization

One important consideration when adding fat to poultry rations is the age of the birds being fed. Carew and coworkers (1972) showed that very young chicks (2 to 7 days of age) are not fully able to absorb fats in the diet, but that absorptive capacity increased rapidly with age. Similar results were found by Renner and Hill (1960), who reported that young chicks had difficulty utilizing tallow, but that absorbability increased from 2 to 8 weeks of age. It has also been shown that growing chicks display variability in their ability to digest and absorb different fat sources (March and Biely, 1957), and that in chicks, absorbability might depend on the fatty acid profile of the individual fat (Young, 1961), with saturated fatty acids being less efficiently utilized than unsaturated fatty acids (Young, 1963). However, Siedler and coworkers (1955) reported that chicks fed diets supplemented with 0, 3, or 6% added animal fats of varying fatty acids profiles utilized the fats equally well, although all were stabilized with an antioxidant. Turkey poults are also less capable of utilizing supplemental

fats (Sell *et al.,* 1986), especially those with a high proportion of saturated fatty acids (Leeson and Atteh, 1995), than older birds.

The Extra Caloric Value of Fat

Added dietary fat exhibits what is known as an extra caloric effect, in which experimentally obtained ME values for a feed containing added fat exceed the expected ME value originally calculated (Jenson *et al.,* 1970; Horani and Sell, 1977). Fat can increase the absorption and nutrient availability of other ingredients in the ration by increasing the intestinal transit time, increasing the overall ME of the diet (Mateos and Sell, 1981; Sell *et al*., 1983). Data supporting this theory was reported from a trial in which the addition of fat increased digestibility of meat and bone meal (Firman and Remus, 1994).

Heat Increment

Another benefit of supplementing fat to poultry rations is a reduction in heat increment. This is the heat produced from the digestion of feed. Added fat can lower the overall heat increment of a diet (Dale and Fuller, 1978; Carew and Hill, 1964), therefore increasing the energetic efficiency of a diet (Fuller and Rendon, 1977; Fuller and Rendon, 1979). This becomes increasingly important during periods of heat stress. Growth depression due

to heat stress has been alleviated in birds consuming diets higher in fat (Dale and Fuller, 1980), and laying hens show a greater ability to overcome heat stress when fed diets supplemented with fat (Reid and Weber, 1975).

Metabolizable Energy

It can be very difficult to assign ME values to individual fat sources. Part of this has to do with previously discussed factors such as the extra caloric value of fat and the heat increment. Additionally, it is possible to obtain a variety of ME values for the same fat depending on the level fed, the type of diet in which they are included, and whether or not they are fed individually or with other fats (Sibbald *et al.,* 1961). However, it has been shown many times that, when supplied in a complete diet, different fat sources will not result in differences in performance (Siedler *et al.,* 1955; Young, 1961; Pesti *et al.,* 2002; Firman *et al*., 2008). Although different fat sources may cause significant differences in ME, those differences may not translate to significant differences in bird performance.

While the benefits of added fats are obvious, it is important to keep several issues of possible concern in mind. Increasing levels of fat in the diet can cause increased body fat deposition in turkeys (Salmon and O'Neil, 1971). One must also be careful when adding fat to layer rations, and major

discrepancies exist within the literature with regard to this topic. Isika and coworkers (2006) reported increasing egg production and egg mass with 5% fat addition to rations of laying hens from 8-22 weeks of age. Research conducted by Bohnsack and others (2002) indicates no increase in egg production but an increase in egg weight at 2 or 6% added fat over three 28-day periods, while early research by Donaldson and Gordon (1960) reported depressed hen-housed egg production, poorer feed conversion, increased mortality, and no effect on egg quality when 3% fat was added to layer rations over a 350-day period. It is evident from the variability in these results that age plays a role in laying hen performance as it relates to fat consumption. Fuller (1996) reports that young birds tend to deposit increased energy in diets in improved egg production and parameters, while older hens tend to deposit extra energy as body weight. It is critical to control energy consumption in laying hens to prevent obesity, which can greatly decrease performance.

A few additional concerns remain over supplementing dietary fat. High levels of fat (greater than 10%) may compromise pellet integrity. In hot weather, high levels of fat may result in greasy bags or equipment. Finally, adding fat to a ration creates a greater potential for hydrolytic or

oxidative rancidity, which can lead to decreased quality and acceptability of feed.

RANCIDITY OF FATS

One of the concerns with using rendered fats such as tallow, blended fats, or other fat-containing animal by-products is that they may be of poor quality due to oxidative rancidity and consequently pose a threat to the performance or health of the birds. While information on rancidity of fats has been available for over 60 years (Gray and Robinson, 1941), relatively little work has been done on the oxidative rancidity of fats and the effects on performance or immune function in poultry.

Hydrolytic Rancidity

Two major forms of rancidity exist, namely hydrolytic and oxidative, both of which cause unpleasant flavors and odors in fats and feeds that contain added fat. Hydrolytic rancidity occurs when triglycerides are hydrolyzed into fatty acids and glycerol. More specifically, this type of rancidity occurs in the presence of moisture and an enzymatic catalyst and results in the liberation of free fatty acids, which have a lower flavor threshold than the parent triglycerides (Rossell, 1994). This is why hydrolytic rancidity causes a distinct rancid flavor, which is a contributing

factor to the decreased quality and acceptability of the fat (Galliard, 1994). The most effective methods to reduce the occurrence of hydrolytic rancidity would be to reduce the amount of moisture in the fat source or store the fat or feed at cold temperatures. Other than the formation of off-flavors and odors, another reason to avoid hydrolytic rancidity is that the reactions of hydrolysis supply free oleic, linoleic, and linolenic acids that could then undergo further oxidative rancidity (Hamilton, 1994).

Oxidative Rancidity

Oxidative rancidity, also termed autoxidation, is certainly the most complex type of rancidity and causes the greatest level of concern among producers. Although all fats are made up of a variety of fatty acids, fats that contain a high level of unsaturated fatty acids tend to be more susceptible to autoxidation, as rancidity typically takes place at a double bond (Hamilton, 1994; Dozier, 2003). Oxidation proceeds at different rates for each of the abundantly occurring unsaturated fatty acids, with the order of reactivity being linolenic > linoleic > oleic (Berger, 1994; Hamilton, 1994). The process of autoxidation consists of three main phases: initiation, propagation, and termination.

Three reactions take place during initiation and propagation, which are shown in Figure 1 (adapted from Talbot, 2004). Initiation takes place when two free radicals are formed through the cleavage of a hydrogen atom from a triglyceride, a reaction that usually requires a heavy metal catalyst such as copper, or energy from heat or light (Talbot, 2004). At this point, oxidation occurs at a relatively slow, uniform rate of speed during what is known as the induction period. Next, in the first of two propagation reactions, a peroxy radical is formed when the triglyceride free radical reacts with an oxygen molecule. In the second propagation reaction, the peroxy radical reacts with another triglyceride, forming a hydroperoxide and regenerating a new free radical that is then available to react with another oxygen molecule, causing an accelerated chain-reaction to occur (Hamilton, 1994). The hydroperoxide concentration can be measured, providing a peroxide value. Oxidation over time as measured by peroxide value (adapted from Coppen, 1994), is shown in Figure 2.

The hydroperoxides that form during the propagation phase 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). Over time, this rate of breakdown becomes equal to the rate of hydroperoxide formation resulting in a temporary equilibrium. Subsequent to this point, hydroperoxide decomposition continues until the double bonds in the fatty acid are destroyed, ending the supply of newly formed hydroperoxides (Shermer and Giesen, 1997). Additionally, termination reactions occur in which two radicals combine and form a product that does not feed those reactions of propagation (Hamilton, 1994; Talbot, 2004). Eventually, this termination phase proceeds until the concentration of hydroperoxides returns to a level near zero (Shermer and Giesen, 1997).

A variety of methods exist to test the level of rancidity in a product, most of which involve testing for levels of products of oxidation or intermediates of the reactions (Grettie and Newton, 1931). These levels are constantly changing during autoxidation, so accurate interpretation of the results can be challenging. Because of this, it is common to utilize at least two different testing methods in order to acquire the necessary information on the stability of the fat in question (Shermer and Giesen, 1997). Two of

the most commonly used tests are Peroxide Value (PV) and the Active Oxygen Method (AOM). PV reveals the current level of oxidative rancidity, measured as milliequivalents of peroxide per kilogram (meq/kg), while the AOM test measure PVs at various time intervals while bubbling air through the fat and is used to predict the ability of a fat in storage to remain stable over time (Pearl, 2004). Recommended maximum levels of PV and oxidative stability vary depending on the source.

It is possible to minimize or delay the development of oxidative rancidity in fat or feeds containing added fat during handling and storage. Berger (1994) reports four main influences on the rate of autoxidation. The first involves the level of contact with air. Oxidation cannot occur without oxygen, and proper storage and handling conditions can reduce the interaction between the fat source and air. The second influence is temperature. Berger (1994) reports that the rate of reaction of oxygen with fat can double with every 10 degree Celsius increase in temperature. By keeping fats in the coolest storage conditions possible or, in situations where heating is required for handling of fats that tend to be in a solid state at room temperature, by avoiding overheating, this rate of reaction can be slowed. The third factor that can increase the rate of oxidation is the presence of

catalysts such as some metals and traces of already oxidized fat. Contact with copper or iron should be avoided, and cleanliness can prevent contact between non-oxidized and oxidized fat. Finally, light can stimulate photo-oxidation, so exposure should be minimized as much as possible.

ANTIOXIDANTS

An alternative method for delaying the development of oxidative rancidity is the use of an antioxidant. The benefits of antioxidant addition to products that are susceptible to oxidation have long been recognized and utilized, and have been discussed in detail as early as the 1940's (Mattill, 1947). An ideal antioxidant possesses several important qualities, including being safe to use for both humans and livestock, effective at low concentrations, easy to incorporate, heat tolerant, (important in the pelleting process), affordable, as well as odorless, colorless, and tasteless (Coppen, 1994). Antioxidants interrupt either the initiation or propagation phase of autoxidation (Hamilton, 1994) by supplying hydrogen atoms to the free radicals, stabilizing them before they are allowed to react further and converting them back to the original fatty acid (Rumsey, 1978). This remains true until the antioxidant has been completely consumed, and it is

important to realize that antioxidants are not capable of preventing oxidation, just delaying it. They are useful for extending the shelf life of fat-containing products, decreasing waste, decreasing nutritional losses due to the oxidation of fat-soluble vitamins, and increasing the number of fats that can be utilized in a diet (Coppen, 1994). The earlier an antioxidant is added, (that is, during initiation versus propagation) the more effective it is, and the effects of adding an antioxidant to a fat early on, (adapted from Coppen, 1994) are displayed in Figure 3. It is also important to keep in mind that antioxidants do not prevent hydrolytic rancidity or the formation of FFAs, and they are not capable of returning an oxidized fat to a non-oxidized state (Coppen, 1994).

EFFECTS OF OXIDIZED FATS AND ANTIOXIDANTS IN POULTRY DIETS ON PERFORMANCE

A large number of antioxidants exist, including natural compounds such as vitamin E (Figure 4) and other tocopherols, and synthetic compounds such as ethoxyquin (Figure 2), butylated hydroxyanisole (BHA) (Figure 3), and butylated hydroxytoluene (BHT) (Cheeke, 2005). All three

of these synthetic antioxidants have demonstrated efficacy in reducing the rate of oxidative rancidity in broiler diets (Njobeh, 2006). A broiler trial conducted by Cabel and others (1988) included diets containing fat oxidized to 0, 50, 100, or 150 meq/kg, each of which were supplemented with 0, 62.5, or 125 ppm ethoxyquin. At 21 and 42 days, birds consuming the feed with a peroxide value of 100 and 175 displayed decreased body weight when compared to those consuming 0 meq/kg, but at 49 days only those birds consuming 175 meq/kg had reduced body weight gain and feed efficiency. Ethoxyquin supplementation resulted in greater body weight gain at 49 days, and as the peroxide levels of the diets increased, supplementation of ethoxyquin reduced the negative affects of the diets with elevated peroxide levels, especially those with 125 ppm.

While severe oxidation has been shown to cause decreased weight gain, feed efficiency, fertility, hatchability, and is associated with health disorders such as encephalomalacia (Cabel *et al.,* 1988, Lin *et al.,* 1989), there are certainly differences in reported effects of feeding oxidized fat and at what levels negative effects will be seen. Performance of poultry has generally not been significantly compromised when oxidized fats with PV levels up to 100 meq/kg were fed (Lea *et al.*, 1966; L'Estrange *et al.,* 1966;

Carpenter, *et al.,* 1966). More recently, experimental data using turkeys showed no deleterious effects on growth performance from feeding oxidized fats (Leeson *et al.,* 1997), and research conducted by Pesti and coworkers (2002) in which various oils with a range of peroxide values were fed to broilers resulted in no deleterious effects on performance, although birds were not taken to typical growout weights.

It has been shown that PV higher than 100 meq/kg can cause depression in performance parameters. A study was conducted in which dietary treatments containing either fresh vegetable oil (PV of 1 meq/kg) or oxidized oil (PV of 156 meq/kg) were fed to broilers. At 35 days of age the trial was concluded, and birds consuming feed with 156 meq/kg PV displayed lower body weight (Enberg, 1996). While a review of the literature certainly produces conflicting opinions concerning the effects of feeding oxidized fat to poultry and the levels at which negative effects may be observed, it is consistently agreed upon that the addition of commercial antioxidants has reduced the impact of oxidized fats (Cabel *et al.,* 1988).

EFFECTS OF OXIDIZED FATS IN POULTRY DIETS ON IMMUNITY

Although from the literature it seems that feeding fats with a peroxide value less than 100 meq/kg should be safe in poultry, concern still exists over feeding such fats. One of these concerns stems from the belief that oxidized fats may compromise immune function. However, a very limited amount of research has been done on this topic in poultry. It has been shown that the presence of unstabilized rancid fat in the intestine increases the number of E. coli and decreases Lactobacilli populations in the small intestine (Dibner, *et al.,* 1995). Despite this lack of research, it is known that the free-radical mechanism of autoxidation leads to the formation of several products that have been previously mentioned 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.

SUMMARY

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 that has been 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 poultry meat and eggs become an increasingly popular protein source. However, fear of decreased quality due to oxidative rancidity and the subsequent effects on performance and immunity stand in the way.

Currently, there are no true industry standards for measuring the level of rancidity of fats, and 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. 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. Continued research is imperative in order to define the acceptable level of rancidity and to determine if high levels of peroxide values affect immune function.

Figure 1. Initiation and propagation reactions of autoxidation

Initiation Reaction	RH ⟶ R . + H . lipid molecule ⟶ lipid free radical + hydrogen free radical
Propagation Reaction 1	R . + O_2 ⟶ ROO . Lipid free radical + oxygen ⟶ peroxy free radical
Propagation Reaction 2	ROO . + RH ⟶ R . + ROOH Peroxy free radical + lipid molecule ⟶ lipid free radical + hydroperoxide

Figure 2. Oxidation over time as measured by peroxide value

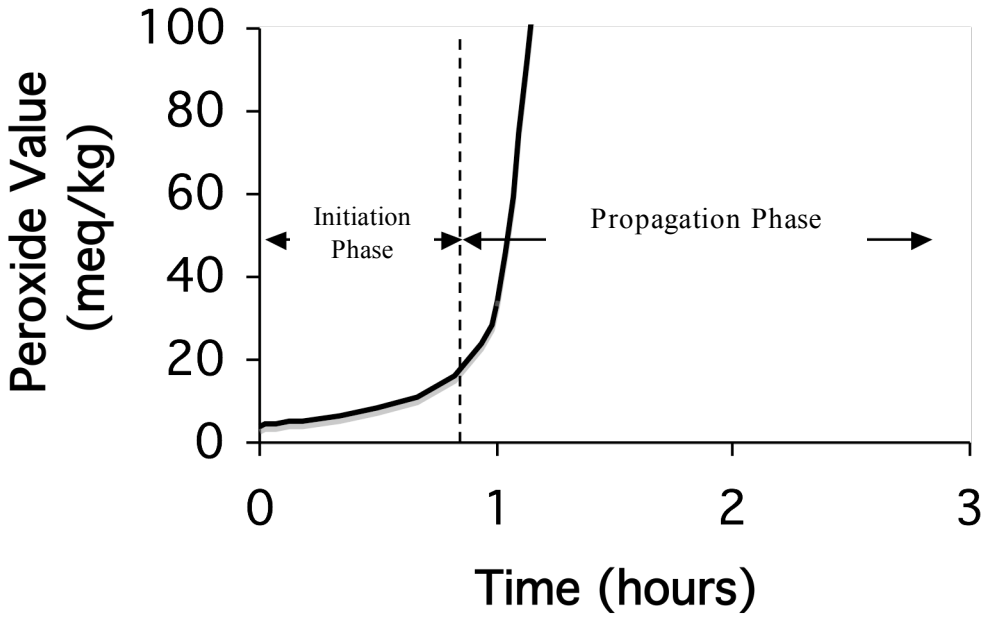


Figure 3. Oxidation over time as measured by peroxide value, with and without the addition of an antioxidant

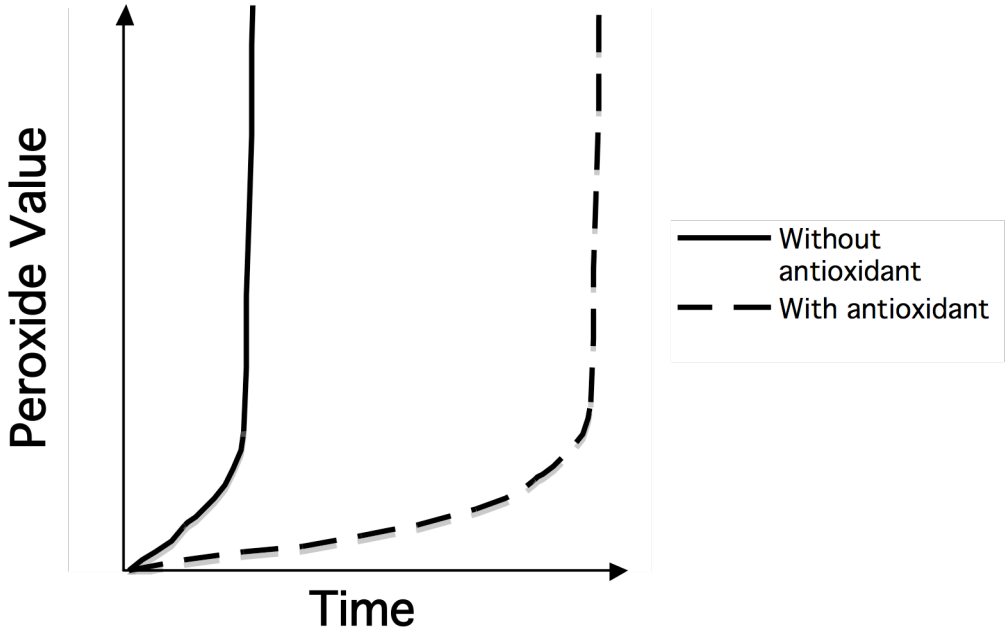


Figure 4. Structure of vitamin E

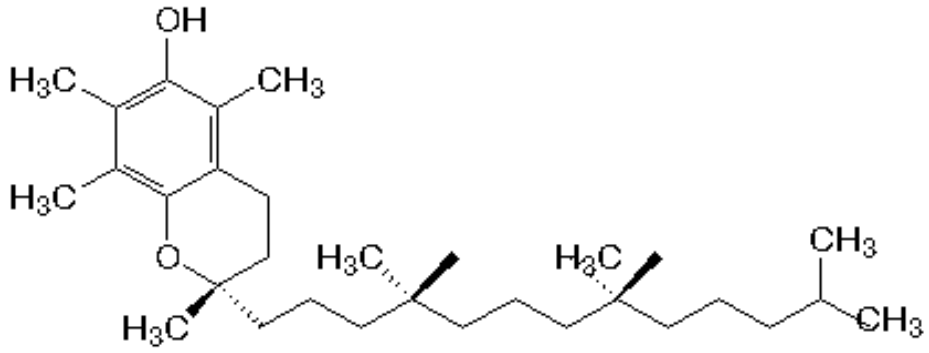


Figure 5. Structure of Ethoxyquin

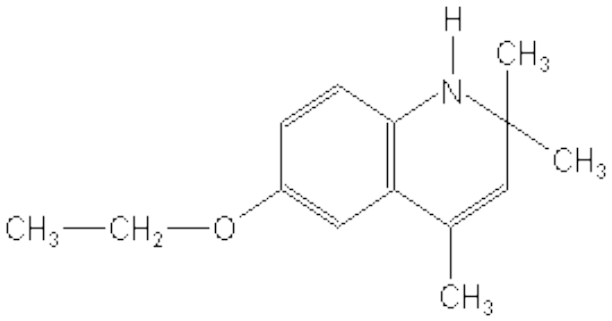


Figure 6. Structure of BHA

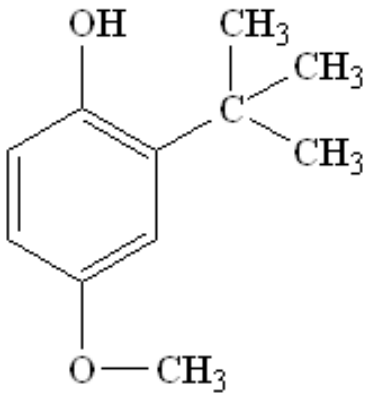
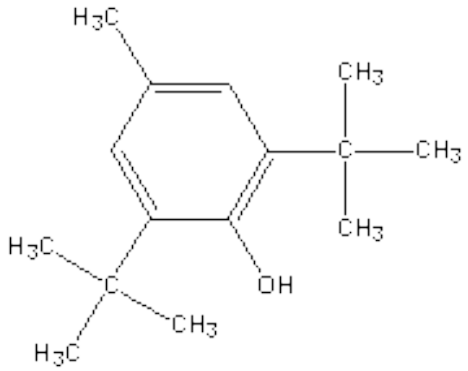


Figure 7. Structure of BHT



CHAPTER 2

EFFECT OF HIGH PEROXIDE VALUE FATS ON PERFORMANCE OF BROILERS IN A NORMAL IMMUNE STATE

ABSTRACT

A floor pen trial was conducted to determine the effect of high peroxide value fats on performance of broilers. One thousand four hundred and forty day-old straight run broilers were obtained from a commercial hatchery and randomly assigned to 48 floor pens. Each floor pen contained 30 broilers. 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, ethoxyquin, at 125 ppm. Six dietary treatments with 8 replicates were fed to Ross 708 broilers from hatch to week 7. Diets were formulated based on standard industry diets meeting all of the NRC requirements 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 1 for each of the 48 pens. 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 in order to obtain a fat pad weight, carcass weight, and percent meat yield. Experimental data were analyzed by analysis of variance using the JMP program. The ANOVA indicated that diets with a peroxide value of 75 or greater exhibit poorer feed conversion than the treatment with an acceptable peroxide value. 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.

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, 2003). 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 grown to market age based on feed intake (FI), weight gain (WG), and feed conversion (F:G).

MATERIALS AND METHODS

One thousand four hundred and forty day-old straight-run broilers were obtained from a commercial hatchery and randomly assigned to floor pens in an environmentally controlled house. The birds were exposed to 24 hours of fluorescent lighting. Six dietary treatments were replicated eight times with thirty birds per replication. Birds were fed diets formulated to resemble standard industry diets that met all of the NRC requirements. Access to experimental diets and water was provided *ad libitum* for the duration of the trial. 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).

Diets were formulated to meet NRC requirements using least-cost formulation software. 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. Each peroxide value treatment was then divided into two, with or without an antioxidant at 125 ppm (Ethoxyquin, Novus Intl., St. Louis, MO).

Birds and feed were weighed on a pen basis on day 0, 21, 35, and 49 to determine weight gain, feed intake, and feed conversion. 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 slaughtered and processed to determine the chilled carcass weight, weight of the fat pad, major cuts such as leg, thigh, wing, pectoralis major, pectoralis minor and percent yield. The birds were cared for using standard husbandry guidelines derived from standard operating procedures.

Analysis of data was performed using pen as the experimental unit. The JMP statistical analysis software package 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 determined 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 (Figure 8). 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 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 (Figure 9).

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 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 (Figure 10) 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 *et al*., 1966; L'Estrange *et al*., 1966; Oertel and Hartfiel, 1982). However, in each of these experiments the authors included an antioxidant to the diets in an effort to keep a steady peroxide level, and it is possible that the harmful effect of the peroxide 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. 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- (1.180 versus 1.84, respectively) was approaching statistical significance, indicating that PV did have an overall negative effect on feed conversion. 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.

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 2x3 factorial to produce 6 treatments; PV0−, PV75−, PV150−, PV0+, PV75+, and PV150+ (Table 1).

[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, 35mg; 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.

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 (kg)	21 – 35 days (kg)	35 – 49 days (kg)	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 (kg)	21 – 35 days (kg)	35 – 49 days (kg)	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 ($P < 0.05$).

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 (kg:kg)	21 – 35 days (kg:kg)	35 – 49 days (kg:kg)	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[ab]
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.

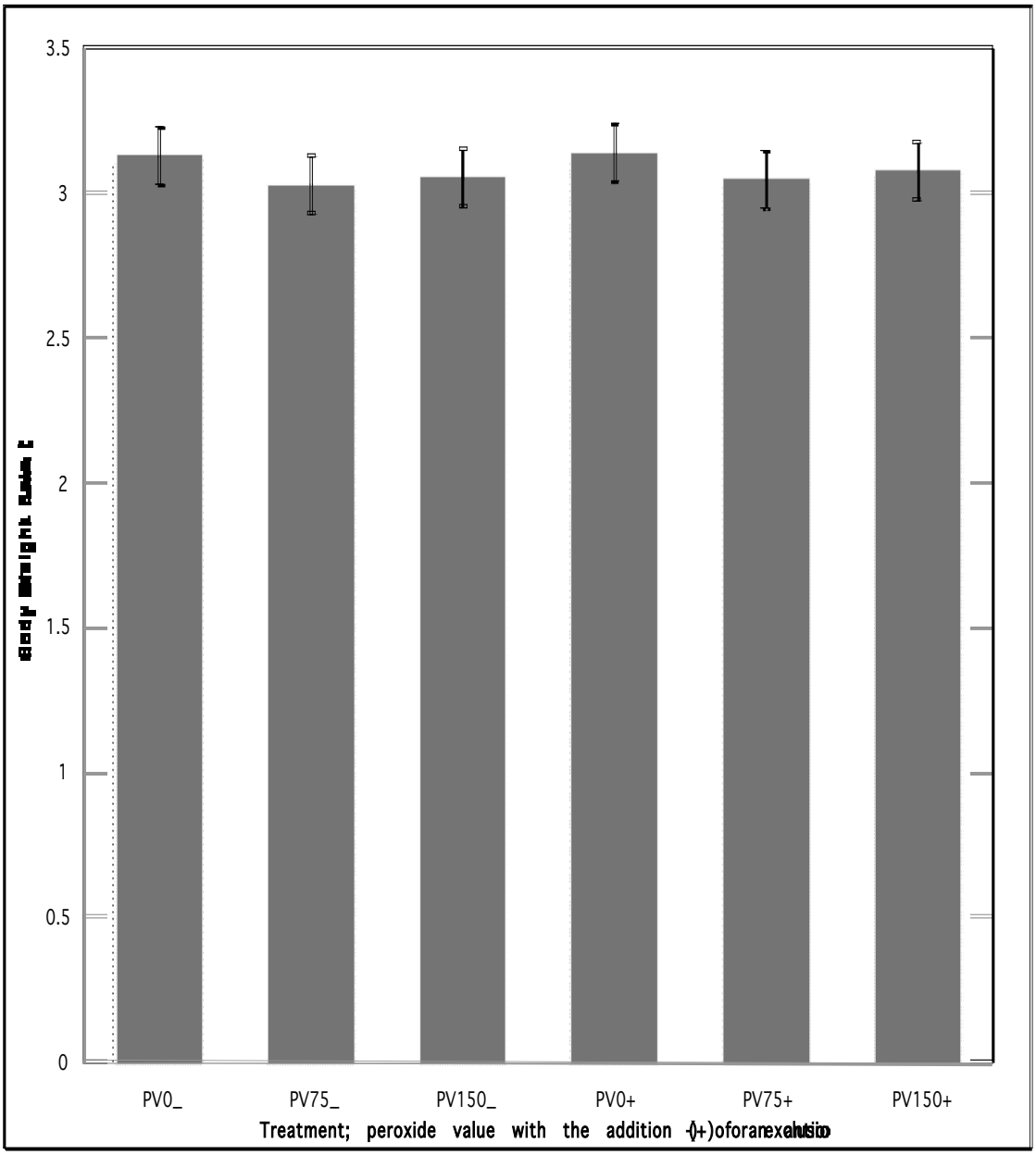


Figure 8: Broiler 0 – 49 day body weight gain (kg) based on dietary fat peroxide level.

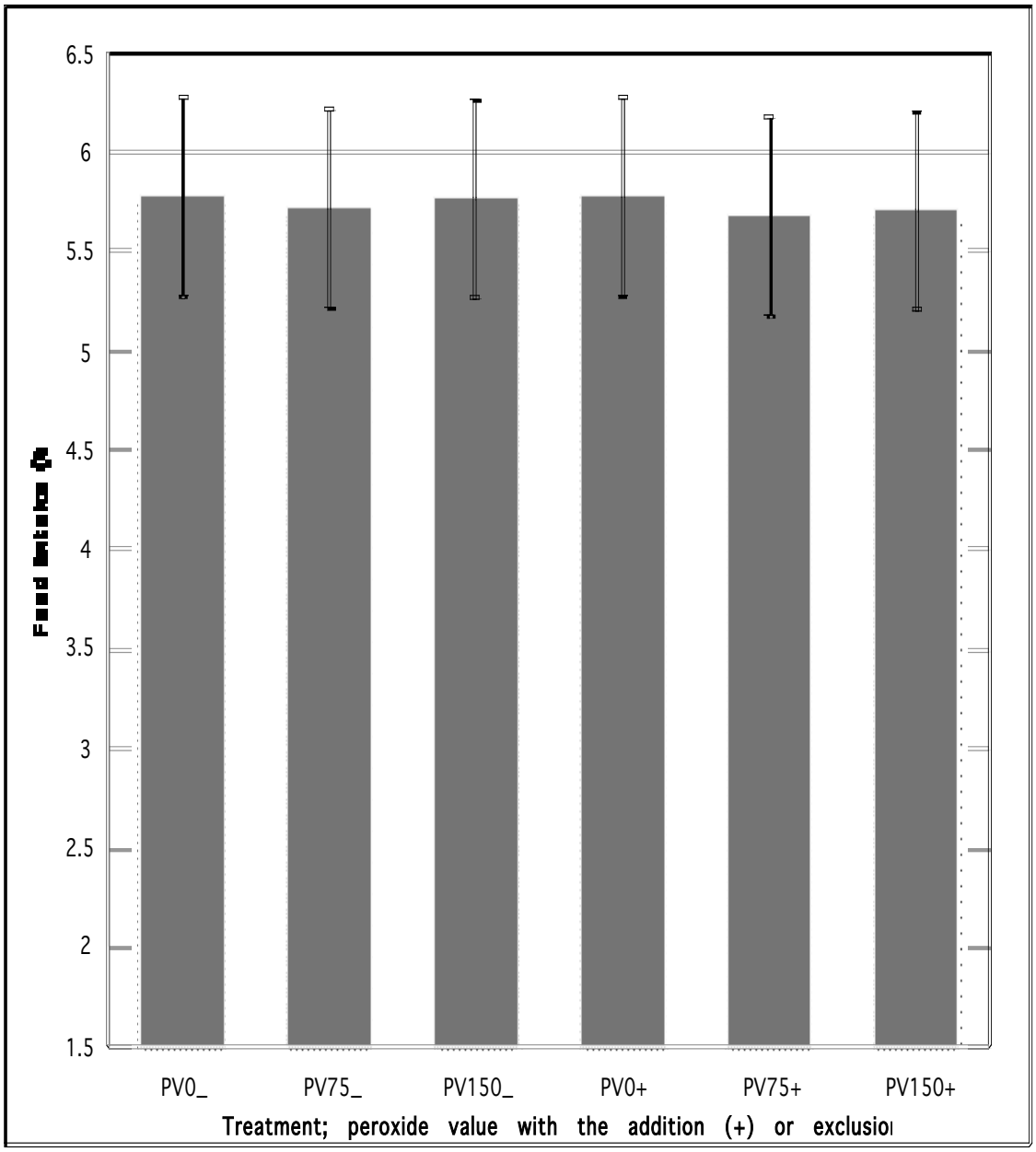


Figure 9: Broiler 0 – 49 day feed intake (kg) based on dietary fat peroxide level.

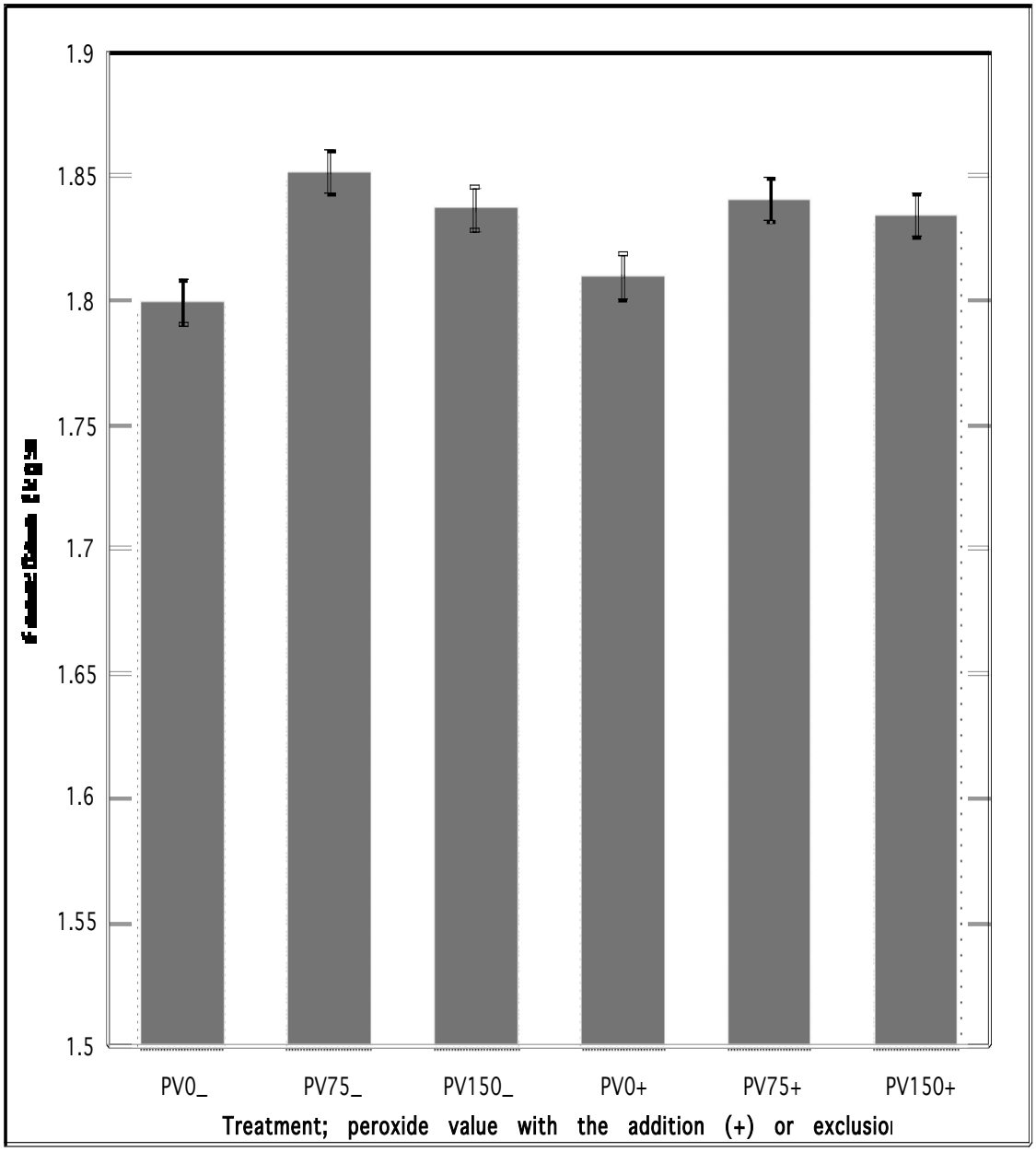


Figure 10: Broiler 0 – 49 day feed:gain (kg:kg) based on dietary fat peroxide level.

CHAPTER 3

EFFECT OF HIGH PEROXIDE VALUE FATS ON PERFORMANCE OF BROILERS IN AN IMMUNE CHALLENGED STATE

ABSTRACT

A floor pen trial was conducted to determine the effect of high peroxide value fats on performance of broilers in an immune challenged state. One thousand four hundred and forty day-old straight run broilers were obtained from a commercial hatchery and randomly assigned to 48 floor pens. Each floor pen contained 30 broilers. 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. The diets were each divided, and one of each of the different peroxide value diets also received an antioxidant, ethoxyquin, at 125 ppm. Six dietary treatments with 8 replicates were fed to Ross 708 broilers from hatch to week 7. Diets were formulated based on standard industry diets meeting all of the NRC requirements 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), weight gain (WG), and feed conversion (F:G). An initial pen weight was taken on day 1 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 in order to obtain a fat pad weight, carcass weight, percent meat yield, and cecal scoring. Experimental data were analyzed by analysis of variance using the JMP program. The ANOVA indicated that birds consuming diets with a peroxide value of 75 or greater exhibit 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.

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, 2003). 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.

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

One thousand four hundred and forty day-old straight-run broilers were obtained from a commercial hatchery and randomly assigned to floor pens in an environmentally controlled house. The birds were exposed to 24 hours of fluorescent lighting. Six dietary treatments were replicated eight times with thirty birds per replication. Birds were fed diets formulated as standard industry diets that met all of the NRC requirements. Access to experimental diets and water was provided *ad libitum* for the duration of the trial. Fat was set to the level of 3% within the starter diet (0 – 3 weeks) and 6% within the grower (3 – 5 weeks) and finisher diet (5 – 7 weeks).

Diets were formulated to meet NRC requirements using least-cost formulation software. A 3 x 2 factorial was the model used for this trial with three levels of fat rancidity with one level based on the peroxide value (PV) of that fat, PV of 7 (PV0), one at 75 (PV75), and one at 150 (PV150). Each peroxide value treatment 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).

Birds and feed were weighed on a pen basis on day 0, 21, 35, and 49 to determine weight gain, feed intake, and feed conversion. Feed:Gain was adjusted for mortality; weight of bird (mortality) was added to the pen weight gain; feed consumed 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 slaughtered and processed to determine the cecal score, chilled carcass weight, weight of the fat pad, major cuts such as leg, thigh, wing, breast major, breast minor and percent yield. The birds were cared for using

standard husbandry guidelines derived from standard operating procedures.

Analysis of data was performed using pen as the experimental unit. The JMP statistical analysis software package 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

Results for weight gain (BWG) are demonstrated in Table 7. 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 (Figure 11).

Feed intake (FI) data are listed in Table 8. When looking at the FI among treatments there were not 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 (Figure 12) 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 listed on Table 9. 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 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 (Figure 13) 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.

The data for mortality are listed in Table 10. 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 11. 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.

Cecal examination revealed little indication of coccidiosis, although signs of mild coccidiosis, such as occasional bloody droppings and ruffled feathers, were seen in the birds near the end of the trial. Ceca were visually scored on a scale from 1 – 4 based on occurrence and 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 consuming the PV75+A treatment consuming the least amount of feed (1.82 kg), and 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 any conclusion 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. It has been shown that the presence of unstabilized rancid fat in the intestine increases the number of *E. coli* and decreases *Lactobacilli* populations in the small intestine (Dibner, *et al.,* 1995), and is known that the free-radical mechanism of autoxidation leads to the formation of several products that have been previously mentioned and that are known to be toxic

(Sanders, 1994) and may compromise immune function and cell wall integrity (Sevanian and Peterson, 1986). The lack of research in this area makes it apparent that additional investigation is needed.

CONCLUSIONS

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. 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. 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 can cause a depression in overall live performance parameters, especially feed conversion, but that the addition of an antioxidant can improve performance in birds consuming diets containing rancid fat. In the case of the second trial, birds also seemed to overcome the immune challenge of the coccidiostat administered at 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.

Table 6. 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[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
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. 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).

[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, 35mg; 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.

Table 7. Effects of Fat Rancidity Level when an antioxidant was added or excluded on Body Weight Gain on day 21, 35, 49, and 0 - 49 in the presence of an immune challenge

PV[1]	A[2]	0 – 21 days (kg)	21 – 35 days (kg)	35 – 49 days (kg)	0 – 49 days (kg)
0	-A	0.71[a]	1.10[a]	0.97[a]	2.86[a]
0	+A	0.75[a]	1.07[a]	0.95[a]	2.84[a]
75	-A	0.73[a]	1.07[a]	0.97[a]	2.84[a]
75	+A	0.72[a]	1.05[a]	0.99[a]	2.79[a]
150	-A	0.73[a]	1.14[a]	0.97[a]	2.81[a]
150	+A	0.74[a]	1.08[a]	1.01[a]	2.84[a]
Pooled SEM		0.008	0.029	0.041	0.032
Source of variation		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
Main effect mean					
PV					
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
A	-A	0.72	1.11	0.97	2.83
	+A	0.73	1.06	0.98	2.82

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

Table 8. Effects of Fat Rancidity Level when an antioxidant was added or excluded on Feed Intake on day 21, 35, 49, and 0 - 49 in the presence of an immune challenge

PV[1]	A[2]	0 – 21 days (kg)	21 – 35 days (kg)	35 – 49 days (kg)	0 – 49 days (kg)
0	-A	0.91[a]	1.87[ab]	2.48[a]	5.36[a]
0	+A	0.98[a]	1.89[a]	2.48[a]	5.45[a]
75	-A	0.98[a]	1.89[a]	2.52[a]	5.49[a]
75	+A	0.95[a]	1.82[b]	2.49[a]	5.32[a]
150	-A	0.96[a]	1.87[ab]	2.52[a]	5.42[a]
150	+A	0.99[a]	1.86[ab]	2.43[a]	5.30[a]
Pooled SEM		0.021	0.016	0.038	0.079
Source of variation		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
Main effect mean					
PV					
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
A	-A	0.95	1.88	2.50	5.42
	+A	0.97	1.86	2.46	5.35

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

Table 9. Effects of Fat Rancidity Level when an antioxidant was added or excluded on Feed Conversions on day 21, 35, 49, and 0 - 49 in the presence of an immune challenge

PV[1]	A[2]	0 – 21 days (kg:kg)	21 – 35 days (kg:kg)	35 – 49 days (kg:kg)	0 – 49 days (kg:kg)
0	-A	1.27[a]	1.68[ab]	2.37[b]	1.82[b]
0	+A	1.31[a]	1.77[a]	2.41[ab]	1.87[a]
75	-A	1.34[a]	1.75[ab]	2.41[ab]	1.87[a]
75	+A	1.32[a]	1.73[ab]	2.38[b]	1.86[ab]
150	-A	1.32[a]	1.67[b]	2.55[a]	1.89[a]
150	+A	1.33[a]	1.72[ab]	2.41[ab]	1.85[ab]
Pooled SEM		0.020	0.032	0.046	0.012
Source of variation			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
Main effect mean					
PV					
0		1.29	1.73	2.39	1.85[b]
75		1.33	1.74	2.39	1.87[a]
150		1.32	1.69	2.47	1.88[a]
A	-A	1.31	1.70	2.44	1.86
	+A	1.32	1.74	2.40	1.86

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

Table 10. Effects of Fat Rancidity Level when an antioxidant was added or excluded on Mortality for 0 - 49 days in the presence of an immune challenge

PV[1]	A[2]	Mortality (birds/pen)
0	-A	1.75[a]
0	+A	1.38[a]
75	-A	1.0[a]
75	+A	1.25[a]
150	-A	1.0[a]
150	+A	0.63[a]
Pooled SEM		0.021
Source of variation		P-value
PV		0.2809
A		0.2368
PV x A		0.9857
Main effect mean		
PV		
0		1.5
75		1.19
150		0.8
A	-A	1.38
	+A	0.96

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

Table 11. 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 in the presence of an immune challenge

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

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

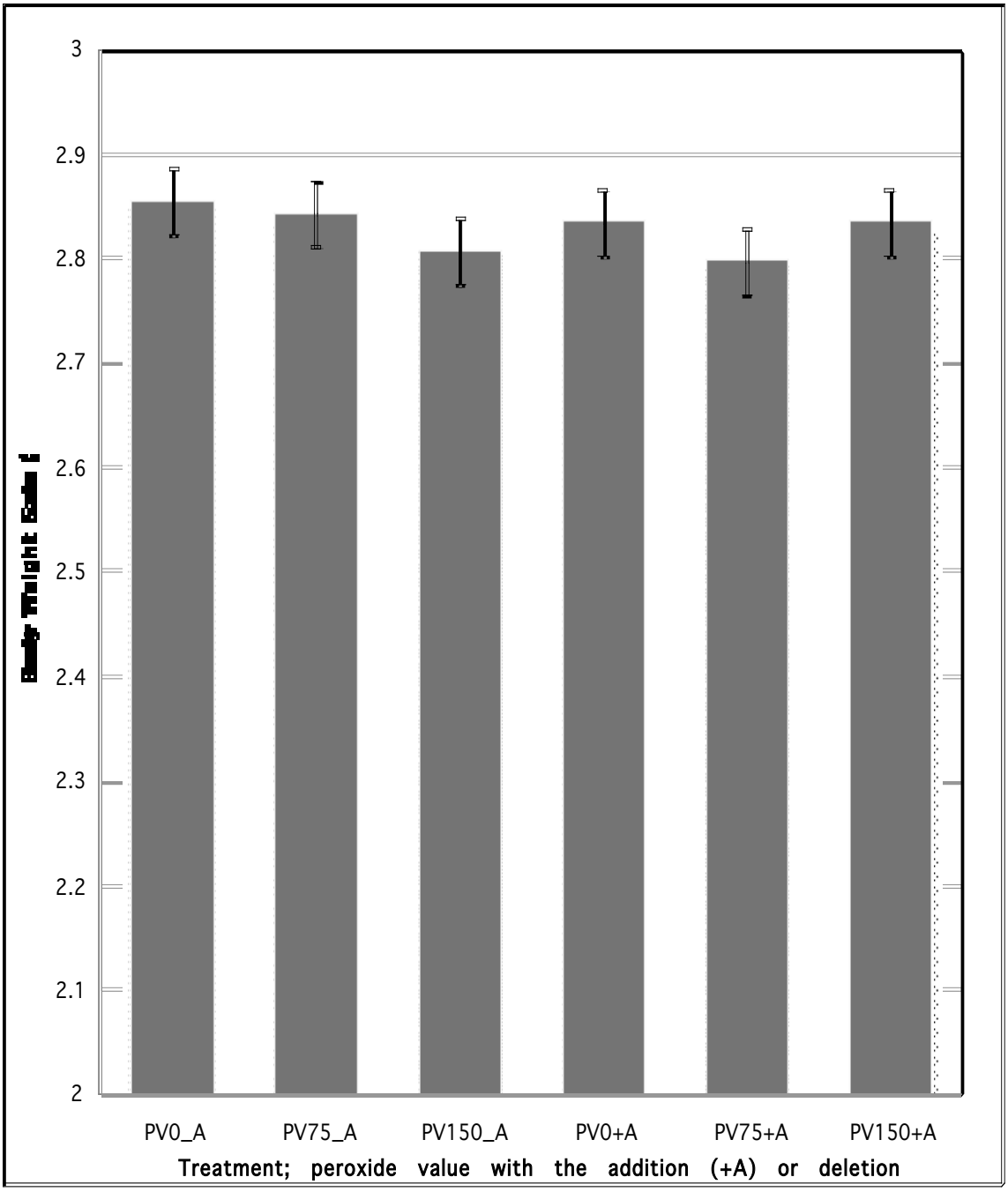


Figure 11: Broiler 0 – 49 day body weight gain (kg) based on dietary fat peroxide level in an immune challenged state.

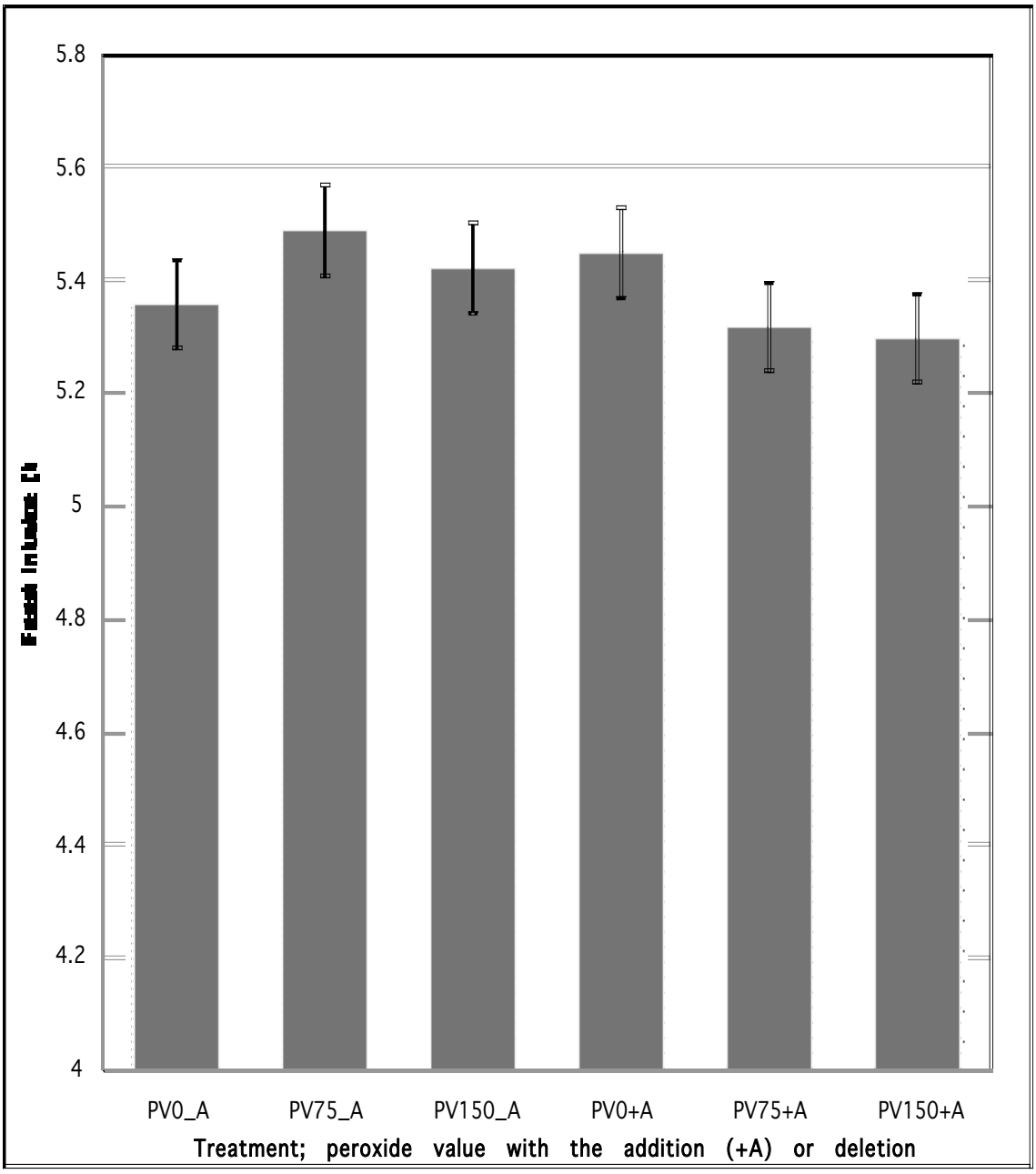


Figure 12: Broiler 0 – 49 day feed intake (kg) based on dietary fat peroxide level in an immune challenged state.

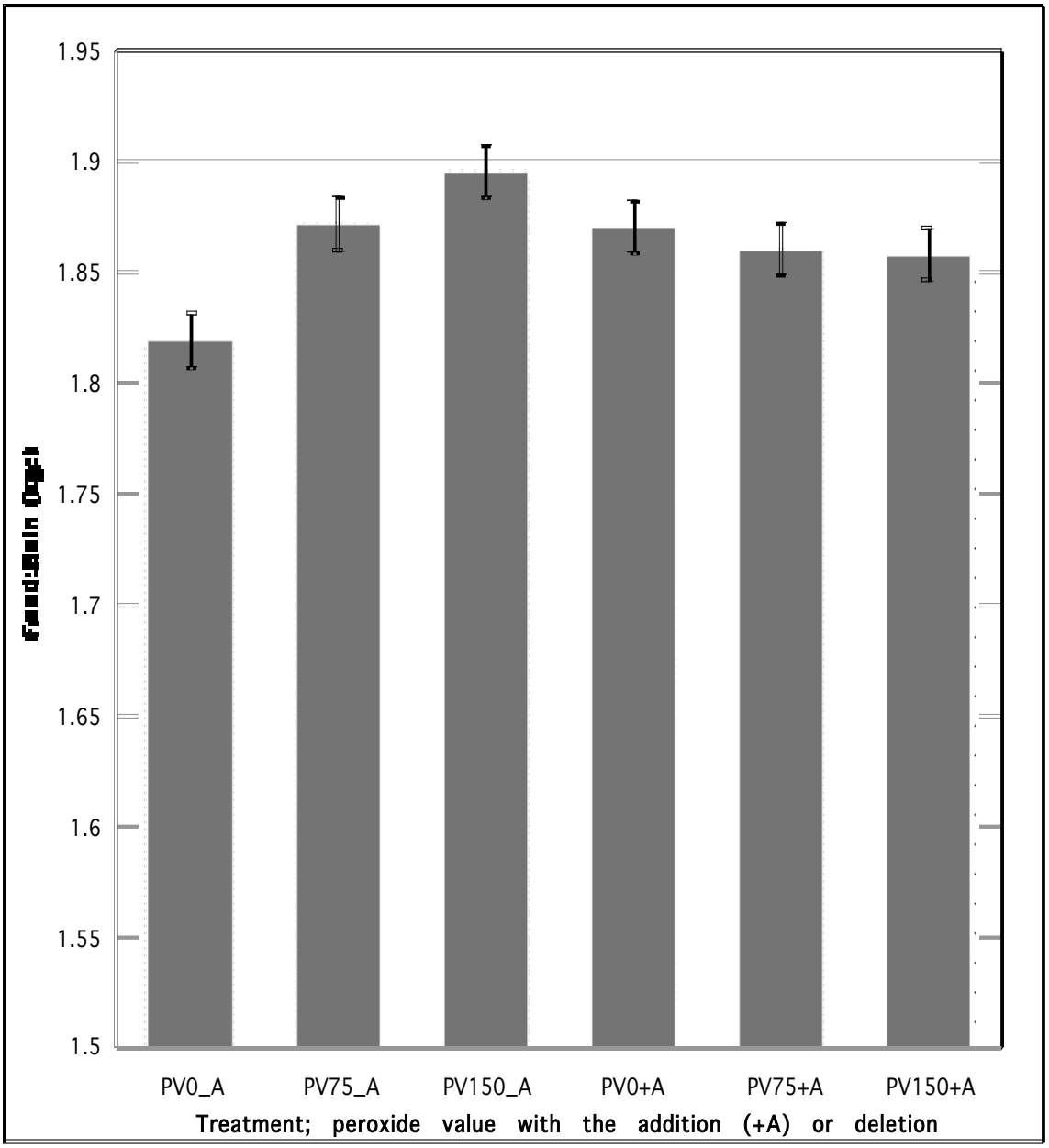


Figure 13: Broiler 0 – 49 day feed:gain (kg:kg) based on dietary fat peroxide level in an immune challenged state.

APPENDIX A

Fat Preparation

1. Locate a 55-gallon metal barrel/drum.

2. Fill the metal barrel half full of animal/vegetable blended fat.

3. Fill the other half of the metal barrel with soy oil.

4. Take into consideration the space required to aerate the fat. Only fill the barrel approximately 5/6 full of fat. This will leave a space for aeration so the fat does not spill over the edge.

5. Place a barrel heat band on the barrel approximately three inches from the ground.

6. Set the heat band to warm the fat to approximately 135-140ºF.

7. Connect four ¼ inch aquarium air lines by using three ¼ inch air line "t" type connectors. Attach the air lines to an air compressor.

8. Attach metal washers (or another source of weight) to the end of the four air lines and insert them into the metal barrel.

9. Turn on the air compressor and adjust to 3 – 5 psi.

10. Test the peroxide value often until the desired level is achieved.

APPENDIX B

Procedure for Measuring Peroxide Values

Reagents

a. Acetic acid in chloroform (3:2 HOAc:$ChCl_3$).

b. 1% Starch solution (1g starch in 100 ml degassed H_2O).

c. Sodium thiosulfate standard solution (0.1N Fisher SS368-1). Prepare a 1/10 dilution of 0.1N $Na_2S_2O_3$ by adding 10 ml of 0.1 N/90 ml degassed H_2O.

d. Saturated Potassium Iodide solution. Excess KI is added to 20 ml H_2O degassed (Store in dark bottle). To check KI, place 100 µl of saturated KI in 6 ml of HOAc:$ChCl_3$, vortex and add 2 drops of starch solution (100 µl). if KI is okay, then no blue color should be observed. If blue color is present, a new batch of KI should be prepared.

Method

1. Thaw fat (may need to run hard fat under hot water to liquefy).

2. Place 1g (+/- 0.02g) of fat in a screw top test tube and label.

Steps 3-6 should be performed in a hood

3. Add 6 ml of the acetic acid:chloroform to the test tube and vortex well.

4. Add 100 µl of KI remembering to prime pipette with some KI. For 1 minute vortex every fifteen seconds. (**TIME IS CRITICAL**)

5. After the 1 minute add 6 ml of degassed H_2O to the test tube. Get all samples to this point before proceeding. (Add H_2O quickly)

6. Shake the starch solution well before using. Add 1.0 ml of starch to the test tube and vortex.

7. A purple color forms between the two layers. Slowly add sodium thiosulfate standard solution (0.1N) by increments of 100 µl. shake the test tube and let stand until settled. Keep adding sodium thiosulfate until the layer of granules no longer has any purple color to it. The second of the duplicates should be titrated more carefully with 0.1N and 0.01N $Na_2S_2O_3$ to provide a more precise peroxide value (PV). The increments can be increased at first in fats known to have high peroxide values. The increments can also be decreased as the purple color is fading to get a closer estimate.

Calculations

$$\text{Peroxide Value (PV)(mEqv/Kg sample)} = \frac{(\text{ml } Na_2S_2O_3)(x\text{N})}{\text{Kg of sample}}$$

APPENDIX C

Weighing of Birds

1. Remove birds from feed 4 hours prior to weighing.

2. Use a functional scale that has the ability to weigh in the desired units (0.01 pounds).

3. Put a container or coop(s) on the scale to restrain the birds and press tare on the scale so the container's weight is not included in the pen weight.

4. Place all of the birds from a single pen into the container(s) that is resting on the scale.

5. Include all of the containers that are figured into the tare weight whether they contain birds or not.

6. Wait until the scale has equilibrated and read the weight so it may be recorded on the weigh sheet.

7. Place the birds back into the assigned pen, and continue to the next pen.

APPENDIX D

Using JMP Statistical Analysis Software

1. Turn on the computer.

2. Click on the JMP icon of select JMP program under start menu.

3. Select new data table under the file menu.

4. Double click on the first column, and type in column heading; for example, average bird gain, intake, feed:gain, etc.

5. Double click on the first cell until a period appears.

6. Copy and paste data from Excel spreadsheet into the appropriate columns.

7. Select fit Y by X under the analyze menu.

8. Select parameters, dependent variable as Y-axis, independent variable as X-axis.

9. Select ok.

10. Click on the red triangle, which will pull down a list of options.

11. Select one-way ANOVA.

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