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Effects of Dietary Vitamin C on Growth Performance, Meat Quality, Immune Function and Anti-Oxidative Capacity of Broilers

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Abstract: This study was intended to assess effects of dietary vitamin C supplementation on growth performance, meat quality, immune function as well as anti-oxidative capacity of broilers. A total of 240, 1 day old Avian broiler chicks divided randomly into three treatments, each of which was composed of eight replicates with ten birds and birds fed a corn-soybean meal basal diet supplemented with 0, 150, 300 mg kg^{-1} vitamin C, respectively. Results showed that dietary vitamin C addition could significantly increased average daily weight gain and feed intake of the broilers ($p<0.05$) but did not have significant effects on their feed to gain ratio; compared with the control treatment, dietary vitamin C addition at 300 mg kg^{-1} could significantly increased the pectoral muscle percentages, abdominal fat percentages and liver weight ratio of broilers ($p<0.05$). Dietary vitamin C additions could significantly increase pectoral muscle b* and leg muscle L* of the broilers and that significantly improved the water holding capacity and tenderness of their leg muscles ($p<0.05$). The thymus index, bursa of Fabricius index, Newcastle disease antibody level of broilers were much higher where vitamin C was added at 300 mg kg^{-1} than in the control treatment indicating that immunity of broilers got effectively improved ($p<0.05$). Dietary vitamin C addition at 300 mg kg^{-1} significantly increased the vitamin C content in blood serum and pectoral muscles ($p<0.05$), also superoxide dismutase and glutathione peroxidase activities and total anti-oxidative capacity in blood serum significantly increased ($p<0.05$), so that dietary vitamin C addition was helpful to eliminating free radicals and raising anti-oxidative capacities of broilers. Dietary vitamin C addition at 300 mg kg^{-1} significantly slowed down lipid per-oxidation and color variation of pectoral muscles while the muscles were refrigerated at 4°C, so that it took effect along with anti-oxidative enzymes to inhibit oxidation-caused meat quality deterioration, stabilize meat colors and prolong meat shelf life. Results of the present study indicated that dietary vitamin C addition at 150 mg kg^{-1} performed better in improving growth performances and immune functions of broilers and dietary vitamin C addition at 300 mg kg^{-1} performed better in improving body and muscle anti-oxidative capacity, reducing lipid per-oxidation in meat storage and prolonging meat shelf life.

Key words: Vitamin C, growth performance, meat quality, immune response, anti-oxidative capacity

INTRODUCTION

In the past several decades when broiler industry has developed very quickly, human consumption for broilers has ranked the second next to pork so that it has become one of the most important and cheapest foods derived from animals worldwide. Nonetheless, unsaturated fatty acids make up high proportion of fat in broiler meat and are extremely liable to lipid preoxidation in storage, processing and cooking which causes meat quality of broilers to deteriorate. Consuming such meat products can increase the risks of diabetes, obesity and cardiovascular diseases in human beings. Improving anti-oxidative conditions and muscle anti-oxidative characters of broilers by feed manipulation is thought as an effective approach for tackling the problems above mentioned.

Vitamin C is the common name for L-ascorbic acid. In humans or animals, the principal role of this molecule is to scavenge reactive oxygen species due to its antioxidant capacity and to serve as cofactor for many enzymes. So, that vitamin C is an essential nutrient for developments, productions and reproductions of living things. Researches show that vitamin C has many physiological

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functions and chicken can employ it to synthesize steroid hormones and amino acids and carry out mineral metabolisms and maintain immune functions. It is capable of promoting antibody formation and white cell phagocytosis capacity by participating in synthesizing dopamine and norepinephrine to enhance immune capacity and reduce disease incidence and thus improve productive capacity and feed efficiency (Zulkifli *et al*., 2000; Young *et al*., 2003; Sahin *et al*., 2003; Panda *et al*., 2008).

Researches show that like VE and VA, VC is a natural antioxidant, able to safeguard cell membrane wholeness so that it plays an important role in somatic anti-oxidative capacity, meat product shelf life and meat quality (Li and Wang, 2005). Currently, frozen fresh broiler's cuts are the major form of broiler meat in China and as result it is of great importance to improve their qualities while they are stored and processed. However, there have been no effective preserving techniques for meat and meat products developed in China. Given this, the study was aimed at investigating effects of dietary vitamin C additions on productive performance, meat quality, immune function and anti-oxidative characters of broilers in order to provide basis for developing anti-oxidizing techniques in broiler industry through feed nutritional regulation.

MATERIALS AND METHODS

Experimental design and bird husbandry: Three dietary treatments that differed in the vitamin C supplementation were used in this study, diets were based primarily on corn, soybean meal and were formulated to meet or exceed NRC nutrient recommendations. Diets were offered in two feeding phases, starter and grower, from 0-21 and 22-42 days of age. Table 1 displays the ingredient and nutrient composition of broilers. The treatments were supplemented vitamin C 0, 150, 300 mg kg^{-1}, respectively. A total of 240, 1 day old Avian broiler chicks divided randomly into three treatments, each of which was composed of eight replicates with ten birds. The VC was of feed grade which purchased from Shanghai Zhongxi Sanve Pharmaceutical Co., Ltd. which contained ω = 95% L-ascorbic acid.

The birds were raised in tiered cages and feed and water were provided *ad libitum*. The photoperiod was set at 22L:2D throughout the whole experimental period. Room temperature was at 35°C at the 1st day and gradually reduced to 25°C by the end of 3rd week. Relative humidity was maintained at about 65%. As required, the vaccination was done as follows: the vaccination with ND-IB combined vaccines by nose dripping were done at the age of 6 days, the vaccination with the Infectious Bursal Disease Vaccine, Live (Strain B87) was done by nose dripping at the age of 14 and 21 days, respectively; the vaccination with ND-IB combined vaccines were done again at the age of 28 days.

Table 1: Composition of basal diets

Ingredients (g kg^{-1})	1-21 days	22-42 days
Corn	608.70	659.70
Soybean meal	300.10	242.80
Corn oil	9.10	14.00
Corn gluten meal	30.00	40.00
Dicalacium phosphate	20.10	13.90
Limestone	12.50	13.00
Salt	3.80	3.30
Choline	1.00	1.80
Lys	1.90	1.00
Met	2.90	0.50
Premix	10.00	10.00
Total	100.00	100.00
Nutrient level		
ME (MJ kg^{-1})	12.15	12.57
CP (g kg^{-1})	200.00	186.00
Ca (g kg^{-1})	10.00	9.00
AP (g kg^{-1})	4.50	3.50
Lys (g kg^{-1})	11.00	9.00
Met (g kg^{-1})	5.86	3.50
Met+Cys (g kg^{-1})	9.00	8.60

The premix (per kg feed) contains (per kg feed): 11000 IU vitamin A, 3740 IU vitamin D_3, 5.1 IU vitamin K_3, 2.2 mg vitamin B_1, 6.6 mg vitamin B_2, 13.5 mg calcium pantothenate, 44 mg nicotinic acid, 1.1 mg folic acid, 0.2 mg biotin, 108 mg Mn, 100 mg Fe, 88 mg Zn, 9.6 mg Cu, 0.3 mg I and 0.23 mg Se

Measurement parameters

Growth performance: At the end of each experiment week, the empty stomach broilers (kept to have empty stomachs for 12 h and free to drink water and weighed at 8:00 am in the following day) and the remaining feeds of all the replicates of all the treatments were weighed and that their feed consumptions were recorded at the same time and these data were employed to calculate their average daily feed intake, weight gain and feed to gain ratio.

Slaughtering parameters: At the age of 42 days, one broiler was taken from each replicate after 12 h fasting, its blood was collected from its wing veins and the broiler was slaughtered. The collected blood was centrifuged at 3000 r min^{-1} for 10 min to isolate its serum which was stored at 0-4°C for future measurement. The pectoral muscle and leg muscle of the slaughtered broiler were stripped and carcass percentage, eviscerated carcass percentage, semi-eviscerated carcass percentages and pectoral muscle percentage and leg muscle percentages and abdominal fat percentage were measured.

Immune function parameters: The thymuses, spleens, bursa of Fabricius, ceca tonsils were taken down and

weighed and its immune organ indexes were measured. The ND-AB in serum were measured (by haemagglutination inhibition or HI).

Meat quality parameters: Meat quality parameters in pectoral and leg muscles including water loss percentage, shear force, pH and meat colors (L*, a* and b*) were measured.

Anti-oxidative parameters: The vitamin C, Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-PX), total Anti-Oxidative Capacity (T-AOC) and Malondialdehyde (MDA) of the serum and pectoral muscles were measured by means of the test kit purchased from Nanjing Jiancheng Bioengineering Institute following the use guide of the test kit.

Shelf life of meat product: After the pectoral muscles were stored at 4°C for 0, 2, 4, 6 and 8 days, their Malondialdehyde (MDA) contents and meat color values (L*, a* and b*) of the pectoral muscles were separately measured, respectively. The meat color parameters (L*, a* and b*) were employed to calculated the total meat color variance (ΔE) of the pectoral muscles:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

$$\Delta L^* = L^*_n - L^*_0; \Delta a^* = a^*_n - a^*_0;$$

$$\Delta b^* = b^*_n - b^*_0 \quad (n = 2, 4, 6, 8)$$

Data processing and statistical analysis: MS Excel and SPSS.V 16.0 were employed to process the data which were expressed in the form of M±SE, the one-way ANOVA was adopted to carry out the analysis of variance and Duncan's multiple range test was adopted to test the difference significances.

RESULTS

Growth performance: Table 2 tells that the average daily weight gains of the broilers got significantly increased by 15.47 and 10.96% when VC was added at both 150 and 300 mg kg^{-1} compared to the control treatment, respectively ($p<0.05$) and did not significantly differed between the VC added treatments ($p>0.05$). The average daily intake of broilers got significantly increased by 4.18% when VC was added to their feeds at 150 mg kg^{-1} ($p<0.05$) and got increased but not significantly when VC was added to their feeds at 300 mg kg^{-1} ($p>0.05$) and did not differed significantly between the VC added treatment. The feed to gain ratio of broilers did not get affected when VC was added to their feeds ($p>0.05$), the feed to gain ratios slightly decreased in the VC added treatments compared to the control treatment ($p>0.05$).

Table 2: Effects of dietary vitamin C on growth performance of broilers

Vitamin C (mg kg^{-1})	Daily gain (g)	Daily feed intake (g)	Feed:Gain
0	48.74±2.01^{b}	104.79±11.95^{b}	2.15±0.20
150	56.28±1.96^{a}	109.18±10.21^{a}	1.94±0.14
300	54.08±2.14^{a}	107.08±9.140^{ab}	1.98±0.21

^{a,b}Means within a column without a common superscript differ significantly ($p<0.05$)

Slaughtering parameters: It can be seen from Table 3 that the carcass percentage of the broilers increased but not significantly ($p>0.05$) when VC was added at 150 and 300 mg kg^{-1} compared to the control treatment and did not significantly differ between the VC added treatments ($p>0.05$). The semi-eviscerated carcass percentage and eviscerated carcass percentages of broilers did not get significantly affected when VC was added at both 150 and 300 mg kg^{-1} ($p>0.05$) but tended to increase as the VC added rates increased. The pectoral muscle percentage of broilers significantly increased by 12.49% when Ve was added to their feeds at 300 mg kg^{-1} ($p<0.05$) compared to the control treatment and somewhat increased but not significantly when VC was added to their feeds at 150 mg kg^{-1} ($p>0.05$) and did not differ significantly between the VC added treatments ($p>0.05$). The leg muscle percentage of broilers did not get affected when VC was added to their feeds at 150 and 300 mg kg^{-1} ($p>0.05$) but the leg muscle percentage in the VC added treatments tended to increase compared with the leg muscle percentage in the control treatment. The abdominal fat percentage of broilers significantly increased by 6.44 and 7.58% when VC was added to their feeds at 150 and 300 mg kg^{-1}. Compared to the control treatment, respectively ($p<0.05$) and did not significantly differ between the VC added treatments ($p>0.05$). The liver weights of the broilers significantly decreased by 16.19 and 15.62% where VC was added to their feeds at 150 and 300 mg kg^{-1} compared to the control treatment, respectively ($p<0.05$) and did not significantly differ between the VC added treatments ($p>0.05$).

Table 3: Effects of dietary vitamin C on carcass yield of broilers

	Vitamin C (mg kg^{-1})		
Items (%)	0	150	300
Carcass percentage	93.21±0.23	94.48±0.48	93.42±0.60
Semi-eviscerated carcass percentage	86.10±0.63	86.25±0.76	88.65±0.78
Eviscerated carcass percentage	73.11±0.79	73.21±0.62	74.08±0.92
Pectoral muscle percentage	10.09±0.32^{b}	11.12±0.37^{ab}	11.35±0.36^{a}
Leg muscle percentage	9.38±0.170	9.98±0.35	9.71±0.30
Abdominal fat percentage	2.64±0.100^{a}	2.47±0.37^{b}	2.44±0.43^{b}
Liver rate	3.52±0.240^{a}	2.95±0.13^{b}	2.97±0.25^{b}

^{a,b}Means within a column without a common superscript differ significantly ($p<0.05$)

Meat quality: It can be seen from Table 4 that the water loss percentage of the broilers did not differed among the treatments but tended to increase where VC was added to their feeds at 300 mg kg^{-1} compared to the control treatment. The meat tenderness of the broilers did not get significantly affected when VC was added at 150 or 300 mg kg^{-1} ($p>0.05$) but the meat tenderness of the broilers tended to increase when VC was added at 150 or 300 mg kg^{-1}. The pectoral muscle pH of broilers did not significantly varied but tended to decline when VC was added at 150 and 300 mg kg^{-1} ($p>0.05$), compared to the control treatment. The pectoral muscle L* and a* of the broilers did not significantly affected but presented an increasing tendency when VC was added at 150 and 300 mg kg^{-1} ($p>0.05$) while the pectoral muscle b* of broilers significantly increased by 18.87 and 12.38% when VC was added at 150 and 300 mg kg^{-1}. Compared to the control treatments ($p<0.05$), respectively and did not significantly differ between the VC added treatments ($p>0.05$).

The water loss percentage of leg muscle of broilers got significantly affected, decreasing by 20.98 and 26.40% when VC was added at 150 and 300 mg kg^{-1} ($p<0.05$), respectively while did not significantly differ between the VC added treatments ($p>0.05$). The shear forces of leg muscle of broilers got significantly affected, decreasing by 16.48 and 15.72% when VC was added at 150 and 300 mg kg^{-1}, respectively ($p<0.05$) but did not significantly differ between the VC added treatments ($p>0.05$). The pH of leg muscle of broilers did not get significantly affected but tended to decrease when VC was added at 150 and 300 mg kg^{-1} ($p<0.05$). The leg muscle L* of broilers significantly decreased by 11.88% where VC was added at 150 mg kg^{-1} compared to the control treatment ($p<0.05$) and tended to decrease but not significantly when VC was added at 300 mg kg^{-1} ($p>0.05$). The leg muscle a* and b* of broilers did not get significantly affected when VC was added at 150 and 300 mg kg^{-1} ($p>0.05$).

Table 4: Effects of dietary vitamin C on meat quality of broilers

	Vitamin C (mg kg^{-1})		
Items	0	150	300
Pectoral muscle			
Water loss (%)	12.77±0.64	12.89±0.16	12.17±0.41
Shear force (N)	18.55±0.93	19.59±1.02	16.07±1.29
pH	6.34±0.06	6.09±0.07	6.25±0.10
L*	45.29±1.43	45.46±0.55	48.11±1.64
a*	11.16±0.38	10.41±0.45	11.68±0.84
b*	20.83±0.75^{b}	24.76±0.56^{a}	23.41±0.55^{a}
Leg muscle			
Water loss (%)	22.31±1.24^{a}	17.63±1.38^{b}	16.42±1.46^{b}
Shear force (N)	27.86±1.13^{a}	23.27±1.26^{b}	23.48±1.54^{b}
pH	6.53±0.08	6.38±0.08	6.46±0.02
L*	52.29±1.91^{a}	46.08±2.01^{b}	47.67±0.85^{ab}
a*	12.32±0.33	12.62±0.60	11.30±0.39
b*	21.12±0.91	22.30±0.41	22.51±0.46

^{a,b}Means within a column without a common superscript differ significantly ($p<0.05$)

Immune function parameters: It can be seen from Table 5 that the thymus index of the broiler was significantly affected increasing by 15.73 and 10.09% when VC was added at 150 and 300 mg kg^{-1}, respectively ($p<0.05$) and did not significantly differ between the VC added treatments ($p>0.05$). The bursa of Fabricius index of the broilers got significantly increased by 23.66% when VC was added at 300 mg kg^{-1} ($p<0.05$), tended to get increased but not significantly when VC was added at 150 mg kg^{-1} ($p>0.05$) and did not significantly differ between the VC added treatments ($p>0.05$). The spleen index and caecum and tonsil index of the broilers did not get significantly affected but tended to decline when VC was added at 150 or 300 mg kg^{-1} ($p<0.05$. The blood serum ND-AB of the broilers got significantly affected, increasing by 15.38% when VC was added at 300 mg kg^{-1} ($p<0.05$) did not get significantly affected but tended to increase when VC was added at 150 mg kg^{-1} and did not significantly differ between the VC added treatments ($p>0.05$).

Anti-oxidative capacity of blood serums: It can be seen from Table 6 that blood serum VC content of the broilers got significantly affected increased by 9.50 or 12.54% when VC was added at 150 or 300 mg kg^{-1}, respectively ($p<0.05$) and did not significantly differ between the VC added treatments ($p>0.05$); blood serum SOD activity of the broilers got significantly increased by 8.38% when VC was added at 300 mg kg^{-1} ($p<0.05$) and got increased but not significantly whe VC was added at 150 mg kg^{-1} and did not significantly differ between the VC added treatments ($p>0.05$). Blood serum GSH-PX activity of the broilers got significantly affected increasing by 10.46% when VC was added at 300 mg kg^{-1} ($p<0.05$) and got

Table 5: Effects of dietary vitamin C on immune organs and newcastle disease antibody of broilers

Vitamin C (mg kg^{-1})	Thymus index	Bursa of Fabricicus index	Spleen index	Caecum and tonsil index	Newcastle disease antibody
0	3.26±0.21^{b}	1.86±0.17^{b}	1.22±0.19	0.24±0.09	6.50±0.34^{b}
150	4.93±0.35^{a}	1.87±0.21^{b}	1.06±0.81	0.18±0.08	7.00±0.25^{ab}
300	4.69±0.33^{a}	2.44±0.06^{a}	1.07±0.17	0.21±0.03	7.50±0.22^{a}

^{a,b}Means within a column without a common superscript differ significantly ($p<0.05$)

Table 6: Effects of dietary vitamin C on anti-oxidative capacity in blood of broilers

Vitamin C rate (mg kg^{-1})	VC (μg/mg Prot.)	SOD (U/mg Prot.)	GSH-PX (U/mg Prot.)	A-TOC (U/mg Prot.)	MDA (nmol/mg Prot.)
0	4.15±1.25^{b}	36.96±0.94	342.30±11.47	9.61±0.94^{b}	0.27±0.03
150	4.95±1.10^{a}	37.79±1.43	347.24±13.04	10.60±1.15^{ab}	0.24±0.02
300	5.27±0.84^{a}	39.72±0.98	344.91±9.820	11.54±1.31^{a}	0.26±0.02

Table 7: Effects of dietary vitamin C on anti-oxidative capacity in the pectoral muscles of broilers

Vitamin C rate (mg kg^{-1})	VC (μg/mg Prot.)	SOD (U/mg Prot.)	GSH-PX (U/mg Prot.)	A-TOC (U/mg Prot.)	MDA (nmol/mg Prot.)
0	4.15±1.25^{b}	36.96±0.94	342.30±11.47	9.61±0.94^{b}	0.27±0.03
150	4.95±1.10^{a}	37.79±1.43	347.24±13.04	10.60±1.15^{ab}	0.24±0.02
300	5.27±0.84^{a}	39.72±0.98	344.91±9.820	11.54±1.31^{a}	0.26±0.02

Table 8: MDA content of pectoral muscle of broilers at different days during refrigeration at 4°C where vitamin added at different dose

	MDA (nmol/mg Prot.)				
Vitamin C rate (mg kg^{-1})	0th day	2nd day	4th day	6th day	8th day
0	0.27±0.03	0.32±0.12	0.39±0.09^{a}	0.45±0.13	0.50±0.11^{a}
150	0.24±0.02	0.29±0.09	0.36±0.11^{ab}	0.41±0.15	0.45±0.17^{ab}
300	0.26±0.02	0.29±0.11	0.32±0.08^{b}	0.36±0.14	0.41±0.13^{b}

$^{a, b}$Means within a column without a common superscript differ significantly ($p<0.05$)

somewhat increased but not significantly when VC was added at 150 mg kg^{-1} and did not significantly differ between the VC added treatments ($p>0.05$). Blood serum A-TOC of the broilers got significantly affected increasing by 27.17% when VC was added at 300 mg kg^{-1} ($p<0.05$) and got somewhat increased but not significantly when VC was added at 150 mg kg^{-1} and did not significantly differ between the VC added treatments ($p>0.05$). Blood serum MDA content did not get significantly affected but tended to decline where VC was added at 150 or 300 mg kg^{-1} ($p<0.05$).

Meat anti-oxidative capacity: It can be seen from Table 7 that the pectoral muscle VC contents of the broilers got significantly increased by 19.28 and 26.99% when VC was added at 150 and 300 mg kg^{-1}, respectively ($p<0.05$) and did not significantly differ between the VC added treatments ($p>0.05$). The pectoral muscle SOD activity of the broilers did not get significantly affected but tended to get increased when VC was added at 150 and 300 mg kg^{-1} ($p<0.05$). The pectoral muscle GSH-PX activity of the broilers did not get significantly affected but tended to increase when VC was added at 150 and 300 mg kg^{-1} ($p<0.05$). The pectoral muscle A-TOC of the broilers got significantly affected increasing by 20.08% where VC was added at 300 mg kg^{-1} ($p<0.05$) and get increased somewhat but not significantly when VC was added at 150 mg kg^{-1} and did not significantly differ between the VC added treatments ($p>0.05$). Pectoral muscle MDA content of the broilers did not get significantly affected but tended to decline when VC was added at 150 or 300 mg kg^{-1} ($p<0.05$).

Anti-oxidative capacity of pectoral muscle stored at 4°C: It can be seen from Table 8 that when VC was added to the feeds of the broilers, the MDA content of pectoral muscle stored at 4°C tended to decrease but not significantly in both the 0 and 2nd refrigeration day ($p>0.05$). When VC was added at 300 mg kg^{-1}, the MDA content of pectoral muscle stored at 4°C got significantly affected, decreasing by 17.95% in the 4th refrigeration day, ($p<0.05$) while the MDA contents of their pectoral muscles stored at 4°C got decreased somewhat but not significantly ($p>0.05$) compared with that of the broilers in the control treatment when VC was added at 150 mg kg^{-1} and the MDA content of pectoral muscle stored at 4°C did not significantly differ among the VC added treatments. In the 6th refrigeration day, VC addition did not affect the MDA content of the pectoral muscle of the broilers stored at 4°C ($p>0.05$). In the 8th refrigeration day, VC addition at 300 mg kg^{-1} significantly reduced the MDA content of the pectoral muscle of the broilers stored at 4°C by 18%, VC addition at 150 mg kg^{-1} did not significantly affect the MDA content of the pectoral muscles of the broilers stored at 4°C compared to the control treatment ($p>0.05$) and the VC added treatments did exert significantly different influences on the MDA content of the pectoral muscle of the broilers stored at 4°C ($p>0.05$).

Table 9: ΔE in pectoral muscle of the broilers on different days of pectoral muscle refrigeration when vitamin C added at different dose

	ΔE			
Vitamin C rate (mg kg^{-1})	2nd day	4th day	6th day	8th day
0	3.68±0.34^{a}	4.13±0.32^{b}	3.56±0.52	4.70±0.52^{a}
150	4.27±0.28^{a}	5.35±0.31^{a}	4.60±0.23	4.98±0.27^{a}
300	2.31±0.48^{b}	4.02±0.17^{b}	3.61±0.13	3.08±0.34^{b}

$^{a, b}$Means within a column without a common superscript differ significantly ($p<0.05$)

Meat colors of pectoral muscle: Table 9 presents the meat color parameters of the broiler pectoral muscles in

the slaughtering day and the refrigeration days after slaughtering which indicated that the higher ΔE were the more the meat colors varied. The results indicated that in the 2nd refrigerating day after slaughtering, the ΔE of the refrigerated pectoral muscle got significantly decreased when VC was added at 300 mg kg^{-1} ($p<0.05$) and got somewhat increased but not significantly when VC was added at 150 mg kg^{-1} ($p>0.05$), compared to the control treatment and did not significantly differ between the VC added treatments ($p<0.05$. In the 4th refrigeration day after slaughtering, the ΔE of the refrigerated pectoral muscles got significantly increased when VC was added at 150 mg kg^{-1} ($p<0.05$) and got insignificantly varied when VC was added at 300 mg kg^{-1} ($p>0.05$) and did not significantly differ between the VC added treatments ($p<0.05$. In the 6th refrigeration day after slaughtering, the ΔE of pectoral muscles got significantly increased when VC was added compared to the control treatment ($p>0.05$). In the 8th refrigeration day after slaughtering, the ΔE of pectoral muscles got significantly decreased when VC was added at 300 mg kg^{-1} ($p<0.05$) and did not get significantly affected when VC was added at 150 mg kg^{-1} ($p>0.05$) and did not significantly differ among the VC added treatments ($p<0.05$).

DISCUSSION

The roles of vitamin C as a natural anti-oxidant always attract attentions from nutritionists. Adding more vitamin C to animal feeds more than required is helpful to their growth and development (Li, 1999). The present research results showed that dietary vitamin C addition at 150 mg kg^{-1} could significantly increase weight gain and average daily feed intake of the broilers but somewhat decrease their feed to gain ratio. Li *et al.* (2008) revealed in their study that dietary vitamin C addition could significantly increase the daily weight gain and decrease the feed to gain ratio of broilers but did not had significant effects on their mortality rate as the VC added rate increased. Li *et al.* (2006) indicated in their study that the body weight and feed conversion rate of broilers were higher where vitamin C was added at 100, 200 and 400 mg kg^{-1} than in the control treatment. Njoku (1986) howed that dietary ascorbic acid addition could increase body weight of broilers linearly ($p<0.05$) or in a quadratic curve ($p<0.001$) and dietary ascorbic acid addition at 200 mg kg^{-1} resulted in the highest body weight and optimal feed/gain ratio of broilers. Sahin *et al.* (2003) also reported that dietary ascorbic acid addition at 250 mg kg^{-1} could increase body weight ($p<0.01$) and feed intake ($p<0.01$) and improve feed conversion efficiency of broilers ($p<0.05$).

The current research results showed, compared with those in the control treatment as the vitamin C added rate increased, the carcass percentage, eviscerated carcass percentage, semi-eviscerated carcass percentage, pectoral muscle percentage and leg muscle percentage of broilers tended to increase and the abdominal fat percentage and liver weight decreased. Dietary vitamin C addition at 300 mg kg^{-1} could significantly increase the pectoral muscle percentage and decrease the abdominal fat percentage and liver weight of broilers indicating that dietary vitamin C addition had improving effects on some slaughtering parameters of broilers.

Strydom (2008) pointed out that besides meat safety factors, there were other meat properties that consumers commonly employ as a whole to judge nutritional values and qualities of meats. Of these other factors, tenderness is investigated to be the most important factor that consumers employ to judge meat qualities as well as the more primary factor affecting all chain components of meat supply. Allen *et al.* (1997) reported that dark color meats have lower L* and b* and higher a* and pH than bright color meats and thus harmful microbes inhibited by lower pH in normal muscles could multiply in dark color meats which have higher pH, thereby causing the latter to deteriorate and even rot and finally shorten their shelf life. The study indicated that dietary vitamin C additions could increase the b* of their pectoral muscles of the broilers but did not significantly affects their other meat quality parameters. Dietary vitamin C additions could reduce the shear forces and water loss percentages of the leg muscles of the broilers, i.e., improving the water holding capacity and tenderness of the muscles thus able to play an active role in meat quality improvement. Dietary vitamin C additions at both 150 and 300 mg kg^{-1} had significant improving effects in meat quality. Dietary vitamin C addition at 150 mg kg^{-1} significantly decreased the leg muscle L*. Zheng (2003) revealed that dietary vitamins C addition could reduce water drip loss percentage of the muscle of the chickens to a varying extent and stabilize the color of their muscles. Present study was in agreement with the previous reports above.

Vitamin C helps immune cells avoid influences of autophagic mechanisms composed of immune reaction-released enzymes, acids and oxidants and thus keep immune cells have high vitamin levels, so that immune cells can keep responding to pathogens for a long time while they are attacked. In biological and medical research, tissue morphology, organ weight, organ index are important indicators to assess animal functions as well and in particular organ weight and size are important indicators of observation. Bursa of Fabricius, thymus, spleen, caecum and tonsil are important immune organs of

animals and thus their weight and indexes are important indicators to assess their immune functions. Immune organ index reflects immune organ development and immune cell functions and indirectly indicate somatic immune level (Guo, 2003). Increase in immune organ index means that immune system can quickly mature and somatic immune functions are strong and decrease in immune organ index means that immune systems deteriorate or slowly mature and somatic immune functions are weak. Acting as an electron provider, vitamin C as an immune regulator is able to prevent bodies from damages by free radices and microbes (Iqbal *et al*., 2004). The present research results shown that thymus index and bursa of Fabricius index of broilers were significantly higher when vitamin C was added at 150 or 300 mg kg^{-1} than that in the control treatment indicating that dietary vitamin C addition was capable of promoting thymus and bursa of Fabricius growth and development and these effects significantly increased as the added vitamin rate increased. But dietary vitamin C additions did not have effects on the spleen index and caecum and tonsil index. Thymus and bursa of Fabricius as the organs in which somatic T and B lymphocytes develop and maure play an important role in immune system operation and somatic immune function. Accordingly, dietary vitamin C additions at 150 and 300 mg kg^{-1} could improve somatic immunities and dietary vitamin C addition at 150 mg kg^{-1} performed best. Meng (2007) added vitamin C to poultry feeds, finding that vitamin C was capable of increasing the immune organ indexes of poultry and promoting spleen, thymus and bursa of Fabricius developments of poultry and enhancing somatic immunities of poultry by increasing its cell immunities and herein dietary vitamin C addition at 400 mg kg^{-1} performed better. Wang (2008) revealed by experimentation that dietary vitamin C addition at 200 mg kg^{-1} could significantly increase the thymus index of 42 days old broilers and there were not significant thymus index differences between dietary vitamin C addition at 100 mg g^{-1} and the control treatment.

Newcastle Disease (ND) is an acute, febrile, septic and highly transmissible disease caused by Newcastle disease virus. ND-AB testing can be employed to monitor ND-AB titers of chickens and determine their optimal vaccination time to prevent ND from occurring. Most fit chicken have an HI range within 5-8 by $\log_2$ and some have a range within 6-10 by $\log_2$.

Immune function parameters shown that dietary vitamin C additions could increase the ND-AB levels of the broilers hence enhancing their somatic immunities and the ND-AB levels tended to increased as the vitamin C added rates increased; the ND-AB levels got significantly increased where vitamin C was added at 300 mg kg^{-1}, compared to the control treatment. Meng (2007) verified that dietary vitamin C addition could raise somatic HI antibody titers and then enhance the immunity and dietary vitamin C addition at 400 mg kg^{-1} performed best for enhancing body defending capacity through humoral immunity pathway.

Vitamin C, a water soluble vitamin that can be synthesized by living organisms, distributes in cellular and extracellular fluids and plays an anti-oxidative role in cellular fluid and capable of transforming oxidized vitamin E into reduced one, kitamin C plays an important role in keeping vitamin E stable in concentration and anti-oxidative capacity in living organisms (Wang, 1995).

Moreover, vitamin C can make GPX transforming into GSH-PX by reviving GPX groups hence regaining the role of resisting free radicals. This study showed that dietary vitamin C addition could increase the vitamin C content in blood serum of broilers. It follows that when vitamin C was added to feed of broilers, the broilers could increase their vitamin E uptakes and extend vitamin C retention in their bodies thus stimulating and promoting their related functions. Dietary vitamin C addition could remarkably increase the blood serum anti-oxidative capacity of living organism. This study showed .that the blood serum SOD and GSH-PX contents and T-TOC tended to increase as the vitamin C added rates increased and the blood serum T-TOC of the broilers increased significantly where vitamin C was added at 300 mg kg^{-1}. Besides, the blood serum MDA contents of the broilers tended to decrease but not significantly when vitamin C was added to diet. Bou *et al*. (2006) revealed in their study that dietary vitamin C addition at 110 mg kg^{-1} could significantly increase the vitamin C content in the muscle of broilers. Wang *et al*. (2008) indicated that dietary vitamin C addition significantly increased SOD and GSH-Px activities and reduced MDA content in serum of broilers and dietary vitamin C addition performed better at 200 than at 100 mg kg^{-1}. Zheng (2003) showed that dietary vitamin C addition could increase SOD and GSH-PX activities in serum of broilers indicating that vitamin C had positive effects on free radical elimination and anti-oxidative capacity enhancement and dietary vitamin C addition at 200 mg kg^{-1} performed better. Aydemir *et al*. (2000) revealed that dietary vitamin C edition at 500 mg kg^{-1} could increase MN-SOD activities in hearts, livers, kidney and brains by about 15%.

Ulu (2004) revealed that during their storages, meat and meat products formed free radicals through their self catalyzing mechanisms which caused their fats to automatically oxidize, forming MDA, an intermediate oxidative product that could be determined with TBA.

CAT, SOD and GSH-PX are anti-oxidative enzymes widely distributing in muscles (Pradhan *et al*., 2000). The present study showed that dietary vitamin C addition could increase vitamin C accumulation in broiler muscles which brought vitamin C into full play in inhibiting fat oxidation thus safe guarding meat quality. However, there were not significant effects on SOD and GSH-PX activities in broiler muscles. Nonetheless, the muscle A-TOC significantly increased when vitamin C added at 300 mg kg^{-1} compared to the control treatment which was a unified indication of the enzymatic system and the non-enzyme system inside the bodies. Recent researches show that A-TOC is a comprehensive indicator employed to assess the function of anti-oxidative systems of living organisms and its magnitude indicates compensating capacity of anti-oxidative enzymatic system and non-enzyme system of living organisms in response to external stresses as well as their free radical metabolisms. Aydemir *et al.* (2000) investigated effects of dietary vitamin C additions on preventing meat and meat products from oxidative damages, finding that dietary vitamin C additions increased the CuZn SOD activity by 20% and the GSH-Px activity by 33%. Ozturk-Urek *et al.* (2001) revealed that dietary vitamin C additions could reduce lipid oxidation products in heart, liver and brain and increase SOD, CAT and GSH-Px activities in these tissues thus raising anti-oxidative capacities in the tissues.

Lipid contained highly unsaturated fatty acid are easy to oxidize into lipid peroxides while exposed to air and during this process, lipid H_2O_2 and various free radicals were generated at the early phase and MDA is formed by chain breaking at the final phase. Vitamin C as one highly reductive antioxidant can reduce peroxyl radical per oxidation in aqueous phase by means of such free radicals as O^{-2}, OH^{-} and H_2O_2 and their oxidants in extracellular fluid to prevent biological membrane from damaging by lipid peroxidation thus indirectly playing the role of meat quality safeguarding (Barja *et al*., 1994). Treatments with exogenous antioxidants can reduce pigment and lipid peroxidation and in particular vitamins C and E can effectively inhibit muscle self-oxidization (Sanchez-Escalante *et al*., 2001). The study revealed that their refrigeration periods prolonged, the MDA contents of the pectoral muscles increased and their peroxidation intensified and by their 4th refrigeration day, the MDA increase of the muscles somewhat intensified. Summarily, the peroxidation of the pectoral muscles did not differed much among the 0, 2nd and 6th refrigeration days of their storage at 4°C and on the 4th and 8th days of their storage, the MDA contents of the muscles significantly decreased where vitamin C was added at 300 mg kg^{-1} compared to the control treatment, so that the lipid peroxidation of the muscles was effectively inhibited and the storage quality of the muscles was safeguarded with consequence that the shelf life of the muscles could be extended. Zheng (2003) revealed that dietary vitamin C addition prevented the peroxidation of stored meats from occurring thus extending their shelf life. Mitsumoto (2000) revealed that vitamin C additions to the daily feed rations of poultry, pork, cattle could keep the pigments and fats of their meats stable for a prolonged period. Mitsumoto *et al.* (2005) indicated in their study that dietary vitamin C additions (at 200 and 400 mg kg^{-1}) had inhibitive effects on lipid peroxidation of chick meat and dietary vitamin C addition performed better at 400 mg kg^{-1} than at 200 mg kg^{-1}. Mielnik *et al.* (2003) comparatively investigated effects of dietary rosemary and vitamins E and C addition on oxidative stabilities of mechanically deboned turkey meat, revealing that their anti-oxidative effects of delaying muscle preoxidation was dependent on antioxidant types and their dose and during the seven month refrigeration, the muscle TBARS increased in all the treatments but more clearly in the control treatment and vitamin C did not performed as well as vitamin E and rosemary but had very significant effects of inhibiting lipid oxidation in a long term refrigeration.

Meat colors are external expressions of physiological, biological and microbiological changes occurring in muscles and employed as one of the most important indicators of muscle appearance assessment as well as important indicators to estimate functional characters of poultry meat (such as water holding capacity) and qualities of processed chicken meat products (Owens *et al*., 2000). Colors of meats and meat products mainly depend on three pigments that determine basic structures of muscle Myohemoglobin (Mb) which along with oxymyoglobin oxidize into brown met-myoglobins thus making meat colors darker, a sign of long meat storages. The colors of muscles differ depending on contents and compositions of these three pigments in them. Vitamin can alleviate meat and meat product oxidation by preventing oxygen from attacking cell membrane with consequence that it is able to reduce fat oxidation in processed meat products; various anti-oxidative enzymes in muscle tissues can eliminate lipid peroxiation-generated free radicals thereby capable of keeping the state of reduction myohemoglobin in its original red color (Sun and Luo, 1993). The present study revealed that for the different storage period, dietary vitamin C addition (at 150 or 300 mg kg^{-1}) had effects on meat colors to a varying extent. In the process of storage, dietary vitamin C addition at 300 mg kg^{-1} could significantly prevent meat color from varying, effectively stabilizing meat color and then prolonging meat shelf life. Wheeler *et al.* (1996) found that spraying vitamin C on

meat surface could very effectively stabilize meat color and compared with the control group the practice could prolong meat shelf life at 9°C as well. Yu *et al.* (2002) investigated meat color parameters of broiler leg muscles at 0th, 48th, 96th and 114th h in their storage at 4°C after vitamin C was spread on their surface, revealing that vitamin played a certain role in stabilizing and maintaining the color of the muscles and capable of improving sensory qualities of the muscles within the limits of their shelf life.

CONCLUSION

The results of present research indicated that dietary vitamin C addition at 150 mg kg^{-1} could significantly increase the growth rate of broilers but did not significantly affected their feed to gain ratio. Dietary vitamin C addition at 150 mg kg^{-1} performed better in improving immune function of broilers while dietary vitamin C addition at 300 mg kg^{-1} performed better in improving body or muscle anti-oxidative capacity of broilers, reducing lipid peroxidation in the process of broiler meat storage, prolonging the shelf life of broiler meat.

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