Effects of Feeding Methods (Water vs. Feed) of Vitamin C on Growth Performance and Carcass Characteristics in Broiler Chickens

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ABSTRACT: This experiment was conducted to compare the effects of vitamin C supplemented in either feed or water on the performance and carcass characteristics of broilers during the hot season. For a 6 week feeding trial, a total of 330 broiler chicks (Ross, 4 d old, average 57 g BW) were alloted to five treatments. The treatments of vitamin C (VC) supplementation were 1)0 ppm VC, 2)10 ppm VC in feed, 3) 20 ppm VC in feed, 4) 5 ppm VC in water and 5) 10 ppm VC in water. During the starter phase (0-3 week), chicks on non-supplemented group grew slower (p<0.01) than the supplemented ones, and a similar trend was also noted during finisher stage and the overall stage. Feed intake was significantly (p<0.05) higher in supplemented groups and higher when fed in feed as compared with water during all stages. But feed conversion efficiency was significantly improved in non-supplemented groups compared to supplemented ones in finisher and overall stage. The digestibility of gross energy and ether extract was significantly (p<0.05) higher during starter phase in supplemented, given in feed, and at higher levels as compared with non-supplemented, given in water, and at lower levels, respectively. The bone resistance was significantly (p<0.05) higher in supplemented, supplied in feed groups as compared with their counterparts. Except breast meat, the dressing percentage and abdominal fat were also higher in supplemented group and the dressing percentage was significantly (p<0.05) higher in VC supplemented in feed as to water, but no effect of supplementation was noticed on meat color when compared between the methods of feeding (feed vs. water). The levels of VC in plasma and liver increased linearly, as the level of supplementation both in feed and water increased and it was significantly (p<0.05) higher in feed group as compared with water group. It can be concluded that, retention and availability of vitamin C in feed was higher than those in water, and supplementation of VC during summer was beneficial for poultry. (Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 8: 1112-1117)

Key Words: Broiler, Vitamin C, Bone, Carcass

INTRODUCTION

Broilers are in continuous stress due to fast growth rate, pathogens and the ever-changing environmental conditions in the broiler houses (McCorkle and Glick, 1980). Birds have the ability to synthesize vitamin C (VC) in their body (McDowell, 2000) and hence there is no recommended requirement established by the NRC (1994). However, environmental and pathological stressors are known to alter VC utilization and synthesis in birds (Pardue and Thaxton, 1986).

Although VC synthesis in the neonatal chick is apparently limited (Horning and Frigg. 1979), it is generally assumed that the endogenous synthesis is adequate to meet biological demands in poultry. But Pardue and Thaxton (1986) accepted that during certain conditions, vitamin C (VC) supplementation provided benefit to poultry.

Researches on the availability of VC either in feed or water are scanty, and sometimes true VC levels are assumed to be higher if rates of its degradation are not considered. It is a normal practice to supplement VC either to feed or water during the period of stress. VC has proven beneficial when supplemented to feed and water during the summer stress. An attempt has been made in the present study to

compare the effects of VC either in feed or water on the performance and carcass characteristics of broilers.

MATERIALS AND METHODS

Three hundred and thirty commercial four-day-old Ross broiler chicks, weighing about 57 g/bird, were raised for a 6 week period in a room with rice hull litter material, under controlled temperature till first week, and air ventilation. The pen size was 2.0 m×2.0 m. The day-old chicks were reared with a commercial starter diet for three days followed by respective experimental diets. The room temperature was not controlled except during the first week (22-30°C) because it was summer season in Korea (June-August). Chicks had *ad libitum* access to diets and water.

Basal diets (mash type) were formulated to contain 22.4 and 20.26% crude protein for starter (0-3 week) and finisher (4-6 week). respectively, as shown in Table 1. The treatments were (1) 0 ppm. (2) 10 ppm VC in feed, (3) 20 ppm VC in feed. (4) 5 ppm VC in water and (5) 10 ppm VC in water. Vitamin C was used as Medivita CTM, which is a vitamin C-polyethylene glycol complex produced by LG Life Sciences (Seoul, Korea). This company has developed Medivita CTM in powder form, which has to be added in feed, as well as in liquid form, which has to be supplemented in water. These levels of VC in feed and water were compared considering the thumb rule and the

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Table 1. Chemical composition of basal diets

	Starter (0-3 wk)	Finisher (4-6 wk)
Ingredients (%)		
Corn	56.06	59.90
SBM (44%)	22.44	20.76
Corn gluten meal	7.00	8.00
Fish meal	6.16	3.00
Animal fat	6.00	5.70
TCP	0.92	1.12
Limestone	0.59	0.87
Vitamin premix ^t	0.10	0.10
Trace mineral premix ²	0.20	0.20
Salt	0.25	0.25
L-lysine HCl	-	0.03
DL-methionine (50%)	0.20	-
Choline chloride (25%)	0.08	0.07
Total	100.00	100.00
Chemical composition calcu	ulated (%)	
ME (keal/kg)	3,200	3,200
CP	22.04	20.26
Ca	0.90	0.90
Avail. P	0.40	0.35
Lysine	1.14	1.00
Methionine	0.53	0.40
Methionine+cystine	0.90	0.75

¹Supplied per kg diet: 9,000 IU vitamin A, 1,800 IU vitamin D₃, 10 mg vitamin E, 1 mg vitamin B₁, 10 mg vitamin B₂, 4 mg vitamin B₆, 0.02 mg vitamin B₁₂, 1 mg vitamin K₃, 12 mg pantothenic acid, 30 mg niacin, 0.03 mg biotin, 0.5 mg folic acid, 3 mg ethoxyquin.

fact that the intake of water is twice the intake of feed as was also mentioned by Leeson and Summers (1991).

For a digestibility trial, thirty chicks (6 birds per treatment) were allocated in individual cages to collect fecal samples. Starter and finisher diets containing 0.25% chromic oxide as an indigestible marker were given to chicks at the age of 15 and 35 days, respectively. Fecal samples from each bird were collected on the 4th day after feeding the respective marked diets. Feces were dried in an

air forced drying oven at 60°C for 3 days and then ground and stored for chemical analysis. Proximate composition of the samples was analyzed according to the methods of AOAC (1990). Gross energy was measured with an adiabetic bomb calorimeter (Model 1241, Parr Instrument Co. Molin, IL). VC with HPLC (Waters, Model 486, USA) and chromium with spectrometer (Jasco Co, Model V-550, Japan).

At the end of the experiment, ten chicks per treatment were sacrificed. Blood samples were taken to analyze VC contents in plasma, and the liver samples were also frozen to be used later. Chicken meat color was measured with a color difference meter (Yasuda Seiko Co. CR 310, Minolta, Japan) and compared with standard color values. Carcass traits like dressing percentage, breast meat and abdominal fat were also measured. Degree of bone mineralisation was studied with tibia. The dried (100°C, 3 h) bone samples were defatted in petroleum ether for 48 h. The right tibia of each bird was used to determine the breaking strength (EZ Test. Shimadzu, Japan), and the left tibia for chemical analysis.

The data were analyzed using the General Linear Model and contrast procedure of SAS (1985). The data were compared in tables between supplemented and non-supplemented group, between feed and water as well as among higher and lower levels.

RESULTS AND DISCUSSION

The supplemental effects of VC on the growth performance of broilers were recorded at 0-3 weeks and 4-6 weeks interval and increase in body weight was noticed at all the phases of measurements (Table 2). The BW gain was significantly (p<0.05) higher in starter, finisher and overall study in supplemented groups over the non-supplemented one. When compared between the supplementing methods, it was higher in feed supplemented groups than that of

Table 2. Effects of feeding methods (feed vs. water) of vitamin C on growth performance in broilers

	Control	F	eed	V	/ater	· SE ¹	Contrast ²		
Vit C, ppm	0	10	20	5	10	· SE		2	3
Starter (0-3 wk)									
Weight gain (g/bird)	478 ^d	504 ^{bc}	516°	498°	510 ^{ab}	3.77	0.0001	NS*	0.0094
Feed intake (g)	934 ^d	$1,009^{b}$	1.041^{a}	974°	1,001 ^b	9.65	0.0001	0.0001	0.0001
Feedigain	1.95	2.00	2.02	1.96	1.96	0.008	NS	0.0128	NS
Finisher (4-6 wk)									
Weight gain (g/bird)	899 ^d	929 ⁶	944°	919°	931 ^b	4.19	0.0001	0.0050	0.0018
Feed intake (g)	$2,283^{d}$	$2,412^{bc}$	2,433a	2,401°	$2,420^{ab}$	14.64	0.0001	0.0207	0.0010
Feed:gain	2.54°	2.60 ^{ab}	2.58 ^{ti}	2.61	2.60 ^{ab}	0.007	0.0001	0.0296	0.0296
Overall (0-6 wk)									
Weight gain (g/bird)	$1,377^{d}$	1,433 ^b	$1,460^{a}$	1,417°	1,441 ^b	7.60	0.0001	0.0008	0.0001
Feed intake (g)	3,217 ^d	3,421 ^b	3,474 ^a	3,375°	3,421 ^b	23.65	0.0001	0.0001	0.0001
Feed:gain	2.34 ^b	2.39 ^a	2.38 ^a	2.38	2.37^{a}	0.005	0.0001	NS	NS

^{*.}b.c.d Means of the same row with different superscripts significantly differ (p<0.05).

²Supplied per kg diet: 80 mg Fe. 80 mg Cu. 100 mg Zn, 120 mg Mn, 2 mg I, 0.1 mg Co. 0.2 mg Se.

¹ Pooled standard error. ² 1: non-supplement vs. supplement, 2: feed vs. water. 3: low vs. high. * Not significant (p>0.05).

Table 3. Water and feed consumption in groups fed vitamin C in water, and feed: water ratio in starter phase

Vitamin C -		Water consump	tion (kg/group)		 Feed consumption (kg/group) 	Feed:water
	1st week	2nd week	3rd week	Total	- 1 ccd consumption (kg/group)	1 cca, water
5 ppm	20	40	60	120	64.28	1:1.87
10 ppm	20	40	60	120	66.07	1:1.82

Table 4. Effects of feeding methods (feed vs. water) of vitamin C on nutrient digestibility (%) in broilers

	Control	Fe	Feed		Water		Contrast ²		
Vit C, ppm	0	10	20	5	10	· SE ¹	1	2	3
Starter (0~3 wk):									
Gross energy	76.20°	77.80°	77.86%	76.62 ^b	77.81^{a}	0.19	0.0001	0.0006	0.0006
Crude protein	73.25 ^b	72.09°	76.46°	70.51 ^d	72.23°	0.53	0.0440	0.0001	0.0001
Ether extract	58.30 ^d	67.86 ^b	72.864	66.61°	67.91 ^b	1.26	0.0001	0.0001	0.0001
Finisher (4~6 wk):									
Gross energy	78.03°	77.83ª	77.38 ^b	77.04°	77.86^{a}	0.10	0.0001	NS*	0.0272
Crude protein	71.11°	78.60^{a}	77.98°	77.01 ⁶	77.94^{a}	0.74	0.0001	0.0055	NS
Ether extract	55.67°	61.20^{a}	61.57*	60.09 ^b	61.33	0.59	0.0001	0.0233	0.0097

^{*.}b.e.d Means of the same row with different superscripts significantly differ (p<0.05).

water-supplemented groups only in finisher and overall study. No such effect was noticed during the starter phase. When compared between the low versus high levels same trend was noticed in body weights. The feed intake was significantly higher (p<0.05) in supplemented over unsupplemented, feed over water and high over low levels of VC. The feed intake was low in starter, finisher and overall phase in un-supplemented group and since the nutrient digestibility of some of the nutrients studied was also low which might have effect on the body weights in this group. Feed intake was increased (p<0.05) linearly as the VC was increased in the feed. Our findings paralleled with some reports that increased feed intake in chicks fed ascorbic acid under heat stress (Kutlu and Forbes, 1993; Kassim and Norzilla, 1995; Mckee and Harrison, 1995) since our research was conducted in hot conditions. The temperature was recorded each day at every four hours interval throughout the experiment and the minimum temperature noted was 19.3°C and the maximum temperature noted was 34.4°C and the average temperature ranged between 26-28°C in our study. But conversely the feed conversion ratio (FCR) was superior in non-supplemented group as compared with others at all the levels of measurements. The bird has adapted itself to utilize efficiently the limited resources of nutrients in this group. FCR was significantly (p<0.05) lower in the water fed groups than the feed in starter phase but it was reversed in the finisher phase and it was not significant in the overall phase. When compared between low and high levels it was significantly (p<0.05) improved only during finisher phase at higher levels. Generally there was a trend to increase weight gains when VC was added. But our results were not in agreement with the results of Sifri et al. (1977) and Pardue et al. (1985) who did not find the improved growth in the broiler chicks. Kassim and Norziha (1995) also reported that VC

supplementation in broilers improved weight gain and feed efficiency in the natural hot humid climate but we did not find any such improvement in FCR in supplemented group, rather in our study reverse was noticed and as the feed intake was higher in the supplemented group, the FCR was affected though there was significantly higher weight gains. It was noticed that as the levels of VC increased in feed and water the body weight increased showing a positive correlation but the VC supplemented in feed had better performance than supplemented in water. During summer it normally happens that the water intake increases. Additional water intake may create the problem of flushing of nutrients from the digestive tract, which may create marginal deficiencies of co-factors necessary for proper metabolic activity. It is a normal practice of supplementing vitamins in water during summer. But there is evidence in birds that the limiting factor for heat tolerance is not water consumption but the rate of evaporation of water. Once the maximum evaporation has been achieved, there is no necessity for additional water intake. In our study we were not able to pinpoint the exact cause of better performance in feed supplemented groups than the water supplemented one. The water intake in the groups where VC was supplemented in water was measured and it was noticed that the water intake was approximately twice the feed intake (Table 3) and hence the groups compared between feed and water were also justified although we have measured the water intake only up to starter phase.

The nutrient digestibility studies were conducted at 15 and 35 days of experimental feeding and the data generated are presented in Table 4. On perusal of data it was noted that in starter phase, the digestibility of all the nutrients measured was significantly (p<0.05) higher in VC supplemented group. The gross energy, crude protein and ether extract digestibility was 76.20, 73.25 and 58.30% in

¹ Pooled standard error. 21: non-supplement vs. supplement, 2: feed vs. water. 3: low vs. high. * Not significant (p>0.05).

Table 5. Effects of feeding methods (feed vs. water) of vitamin C on carcass traits and chicken meat quality in broilers

	Control	Feed		Wa	Water		Contrast ²		
Vit C, ppm	0	10	20	5	10	SE	1	2	3
Carcass traits (%)									
Dressing percentage	73.21 ^b	74,44 ^{ab}	75.02°	73.40^{6}	74.12 ^{ab}	0.24	0.0444	0.0377	NS*
Breast meat	13.13	13.24	13.45	13.05	13.23	0.14	NS	NS	NS
Abdominal fat	3.46°	2.70^{b}	2.94^{ab}	2.51 ^b	$2.67^{\rm b}$	0.11	0.0052	NS	NS
Meat color									
L	57.76	59.93	60.01	59.01	59.46	0.37	NS	NS	NS
a	9.92^{b}	11.20 ^a	11.61^{a}	10.91^{a}	11.13	0.18	0.0008	NS	NS
Ъ	8.51	9.17	9.19	8.21	9.24	0.18	NS	NS	NS

^{8.6} Means of the same row with different superscripts significantly differ (p<0.05).</p>

Table 6. Vitamin C contents in plasma and liver in broilers as affected by feeding methods (feed vs. water) of Vitamin C

	Control	Feed		W	Water		Contrast ²		
Vit C, ppm	0	10	20	5	10	- DL	1	2	3
Plasma (mg/dl)	0.76 ^d	1.08 ⁶	1.28ª	1.02°	1.10 ^b	0.05	0.0001	0.0001	0.0001
Liver (ppm)	28.71 ^d	51.14 ⁶	54.76°	48.51°	50.96 ^b	2.47	0.0001	0.0001	0.0001

a.b.c.d Means of the same row with different superscripts significantly differ (p<0.05).

non-supplemented group which was significantly lower as compared with 77.86, 76.46 and 72.86% in 20 ppm fed VC level, respectively. VC supplemented in feed showed higher (p<0.05) nutrient digestibilities as compared with that supplemented in water which has ultimately reflected in the higher weight gains in these groups when compared with their counterparts. Comparison between VC levels also showed lower values at low levels of supplementation. Same trend of higher nutrient digestibilities in VC supplemented groups over non-added groups was also noticed in finisher phase. Except gross energy, the digestibility of CP. EE was higher (p<0.05) in feed supplemented groups than when VC was added in water. The CP digestibility was comparable when compared between the higher and lower levels and the digestibility of gross energy and ether extract were significantly (p<0.05) higher at higher levels as compared to lower levels. The improvement in the nutrient digestibilities had culminated into the increased weight gains in supplemented groups but however there was no evidence why nutrient digestibility was improved when VC was added in the diet. Sahin and Kucuk (2001) also reported greater (p<0.02) digestibility of DM. OM. CP and EE when fed higher dietary vitamin C at the level of 200 mg/kg diet to Japanese quails reared under chronic heat stress (34°C). Sahin and Sahin (2002) found increased N retention and decreased N excretion of laving hens reared at a low ambient temperature (7°C) when fed VC at a level of 250 mg/kg diet when compared with nonsupplemented diet. Scanty reports are available with respect to effect of VC on nutrient digestibility.

Ten chicks in each group were subjected to cervical dislocation in order to study the effect of VC supplementation on carcass traits. The data generated are presented in Table 5. The data revealed that dressing

percentage was significantly (p=0.0444) supplemented groups than non-added group, but dressing percentage was significantly (p<0.05) higher when VC was supplemented in feed as compared with water The abdominal fat percent was supplementation. significantly (p=0.0052) higher in non-supplemented group than supplemented one. Breast meat and abdominal fat was not influenced by dietary VC supplementation either in water or feed when compared. The same non-influential effect was also noted when higher and lower levels were compared. The effect of VC on meat color revealed nonsignificant higher values in supplemented groups when compared with standard colors but the 'a' value for redness was significantly (p<0.05) higher in supplemented groups and 'b' value for brownness remained unaffected. The 'a' values was linearly, though non-significantly, increased as the level of VC increased both in feed and water. This means that the color stability was improved by the addition of VC as an antioxidant agent. The 'b' value was numerically lower in the non-supplemented diets and little higher in the supplemented groups. It was suggestive of lipid peroxidation has somewhat progressed. This mean that muscle oxymyoglobin oxidised in the presence of oxidising lipids and undesirable brown discoloration results which was not prevented by the supplemental VC levels. No significant effect of VC on storage or meat color of breast meat was noticed when methods of supplementation (feed vs. water) or higher versus lower levels were compared for all L, a, and b values.

The data of VC content in plasma and liver revealed a significantly (p<0.05) higher levels in VC supplemented diets as compared with un-supplemented one (Table 6). Plasma ascorbic acid level in the fowl was easily influenced by exogenously administered ascorbic acid (Pardue and

¹ Pooled standard error. ² 1: non-supplement vs. supplement, 2: feed vs. water, 3: low vs. high. * Not significant (p>0.05).

¹ Pooled standard error. ² 1: non-supplement vs. supplement, 2: feed vs. water, 3: low vs. high.

Table 7. Effects of feeding methods (feed vs. water) of vitamin C on tibia characteristics and its composition in broilers

	Control Feed		Wa	ter	SE ¹	Contrast ²			
Vit C, ppn	n 0	10	20	5	10	3L	l	2	3
Tibia characteristics									
Resistance (kg)	17.18 ^d	21.61 ^b	25.68°	20.13°	21.11 ^b	0.73	0.0001	0.0001	0.0001
Chemical composition ((%)								
Dry matter	76.48	76.97	77.17	74.94	76.48	0.25	NS*	0.0015	0.0063
Crude Ash	46.21°	55.94ª	55.62°	52.14 ^b	54.18 ^a	0.98	0.0001	0.0002	0.0170
Calcium	17.00°	19.94°	18.51 ^b	17.88 ^b	19.63	0.30	0.0001	NS	NS
Phosphorus	7.98	8.45	8.39	7.94	8.03	0.14	NS	NS	NS

 $^{^{}a.b.\circ}$ Means of the same row with different superscripts significantly differ (p<0.05).

Thaxton, 1986). The VC levels in plasma were 0.76, 1.08, 1.28, 1.02 and 1.10 mg/dl in un-supplemented, 10 and 20 ppm in feed and 5 and 10 ppm in water, respectively and that of liver were 28.71, 51.54, 54.76, 48.51 and 50.96 ppm respectively. The plasma ascorbic acid levels were within the range as measured by Mckee et al. (1997) in their studies with broilers fed 0 and 400 ppm ascorbic acid. In our study, as the level of supplementation increased both in feed and water, the levels in plasma and liver also increased linearly. A significant positive trend was noticed with the levels supplied and their levels in plasma and liver. When feed and water supplemented groups were compared with respect to plasma and liver VC content, higher values were noted in feed supplemented groups and same case was noticed when compared between higher and lower levels since the bio-availability in feed was improved which may be because of more stability of product in the feed form than that of water. Though we have not measured the content of VC in feed and water after supplementation but the reason for such results remained obscure or the conditions required for better absorption of VC and its bioavailability was more severely affected in water supplemented groups because of its quick flushing from digestive tract. This might have been the reason of lower availability of VC in water supplemented groups. Because ascorbic acid is actively transported into tissues and its utilization increases during period of stress like heat, the birds synthetic capacity may become inefficient reducing plasma ascorbic acid concentrations which was found in unsupplemented groups. The efficacy of supplementing birds with VC under stressful conditions therefore depends on its ability to elevate plasma ascorbic acid concentrations. thereby preventing tissue depletion. Ascorbic acid either decreases heat load by lowering heat production or increase heat loss by influencing avenues of thermal exchange between the body and the environment as suggested by Chang et al. (1993). It has also been postulated that VC ameliorates the ability of birds to perceive higher heat loads (Njoku et al., 1990).

The bone breaking strength of tibia was significantly (p<0.05) increased when the VC was supplemented either

in feed or water as was found in the Table 7. The maximum strength was recorded as 25.68 kg in 20 ppm VC supplemented in feed treatment followed by 21.61, 21.11. 20.13 and 17.18 kg in 10 ppm (feed) and 10 ppm, 5 ppm (water) and 0 ppm supplemented groups respectively. A positive correlation was noted with respect to bone breaking strength with the levels supplemented. As the levels increased the strength increased. Vitamin C is necessary for bone development and egg shell quality, as a co-factor for the bio-conversion of vitamin D3 to its active form of 1.25 (OH)₂D₃ (McDowell, 2000). Sergeev et al. (1990) reported first time that ascorbic acid plays a critical role in vitamin D metabolism and binding in guinea pigs. The effects occur both at a level of vitamin D hormone formation in kidneys and it's binding in target tissue. Significant higher strength was also noticed in feed supplemented group than water supplemented ones and at higher levels than the lower levels. This showed the availability status of VC in bone. From this data it can be revealed that VC in the feed form was more bio-available for the synthesis of collagen in the bone and hence the strength must have been increased. Vitamin C has been reported to be required for hydroxylation of proline residues necessary for the synthesis of procollagen, which is a precursor to bone formation (Leeson and Summers. 2001). The chemical composition of the tibial bone showed non-significant difference in dry matter and phosphorus content when compared between supplemented and un-supplemented groups (Table 7). But calcium and crude ash was significantly (p<0.05) higher in VC supplemented group as compared to its counterpart. Bone calcium and phosphorus were comparable when VC was supplemented in either feed or water or when compared between higher and lower levels. But dry matter and crude ash was significantly higher in feed supplemented groups than in water supplemented groups and in higher levels fed group when compared to lower levels. Since the intake and digestibility of the nutrients was higher in VC supplemented groups the bioavailability and its content increases in the bone and same was also reflected in the plasma and liver content of vitamin C (Table 6).

¹ Pooled standard error. ² 1: non-supplement vs. supplement, 2: feed vs. water, 3: low vs. high. * Not significant (p>0.05).

From the above results it could be concluded that 20 ppm vitamin C as Medivita CTM in feed was beneficial to improve growth performance and carcass traits in broilers during heat stress.

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REFERENCES

- AOAC. 1990. Official Method of Analysis (15th ed). Association of Official Analytical Chemists. Arlington, VA.
- Chang, K. C., W. S. Chong, D. Sohn, B. H. Kwon, I. J. Lee, C. Y. Kim, J. S. Yang and J. I Joo. 1993. Endothelial potentiation of relaxation response to ascorbic acid in rat and guinea pig thoracic aorta. Life Sci. 52:37-42.
- Horning, M. P. and M. Frigg. 1979. Effects of age on biosynthesis of ascorbate in chicks. Archy. Fur. Gefluegel, 43:108-112.
- Kassim, H. and J. Norziha. 1995. Effects of ascorbic acid (vitaminC) supplementation in layer and broiler diets in the tropics.Asian-Aust. J. Anim. Sci. 8:607-610.
- Kutlu, H. R. and J. M. Forbes. 1993. Effect of changes in environmental temperature on self-selection of ascorbic acid in coloured feeds by broiler chicks. Proceedings of the Nutrition Society, 52:29A.
- Leeson, S. and J. D. Summers. 1991. Commercial Poultry Nutrition. (1st Edition). University Books Publication, Guelph, Canada.
- Leeson, S. and J. D. Summers. 2001. Nutrition of the Chicken. (4th Edition). University Books Publication, Guelph, Canada.
- McCorkle, F. M. and B. Glick. 1980. The effect of ageing on immune competence in the chicken: antibody mediated immunity. Poult. Sci. 59:669-672.
- McDowell, L. R. 2000. Vitamins in Animal and Human Nutrition. (2nd Edition). Iowa State University Press, Ames, Iowa.

- Mckee, J. S. and P. C. Harrison. 1995. Effects of supplemental ascorbic acid on the performance of broiler chickens exposed to multiple concurrent stressors. Poult. Sci. 74:1772-1785.
- Mckee, J. S., P. C. Harrison and G. L. Riskowski. 1997. Effects of supplemental ascorbic acid on the energy conversion of broiler chicks during heat stress and feed withdrawal. Poult. Sci. 76:1278-1286.
- Njoku, P. C., C. C. Whitehead and M. A. Mitchell. 1990. Heat stress and ascorbic acid effects on the production characteristics of chickens under controlled and uncontrolled temperature conditions. pp. 251-261 in: Ascorbic acid in domestic animals. (Ed. C. Wenk, R. Fenster and L. Volker). Schriftenreihe aus dem, Institut fur Nutztierwissen Schaften, Gruppe Emahrung. Kartause Ittingen, Switzerland.
- National Research Council. 1994. Nutrient requirements of Poultry. 9th rev. ed. National Academy Press, Washington, DC.
- Pardue, S. L., J. P. Thaxton and J. Brake. 1985. Influence of supplemental ascorbic acid on broiler performance following exposure to high environmental temperature. Poult. Sci. 64:1334-1338.
- Pardue, S. L. and J. P. Thaxton. 1986. Ascorbic acid in poultry: A review. World's Poult. Sci. J. 42:107-123.
- Sahin, K. and O. Kucuk. 2001. Effects of vitamin C and vitamin E on performance, digestion of nutrients and carcass characteristics of Japanese quails reared under chronic heat stress (34°C). J. Anim. Physiol. Anim. Nutr. 85:335-342.
- Sahin, K. and N. Sahin. 2002. Effects of chromium picolinate and ascorbic acid dietary supplementation on nitrogen and mineral excretion of laying hens reared in a low ambient temperature (7°C). Acta Vet. Brno. 71:183-189.
- SAS. 1985. SAS. User's Guide: Statistics, SAS Inst. Inc. Cary. NC.
 Sergeev, I. N., Y. P. Arkhapchev and V. B. Spirichev. 1990.
 Ascorbic acid effects on vitamin D hormone metabolism and binding in Guinea pigs. J. Nutr. 120:1185-1190.
- Sifri, M., F. H. Kratzer and L. C. Norris. 1977. Lack of effect of ascorbic and citric acids on calcium metabolism of chickens. J. Nutr. 107:1484-1492.