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Influence of Composition and Storage Conditions on the Concentrations of Free Fatty Acids and Peroxides in Broiler Diets

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Abstract: An experiment was conducted to determine the effects of diet composition and varying storage temperature and relative humidity (RH) on the concentrations of free fatty acids (FFA) and peroxides. Diets were supplemented with or without feed preservative mixture of Mold-Zap (a mold inhibitor) and Banox E (an antioxidant) and stored for a period of sixty days. Lipid deterioration took place in all the experimental diets after two months of storage. This was much more noticeable in diets stored in high temperature (30°C) environments and in diets devoid of the preservative mixture. Storage of feed A (low fat) at high temperature (30°C) and RH (80%) resulted in a tremendous increase in the concentration of FFA, while in feed B (high fat), Free fatty acids concentration significantly increased after sixty days. The presence of the preservative mixture significantly increased and decreased the concentrations of FFA and peroxides, respectively in feed A, while in feed B, FFA content increased and peroxide value decreased. This study clearly demonstrated the relative importance of the preservative mixture used in reducing feed deterioration in regards to oxidative rancidity. Further studies are required to assess the effects of mold inhibitors as well as antioxidants, singly or in combinations on diets high in fats.

Key words: Antioxidants, broiler diets, free fatty acids, mold inhibitor, peroxides, relative humidity

Introduction

The prevailing climatic conditions in the tropics and most parts of southern Africa within the year (temperature above 25°C and RH greater than 70%) are ideal for feed spoilage. Such conditions are usually favourable for lipid deterioration (Berger, 1989; Coppen, 1989; van den Berghe *et al*., 1990). Rancidity results in loss in quality and acceptability (Galliard, 1989), causing reduced feed intake (Sanders, 1989) in broilers (Lin *et al*., 1989). The economic implication of spoilage of livestock feeds cannot be overemphasized. Furthermore, according to Shermer and Calabotta (1985), the consequences of consuming rancid feeds may cause both acute and chronic adverse biological effects in animals. Cabel *et al*. (1988), Sanders *et al*. (1989), Sanders (1989), Hamilton (1989) and Cheeke (1999) reported the oxidation of vitamins A and E in diets at high temperature, which may result in hypo-vitaminoses A and E, respectively (Lin *et al*., 1989). Encephalomalacia is a common illness among broilers that consume feeds that have undergone severe oxidation (Cabel *et al*., 1988; Lin *et al*., 1989).

Although much work has been done in preventing fat deterioration in other parts of the world with varying degree of success, studies conducted to determine the effects of antioxidants as influenced by climate on rancidity in Southern Africa are limited. Microbial growth is favoured by an increase in internal temperature and moisture content of feedstuff (Chow, 1978). This may enhance the activity of extracellular lipase in biological materials, leading to increased lipid degradation (Kinderlerer and Kellard, 1984; Kinderlerer, 1994). There are no reports on the possibility of controlling these changes with commercial mold inhibitors. The present study is designed to present findings obtained from using a combination of antioxidant and mold inhibitor with respect to their antioxidant effects on both oxidative and hydrolytic rancidities in diets stored under varying conditions.

Materials and Methods

Experimental diets: This experiment was conducted with commercial broiler starter diets obtained from a commercial feed producing company-Epol, Pietermaritzburg, Kwazulu-Natal, South Africa. Materials were received on the same day of production. The composition of the diet is shown in Table 1. Nitrogen corrected apparent metabolizable energy (AME_n) and true metabolizable energy (TME_n) were determined on roosters (Sibbald, 1986).

Feed treatment and storage: A preservative mixture composed of a mold inhibitor (Mold-Zap; principally propionic acid, sorbic acid, tartaric acid, vermiculite and ammonium hydroxide) and an antioxidant (Banox E; principally butylated hydroxyanisole, butylated

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Table 1: Ingredient and nutrient composition of experimental diet

Ingredient (g/kg)	Feed A	Feed B
Yellow maize	615.3	556.2
Soya Hi Pro (46%)	231.5	250.0
Wheat middling	-	50.0
Sunflower oilcake	51.0	-
Fishmeal (Peruvian)	-	16.0
Fishmeal (Chilean)	72.5	20.0
Poultry byproduct	-	30.0
Limestone powder	13.0	-
Blended vegetable oil	-	10
Monocalcium phosphate	9.7	-
Bone meal	-	48.5
Salt (NaCl)	1.7	2.6
Molasses	-	9.0
Choline chloride	0.7	0.7
Premix (Starter)[1]	1.5	1.5
DL-Methionine	2.25	3.08
L-Lysine Hcl	-	1.2
Threonine	-	0.3
Cycostat (Robenidine)	0.5	0.5
Zinc bacitracin	0.4	0.4
Nutrient composition (g/kg)		
Dry matter	886.0	83.0
Crude protein	193.0	204.0
Fat	34.0	49.0
Crude fibre	36.0	28.0
Calcium	12.0	15.0
Phosphorus	7.0	8.0
Methionine	3.0	2.0
Lysine	10.0	9.0
AME_n (MJ/kg)	12.6	12.5
TME_n (MJ/kg)	13.0	12.9

1. Active ingredients (g/kg): Vitamin B_1 (1.0), B_2 (3.0), B_6 (2.5), E (25), K_3 (1.5), folic acid (1.5), choline (150.0), niacin (35.0), pantothenic acid (7.5), iodine (1.0), manganese, 50.0), copper (5.0), zinc (50.0) and iron (20.0). Other active ingredients were vitamin A (6 MIU), D_3 (2 MIU), B_{12} (10 mg), selenium (150.0 mg) and Cobalt (250 mg) (Nutec S.A. (Pty) Ltd., Pietermaritzburg, South Africa).

hydroxytoluene, propyl gallate, ethoxyquin and citric acid) at a ratio of 1:3, i.e. 250 g/750 g per tonne of feed, were obtained from Alltech Inc. and thoroughly mixed with one half of the diets. The combined use of both the antioxidant and mold inhibitor is to simultaneously prevent fat oxidation as well as inhibit mold growth, which may enhance lipid degradation through extracellular lipase production in feeds. The feeds were stored in 50 kg bags placed adjacent to one another (1 m apart) on wooden bases (10 cm high). Four environment-controlled chambers, each 12 x 4 x 8 m, at Ukulinga Research Farm, University of KwaZulu-Natal, Pietermaritzburg, South Africa, were used for storage. The four storage conditions were 15°C and 50% RH; 15°C and 80% RH; 30°C and 50% RH, and 30°C and 80% RH. The environmental conditions were adjusted to meet the requirements of the research. Room temperature and RH were monitored and adjustments made on control heaters and humidifiers where necessary 10 days prior to the commencement of storage. Data on daily temperature and RH readings were recorded between 09:00 and 11:00 (Fig. 1) with a hygro-thermometer suspended just above the bags throughout the storage period.

Laboratory analyses: The feeds were thoroughly mixed and duplicate sub-samples of each treatment were obtained, put in sealed plastic bottles at the start and again 30 and 60 days after storage. Samples were freeze-dried in liquid nitrogen and stored at -15°C until analyzed at the Department of Animal and Poultry Science, University of Kwazulu-Natal, Pietermaritzburg, South Africa. Concentrations of FFA and peroxides were determined by titration according to the methods described by AOAC (1990).

Statistical analysis: The results presented in this experiment are the averages of two measurements from one treatment. The experimental structure was a 2 x 2 x 2 x 3 factorial where data obtained were analyzed by the general linear model (GLM) of Minitab as well as multiple regression (Minitab, 1998). A one-way analysis of variance (ANOVA) was used to derive mean values, which were compared by least significant difference. Mean values were deemed to be significantly different if the level of probability was = 0.05.

Results

All the experimental diets, regardless of storage temperature and RH, deteriorated during storage. The FFA contents of the diets (basal feed A) increased ($p<0.001$, $R^2 = 0.73$) from an initial value of 3.5 to an average value of 12.5% after two months, while in feed B, it increased ($p<0.001$, $R^2 = 0.84$) from 3.8 to about 14%. The peroxide value (Pv) of the diets rapidly increased from an initial value of 0.6 to an average of 6 in feeds A ($p<0.001$, $R^2 = 0.84$) and 0.6 to 6.5 mEq O_2/kg fat in feed B ($p<0.001$, $R^2 = 0.89$), over time. The highest concentrations of FFA were obtained in diets stored at high temperature and RH chambers with or without the preservative mixture. On the other hand, high peroxide values were found in diets without inhibitors stored in high temperature and RH chambers. Storage of diets (feeds A and B) at high temperature increased ($p<0.001$) FFA content with increasing length of storage period. The rates of hydrolytic and oxidative rancidities in diets determined during the first month of storage were quite similar when compared to those in the second month. At low temperature, the rate of fat hydrolysis in diets was much slower than at high temperature.

Free fatty acid content as well as peroxide value were

Table 2: Effects of varying feed storage conditions and antioxidant inclusion on the concentration of free fatty acids (%) and peroxides (mEq O_2/kg fat) for feed A

Temp	RH	Inhibitor	Period	FFA	PV
15	50	-	1	3.5^{j}	0.6^{i}
			2	6.5^{h}	3.1^{f}
			3	8.6^{f}	5.7^{c}
		+	1	3.5^{j}	0.6^{i}
			2	5.7^{i}	1.6^{h}
			3	9.3^{ef}	4.2^{e}
15	80	-	1	3.5^{j}	0.6^{i}
			2	7.6^{g}	3.5^{f}
			3	10.9^{d}	6.0^{c}
		+	1	3.5^{j}	0.6^{i}
			2	6.7^{h}	2.5^{g}
			3	11.8^{c}	5.0^{d}
30	50	-	1	3.5^{j}	0.6^{i}
			2	10.0^{ef}	4.4^{de}
			3	8.0^{fg}	4.1^{e}
		+	1	3.5^{i}	0.6^{i}
			2	10.4^{de}	2.6^{fg}
			3	12.9^{b}	6.3^{bc}
30	80	-	1	3.5^{j}	0.6^{i}
			2	10.4^{de}	4.8^{d}
			3	15.7^{a}	8.2^{a}
		+	1	3.5^{j}	0.6^{i}
			2	10.0^{de}	3.1^{f}
			3	16.5^{a}	6.6^{b}
SEM				0.23	0.13
Source of variation					
Temp				***	***
RH				***	***
Inhibitor				***	***
Period				***	***
Temp*RH				***	***
Temp*Inhibitor				***	***
Temp*Period				***	***
RH*Inhibitor				***	***
RH*Period				***	***
Inhibitor*Period				***	***
Temp*RH*Inhibitor				***	***
Temp*RH*Period				***	***
Temp*Inhibitor*Period				***	***
RH*Inhibitor*Period				***	***
Temp*RH*Inhibitor*Period				***	***

***a,b,c,d,e,f,g,h,i,j Mean values in the same column not sharing a superscript are significantly different ***p<0.001 NS: not significant. SEM is the standard error of difference between the mean values.

Table 3: Effects of varying feed storage conditions and antioxidant inclusion on the concentration of free fatty acids (%) and peroxides (mEq O_2/kg fat) for feed B

Temp	RH	Inhibitor	Period	FFA	PV
15	50	-	1	3.8^{j}	0.6^{k}
			2	6.7^{h}	3.2^{h}
			3	10.7^{f}	6.1^{d}
		+	1	3.6^{j}	0.6^{k}
			2	5.6^{i}	2.1^{j}
			3	10.7^{f}	4.7^{f}
15	80	-	1	3.8^{j}	0.6^{k}
			2	8.3^{g}	3.6^{g}
			3	12.9^{cd}	6.1^{d}
		+	1	3.6^{j}	0.6^{k}
			2	8.3^{g}	2.6^{i}
			3	12.8^{d}	5.2^{e}
30	50	-	1	3.8^{j}	0.6^{k}
			2	10.6^{f}	4.5^{f}
			3	13.6^{c}	7.9^{b}
		+	1	3.6^{j}	0.6^{k}
			2	10.3^{f}	2.7^{i}
			3	14.3^{b}	6.5^{c}
30	80	-	1	3.8^{j}	0.6^{k}
			2	11.5^{e}	4.8^{f}
			3	16.9^{a}	8.5^{a}
		+	1	3.6^{j}	0.6^{k}
			2	10.8^{ef}	3.2^{h}
			3	17.5^{a}	6.6^{i}
SEM				0.20	0.05
Source of variationTemp				***	***
RH				***	***
Inhibitor				*	***
Period				***	***
Temp*RH				NS	NS
Temp*Inhibitor				*	***
Temp*Period				***	***
RH*Inhibitor				NS	NS
RH*Period				***	***
Inhib*Period				***	***
Temp*RH*Inhib				*	***
Temp*RH*Period				***	NS
Temp*Inhibitor*Period				*	***
RH*Inhibitor*Period				NS	NS
Temp*RH*Inhibitor*Period				*	***

***a,b,c,d,e,f,g,h,i,j Mean values in the same column not sharing a superscript are significantly different * p<0.05, ** p<0.01 & *** p<0.001 NS, not significant. SEM is the standard error of difference between the mean values.

decreased (p<0.001) by storage at low RH and temperature in both feeds. The use of the preservative mixture decreased (p<0.001) the concentrations of peroxides in feed A and B, irrespective of storage temperature and RH. On the other hand, FFA content increased and in feed A (p<0.001) and B (p<0.05), in the presence of the preservative mixture. In feed A, all the interactions tested were found to have influenced (p<0.001) feed quality but this was not the case for feed B (Table 3). Few interactive effects of inhibitor with other factors were significant in influencing quality of feed B.

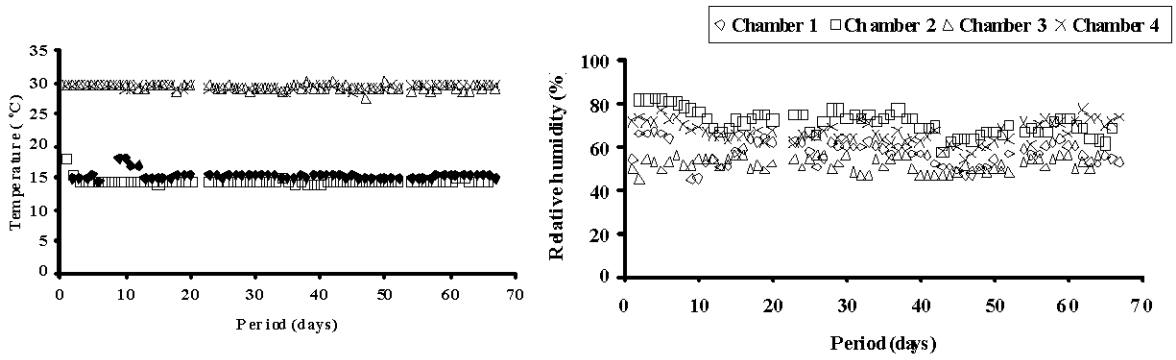

Fig. 1: Graph of daily temperature (left) with standard deviation of ± 0.63 and relative humidity (right) with standard deviation of ± 5.65 readings against storage period

Discussion

In agreement with other authors (Bautista *et al.*, 1992; Ramezanzadeh *et al.*, 1999), storage at high temperature resulted in an increase in both oxidative and hydrolytic rancidities with possible loss in quality. The higher concentrations of FFA and peroxides in diets stored at high temperature and RH in this study clearly demonstrate this hypothesis. Previous studies by Hamilton (1989), Sanders (1989), van den Berghe *et al.* (1990) and Ruiz *et al.* (2000) indicate that fats are intrinsically unstable when subjected to high temperature (=30°C). Under such conditions, fats are hydrolysed to release keto acids, which further undergo auto-oxidation with possible generation of rancid products (Hamilton, 1989).

Diets stored in high RH environments had higher concentrations of peroxides than in low RH environments, probably due to the fact that there was high potential for O_2 to diffuse into the diets with high water activity (water being absorbed from the atmosphere). The rate of lipid peroxidation is highly influenced by the presence of antioxidants (Rumsey, 1978; Cabel *et al.*, 1988) that inhibit peroxides production. The mechanism of preventing fat oxidation may be through deactivation of free radicals or possibly through rendering transition metals (pro-oxidants) unavailable during the first phase of oxidation (Decker and Zhimin, 1998).

At high temperature, it was found that the anti-oxidative strength of the preservative mixture to prevent oxidative rancidity was reduced during the last month of storage. It has been clearly demonstrated in other studies that antioxidants are themselves unstable and undergo oxidation (Paster *et al.*, 1985; Lin *et al.*, 1989; Khalil and Mansour, 1998) or even leach away (Lang, 1970) with time. This caused significant increase in the rate of peroxidation in the diets tested. This was one of the major observations made in this study.

Another observation in this study was that the presence of the preservative mixture in the diets tested failed to reduce the rate of hydrolytic rancidity irrespective of temperature and RH conditions. Antioxidants are unable to reduce the rate of FFA production (Coppen, 1989) as opposed to peroxides. In this study, most of the diets supplemented with the preservative mixture experienced a significant increase in the concentration of FFA. The presence of mold inhibitor in the preservative mixture may not have been effective in preventing the release and activity of extracellular lipase, as can be seen from the continued increase in the concentration of FFA during storage. However, this may not be detrimental to product quality except at very high concentrations, when diet palatability may be compromised. Lipids undergo such enzymatic hydrolysis in the gastro-intestinal tract (Cheeke, 1999).

The rate of lipid hydrolysis and oxidation was higher in feed B (contained 4.9% fat) than feed A (3.5% fat). Diets high in fat are less stable and undergo deterioration faster than those low in fats according to Ruiz *et al.* (2000). Free fatty acid contents and peroxide values on day 0 were quite similar in both basal feeds and their concentrations increased almost at the same rate probably due to lack of drastic differences in fat level.

Conclusion: In conclusion, this experiment showed that fats in diets deteriorated with time, which was further compounded by storage at high temperature and RH. However, addition of the preservative mixture in the diets reduced the rate of oxidative rancidity by reducing peroxide production, but failed to hinder hydrolysis. Further studies are required to test for antioxidants and mold inhibitors, singly or in combinations in high-fat diets to evaluate their efficiency in preventing feed deterioration.

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