EFFECT OF VARIOUS LEVELS OF FAT AND ANTIOXIDANT ON THE QUALITY OF BROILER RATIONS STORED AT HIGH TEMPERATURE FOR DIFFERENT PERIODS

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ABSTRACT

Broiler rations containing 2, 3 and 4% corn oil were supplemented with normal (125 mg/kg) and higher (175 mg/kg) levels of an antioxidant, santoquin. The rations were stored at high temperature (40°C) for upto 42 days. The extent of fat oxidation, in term of thiobarbituric acid (TBA) value, of stored rations was determined at weekly intervals from day one till the end of 6th week storage period. The highest TBA values (0.807 ± 0.017) were observed in rations containing 4% corn oil, whereas the lowest (0.508 ± 0.018) were recorded in the rations containing 2% corn oil. Oxidation of oil in rations increased significantly ($P<0.05$) with increasing storage period. High storage temperature also significantly ($P<0.05$) affected the oxidation of fat. Supplementation of rations with higher levels of santoquin (175 mg/kg) significantly ($P<0.05$) improved the stability of corn oil against fat oxidation in rations stored at 40°C.

Key words: Fat, antioxidant, temperature, storage time, thiobarbituric acid value.

INTRODUCTION

Poultry diet formulation is based on the concept that poultry birds eat primarily to meet their energy needs (NRC, 1994). The interaction between environmental temperature and feed intake is of utmost importance in the formulation of poultry diets for different climatic and geographical locations. Feed intake in chicken is inversely related to environmental temperature (Hurwitz *et al*., 1980). During hot summer months, feed intake of birds is substantially reduced because of more heat production in the body. Under such circumstances, adjustments need to be made in feed formulation by using more dietary fat as energy source instead of carbohydrates. Chicken fed diets containing high fat content (5-10%) deposit 10-15% more energy in their body compared with similar diets low in fat (Hurwitz *et al*., 1980). The high fat contents diet can minimize the apparent energy requirements of the birds due to the fact that fats have low specific dynamic action (Devegowda and Ramappa, 1986).

Recently, it has become a common practice to add more fat to commercial broiler diets to increase energy density (Squires *et al.*, 1991). Vegetable oils rich in polyunsaturated fatty acids (PUFA) are more commonly used in broiler diets. The use of high quality fats and oils in animal feeds is important if high levels of growth and production are to be achieved especially during the hot climate (Bishawi, 1993). The digestibility of corn oil is approximately 90% and contains high proportion of fatty acids compared to poultry fat and commercial animal-vegetable blend (Squires *et al.*, 1991).

Fats with polyunsaturated fatty acids are highly sensitive to oxidation reactions during storage and are likely to turn rancid at high environmental temperature. The presence of fats and oils in poultry rations provides a suitable medium for rancidity and feed nutrients get capacity to react with oxygen to form free radicals (Bishawi, 1993). Rancidity deteriorates the nutritive value of feeds and thus causes economic losses by adversely affecting performance and health of broiler birds (Engberg *et al.*, 1996). Award *et al.* (1983) reported that the consumption of poultry diets containing rancid fat (0.2 - 6.0%) was associated with high mortality (65%), diarrhoea, reduced feed intake and reduced body weight gains.

Rancidity can be checked by the addition of antioxidants in feeds. Antioxidants retard the oxidative spoilage while light, air and high temperature accelerate spoilage process (Warraich, 1972). In the presence of oxygen, light, heat or metals, unsaturated fatty acids are converted to fatty acid radicals which undergo further reduction, to produce more free radicals or peroxides which in turn act on fatty acids. Antioxidants inhibit oxidation by reacting with free radicals, which are

formed early in oxidation process, blocking the formation of fatty acid radicals and terminate the chain reaction. The relative protective effect of 0.02% antioxidant against oxidative rancidity was found to be dependent on storage time (Chahine, 1978). However, with increasing amounts of antioxidants within a specific storage period, fat oxidation was less intensive in mixed poultry feeds. Increase in storage temperature increased the oxidation of vegetable oils (Villwock and Hartfiel, 1982). The oxidative rancidity leads to formation of malonaldehyde or derivatives of this compound. Malonaldehydes can be measured by thiobarbituric acid (TBA) test, using tetra ethoxy propane as standard (Sinnhuber and Yu, 1958).

The beneficial effects of Ethoxyquin (EQ) as antioxidant, delaying the oxidative rancidity, have been well documented (Franic, 1985). Santoquin (Ethoxy-quin-1,2-dihydro-6-ethoxy-2,24-trimethyl-quinoline, a synthetic antioxidant approved for use in poultry feeds at 125 ppm, is a widely used feed grade antioxidant that can prevent the oxidation of lipids and lipid soluble components in the feed (Cable and Waldroup, 1988).

The present study was conducted to determine the optimum levels of antioxidant (Santoquin) in broiler diets containing high levels of added fat and stored under conditions similar to those found locally during hot environment.

MATERIALS AND METHODS

Three basal isocaloric and isonitrogenous broiler rations containing 2, 3 or 4 % corn oil were formulated (NRC, 1994) for broilers nutrient requirements. Each of these rations were supplemented with a normal (125 mg/kg) and a higher (175 mg/Kg) level of santoquin (Table 1).

Mixed rations were packed in 126 small bags of polythene, which is traditionally used for feed packing. These bags were then stored in a chamber fitted with a thermostatically controlled heater. The temperature of the chamber was fixed at 40°C. At the start of experiment, and thereafter at weekly intervals triplicate bags of each ration were drawn and analysed in duplicate for thiobarbituric acid (TBA) values as rancidity parameter (Pearson, 1976).

The data thus obtained were analysed by using analysis of variance technique in completely randomised design with 7x3x2 factorial arrangement. Means of significant results were compared by Duncan's Multiple Range test (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

The results showed significant ($p<0.05$) effect of fat percent and storage time on TBA values of rations stored at 40ºC (Table 2). An obvious difference was observed in TBA values of the rations containing different levels of fat at the start of the study, which indicates the presence of differential peroxides already present in the fat mixed with the feed. Due to that reason increased levels of fat ultimately resulted in higher TBA values in the feed with the passage of storage time.

TBA values of the feeds containing different levels of fat and supplemented with synthetic antioxidant (santoquin) were significantly affected by the storage time ($p<0.05$) at high temperature (Tables 2 and 3). TBA value was considerably less during first 7 days of storage but after that a pronounced increase was seen in TBA values with the passage of storage time and was maximum at 42 day of storage. Fat percent had a significant ($p<0.05$) effect on TBA values of the feed. Highest TBA values were found at 4 percent fat level. These results differ from the results of Ramzan (1993), who found an increase in TBA values with the increasing storage period but fat percent had a non-significant effect on TBA values. According to him, TBA values at 2% fat and 0, 75, 125 and 175 g/ton Endox were 8.868, 7.991, 7.882 and 7.90 for starter and 6.95, 6.281, 6.125 and 6.080 in finisher feeds after 40 day storage period, while at 4% fat level these values were 9.334, 8.213, 8.082 and 7.456 for starter and 7.459, 6.035, 6.483 and 6.457 respectively for finisher rations. These difference in TBA values might be due to the fat source used in the feeds. In the present study, corn oil was used that had a lower TBA value compared to other fat sources. Squires *et al.* (1991) reported that the corn oil had lowest value (0.43 ± 0.06) in TBA test compared to poultry fat and commercial animal vegetable blend. However, rapid increase in TBA value in this study might be due to high storage temperature.

TBA values of the rations containing santoquin at the rate of 125 and 175 g/ton of feed was almost similar and minimum at the start of the experiment (Table 3). However, these values increased significantly ($p<0.01$) with the passage of storage time. The level of santoquin had a significant effect ($p<0.05$) on the TBA values of rations. The lower was the level of santoquin, the higher was the TBA value. The increase in TBA value of the feeds containing two levels of santoquin was less pronounced ($p>0.05$) till 7 days of storage but after that it started increasing at a significant rate. Mean TBA values showed increasing trend with the increase in storage time. The effect of increased santoquin level was not so pronounced to control the increasing TBA values with the passage of time which might be due to the effect of high storage temperature.

Mean TBA values of the feeds containing 2, 3 and 4% fat and santoquin at 125 and 175 g/ton feed have

Table 1: Composition of experimental rations (%)

Ingredients	Rations*					
	AS_1	AS_2	BS_1	BS_2	CS_1	CS_2
Corn	31	31	28	28	25.5	25.5
Rice broken	20	20	20	20	20	20
Rice polish	3	3	3	3	3	3
Wheat bran	0.5	0.5	2	2	3	3
Cottonseed meal	6	6	6	6	6	6
Rapeseed meal	2	2	3.5	3.5	5	5
Corn gluten 60%	6	6	4	4	2	2
Soybean meal	17	17	17	17	17	17
Fish meal	8	8	8	8	8	8
Blood meal	1	1	2	2	3	3
Corn oil	2	2	3	3	4	4
Antioxidant (santoquin)	0.0125	0.0175	0.0125	0.0175	0.0125	0.0175
Di-calcium phosphate	0.75	0.75	0.75	0.75	0.75	0.75
Limestone	1.25	1.25	1.25	1.25	1.25	1.25
Molasses	1	1	1	1	1	1
Vitamin and mineral premix	0.5	0.5	0.5	0.5	0.5	0.5
Nutrients (%)						
Crude protein	23.01	23.01	23.02	23.02	22.99	22.99
Metabolizable energy (Kcal/kg)	3003	3003	2998	2998	3003	3003
Crude fiber	3.8	3.8	4.0	4.0	4.25	4.25
Calcium	1.04	1.04	1.06	1.06	1.08	1.08
Phosphorus	0.48	0.48	0.48	0.48	0.48	0.48
Lysine	1.10	1.10	1.17	1.17	1.23	1.23
Methionine	0.65	0.65	0.61	0.61	0.57	0.57

*A, B and C denotes the level of corn oil as 2, 3 and 4% respectively, whereas S_1 and S_2 indicates the levels of antioxidant (Santoquin) as 125 and 175 mg/kg of the ration.

Table 2: Average TBA values as affected by fat percentage and storage period

Days of storage	Fat percentage			Mean
	2%	3%	4%	
0	0.371 ± 0.006	0.478 ± 0.006	0.638 ± 0.007	0.495 ± 0.019^{f}
7	0.399 ± 0.006	0.499 ± 0.005	0.673 ± 0.009	0.523 ± 0.020^{f}
14	0.437 ± 0.006	0.536 ± 0.006	0.731 ± 0.016	0.568 ± 0.026^{e}
21	0.502 ± 0.010	0.580 ± 0.009	0.736 ± 0.062	0.606 ± 0.026^{d}
28	0.571 ± 0.010	0.653 ± 0.012	0.840 ± 0.018	0.688 ± 0.021^{c}
35	0.624 ± 0.007	0.686 ± 0.056	0.867 ± 0.011	0.726 ± 0.026^{b}
42	0.690 ± 0.008	0.792 ± 0.010	0.913 ± 0.008	0.798 ± 0.016^{a}
Mean	0.513 ± 0.013^{c}	0.603 ± 0.014^{b}	0.771 ± 0.014^{a}	0.629 ± 0.010

Means within a column or a row with different superscripts differ significantly (P<0.05).

been shown in Table 4. It is evident that santoquin level had a significant ($p<0.05$) effect on fat stability. The increase in TBA value of feed containing two levels of santoquin was however non significant ($p<0.05$) at 2 and 3% fat levels. The most conspicuous effect of santoquin level was observed at 4% fat level, as the lower was the santoquin level, the higher was the TBA value.

Average TBA value as influenced by santoquin (125 and 175 mg/kg.), storage time and high temperature was the minimum (0.495 ± 0.018) at the start of the experiment. However, it started increasing

(p>0.05) at a considerable rate with the passage of storage time. The increase in TBA value was less in case of feed containing 2% fat level. However, increase in TBA value was more pronounced in feeds containing the highest level of fat. The increase in TBA value was slow after first 7 days of storage, but afterwards the values started increasing at higher rate with the increase in storage time.

Increased TBA value in this study may be compared with the results of Trebusiewicz *et al*. (1980), who found that absorbency in TBA test increased from 0.095 to 0.36 and 0.39 in light and dark, and from 0.075 to 0.265 and 2.270 in starting and finisher rations. However, higher absorbency values may be due to fat source used or too much prolonged storage of feed. Desikacher (1977) reported that if freshly milled rice bran was not immediately defatted, there would be increased peroxides and TBA value within 2 or 3 weeks of storage. Villwock and Hartfiel (1982) also reported increased fat oxidation in terms of peroxide value with increasing length of storage and high temperature. TBA values of fish meal stored at high temperature increased significantly due to excessive moisture and prolonged storage (Franic, 1985).

both levels of santoquin with increasing fat percent and storage period. Ramzan (1993), also concluded that with increase in endox level, the TBA value decreased from 11.668 to 11.048, however, 75 and 125 g/ton endox level had similar effects on TBA value while 175 g/ton gave lower TBA values. The results of present the study are also in agreement with the results of Berg *et al.* (1990), who found that 0.01% level of antioxidant had significantly favorable effect on the TBA value for the starter rations at 2,4 and 6 weeks of storage. The finding of present study are also in accordance with the results of El-Lakany and March (1974), who found that 0.025 percent ethoxyquin stabilized the meal lipids against oxidative changes and malonaldehyde formation. Villwock and Hartfiel (1982) also reported that with increasing amount of antioxidants fat oxidation was less intensive in the mixed feeds. Ethoxyquin significantly improved the TBA values of liver lipids (Bartov and Bornstein, 1972). Travis and Pilbeam (1978) concluded that santoquin at the rate of 170 g/ton was effective antioxidant against oxidative rancidity and in the control of peroxide value from 7.36

Table 3: Average TBA values as affected by santoquin level and storage period

Days of storage	Santoquin level (g/ton)		
	125	175	Mean
0	0.502 ± 0.027	0.488 ± 0.027	0.495 ± 0.019 ^{g}
7	0.531 ± 0.029	0.516 ± 0.027	0.524 ± 0.020 ^{f}
14	0.580 ± 0.034	0.556 ± 0.028	0.568 ± 0.021 ^{e}
21	0.647 ± 0.036	0.566 ± 0.037	0.606 ± 0.026 ^{d}
28	0.703 ± 0.034	0.673 ± 0.023	0.688 ± 0.020 ^{c}
35	0.715 ± 0.046	0.737 ± 0.023	0.726 ± 0.026 ^{b}
42	0.807 ± 0.024	0.790 ± 0.022	0.798 ± 0.016 ^{a}
Mean	0.641 ± 0.015 b	0.618 ± 0.014 a	0.629 ± 0.010

Means within a column or a row with different superscripts differ significantly (P<0.05).

Table 4: Average TBA values as affected by fat percentage and santoquin level

Santoquin level (g/ton)	Fat Percentage			
	2	3	4	Mean
125	0.519±0.018^{d}	0.596±0.021^{c}	0.807±0.017^{a}	0.64078±0.01532^{a}
175	0.508±d0.018^{d}	0.611±0.018^{c}	0.735±0.022^{b}	0.61787±0.01387^{b}
Mean	0.513±0.013^{c}	0.603±0.014^{b}	0.771±0.010^{a}	0.62932±0.01034

Means within a column or a row with different superscripts differ significantly (P<0.05).

The results also revealed that santoquin level had a significant effect on fat stability in TBA test. Santoquin level at 2 and 3% fat had a non significant (p>0.05) effect but at 4% fat, level of santoquin had a significant effect. TBA values increased significantly (p<0.05) at

to 2.52 in control and santoquin group, respectively.

Pelevin and Novicov (1976) reported that santoquin was more active lipid antioxidant than diludin. Similarly, Cable and Waldroup (1989) reported that rancidity development in stored rice bran can be

slowed by the addition of ethoxyquin or EDTA or both. In the same lines Franic (1985) tested the effect of storage on fish meal for 4-10 months at different temperatures (18-40°C) TBA value increased from 10 to 15 during prolonged storage in feeds with and without antioxidants. Kntekhtsyan *et al.* (1985) reported that santoquin and propionic acid in grass meal stored for 8-10 months significantly decreased lipid peroxides compared to control. Bautista *et al.* (1992) reported non significant effect on TBA values in the feeds with and without antioxidants after 0, 30, 60 and 90 day storage but feed deteriorated significantly at 120th day storage. McGeachin *et al.* (1992) concluded that feed treated with Termox and santoquin had significantly lower lipid oxidation compared to control. Aziz (1997) also reported that santoquin was most effective antioxidant to control oxidation in rice polishing.

This study showed that rancidity (TBA) values significantly increased due to prolonged storage and inclusion of higher levels of fat. Santoquin level improved the stability of fat in feeds stored at high temperature. High temperature affected TBA values at an increasing rate with the passage of storage time.

REFERENCES

Award, Y. L., A. M. El-Baggouri and O. E. Mohamed, 1983. Deterioration of rancidity in practical poultry diets and related production problems. J. Egyptian Vet. Med. Assoc., 43: 401-404.

Aziz, M. 1997 Effect of different antioxidants in rice polishing on the performance of broiler chicks. M.Sc Thesis. Dept. Animal Nutrition, College of Vet. Sci., Lahore. Univ. Agri., Faisalabad.

Bartov, I. and S. Bornstein, 1972. Comparison of BHT and ethoxyquin as antioxidants in the nutrition of broilers. Poult. Sci., 51: 859-868.

Bautista, M. N., P. F. Subosa and C. R. Lavila-Pitogo, 1992. Effects of antioxidants on feed quality and growth of penaeus monodon juveniles. J. Sci. Food and Agri., 60: 55-60.

Berg, C.H.V., P.O. Ahovangninou and E.K. Deka, 1990. The effect of antioxidant and mould inhibitor on feed quality and performance of broilers under tropical conditions. Trop. Sci., 30: 5-13.

Bishawi, K., 1993. Antioxidants in poultry feeds. Zootecnica International. July/August: 44-47.

Cable, M. C. and P. W. Waldroup, 1988. Effect of ethoxyquin preservative and peroxide level on broiler performance. Poult. Sci., 67: 1725-1730.

Cable, M. C. and P. W. Waldroup, 1989. Ethoxyquin and ethylenediaminetetraacetic acid for the prevention of rancidity in rice bran stored at elevated temperature and humidity for various length of time. Poult. Sci., 68: 438-442.

Chahine, M. H., 1978. Antioxidants to stabilise fish meal. Feedstuffs, 50: 28-29.

Desikacher, H.S.R., 1977. Preservation of by-products of rice milling In: Proc. Rice By-products Utilization, Intl. Conf. Valencia, pages 1-32.

Devegowda, G. and B. S. Ramappa, 1986. The extra caloric value of fat and high energy diets for poultry. Poult. Advisor, 19: 21-23.

El-Lakany, S. and B. E. March, 1974. Chemical and nutritive changes in herring meal during storage at different temperatures with and without antioxidant treatment. J. Sci. Food and Agri., 75: 899-906.

Engberg, R. M., C. Lauridsen, S. K. Jensen and K. Jakobseon, 1996. Inclusion of oxidised vegetable oil in broiler diets. Its influence on nutrient balance and on the antioxidant status of broiler. Poult. Sci., 75: 1003-1011.

Franic, I., 1985. Control of rancidity developing in fish meals. Obiettivi e Documenti Veterinari. 6: 43-48 (Nutr. Abst. Rev., 56: 68, 1986).

Hurwitz, S., M. Weiselberg, U. Eisner, I. Bartov, G. Riesenfeld, M. Sharvit, A. Niv and S. Bornstein, 1980. The energy requirements and performance of growing chickens and turkeys as affected by environmental temperature. Poult. Sci., 59: 2290-2299.

Kntekhtsyan, A.A., L. Y. Davidchik and V. A. Pleshko, 1985. Use of a mixture of santoquin and propionic acid in stabilizing carotene in grass meal. Khimiya v Selskom Khozyaistive. 23: 30-32 (Nutr. Abst. Rev., 55: 5459, 1985).

McGeachin, R. B., L. J. Srinivasan and C. A. Bailey, 1992. Comparison of the effectiveness of the two antioxidants in the broiler type diet. J. Appl. Poult. Res., 1: 355-359 (Nutr. Abst. Rev., 63: 492, 1993).

NRC, 1994. Nutrient requirement of poultry. National Research Council. National Academy Press, Washington.

Pearson, D., 1976. The Chemical Analysis of Foods. 7th Ed. Churchill Living stone, New York USA.

Pelevin, A. and S. S. Novicov, 1976. Stabilisation of concentrate mixtures for broilers. Khimiya a sel'skom Khozyaistve, 14: 69-71 (Nutr. Abst. Rev., 47: 2455, 1977).

Ramzan, M., 1993. Effect of different levels of endox and fat on the quality of stored feed and performance of broiler chicks. M.Sc Thesis. Dept. Animal Nutrition. Univ. Agri., Faisalabad, Pakistan.

Sinnhuber, R. O. and C. T. Yu, 1958. 2-Thiobarbituric acid method for the measurement of rancidity in fishery product. Food Tech., 1: 9-11.

Squires, E. J., E. V. Valdes, J. Wu and S. Leeson, 1991. Utility of the thiobarbituric acid test in the determination of quality of fats and oils in feeds. Poult. Sci., 70: 180-183.

Steel, R. G. D. and J. H. Torrie, 1984. Principles and Procedures of Statistics: A biometerical approach. 2nd ed. McGraw-Hill Book Company, New York.

Travis, H. F. and T. E. Pilbeam, 1978. Effects of storage on vitamin E and oxidative rancidity levels of feeds. Fur. Rancher. Feb., 10-11 (Nutr. Abst. Rev., 46: 2025, 1979).

Trebusiewicz, B., B. Chelmonska., T. Gwara., D. Jamroz and A. Mazanowska, 1980. Use of the waste animal fat in feeds for broiler chickens. Stability of fats in feeds for broiler chickens. Przemyslu Praszowego, 19: 16 (Nutr. Abst. Rev., 52: 2617, 1982).

Villwock, U. and W. Hartfiel, 1982. Effect of different types and amounts of fat and antioxidants, and temperature and duration of storage on peroxide formation and content of antioxidant residues in mixed feeds. Zeitschrift fur Tierphysiologie, 48: 130-138 (Nutr. Abst. Rev., 53: 2316, 1983).

Warraich, M.A., 1972. Stability of sunflower oil as affected by antioxidants under varying conditions. M.Sc. Thesis., Univ. Agri. Faisalabad, Pakistan.