COMPARATIVE EFFICIENCY OF DIFFERENT ANTIOXIDANTS ON FAT STABILITY IN BROILER RATIONS: THIOBARBITURIC ACID VALUES

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ABSTRACT

Thiobarbituric acid (TBA) value was significantly ($P<0.05$) affected by storage period, fat levels and antioxidants but the interaction of these factors was non significant. TBA value increased with the increase in storage period, however, the increase was relatively less during first 14 days of storage then a significant increase in TBA was observed as the storage period prolonged. Rations containing 4% fat have greater TBA value than the rations containing 2 or 3% fat. There was also a significant difference on TBA value due to antioxidant and their levels. TBA value was lower in the rations containing ethoxyquin than BHT containing rations and the rations supplemented with oxistat had greater TBA value. At higher level of any antioxidant, TBA value decreased, however, the difference between TBA values at both levels is non significant. With the increase in storage period, there was increase in TBA value at both the antioxidant level. Antioxidant had a significant effect on fat stability in TBA test. Antioxidant level at 2 and 3% fat had a non significant effect but at 4% fat level, antioxidant level had a significant effect. However, TBA values increased significantly at both levels of antioxidant with the increase in fat levels.

Key Words: Antioxidants, fat stability, broiler feeds, thiobarbituric acid value.

INTRODUCTION

For more than 30 years, fats and oils have been supplemented to commercial poultry feeds, particularly to broilers rations, to increase the energy density. The use of high energy feeds proved that added fats and oils in broiler diets improved the weight gain and feed efficiency and reduced cost of broiler production (Rowland, 1992). The quality of the fat used in feeds is important, particularly with regard to the degree of oxidative rancidity that has occurred in the fat. During storage of feed, considerable loss of fats and vitamins occur due to oxidation. The loss of vitamins can be compensated by overdosing with vitamin supplements but the rancid fats cause more damage than the benefits of additional fats or oils as source of energy. Poultry diets containing rancid fat (0.2-0.6%) was associated with high (65%) mortality, diarrhea, reduced feed intake and reduced body weight gains (Awad *et al.*, 1983).

Prevention of economic losses to dietary fats due to oxidation depends upon the presence of inherent/natural or added/synthetic antioxidants. These days the antioxidants have been adopted almost universally as preservatives in animal feeds and components there of. Vitamin E acts as natural oxidant but at high storage temperature it itself is oxidized. Synthetic antioxidants are used to prevent oxidation of fats and destruction of fat soluble vitamins, however, light, air, humidity and high temperature accelerate the spoilage process (Warraich, 1972). A number of synthetic antioxidants are now available under different trade names, each one of them is claimed to be the best. Oxidative rancidity in fat containing foods leads to the formation of malonaldehyde or derivatives of this compound. Malonaldehyde can be measured quanitatively by thiobarbituric acid (TBA) test using tetra ethoxy propane (TEP) as standard. The present research was conducted to determine the comparative efficiency of different antioxidants (Oxistat, Butylated hydroxytoluene and ethoxyquin) in broiler rations stored at high temperature (40°C) and to determine the optimum level of inclusion of antioxidants in broiler rations containing high levels of fat and stored at high temperature (40°C).

MATERIALS AND METHODS

This study was conducted in the laboratory of Animal Nutrition, Department of Animal Nutrition, University of Agriculture, Faisalabad. Three basal isocaloric and isonitrogenous broiler rations containing 2, 3 or 4 percent fat were formulated and designated as A, B or C. Proximate analysis of these rations was done before the start of the experiment. Each of these rations was supplemented with a normal (125 mg/kg) or higher (175 mg/kg) level of an antioxidant (oxistat) and called as AO_1, AO_2, BO_1, BO_2, CO_1 and CO_2 respectively.

The basic rations (A. B or C) were taken separately and supplemented. in the same way. with a normal (125 mg/kg) or higher (175 mg/kg) level of another antioxidant butylated hydroxy toluene (BHT) and called as AT_1. AT_2. BT_1. BT_2. CT_1 and CT_2 respectively. Basic rations were supplemented. separately. with the same levels of the third antioxidant (ethoxyquin) and called as AE_1. AE_2. BE_1. BE_2. CE_1 and CE_2 respectively.

Each of the eighteen mixed rations were packed in 378 small bags (about 250 gm feed per bag) of the same polyethyline material which is traditionally used for feed packing. These bags containing rations were stored in a wooden chamber fitted with a thermostatically controlled heater. The temperature of the chamber was fixed at 40°C. Triplicate bags of each type of rations were drawn at first day and then at weekly interval (up to 6th week) and analyzed for thiobarbituric acid (TBA) value (Pearson. 1976). a rancidity parameter.

Thiobarbituric acid (TBA) values of the rations containing 2. 3 or 4 percent fat. mixed with stabilizers (oxistat. butylated hydroxytoluene and ethoxyquin and stored at high temperature (40°C) were estimated as influenced by storage period i.e. 0. 7. 14. 21. 28. 35 and 42 days.

The data were subjected to statistical analysis for interpretation of results. using analysis of variance technique in completely randomized design with 7 × 3 × 3 × 2 factorial arrangement. Means of significant results were compared by Duncan's multiple range (DMR) test (Montgomery. 1997).

RESULTS AND DISCUSSION

The analysis of variance of data on TBA value is shown in Table 1. Average TBA values of rations containing various levels of fat and antioxidants (125 and 175 mg/kg) as affected by storage time and high temperature are also tabulated (Tables 1 to 5).

Mean TBA value of the rations having different levels of fat was minimum (0.523) at zero day or start of the experiment. However. the value began to increase significantly ($P<0.05$) at a considerable rate with the passage of storage period. The TBA value increased slowly in case of ration having 2% fat level. While the TBA value increased rapidly ($P<0.05$) in feeds containing the highest level of fat. During first week of storage. the TBA value increased very slowly but after that the values started increasing significantly ($P<0.05$) with the increase in storage period (Table 2). Analysis of variance showed significant ($P<0.05$) effect of fat percentage and storage period on TBA values (Table 1).

TBA values of the rations containing different antioxidants. as influenced by storage period. were minimum and had less difference at the start of the study. However. the values increased significantly ($P<0.05$) as the time of storage prolonged. All the three antioxidants had a significant ($P<0.05$) effect on the TBA values of feeds. The increase in TBA value of the rations containing three antioxidants was less pronounced ($P<0.05$) till 7th day of storage but after that it started increasing at significant rate (Table 1).

Mean TBA values of the rations containing 2. 3 or 4% fat and 3 antioxidants are shown in Table 2. The results of statistical analysis showed that antioxidants had a significant ($P<0.05$) effect on fat stability in TBA test. The TBA values increased significantly ($P<0.05$) with the increase in fat %age of the feeds containing three antioxidants. The results shown in Table 2 also indicate that the feeds containing 2. 3 and 4% fat levels and mixed with oxistat had greater TBA values than the feeds mixed with BHT. The feeds with same fat levels and mixed with ethoxyquin had lower TBA values than the other two feeds.

Different antioxidants behaved differently ($P<0.01$) as shown by analysis of variance. The highest TBA value was observed in Oxistat (0.694). then was BHT (0.650) and the lowest was in ethoxyquin (0.621) (Table 3).

Average TBA values of the rations containing 2. 3 or 4 percent fat and antioxidant at the rate of 125 and 175 mg/kg feed are shown in Table-4. The analysis of variance indicated that antioxidant level had a significant ($P<0.05$) effect on fat stability in TBA test. The increase in TBA value of feeds containing two levels of antioxidant was. however. non-significant ($P<0.05$) at 2 and 3% fat levels. The most eminent effect of antioxidant levels was observed at 4 percent fat level as the higher the antioxidant level. the lower was the TBA value.

Mean TBA values. as influenced by fat percentage. various antioxidants and different levels of these antioxidants are shown in Table-5. TBA value was minimum (0.505) at 2 percent fat with ethoxyquin at the rate of 175 mg/kg and was maximum (0.826) at 4 percent fat with oxistat at the rate of 125 mg/kg of feed. TBA values increased significantly ($P<0.05$) as the level of fat increased. It is clear from the data that in case of each antioxidant and fat level. higher the level of antioxidant. the lower the value of TBA was and vice versa. Ethoxyquin at the rate of 175 mg/kg of feed is effective in minimizing the fat oxidation.

The analysis of variance showed non-significant ($P<0.05$) effect due to the interaction of storage period. fat percentage. antioxidants and level of antioxidants on the TBA value. However, storage period, fat percentage, antioxidants and levels of antioxidants individually had significant effects on TBA values. The analysis of the data regarding the TBA values showed significant ($P<0.01$) difference due to storage period, fat levels and antioxidants but the interaction of these three factors was non significant. Some two factor level interactions (A×B, B×C) were significant ($P<0.01$)

Further analysis also showed that with the increase in the storage period there was increase in TBA value. Increase in TBA value was considerably less during first 14 days of storage but after that there was considerable increase in TBA value was observed as the storage time prolonged. It is also clear from Table 1 that with the increase in storage period there was increase in TBA value at all the fat levels, rations

Table 1: Analysis of variance of TBA values of stored rations at high temperature containing various fat and antioxidant levels.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	F-value
Factor A	6	4.349	0.725	1130.40**
Factor B	2	3.720	1.860	2900.43**
A X B	12	0.032	0.003	4.14**
Factor C	2	0.342	0.171	266.67**
A X C	12	0.013	0.001	1.73 NS
B X C	4	0.014	0.004	5.62**
A X B X C	24	0.012	0.001	0.80NS
Factor D	1	0.019	0.019	30.07**
A X D	6	0.001	0.000	0.23 NS
B X D	2	0.003	0.001	2.11 NS
A X B X D	12	0.003	0.000	0.45 NS
C X D	2	0.001	0.000	0.74 NS
A X C X D	12	0.003	0.000	0.41 NS
B X C X D	4	0.002	0.000	0.59 NS
AXBXCXD	24	0.007	0.000	0.47 NS
Error	252	0.162	0.001	
Total	377	8.684		

**= Highly significant, * = Significant, N. S.= Non-significant

A = Storage period

B = Fat levels

C = Antioxidants

D = Levels of antioxidants

Table 2: Average TBA values of rations as influence by storage period and fat percentage.

Days of storage	Fat percentage 2%	3%	4%	Mean
0	0.397^{n}	0.505^{k}	0.667^{fg}	0.523^{g}
7	0.427^{m}	0.528^{j60}	0.687^{f}	0.547^{f}
14	0.471^{l}	0.560^{i}	0.727^{e}	0.586^{e}
21	0.527^{j}	0.608^{h}	0.770^{d}	0.635^{d}
28	0.594^{h}	0.671^{fg}	0.820^{c}	0.695^{c}
35	0.661^{g}	0.742^{e}	0.880^{b}	0.761^{b}
42	0.745^{e}	0.817^{c}	0.952^{a}	0.838^{a}
Mean	0.546^{c}	0.633^{b}	0.786^{a}	0.655

Values not sharing a letter in common differ significantly ($P<0.05$)

Table 3: Average TBA values of rations as affected by fat percentage and antioxidants.

Days of storage	Antioxidants Oxistat	BHT	EQ	Mean
2	0.583^{f}	0.543^{g}	0.512^{h}	0.546^{c}
3	0.683^{d}	0.620^{e}	0.596^{f}	0.633^{b}
4	0.816^{a}	0.787^{b}	0.755^{c}	0.787^{a}
Mean	0.694^{a}	0.650^{b}	0.621^{c}	0.655

Values not sharing a letter in common differ significantly ($P<0.05$)

containing 4% fat had greater TBA value than the ration containing 2 or 3 percent fat.

Interaction of storage period and fat levels (Table 1) showed a significant difference in TBA values of the feeds containing 2, 3 or 4% fat levels at the 0 day of feed storage. This difference might be due to the presence of peroxides or total fatty acids which would have been there in the crude oil that was mixed in the rations during feed preparation. This may be the reason that the increased level of fat ultimately resulted in higher (P>0.05) TBA value in the respective feeds at 0 day of storage.

These results differ, as far as the fat levels are concerned, from the results of Ramzan (1998), who found an increase in TBA value as the storage period prolonged but the fat level had a non-significant difference on TBA value. According to him, at 2% fat level, the TBA values increased from 5.707 to 10.314 and 5.325 to 7.297 in starter and finisher rations respectively, while at 4% fat level, the values increased from 5.621 to 10.675 and 5.711 to 7.742, respectively, after 40 days of storage. These differences in TBA values, as compared to present study, might be due to the method used for the determination of TBA values or due to the fat source mixed in the rations. Crude corn oil, used in the present study, has the lower TBA value (0.43 ± 0.06) as compared to other fat sources, e.g., poultry fat (23.11 ± 8.82), commercial animal-vegetable blend (79.92 ± 17.80), etc. as reported by Squires *et al.* (1991). So higher TBA values in this study might be due to high storage temperature.

Villwock and Hartfiel (1982) also concluded that oxidation of vegetable oils, in terms of peroxide value, increased with the increasing length of storage and temperature.

The analysis showed significant difference due to antioxidants and their levels. It may also be seen (Table 4) that with the increase of antioxidant levels, the TBA value decreased. However, the difference between TBA values at both levels was non significant, but higher level (175 mg/kg) of antioxidant gave lower TBA value as compared to lower level (125 mg/kg). It is also clear that with the increase in storage period, there is increase in TBA value at both the antioxidant levels. The results also indicated that antioxidant level had a significant (P<0.05) effect on fat stability in TBA test. Antioxidant level at 2 and 3% fat had a non-significant effect but at 4% level of fat, antioxidant level had a significant effect. However, TBA values increased significantly (P<0.05) at both levels of antioxidants with the increase in fat levels.

Table 4: Average TBA values of rations as affected by fat percentage and antioxidants levels

Anitoxid-ant level (mg/kg)	Fat Percentage			Mean
	2	3	4	
125	0.552	0.637	0.797	0.662[a]
175	0.540	0.629	0.735	0.648[b]
Mean	0.546[c]	0.633[b]	0.786[a]	0.655

Values not sharing a letter in common differ significantly (P<0.05)

It may be a possibility that the effectiveness of higher level of an antioxidant than the lower level might have been due to the storage of feed at high temperature. At low storage temperature, lower level of antioxidant may be better than the higher level to help avoiding the increase in acid /TBA value.

The results of present study are in agreement with the results of Ramzan (1998), who found that with the increase in endox levels, the TBA value decreased, however, 75 and 125 mg/kg endox levels had similar effect while 175 mg/kg level gave low TBA value than others. Villwock and Hartfiel (1982) concluded that with increasing amounts of antioxidants, fat oxidation was less intensive in the mixed rations to which the vegetable fat products were supplemented.

Travis and Pilbeam (1978) concluded that santoquin at the level of 170 mg/kg was an effective antioxidant against oxidative rancidity and in the control of peroxide value from 7.36 to 2.52 in control and santoquin groups, respectively.

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Table 5: Average TBA values of rations as affected by storage period, fat percentage, antioxidants and levels of antioxidant .

Days of storage	Fat %age with antioxidants and levels of antioxidants (mg/kg)																		Mean
	2%						3%						4%						
	OXI		BHT		EQ		OXI		BHT		EQ		OXI		BHT		EQ		
	125	175	125	175	125	175	125	175	125	175	125	175	125	175	125	175	125	175	
0	0.430	0.410	0.410	0.390	0.380	0.360	0.540	0.550	0.510	0.500	0.470	0.460	0.700	0.680	0.670	0.670	0.650	0.630	0.523
7	0.460	0.440	0.430	0.420	0.410	0.400	0.570	0.560	0.530	0.520	0.490	0.500	0.730	0.700	0.690	0.680	0.670	0.650	0.547
14	0.500	0.510	0.470	0.467	0.450	0.430	0.610	0.590	0.560	0.540	0.530	0.530	0.770	0.740	0.730	0.740	0.700	0.680	0.586
21	0.570	0.560	0.520	0.533	0.500	0.480	0.670	0.650	0.600	0.580	0.580	0.570	0.810	0.790	0.780	0.760	0.760	0.720	0.635
28	0.640	0.633	0.600	0.580	0.560	0.550	0.733	0.720	0.650	0.660	0.640	0.620	0.850	0.860	0.830	0.800	0.810	0.770	0.695
35	0.710	0.690	0.660	0.640	0.630	0.637	0.810	0.820	0.730	0.710	0.700	0.680	0.930	0.900	0.890	0.850	0.880	0.830	0.761
42	0.820	0.790	0.750	0.730	0.700	0.680	0.880	0.863	0.790	0.800	0.790	0.780	0.990	0.970	0.960	0.970	0.930	0.890	0.838
Mean	0.590	0.576	0.549	0.537	0.519	0.505	0.688	0.679	0.624	0.616	0.600	0.591	0.826	0.806	0.793	0.781	0.771	0.739	0.655

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