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Influence of Dietary Rapeseed Oil, Vitamin E, and Copper on the Performance and the Antioxidative and Oxidative Status of Pigs¹

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ABSTRACT: We investigated the effects of dietary copper and vitamin E in diets containing 6% rapeseed oil on the performance and the antioxidative and oxidative status of growing pigs. The 10 dietary treatments consisted of a basal diet (9 mg of vitamin E/kg feed, 15 mg of Cu/kg feed), the basal diet + 6% rapeseed oil (Diet 1; 18 mg of vitamin E/kg feed, 15 mg of Cu/kg feed), and Diet 1 plus supplements of vitamin E (0, 100, and 200 mg of dl- α -tocopheryl acetate/kg feed) and copper (0, 35, and 175 mg of Cu/kg feed) in a 3 \times 3 factorial arrangement of treatments. Eight or nine pigs were given ad libitum access to each diet from 25 to 100 kg of live weight. The inclusion of rapeseed oil tended ($P < .10$) to improve ADG and feed utilization. Compared with the addition of 35 mg of Cu/kg, the addition of 175 mg/kg improved growth rate and increased feed intake early in the experiment, but, over the total experiment, neither 35 nor 175 mg of Cu/kg affected performance. Compared with the addition of 100 mg of vitamin E/kg or no addition, the addition of 200 mg/kg reduced ADG over the total experiment ($P = .05$). The antioxidative and oxidative status of the pigs was evaluated in terms of blood and liver concentrations of antioxidants

(α -tocopherol, ascorbic acid, vitamin A, superoxide dismutase, glutathione peroxidase), prooxidants (Cu), concentrations of lipids (triglycerides and cholesterol), fatty acid composition, thiobarbituric acid-reactive substances (TBARS), and clinical chemical (creatinase kinase and glutamate-oxaloacetate-transaminase) and hematological variables that indicate the level of oxidative stress. There were no vitamin E deficiency signs or increased oxidative stress in pigs fed low dietary vitamin E levels, and no prooxidative effect of Cu was found. Increasing dietary levels of vitamin E increased the concentration of α -tocopherol in plasma and liver. Supplementation with Cu increased liver concentrations of Cu and α -tocopherol. The progression in liver TBARS was reduced by the addition of vitamin E and Cu. The addition of rapeseed oil changed the fatty acid composition of liver, increased α -tocopherol concentration in plasma and Cu concentration in liver, and reduced the rate of lipid oxidation in liver. In conclusion, even though the effects were minor, vitamin E, Cu, and rapeseed oil improved the antioxidative status of the live pigs.

Key Words: Pigs, Rapeseed Oil, Copper, Vitamin E, Performance, Antioxidants

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Introduction

There has been a growing desire over the last few years to maintain by dietary means the balance of prooxidants and antioxidants in the live animals. This balance determines the susceptibility of a given tissue to lipid oxidation. Oxidation of polyunsaturated fatty acids present in cell membranes may lead to cell injury due to the disruption of the normal membrane

structure and function. The dietary intake of unsaturated fat is, therefore, known to increase the requirement for vitamin E, which is a membrane-associated antioxidant that effectively protects the organism against free radical species capable of initiating and propagating lipid oxidation (Schaefer et al., 1995). However, vitamin E does not function as the only antioxidant in vivo; it is an integrated part of a network of antioxidants (e.g., superoxide dismutase [SOD] and glutathione peroxidase [GSH-Px]).

Besides the presence of unsaturated fatty acids and the availability of antioxidants, the susceptibility to lipid oxidation depends on the presence of transition metals such as Fe and Cu. Copper promotes oxidation of low-density lipoproteins (LDL) in vitro (Strain, 1994). The addition of dietary Cu at growth-promotion levels has improved fat digestion and increased

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the proportion of unsaturated fatty acids in carcass fat (Amer and Elliot, 1973ab); this may increase the risk of lipid oxidation.

This study was conducted to determine the effect of supplementing diets with vitamin E and Cu in combination with a high level of monounsaturated fatty acids (6% rapeseed oil) on the antioxidative and oxidative status of pigs. This paper presents the results obtained for pig performance, and antioxidative and oxidative status in the live pigs and in liver at slaughter. The antioxidative and oxidative status of muscles from these same pigs is described in another article (Lauridsen et al., 1999).

Materials and Methods

Animals

At approximately 25 kg of live weight, 82 Landrace × Yorkshire gilts from 22 litters were assigned to one of nine dietary treatments in a 3 × 3 factorial design: three levels of vitamin E and three levels of copper added to a diet with 6% rapeseed oil (Diets 1 to 9). To study the effect of rapeseed oil, a diet without rapeseed oil (basal diet) was added to the experimental design, giving a total of 10 dietary treatments (Table 1).

Pigs were housed individually in facilities at Research Centre Foulum. Pen size was 2.4 × 1.0 m with a combined concrete and slatted floor. The temperature was 19 to 20°C, and the humidity was approximately 65%. The pigs were given ad libitum access to feed and water. Individual weight and feed intake were recorded weekly.

Diets

As shown in Table 1, the diet with 6% rapeseed oil was supplemented with increasing amounts of vitamin E (0, 100, 200 mg of dl- α -tocopheryl acetate/kg feed) or copper (0, 35, 175 mg of Cu/kg feed, provided as Cu[II]-sulphate, pentahydrate). The vitamin E and copper premixes as well as the vitamin-mineral premix were provided by KFK (Korn-og foderstofkompagniet, Viby, Denmark). All batches of feed were analyzed for the total content of fat, vitamin E, and copper. These results are presented in Table 1. The diets (Table 2) were based on soybean meal, barley, and wheat. Rapeseed oil was provided by Aarhus Oil Co. (Aarhus C, Denmark). All experimental diets were mixed and pelleted at Research Centre Foulum. The chemical composition of the diets (Table 2) was determined according to AOAC procedures (1990) except for the starch and sugar contents, which were analyzed after the methods of Aaman and Hesselman (1984) and of Jacobsen (1981), respectively. Selenium analysis was carried out according to the fluorometric method of Michie et al. (1978). Dietary lipids were extracted by the method of Stoldt (1952) using petroleum ether, and the long-chain fatty acids (> C:8) were determined by GLC (capillary) after saponification and methylation as described by Rothenberg and Andersen (1980) with substitution of hexane with heptane, and with C-17:0 as the internal standard. Fatty acid analyses of the diets are shown in Table 3.

Sampling and Analytical Procedures

Littermates were slaughtered on the same day at a live weight of approximately 100 kg. Three days before

Table 1. Number of pigs in experiment and mean concentration (SD) of fat, vitamin E, and copper (Cu) in diets

| Dietary treatments ^a | n | Analyzed concentrations in feed | | |
|--|---|---------------------------------|-------------------------------|-----------|
| | | Fat, % | Vitamin E, mg/kg ^b | Cu, mg/kg |
| Basal diet | 8 | 2.7 (.5) | 9 (2) | 14 (5) |
| 1) Basal diet + 6% rapeseed oil | 8 | 8.5 (.3) | 18 (1) | 17 (6) |
| 2) Basal diet + 6% rapeseed oil + 100 mg vitamin E | 8 | 9.2 (1.0) | 78 (7) | 14 (8) |
| 3) Basal diet + 6% rapeseed oil + 200 mg vitamin E | 9 | 9.3 (.2) | 131 (8) | 15 (8) |
| 4) Basal diet + 6% rapeseed oil + 35 mg Cu | 8 | 9.1 (.7) | 23 (4) | 42 (5) |
| 5) Basal diet + 6% rapeseed oil + 35 mg Cu + 100 mg vitamin E | 8 | 9.2 (.9) | 77 (9) | 43 (5) |
| 6) Basal diet + 6% rapeseed oil + 35 mg Cu + 200 mg vitamin E | 9 | 8.7 (.6) | 133 (12) | 45 (7) |
| 7) Basal diet + 6% rapeseed oil + 175 mg Cu | 8 | 8.9 (.7) | 19 (6) | 153 (13) |
| 8) Basal diet + 6% rapeseed oil + 175 mg Cu + 100 mg vitamin E | 8 | 9.0 (.7) | 81 (10) | 154 (14) |
| 9) Basal diet + 6% rapeseed oil + 175 mg Cu + 200 mg vitamin E | 8 | 9.3 (1.1) | 139 (11) | 153 (15) |

^aVitamin E was provided as dl- α -tocopherol acetate and copper as Cu[II]-sulfate, pentahydrate.

^bConcentration of α -tocopherol.

slaughter, a blood sample was collected from the vena jugularis by puncture into vacuum tubes, which were either heparinized (for plasma) or not (for serum). After centrifugation at $1,000 \times g$ for 10 min at 4°C , the plasma and serum samples were stored at -80°C until analysis. Packed cell volume (**PCV**) was determined using heparin-stabilized blood in capillary tubes centrifuged at 1,200 rpm for 5 min (Sigma 201 m, B. Braun, Osterode/Harz, Germany). Concentration of hemoglobin was measured using a spectrophotometric determination of the hemoglobincyanide (J. T. Baker BV, Deventer, The Netherlands). Erythrocyte hemolysis in vitro was performed photometrically (540 nm) after incubation of red blood cells with .9% NaCl, .6% NaCl, and distilled water, each for 30 min at 25°C .

An erythrocyte lipid peroxidation test (**ELP**) was carried out as described by Fontaine and Vally (1977) and modified by Jensen et al. (1979). The enzyme activity of creatine kinase (**CK**; E.C.2.7.3.2) and glutamate-oxaloacetate-transaminase (**GOT**; E.C.2.6.1.1) were measured in serum at 365 nm using commercial test kits (Merck, Darmstadt, Germany). The former was based on the measurement of the rate of increase in NADPH, which is directly proportional

Table 2. Composition of diets

| Item | Basal diet | Diets 1-9 |
|---|------------|-----------|
| Ingredients, % | | |
| Soybean meal (49.5% CP) | 21.0 | 27.82 |
| Barley | 37.60 | 31.00 |
| Wheat | 37.60 | 31.00 |
| L-lysine ^a | .20 | .10 |
| DL-methionine ^b | .00 | .10 |
| Dicalcium phosphate | 1.25 | 1.70 |
| Calcium carbonate | .95 | .88 |
| Sodium chloride | .40 | .40 |
| Rapeseed oil | .00 | 6.00 |
| Mineral and vitamin premix ^c | 1.00 | 1.00 |
| Analyzed chemical composition, % | | |
| Dry Matter | 88.44 | 89.61 |
| CP ^d | 26.24 | 26.58 |
| Crude fat | 4.15 | 10.56 |
| Sugar | 6.92 | 6.90 |
| Ash | 6.69 | 7.25 |
| Starch | 50.14 | 41.02 |
| Selenium, mg/kg | .26 | .27 |
| ME, Mcal/kg | 3.80 | 4.10 |

^aL-Lysine hydrochloride in wheat bran, containing 320 g pure L-lysine/kg.

^bDL-Methionine in wheat bran, containing 400 g pure DL-methionine/kg.

^cMinerals and vitamins were mixed in soybean meal and provided per kilogram feed: potassium, 2.5 g; iron, 80 mg; zinc, 100 mg; iodine, .2 mg; selenium, .2 mg; vitamin A, 4,000 IU; vitamin D₃, 400 IU; thiamin, 2 mg; riboflavin, 2 mg; vitamin B₆, 3 mg; niacin, 20 mg; biotin, .05 mg; pantothenic acid, 10 mg; vitamin B₁₂, 20 µg; vitamin K₃, 2 mg. For diets with added vitamin E and copper, levels were added as described (Table 1).

^dPercentage content of basal diet and Diets 1-9 on a DM basis were, respectively: cysteine, .41 and .39; lysine, 1.15 and 1.16; methionine, .34 and .38; threonine, .82 and .84.

Table 3. Analyzed dietary fatty acid composition^a

| | Basal diet | Diets 1-9 |
|-----------------------|------------|-----------|
| C14:0 (myristic) | .4 | .2 |
| C16:0 (palmitic) | 20.6 | 9.6 |
| C16:1 (palmitoleic) | .2 | .2 |
| C18:0 (stearic) | 2.5 | 2.1 |
| C18:1 (oleic) | 16.8 | 47.7 |
| C18:2 (linoleic) | 53.2 | 30.5 |
| C20:0 (arachidic) | .1 | .4 |
| C18:3 (linolenic) | 5.4 | 7.9 |
| C20:1 (eicosenic) | .6 | 1.0 |
| Others | .2 | .3 |
| Unsaturated:saturated | 3.2 | 7.1 |

^aValues are the percentage of total analyzed fatty acids.

to the CK activity in the serum, and the latter was based on the rate of NADH consumption, which is directly proportional to the GOT activity in the serum. The activity of GSH-Px (E.C.1.11.1.9) was determined spectrophotometrically in blood plasma by the method of Günzler et al. (1974) as modified by Agergaard and Jensen (1982) at 366 nm and 37°C . The results were expressed in millikatal per milliliter of plasma (mkat/mL). Lithium-heparin-stabilized blood was used for determination of Na⁺ and K⁺ in plasma using flame photometry (FLM 3, Radiometer, Copenhagen, Denmark). Plasma concentrations of triglycerides were determined using enzymatic hydrolysis of triglycerides with subsequent determination of liberated glycerol by colorimetry (Boehringer Mannheim, Mannheim, Germany). The plasma concentration of cholesterol was determined after oxidation to cholestenone and hydrogen peroxide, which, in the presence of catalase, forms formaldehyde. The latter reacts with acetylacetone, forming a yellow lutidine-dye (3,5-diacetyl-1,4-dihydrolutidine). The concentration of lutidine-dye formed is stoichiometric with the amount of cholesterol and is measured by the increase of absorbance at 405 nm (Boehringer Mannheim).

The susceptibility of plasma to copper-induced lipid oxidation was determined measuring thiobarbituric acid-reactive substances (**TBARS**) (Beuge and Aust, 1978). Plasma samples were incubated in CuCl₂ and Tris-maleate buffer for 0, 40, 80, and 120 min. Following incubation, 2 mL of thiobarbituric acid-trichloroacetic acid-hydrochloric acid reagent was added, and, after boiling and centrifugation for 15 min, the absorbance was read at 535 nm. The results are expressed as nanomoles of malonaldehyde (**MDA**) per milliliter of plasma as well as per milligram of protein. Protein concentration in plasma was determined spectrophotometrically by the Pierce BCA protein assay (Pierce, Rockford, IL).

After stunning the pigs with CO₂, a sample of approximately 20 mL of blood from the carotid artery was collected and immediately chilled. The concentration of vitamin C was determined in plasma obtained

after centrifugation at $1,000 \times g$ (10 min, 4°C) within 6 h after slaughter according to the method of Zannoni et al. (1974). After bleeding of the animals and evisceration of the carcasses, the liver was removed and weighed. Samples of liver were taken from each carcass and stored at -20°C until analysis.

The liver was prepared for the determination of the activities of Se-GSH-Px, non-Se-GSH-Px, and SOD as described by Lauridsen and Jakobsen (1993). The activity of SOD was measured spectrophotometrically at 418 nm following inhibition of cytochrome C reduction according to McCord and Fridovich (1969). The reaction mixture contained $1.5 \times 10^{-4} M$ xanthine, $6.25 \times 10^{-9} M$ xanthine oxidase, and $1 \times 10^{-2} M$ cytochrome C. One unit of SOD activity was defined as the activity that inhibits the reaction by 50%.

Concentration of vitamin E in feed, plasma, and liver was determined according to Jensen et al. (1997). Concentration of copper in feed, liver, and backfat was determined by atomic absorption spectrophotometry (King, 1984). Fatty acid profiles of liver were analyzed as described by Jakobsen et al. (1995). The susceptibility of liver tissue homogenates to iron-induced lipid oxidation was determined by a modification of the method of Kornbrust and Mavis (1980). Liver homogenates were incubated in 40 mM Tris-maleate buffer (pH 7.4) with 1 mM FeSO_4 in a total volume of 1 mL. At fixed time intervals, aliquots were removed for the measurement of TBARS with the method of Buege and Aust (1978).

Statistical Analysis

For growth and feed intake data, repeated measurements were made on the same experimental unit (i.e., pig), and data were, therefore, expected to exhibit some sort of correlation. This was accounted for by considering statistical models in which performance was modeled as a function of time; all measurements, not only total weight gain or feed intake at the end of the experimental period, were used in the statistical analysis. Furthermore, data from pigs within a litter were expected to be correlated.

The weekly increase in growth is given by $G_{ecti} = X_{ecti} - X_{ec(t-1)i}$, where X_{ecti} denotes the total weight gain from the beginning of the experiment until time t of pig i during vitamin E treatment e and copper treatment c . A profile diagram (i.e., a plot of the average weight gain in group ect against t) showed a large weight gain during the 1st 2 wk of the experiment, and, thereafter, the weight gain was approximately linear in time. Effect of treatments at specific timepoints was investigated using a standard ANOVA model with a random litter effect. The effect of different levels of the same treatment was investigated by pairwise comparisons.

Clearly, at the beginning of the experiment, there is no treatment effect (Figure 1). However, a treatment effect began to appear after 3 wk. Therefore, the

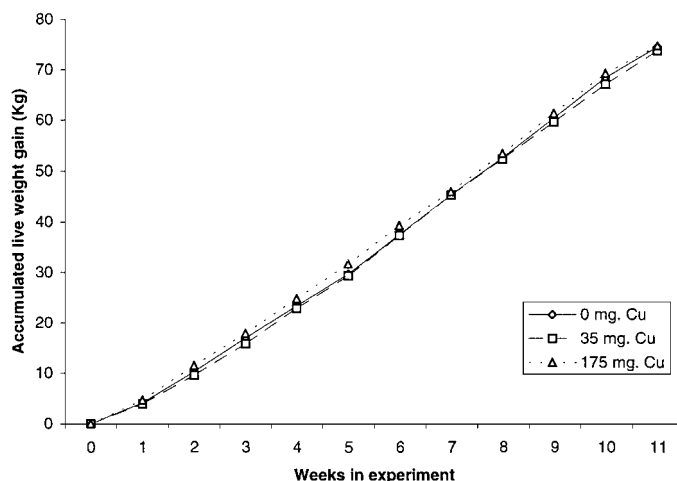


Figure 1. Accumulated weight gain over time for each level of copper supplementation. In wk 3, a significant effect of 175 ppm copper on the traits appeared.

weight gain from wk 4 to 12 was modeled with a third-degree polynomial, with the intercept depending on the treatment and random linear regression coefficients:

$$X_{ecti} = \mu + \mu_{ec} + (\beta_1 t + B_{eci})t + \beta_2 t^2 + \beta_3 t^3 + U_{1(i)} + \epsilon_{ecti}, \quad t \geq 4, \quad U_{1(i)} \sim N(0, \omega^2), \\ W_{eci} \sim N(0, \tau^2), \quad \epsilon_{ecti} \sim N(0, \sigma^2) \quad [1]$$

The term $U_{1(i)}$ refers here and henceforth to a random litter effect accounting for dependency among pigs from the same litter, and B_{eci} is a random effect accounting for correlation between repeated measurements on the same animal.

According to a profile diagram of the weekly feed intake (not shown), a large change occurred during the 1st 2 wk of the experiment, and thereafter the feed intake increased approximately linearly in time, with some variation toward the end of the experiment. Feed utilization was defined as the weight gain per unit of feed (i.e., $E_{ecti} = G_{ecti}/D_{ecti}$). A profile diagram showed an approximately linear decrease in utilization over time (except in wk 12, when the decrease seemed to be larger). To study whether any treatment effect occurred, this linear decrease was investigated, allowing slope and intercept of the curves to depend on the treatments.

For investigating the influence of rapeseed oil on the performance of pigs, analyses similar to those described above were conducted on the basis of the basal diet and Diet 1.

The effects of vitamin E and copper on performance traits for the entire experimental period, as well as on blood and liver traits, were analyzed using an ANOVA model with a random litter effect. Because the interaction between dietary vitamin E and copper was

not statistically significant for most of the traits, the following model was used:

$$Y_{eci} = \mu + \alpha_e + \beta_c + U_{I(i)} + \epsilon_{ec}, \epsilon_{ec} \sim N(0, \sigma_{ec}^2) \quad [2]$$

Results

Performance

Table 4 shows the influence of dietary treatments on the performance of the pigs. For all performance traits, no statistically significant differences were found between treatments, except between dietary vitamin E levels with regard to ADG, for the entire period (i.e., performance measured at the end of the experiment).

However, when the individual growth curves were analyzed (not shown), a copper effect on weekly weight gain was found for wk 3. Pigs fed 175 ppm copper grew faster ($P = .02$) than did pigs fed 35 ppm copper, but no differences were found between pigs fed 175 and 0 ppm of supplemental copper. The profile diagram (Figure 1) of the accumulated weight gain against time for each level of copper shows that the copper effect found in wk 3 was maintained throughout the rest of the experimental period in the sense that the growth curves were parallel ($P = .0001$), based on analyses described in Eq. [1]. Thus, the growth curves for 175 and 35 ppm copper differed ($P = .0001$) and so did those for 175 and 0 ppm ($P = .0001$), but those for 0 and 35 ppm did not differ.

Differences on feed intake among treatments were consistent with those found for weight gain; the only treatment effect was the supplementation with copper, which caused an increase in the feed intake in wk 3 (P

< .05 for 175 vs 35 ppm). Feed utilization was not influenced by copper treatments. No effect of vitamin E was observed on weekly performance data.

For pigs fed rapeseed oil, the rate of growth tended to increase and the feed intake tended to be less until about wk 6 compared with pigs fed the basal diet ($P < .10$). However, differences were not statistically significant, and the results are, therefore, not shown.

Blood Responses

Supplementation with vitamin E increased the concentration of this vitamin in plasma ($P < .001$), but the increase did not differ between the two levels of supplementation ($P > .10$) (Table 5). Pigs fed rapeseed oil had a higher concentration of vitamin E in plasma ($P = .004$) than pigs fed the basal diet. There was no interaction between dietary vitamin E and copper for this trait. The concentration of vitamin C, the activity of the antioxidative enzymes in the plasma, and the hematological traits (PCV, concentration of hemoglobin, spontaneous hemolysis in vitro, and plasma concentrations of Na^+ and K^+) were not influenced by the dietary treatments. Increasing levels of copper in the diet tended to increase ($P = .08$) the activity of GOT, and the activity of GOT in plasma of pigs fed rapeseed oil was slightly lower than that of pigs on the basal diet ($P = .06$) (Table 5). No influence of dietary vitamin E was observed on the plasma activity of GOT. The activity of CK did not differ between treatment groups. Dietary treatments influenced neither ELP nor the susceptibility of the plasma to lipid oxidation measured as TBARS. However, there was a tendency ($P = .11$) for a decreased level of plasma TBARS at 0 min of pigs fed dietary copper.

Table 4. Performance of pigs over the entire experimental period^a

| Diets | No. of pigs | ADG, kg | ADFI, kg | Gain:feed |
|-------------------|-------------|-------------------|----------|-----------|
| Fat | | | | |
| Basal diet | 8 | .917 | 2.307 | .398 |
| + 6% rapeseed oil | 9 | .987 | 2.314 | .433 |
| SE | | .027 | .111 | .013 |
| <i>P</i> -value | | .09 | .97 | .08 |
| Vitamin E | | | | |
| 0 mg/kg feed | 24 | .966 ^b | 2.221 | .438 |
| 100 mg/kg feed | 24 | .990 ^b | 2.262 | .446 |
| 200 mg/kg feed | 24 | .933 ^c | 2.139 | .438 |
| SE | | .020 | .061 | .010 |
| <i>P</i> -value | | .05 | .31 | .77 |
| Copper | | | | |
| 0 mg/kg feed | 25 | .966 | 2.120 | .456 |
| 35 mg/kg feed | 25 | .956 | 2.220 | .433 |
| 175 mg/kg feed | 24 | .969 | 2.264 | .433 |
| SE | | .020 | .059 | .009 |
| <i>P</i> -value | | .89 | .21 | .13 |

^aPigs were housed individually and fed from 25 to 100 kg live weight.

^{b,c}Within a column, means lacking a common superscript letter differ ($P = .05$).

Table 5. Blood traits of pigs fed different dietary levels of rapeseed oil (RO), vitamin E, and copper: concentration of vitamin E and vitamin C; activities of glutathione peroxidase (Se-GSH-Px) and superoxide dismutase (SOD); packed cell volume (PCV); concentration of hemoglobin, K⁺, and Na⁺; hemolysis; activities of creatine kinase (CK) and glutamate-oxaloacetate transaminase (GOT); erythrocyte lipid peroxidation (ELP); and thiobarbituric acid-reactive substances (TBARS)

| Traits | Fat | | Vitamin E, mg/kg feed | | | Copper, mg/kg feed | | | SE ^a |
|------------------------------------|------------------|-------------------|--------------------------|-------------------|-------------------|-----------------------|-------|-------|-----------------|
| | Basal diet | RO | 0 | 100 | 200 | 0 | 35 | 175 | |
| No. of pigs ^b | 8 | 9 | 24 | 24 | 26 | 25 | 25 | 24 | — |
| Vitamin E, $\mu\text{g}/\text{mL}$ | .97 ^c | 2.09 ^d | 2.20 ^c | 3.15 ^d | 3.22 ^d | 2.80 | 3.00 | 2.79 | .23 |
| Vitamin C, $\mu\text{g}/\text{mL}$ | 17.0 | 18.1 | 19.2 | 19.3 | 16.1 | 17.1 | 20.0 | 17.8 | 6.18 |
| Se-GSH-Px, mkat/L | .072 | .074 | .082 | .078 | .072 | .074 | .077 | .081 | .018 |
| SOD, U/g protein | 89 | 98 | 92 | 93 | 95 | 93 | 91 | 95 | .11 |
| PCV, % | 41.0 | 42.0 | 41.7 | 40.7 | 41.0 | 40.8 | 41.0 | 41.5 | .09 |
| Hemoglobin, mmol/L | 8.3 | 8.5 | 8.4 | 8.2 | 8.3 | 8.3 | 8.4 | 8.3 | .49 |
| Hemolysis, % (.6% NaCl) | 87.9 | 78.3 | 83.0 | 83.7 | 88.8 | 91.0 | 79.3 | 85.9 | .24 |
| Hemolysis, % (.9% NaCl) | .33 | .25 | .24 | .24 | .26 | .24 | .25 | .25 | .08 |
| K ⁺ , mmol/L | 4.1 | 4.2 | 4.0 | 4.1 | 4.1 | 4.1 | 4.1 | 4.1 | .09 |
| Na ⁺ , mmol/L | 128.8 | 126.5 | 124.5 | 123.6 | 123.1 | 124.2 | 124.1 | 122.9 | 2.7 |
| CK, U/L | 686 | 683 | 648 | 650 | 940 | 597 | 842 | 802 | 564 |
| GOT, U/L | 46.4 | 34.6 | 48.3 | 46.0 | 44.2 | 39.2 | 48.3 | 50.8 | 14.4 |
| ELP, extension at 562 nm | .056 | .041 | .038 | .049 | .049 | .043 | .047 | .047 | .009 |
| TBARS, nmol MDA/mg protein | | | | | | | | | |
| 0 min | .160 | .216 | .189 | .191 | .185 | .205 | .183 | .176 | .023 |
| 20 min | .888 | .663 | .683 | .715 | .747 | .756 | .716 | .677 | .117 |
| 40 min | 1.001 | .878 | .886 | .909 | .920 | .935 | .933 | .848 | .113 |
| 80 min | 1.097 | .944 | .973 | 1.032 | 1.048 | 1.036 | 1.072 | .948 | .103 |
| 120 min | 1.134 | .992 | 1.031 | 1.073 | 1.105 | 1.076 | 1.136 | 1.001 | .110 |

^aSE based on the model given in Eq. [2].

^bNumber of pigs per mean. Pigs were individually fed from 25 to 100 kg.

^{c,d}Means within a column of equal treatments with different letters are different ($P < .05$).

Total cholesterol ($20 \pm .46$ mg/dL) and triglyceride (29 ± 1.69 mg/dL) content of plasma were not influenced by supplemental dietary vitamin E or copper (Diets 1 to 9). Pigs fed the basal diet had a lower content of total cholesterol (17 mg/dL, $P = .023$) and triglycerides (16 mg/dL, $P = .005$) in plasma than pigs fed rapeseed oil (21 and 29 mg/dL for cholesterol and triglycerides, respectively). Correlation between plasma vitamin E and plasma triglycerides ($r = .90$, $P = .0002$) and of plasma vitamin E and plasma cholesterol ($r = .64$, $P = .01$) were found. Thus, when the vitamin E concentration was expressed in relation to the content of triglycerides in plasma instead of per milliliter of plasma, as in Table 5, the difference between the two dietary treatments was smaller but still significant ($P = .03$) (6.5 μg of vitamin E per milligram of triglycerides per milliliter of plasma from pigs on the basal diet and 7.9 μg of vitamin E per milligram of triglycerides per milliliter of plasma from those on the rapeseed oil diet).

Liver Responses

The weight of the liver was not influenced by the dietary treatments. Table 6 shows the effects of dietary treatments on the concentration of vitamin E, copper, and vitamin A in the liver and the activities of SOD, Se-GSH-Px, and non-Se-GSH-Px in the liver cytosolic fraction. The liver concentration of vitamin E

increased with addition of vitamin E to the diets ($P < .001$), whereas no differences were found between the basal diet and Diet 1 with respect to this trait. An interaction was found between dietary vitamin E and copper ($P = .05$); addition of 35 or 175 ppm copper to the feed increased the vitamin E concentration in the liver, but the increase in vitamin E was similar for both 35 and 175 ppm copper. No interaction between dietary vitamin E and copper was observed for the other traits presented in Table 6.

Supplementation with 175 mg of copper increased ($P < .01$) the concentration of copper in the liver. Addition of rapeseed oil to the feed increased ($P = .04$) the concentration of copper in the liver, whereas no influence of dietary vitamin E was observed with regard to the copper concentration in the liver. Dietary treatments did not influence the activity of SOD, Se-GSH-Px, and non-Se-GSH-Px measured in the liver cytosolic fraction (Table 6).

The concentration of fat in the liver ($2.9 \pm .98$ g/100 g) was not influenced by the dietary treatments. However, addition of rapeseed oil to the feed affected the fatty acid composition of the liver and elevated ($P = .06$) the total concentration of fatty acids (Table 7). Overall, the content of saturated fatty acids was unaffected, but the content of monounsaturated fatty acids and that of polyunsaturated fatty acids were

Table 6. Hepatic concentration of vitamin E, copper, vitamin A, and activities of SOD, non-Se-GSH-Px, and Se-GSH-Px of pigs fed different dietary levels of rapeseed oil, vitamin E, and copper

| Diets | n ^a | Vitamin E, μg/g tissue | Vitamin E, μg/g fat ^b | Copper, μg/g DM | Vitamin A, μg/g tissue | SOD, U/mg protein | Non-Se-GSH-Px, nmol NADPH/ min/mg protein | Se-GSH-Px, nmol NADPH/ min/mg protein |
|------------------------------|----------------|---------------------------|-------------------------------------|--------------------|---------------------------|----------------------|---|---|
| Fat | | | | | | | | |
| Basal diet | 8 | 1.71 | .18 | 21.47 | 85 | 24 | 247 | 137 |
| + 6% rapeseed oil | 9 | 2.50 | .26 | 27.77 | 81 | 27 | 188 | 127 |
| <i>P</i> -value | | .26 | .29 | .037 | .72 | .19 | .17 | .57 |
| Vitamin E, mg/kg feed | | | | | | | | |
| 0 | 24 | 4.73 ^d | 1.64 ^d | 28.28 | 82 | 26 | 191 | 137 |
| 100 | 24 | 11.80 ^e | 4.95 ^e | 27.53 | 83 | 27 | 172 | 141 |
| 200 | 26 | 21.44 ^f | 7.49 ^f | 31.30 | 80 | 27 | 167 | 139 |
| <i>P</i> -value | | <.001 | <.001 | .77 | .91 | .53 | .60 | .59 |
| Copper, mg/kg feed | | | | | | | | |
| 0 | 25 | 11.15 ^d | 3.81 ^d | 24.15 ^f | 79 | 28 | 160 | 139 |
| 35 | 25 | 13.07 ^{de} | 4.39 ^{de} | 26.30 ^f | 83 | 26 | 188 | 140 |
| 175 | 24 | 14.52 ^e | 5.26 ^e | 38.12 ^g | 82 | 26 | 181 | 139 |
| <i>P</i> -value | | .03 | .04 | <.001 | .70 | .12 | .48 | .73 |
| SE ^c | | .47 | .48 | .34 | 19.4 | 3.1 | 22 | 11 |

^aNumber of pigs per mean. Pigs were individually fed from 25 to 100 kg.

^bTotal fat (g) per 100 g of tissue.

^cSE based on the model given in Eq. [2].

^{d,e}Means within a column of equal treatments with different letters are different ($P < .05$).

^{f,g}Means within a column of equal treatments with different letters are different ($P < .01$).

higher ($P = .01$) in the liver of pigs fed rapeseed oil than in liver of pigs fed the basal diet.

Dietary vitamin E and copper caused no significant effects on the fatty acid composition of the liver. A significant interaction ($P = .01$) between dietary vitamin E and copper was observed for the hepatic

concentration of C22:6, but, because a similar effect was not found for the other fatty acids, means of this fatty acid were pooled within treatments as for other fatty acids.

The rates of Fe²⁺-induced lipid oxidation in livers of pigs on the different feeding regimens are presented in

Table 7. Concentration of selected fatty acids (μg/g wet tissue) from total lipids extracted from the liver of pigs fed different levels of dietary rapeseed oil (RO), vitamin E, and copper

| Fatty acids | Fat | | | Vitamin E, mg/kg feed | | | Copper, mg/kg feed | | | SE ^a | <i>P</i> -value | |
|-------------------|------------|--------|-----------------|-----------------------|------------------|------------------|--------------------|------|------|-----------------|-----------------|--------|
| | Basal diet | +6% RO | <i>P</i> -value | 0 | 100 | 200 | 0 | 35 | 175 | | Vitamin E | Copper |
| n ^b | 8 | 8 | | 24 | 24 | 26 | 25 | 25 | 24 | | | |
| C16:0 | 4.00 | 3.21 | .003 | 3.03 | 3.28 | 3.10 | 3.11 | 3.03 | 3.29 | .044 | .41 | .37 |
| C18:0 | 5.94 | 6.91 | .02 | 6.56 | 6.62 | 6.64 | 6.77 | 6.42 | 6.63 | .064 | .90 | .24 |
| C18:1*9 | 3.05 | 4.01 | .01 | 3.99 | 4.26 | 4.24 | 4.16 | 4.14 | 4.20 | .059 | .21 | .92 |
| C18:1*7 | .31 | .44 | .01 | .39 | .44 | .43 | .45 | .40 | .42 | .007 | .28 | .37 |
| C18:2 | 3.61 | 4.80 | .001 | 4.67 | 4.84 | 4.75 | 4.81 | 4.66 | 4.80 | .049 | .49 | .42 |
| C18:3 | .18 | .30 | .05 | .28 | .34 | .32 | .35 | .30 | .30 | .303 | .23 | .50 |
| C20:4 | 4.01 | 3.61 | .17 | 3.57 | 3.66 | 3.66 | 3.63 | 3.60 | 3.66 | .040 | .64 | .73 |
| C20:5 | .15 | .69 | .001 | .52 | .51 | .57 | .60 | .50 | .50 | .01 | .45 | .07 |
| C22:5 | .66 | .71 | .34 | .69 ^d | .78 ^e | .78 ^e | .76 | .73 | .75 | .008 | .02 | .52 |
| C22:6 | .54 | .60 | .64 | .41 | .44 | .44 | .49 | .37 | .43 | .015 | .91 | .19 |
| SAFA ^c | 9.9 | 10.1 | .75 | 9.6 | 9.9 | 9.7 | 9.9 | 9.5 | 9.9 | .097 | .70 | .19 |
| MUFA ^c | 3.4 | 4.4 | .01 | 4.4 | 4.7 | 4.7 | 4.6 | 4.5 | 4.6 | .064 | .17 | .89 |
| PUFA ^c | 9.1 | 10.7 | .01 | 10.1 | 10.5 | 10.5 | 10.6 | 10.2 | 10.4 | .100 | .33 | .28 |
| Total | 22.4 | 25.3 | .06 | 24.7 | 25.9 | 24.9 | 25.1 | 24.2 | 25.0 | .260 | .36 | .31 |

^aSE based on the model given in Eq. [2].

^bNumber of pigs per mean. Pigs were individually fed from 25 to 100 kg.

^cSAFA = saturated fatty acids, MUFA = monounsaturated fatty acids, and PUFA = polyunsaturated fatty acids.

^{d,e}Means within a column of equal treatments with different letters are different ($P < .05$).

Figure 2. The oxidative changes were larger ($P < .01$, at 40 to 120 min) in the livers of pigs fed the basal diet compared with pigs fed diets with rapeseed oil (Figure 2a). The addition of either vitamin E ($P = .002$) or copper ($P = .02$) to the feed reduced the oxidative stress of the liver even before Fe^{2+} -induced lipid oxidation (Figure 2b). No differences between the two levels of supplementation of either vitamin E or copper were seen on the inhibition of the Fe^{2+} -induced lipid oxidation, and no significant interactions between dietary vitamin E and copper were observed on the rate of lipid oxidation.

Discussion

The analyzed dietary content of vitamin E (α -tocopherol) and copper show the clear differences between treatment groups. However, the analyzed concentrations of α -tocopherol and copper in the diets were generally lower than planned, even though the analyzed concentrations of the premixes were as declared. No obvious explanation for the difference between planned and analyzed contents of the dietary supplements is apparent. With regard to α -tocopherol, subsequent analyses in our lab revealed difficulties in the quantification of α -tocopherol in fat-rich feed samples.

Rapeseed has a lower concentration of saturated fatty acids, especially C16:0 and of C18:2n-6, than do wheat and barley. The inclusion of rapeseed oil in the diets changed the fatty acid pattern of the basal diet (18:2 > 16:0 > 18:1 > 18:3) in the direction of the pattern in rapeseed oil (18:1 > 18:2 > 18:3 = 16:0). Rapeseed oil is rich in vitamin E, which consists of α -, γ -, and δ -tocopherol and α -tocotrienol, and the inclusion of 6% of this oil resulted in a doubling of the amount of α -tocopherol in the feed.

Analysis of weekly data showed that the addition of 175 ppm copper improved growth rate and feed intake for a short period but did not affect feed utilization. In the European Union, a copper supplementation at 175 mg per kilogram feed is permitted until pigs are 16 wk of age, and, from 17 wk of age, 35 mg copper per kilogram feed is permitted. In the present study, the increase in accumulated growth rate was observed within the 1st 3 wk of the experiment, and the difference was maintained during the rest of the experiment. Several studies demonstrated a growth-stimulating effect of feeding high dietary levels of copper (100 to 250 mg/kg feed) to weanling pigs (Bunch et al., 1961; Burnell et al., 1988; Dove and Haydon, 1992; Zhou et al., 1994; Apgar et al., 1995). Regarding the growth-promoting potential of copper when fed to growing-finishing pigs, results are more unclear. Hawbaker et al. (1961) found that 100 ppm $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was the optimum dietary level for rapid growth and efficient feed utilization in growing swine.

In a Danish study (Madsen et al., 1982), an increase in the copper supplementation from 30 to 120 ppm increased the daily gain and feed efficiency by about 3% in the weight range from 20 to 50 kg. The increase was slightly larger with supplementation of 240 ppm compared with supplementation of 120 ppm (Madsen et al., 1982). However, an influence on animal performance of dietary copper at a level of 250 ppm was not found in other studies (Amer and Elliot, 1973b; Myer et al., 1992).

Blood, and especially the erythrocytes, may reflect the susceptibility of the animal to oxidative conditions (Fraga et al., 1990). The lack of differences between dietary groups with respect to hematological traits and the plasma enzymatic traits indicates that the natural vitamin E in the diets without supplemental vitamin E was sufficient to protect the live animal against oxidation processes. The critical level of vitamin E of a given system apparently depends on lipid content, lipid oxidizability, and the nature and intensity of the initiating oxidative challenge (Fukuzawa et al., 1985). The addition of rapeseed oil

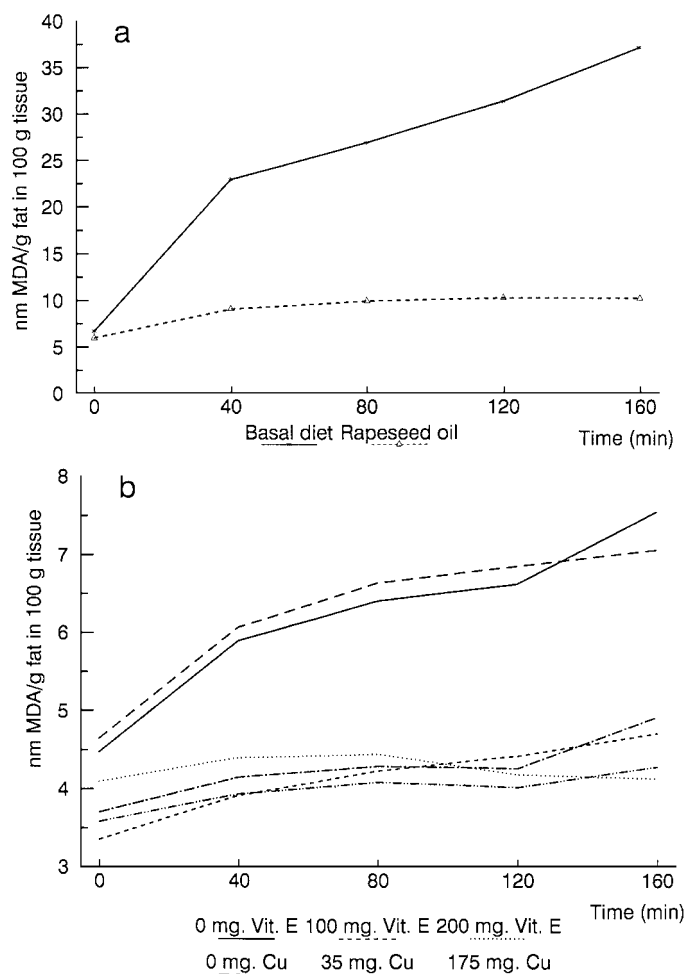


Figure 2. The effect of dietary rapeseed oil (a) and dietary vitamin E and copper (b) on Fe^{2+} -induced lipid oxidation of pig liver.

elevated the plasma concentration of triglyceride, and, when the vitamin E concentration was expressed relative to plasma triglyceride as suggested by several works (Morrissey et al., 1993), the difference in plasma vitamin E between the basal diet and the basal diet with 6% rapeseed oil was reduced.

The leakage of enzymes, such as GOT and CK, to serum may be associated with the injury of membranes of the liver and of muscle tissue, respectively. A high activity of these enzymes, along with increased hemolysis *in vitro*, leakage of potassium ions, and release of hemoglobin due to impaired integrity of the erythrocyte membrane, as well as increased plasma TBARS, have been attributed to vitamin E deficiency (Machlin, 1984; Engberg et al., 1993) and to feeding oxidized lipids (Hyam et al., 1993). Even though the concentration of dietary vitamin E in the basal diet was slightly lower (9 mg/kg feed) than recommended by NRC (1998) (11 mg/kg feed for slaughter pigs), no vitamin E deficiency symptoms or reduced resistance of the blood cells against oxidative stress were observed.

No indication of any prooxidant effects of dietary copper was found on the blood traits. Even though the serum activity of GOT slightly increased with increasing dietary copper, this result may not reflect a decreased membrane stability of hepatocytes. The values were within ranges similar to those obtained by Myer et al. (1992), who also observed an increased level of GOT in serum of pigs fed 250 ppm dietary copper. Myer et al. (1992) found that serum tocopherol levels increased despite the presence of copper in the diets. In our study, dietary copper did not influence the plasma concentration of vitamin E, but it did indeed increase the concentration in the liver.

Copper supplementation decreased plasma triglycerides in chickens (Bakalli et al., 1995) and in pigs (Amer and Elliot, 1973a) as well as cholesterol in chicken plasma (Bakalli et al., 1995). A linkage may therefore exist between copper assimilation and dietary fat and lipid metabolism. However, in the present experiment neither dietary copper nor vitamin E influenced the concentration of triglycerides and cholesterol in the plasma. Due to the inclusion of rapeseed oil, which mainly consists of triglycerides, the elevated concentration of triglycerides in the plasma of pigs fed rapeseed oil was expected. However, the increased concentration of cholesterol in the plasma of pigs fed rapeseed oil may be ascribed to an increase in LDL, whereby more transport of endogenously formed cholesterol is facilitated.

Treatment effects with respect to liver concentrations of vitamin E and copper and fatty acid profile were not paralleled by significant changes in the antioxidant enzyme activities of the liver cytosolic fraction. In contrast to this, Dove and Ewan (1991) found influences of supplemental vitamin E and

copper on the activity of GSH-Px in the liver. It is well known that both vitamin E (Jensen et al., 1988) and copper (Hedges and Kornegay, 1973; Madsen et al., 1982; Luo and Dove, 1996) accumulate in the porcine liver in response to dietary vitamin E and copper. In the present study, the increase in the liver concentration of vitamin E with increasing dietary copper could be ascribed to a reduced liver turnover of vitamin E due to a fortified antioxidative system. An increased availability of copper may increase the activities of enzymes such as SOD, ceruloplasmin, and catalase, because the activity of these enzymes is known to be reduced during copper deficiency followed by an increased incidence to oxidative damage (Paynter et al., 1979; Taylor et al., 1988). In agreement with other studies (Van den Berg et al., 1993), supplemental vitamin E had no effect on hepatic copper concentration. Previous results have shown that dietary copper supplementation alters lipid metabolism and changes the fatty acid composition of depot and serum lipids of swine (Elliot and Bowland, 1968; Amer and Elliot, 1973b; Dove and Haydon, 1992). These studies are in agreement with our result that dietary copper increases the liver concentration of vitamin E.

Addition of vitamin E and of copper to the feed reduced the oxidation rate of the liver, indicating an antioxidative effect of both nutrients. It is well known that vitamin E inhibits oxidative progression (Monahan et al., 1990), whereas experiments *in vitro* indicate that copper, in its unbound form, is a prooxidant (Estebauer et al., 1992). It is accepted that the bulk of hepatocellular copper is not present in its free ionic form, but it is associated with high-capacity copper-binding proteins so it is unable to act as a prooxidant (Luzy and Speisky, 1996). In addition, giving mice a subcutaneous injection of copper (.2 mg/kg) did not disturb the natural antioxidant defense system (Kukhtina and Glushchenko, 1996). In the present experiment, the reduced oxidation rate of the liver after the copper treatments might also be ascribed to the slightly higher vitamin E concentration or increased activities of non-GSH-Px in the liver (or other antioxidants, not measured in the present experiment).

It is difficult to explain the elevated oxidation rate of the liver from pigs fed the basal diet compared with liver from pigs fed rapeseed oil. The amount of oxidizable fatty acids (i.e., PUFA) was higher in liver of pigs fed rapeseed oil. Despite different dietary concentrations of vitamin E (9 vs 18 mg/kg of feed), the liver concentration of vitamin E of pigs fed 18 mg of vitamin E per kilogram of feed was only slightly higher than that of pigs fed the basal diet. However, it cannot be excluded that the higher level of liver α -tocopherol in pigs fed the 18-mg-per-kilogram feed exceeds the critical level for antioxidative protection.

Implications

Vitamin E supplementation of diets for growing pigs improved the antioxidative status of live pigs. Diets containing at least 9 mg/kg of vitamin E can be supplemented with copper and rapeseed oil without any detrimental effects on the prooxidative/antioxidative status of the animal. In fact, the two latter components improved the antioxidative status to some extent. Based on the analysis of the entire experimental period, dietary copper had no influence on the performance of pigs, but analysis of weekly data showed that the addition of 175 ppm copper increased the gain and feed intake at the beginning of the experiment.

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