VALIDATION OF THE RELATION BETWEEN *IN VIVO* ANTIOXIDANT STATUS AND SHELF LIFE OF RABBIT MEAT BY THE KRL METHOD

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ABSTRACT

The aim of this study was to test the effect of vitamin E concentration in fattening rabbit diet on antioxidant status of animals and meat quality. A total of 120 rabbits were divided into 3 groups at weaning (36 days of age) till slaughtering (70 days of age) according to their live weight the day before weaning and their litter origin. Each group received a different diet with increasing levels of vitamin E: 5, 35 and 105 ppm. Blood samples were taken from 16 rabbits per group at the end of the fattening period to analyze blood and red blood cells antioxidant status by KRL method. Among these 16 rabbits, the two hind legs of 12 rabbits were kept after slaughtering to measure pH, color and water loss. Malondialdehyde (MDA), oxidized glutathione (GSSG) and reduced glutathione (GSH) concentrations in the meat of the 2 hind legs of 4 rabbits per group were measured after 1 and 8 days of storage at 4°C. Results showed that antioxidant protection of animals significantly increased with vitamin E level in the diet due to higher antioxidant properties of plasma. After 8 days of storage, MDA was less concentrated in the meat when rabbits were fed higher vitamin E rates showing a slowing down of lipid peroxidation. MDA evolution and GSSG concentration after 8 days of storage showed similar results. Color, water loss and pH were not modified by vitamin E level in the diet. As a conclusion, an increase of 30 ppm and 100 ppm in vitamin E in the diet linearly improves antioxidant status of fattening rabbits and rabbit meat shelf life.

Key words: Antioxidant, rabbit meat, Vitamin E, oxidation.

INTRODUCTION

Oxidative rancity represents one of the main causes of meat deterioration during storage (Fellenberg *et al.,* 2006). An increase in antioxidant level such as vitamin E (vit E) in rabbits' diet leads to an increase in vit E concentration in plasma and in meat, inducing a slowing down of lipid peroxidation (Bernardini *et al.,* 1996; Castellini *et al.,* 2001). These results were obtained with high increases in vit E levels in the diet (till 300 ppm). Such increases require important costs and we can wonder if slighter increases also lead to an improvement of antioxidant status of animals and meat quality. The aim of this study was to test the effect of lower dietary supplementation in vit E (5, 35, 105 ppm) on rabbit status and meat quality to know if vit E is effective at lower rates, more compatible with economic pressures. Rabbits' antioxidant status was measured by the KRL method which measures a global biologic response of antioxidant defenses of the blood. Malondialdehyde and glutathione concentrations in the meat before and after storage were used as quality indicators.

MATERIALS AND METHODS

Animals and experimental design

One hundred and twenty rabbits were divided into three groups from weaning (36 days of age) till slaughtering (70 days of age) according to their live weight the day before weaning and their litter

origin. Each group was fed with a different diet. The three diets were similar on a nutritional and composition point of view and differed only by the level in vit E. They contained 14.5% of protein, 18.9% of cellulose and 9.0% of starch. The E5 group was fed a diet containing 5 ppm of vit E, the E35 received a diet containing 35 ppm of vit E and E105 one ate a diet containing 105 ppm of vit E. Additional vit E was brought by all-rac-alpha-tocopheryl acetate. Rabbits were feed restricted in all groups from weaning till slaughtering to be in accordance with French breeding conditions. They had free access to water.

Measurements

At the end of the fattening period, blood samples were taken from 16 healthy rabbits per group to measure their antioxidant status by the KRL method. The KRL method consists in submitting a blood sample or a Red Blood Cells (RBC) sample to a stress (UV light) in order to induce an oxidative stress till the complete destruction of red blood cells. Time when 50% of the RBC are lysed is measured and compared between groups. This time is called Half Lysis Time (HLT) and is expressed in minutes. A HLT is calculated for plasma by the difference between HLT of blood and HLT of RBC and represents antioxidant protection brought through the plasma. Longer the HLT is, higher the antioxidant protection of the animal is. The interest of this method is to measure a global biological response to an oxidative stress. The 2 hind legs of 12 animals per group were kept at slaughtering. The day after, pH and color were measured on the first leg before being frozen to stop oxidation process and being sent for analysis. Color and weight of the second leg were measured before being stored during 7 days in the fridge at 4°C. The eighth day, color and weight of the second leg were measured again. The pH was also measured on the second leg before freezing it and sending it for analysis. Color was measured by a chroma meter from Konica Minolta which measures L* (lightness), a* (redness), b* (yellowness). The pH was measured by a meat pH meter (HI99163) from Hanna. In order to measure meat lipid oxidation, concentration of meat in malondialdehyde (MDA) was analyzed as well as concentration in reduced glutathione (GSH) and in oxidized glutathione (GSSG). Analysis were performed on 4 hind legs frozen the day after slaughtering and 4 hind legs frozen after 8 days of storage in the fridge in each group. These 8 hind legs came from 4 rabbits in each group.

Statistical Analysis

Results were analyzed according to a general linear model testing the effect of the level of vit E in the diet as a quantitative data on the different criteria measured (antioxidant status results, color parameters, pH, MDA concentrations, GSH and GSSG concentrations). The software used was SAS System V8.

RESULTS AND DISCUSSION

Antioxidant status of rabbits

KRL results showed a significant improvement in rabbits' antioxidant status when vit E rate increased in the diet (Table 1 and Figure 1). HLT increased linearly with the addition of vit E, particularly in blood thanks to the contribution of the plasma more than the red blood cells.

Table 1: Antioxidant status measured by KRL method

	5 ppm	35 ppm	105 ppm	P. vit E
	Half lysis time (min)			
Blood	78.65±8.7	88.85±14.6	118.08±23.1	<0.0001
Red Blood Cells	70.5± 9.8	75.7± 8.2	77.6± 8.2	0.0835
Plasma	8.1± 9.7	13.1± 16.0	40.5± 21.9	<0.0001

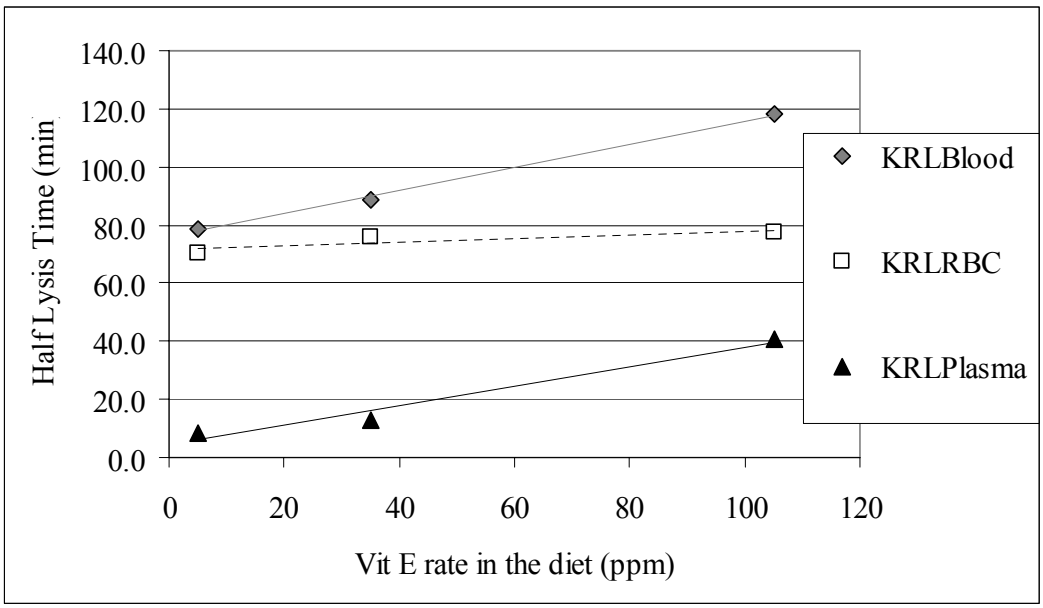

Figure 1: Antioxidant status of rabbits measured by KRL method according to the level of dietary vit E

Meat characteristics

Results did not show significant differences in percentages of water loss (Table 2). Hind legs lost between 1.65 and 2.08% of their weight after 7 days of storage at 4 °C. Meat pH did not differ between groups. It slightly decreased during storage, showing a meat acidification of 0.09 to 0.16 pH unit (Table 2).

Table 2: Water loss (%) during storage (7 days at 4°C) and meat pH after 1 and 8 days of storage

	5 ppm	35 ppm	105 ppm	P. vit E
Weight loss (%)	2.08 ± 0.35	1.65 ±0.36	1.95 ±0.25	0.8995
pH Day 1	6.20 ±0.27	6.26 ±0.17	6.06±0.23	0.19
pH Day 8	6.13±0.21	6.10±0.32	5.97±0.15	0.24

Vit E level in the diet slightly influences meat color the day after slaughtering (a* parameter only) but not after 8 days of storage at 4 °C. L and a* parameters values increased during storage, meaning that meat became lighter and more red during storage. The third color parameter (b*) seemed to decrease, meaning that meat turned a little bit to a bluish color during storage (Table 3).

Shelf life indicators of rabbit meat

Just after slaughtering, MDA concentration in rabbit meat was similar. An increase in vit E in the diet significantly avoided MDA increase, and consequently lipid oxidation, in rabbit meat during storage at 4°C (table 4). Even if reduced glutathione (GSH) decreased less in the meat during storage when rabbits are fed 35 or 105 ppm of vit E, this evolution did not depend significantly on vit E concentration. Oxidized glutathione (GSSG) on day 1 could not be always analyzed because it was under detection level in some legs. On day 8, concentration in GSSG tended to decrease when vit E level in the diet increased (P=0.079).

Table 3: Meat color (L*a*b*) the day after slaughtering and after 8 days of storage

	5 ppm	35 ppm	105 ppm	P. vit E
L* Day 1	54.3±2.6	54.6±2.3	54.3±1.7	0.998
a* Day 1	2.9±0.9	2.5±1.3	1.8±0.8	0.031
b* Day 1	3.1±0.9	2.9±1.5	2.2±1.4	0.108
L* Day 8	60.2±3.3	59.4±2.4	61.3±1.2	0.328
a* Day 8	3.3±1.4	3.4±1.9	2.6±1.0	0.387
b* Day 8	1.9±0.9	1.5±2.0	2.8±1.5	0.231

Table 4: MDA and GSH concentrations in rabbit meat after 1 or 8 days of storage at 4 °C and GSSG concentration after 8 days of storage at 4 °C.

	5 ppm	35 ppm	105 ppm	P. vit E
MDA Day 1 (mg/100g)	0.127±0.040	0.127±0.017	0.104±0.018	0.193
MDA Day 8 (mg/100g)	0.1686±0.026	0.1205±0.031	0.0952±0.050	0.0129
MDA evolution (mg/100g)	0.0373±0.014	-0.0055±0.024	-0.0243±0.030	0.022
GSH Day 1 (mg/100g)	39.475±8.062	30.650±3.987	34.250±4.810	0.478
GSH Day 8 (mg/100g)	28.980±7.660	26.567±4.320	26.340±5.155	0.538
GSH evolution (mg/100g)	-12.05±3.961	-5.15±2.213	-7.85±3.584	0.3663
GSSG Day 8 (mg/100g)	4.160±1.238	3.220±0.858	2.700±0.700	0.079

DISCUSSION

The addition of vit E in the diet induced an improvement of antioxidant status of rabbits. According to KRL results, blood, and more precisely plasma, contained a higher quantity of antioxidants, leading to a better defense of the animal against oxidative stress. These results confirmed those found by Bernardini *et al.* (1996) and Castellini *et al.* (2001). The first ones showed an improvement in trapping antioxidant parameter of rabbits' plasma when vit E in the diet rose from 50 to 200 ppm. The second ones showed an increase in α-tocopherol levels in plasma between rabbits fed 50 or 200 ppm of vit E. Our results showed that a better antioxidant status of the animal led to a better meat quality and a longer shelf-life (Figure 2). There is a linear relation between KRL results on blood and MDA concentration in the meat after 8 days of storage at 4°C. MDA concentration during storage increased with 5 ppm of vit E in the diet whereas it did not from 35 ppm, showing that 35ppm could be enough to prevent rabbit meat from lipid oxidation. Oxidized glutathione concentration was also a good criterion to detect meat oxidation. An improved antioxidant status of animals induced better antioxidant properties of meat. The improvement in meat quality is probably due to an increase in vit E level in the meat as described by Zsedely *et al.* (2008). Castellini *et al.* (2001) also showed that an increase of vit E in the diet induced an increase of vit E in plasma and in meat. In Zsedely *et al.* study, an increase from 60 to 300 ppm of vit E in the diet limited the increase in MDA in the meat during storage and significantly increased vit E concentration in the meat. An improvement of antioxidant status of the meat also limited the loss of vit E in the meat during storage (Selim *et al.*, 2008). Most of the studies tested high levels in vit E. According to our results, meat conservation is improved linearly with the increase of vit E in the diet and differences are observed from an increase of 30 ppm of vit E. No study could show an improvement in rabbit meat sensory quality (Bielanski *et al*., 2008). Technological criteria such as water loss during storage and cooking were improved when vit E was raised from 50 to 200 ppm (Dal Bosco *et al.*, 1998). This result was not observed with our vit E levels. Higher concentration of vit E (300 ppm *vs* 150 ppm) increased pH 24 hours after slaughtering in loin but not in thigh (Virag *et al.*, 2008). Results on meat color are different between authors. Virag *et al.* (2008) did not see any effect of vit E the day after slaughtering but Dal Bosco *et al.* (1998) observed a difference after 7 days at 4 °C and 30 days at -18 °C.

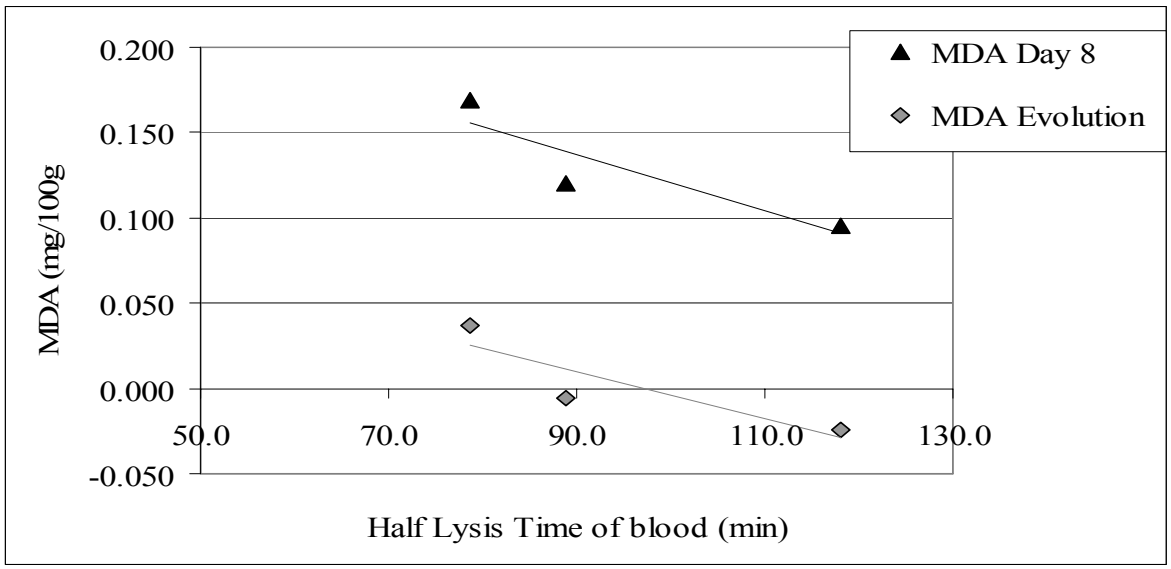

Figure 2: Relation between Half Lysis Time of blood (KRL measure) and MDA concentration in rabbit meat after 8 days of storage at 4 °C or MDA evolution during storage.

CONCLUSIONS

As a conclusion, our study showed the relation between *in vivo* results and meat quality. A linear improvement of rabbits' antioxidant status measured by KRL method is observed when vit E level in the diet increases between 5, 35 and 105 ppm. This result induces a better meat quality and meat *shelf life*. Our results led to the conclusion that a vit E supplementation is beneficial for rabbits' health as well as meat quality, even from 35 ppm and confirm results showed by previous studies with higher levels of vit E.

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