Influence of supplemental magnesium, tryptophan, vitamin C, vitamin E, and herbs on stress responses and pork quality

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ABSTRACT: This study investigated the effects of supplemental Mg, Trp, vitamin C, vitamin E, and herbs on stress responses, skin lesions, and meat quality of slaughter pigs (106.0 ± 8.6 kg of BW). These 5 supplements were tested in 4 similar experiments. In Exp. 1, 2 treatments were tested: 1) control treatment without supplementation, and 2) supplementation of an herbal product (2.5 g/L of drinking water for 2 d). Eighty-eight pigs per treatment were involved, of which 40 were selected for meat quality measurements (over 4 replicates). The experimental design of Exp. 2 and 3 was the same as Exp. 1, except that in Exp. 2 Mg (3 g/L of drinking water for 2 d) was supplemented and in Exp. 3 Trp (6 g/kg of feed, as-fed basis) was supplemented. In Exp. 4, 3 treatments were tested: 1) control treatment without supplementation, 2) supplementation of vitamin C (300 mg/kg of feed for 21 d, as-fed basis), and 3) supplementation of vitamin E (150 mg/kg of feed for 21 d, as-fed basis). In Exp. 4, 66 pigs per treatment were used, of which 42 were evaluated for meat quality (over 6 replicates). Pigs supplemented with vitamin E ate less than control (P = 0.03) or vitamin C-supplemented pigs (P = 0.03). Pigs were transported to a commercial slaughterhouse and were slaughtered after a lairage period. Blood sampling at slaughter revealed no differences between the control and supplemented pigs in plasma cortisol, glucose, lactate, or creatine kinase concentrations. Pigs provided with Mg (P = 0.002) or Trp (P = 0.04) had lower plasma NEFA concentrations than control pigs, and pigs supplemented with vitamin C had greater concentrations than the control (P = 0.03) or vitamin E-supplemented pigs (P = 0.01). Supplementation of the herbal product increased the frequency of pigs with shoulder (P = 0.05) and loin lesions (P = 0.03), whereas Mg lowered the incidence of loin lesions (P = 0.01). Measurements of pH and temperature in the LM and biceps femoris 45 min postmortem revealed no differences among treatments, and no influence of treatments on LM pH, electrical conductivity, and water holding capacity was observed 48 h postmortem. Compared with the control loins, loins of pigs supplemented with vitamin C (Japanese color scale, L*, and a* value; P < 0.05) or vitamin E (Japanese color scale and a* value; P < 0.03) were redder and less pale, and the loin of vitamin E-supplemented pigs was more yellow (b* value; P = 0.04). Generally, Mg could lower loin damage, whereas vitamin C and vitamin E supplementation resulted in a color improvement of the loin.

Key words: herb, nutrient supplementation, pig, pork quality, skin lesion, stress

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doi:10.2527/jas.2005-600

INTRODUCTION

Nowadays, consumers are more aware of pork quality including animal welfare during housing, transport, and slaughter. Pigs transported to the slaughterhouse are exposed to different stressors (Lambooij and van Putten, 1993), increasing the risk of impaired welfare and economic losses related to carcass damage, inferior meat quality, and even mortality. In the past, pharmaceutical tranquilizers were commonly used to calm down the pigs; however, the use of these sedatives has been banned by the European Community to improve food safety. The use of legal feed additives,
known for their influence on stress responses and meat quality, can be a viable alternative. Magnesium is able to reduce plasma corticosteroids and catecholamines and neuromuscular stimulation (Kietzmann and Jablonski, 1985). Tryptophan is converted to brain serotonin, influencing temperature regulation, arousal, pain sensitivity, feeding, sexual behavior, and aggression (Leathwood, 1987). Vitamin E and vitamin C are mainly known for their antioxidant properties (Packer et al., 1979). Also, the use of natural herbs, gaining more attention in the last decades, is worth trying. Valeriana officinalis L. and Passiflora incarnata L., the active components of the used herbal product are known for their sedative and anxiolytic properties (Houghton, 1999; Cropley et al., 2002).

These supplements were tested in laboratory conditions on pig’s heart rate, behavior, saliva cortisol, urinary catecholamines, and plasma intermediary metabolites (Peeters et al., 2004, 2005). The results indicated that supplementation of Mg, Trp, vitamin C, and vitamin E improved the coping ability during transport simulation compared with a control treatment. The aim of the current study is to determine the effect of supplemental Mg, Trp, vitamin C, vitamin E, and herbs on stress responses, skin damage, and meat quality of slaughter pigs in field conditions.

**MATERIALS AND METHODS**

**Animals**

The experimental pigs were treated in accordance with Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes (OJEU, 1986). Crossbred (Piétrain × Hypor) pigs (106.0 ± 8.6 kg of BW) that were heterozygous for the halothane gene were housed on a concrete, slatted floor in standardized housing conditions with 6 pigs (3 barrows, 3 gilts) in 1 pen (1.9-m width ×2.5-m length). The number of pigs within each experiment was 352 in Exp. 1 to 3 and 396 in Exp. 4.

**Treatments**

The pigs were given free access to drinking water (via a nipple) and a commercial feed (25% wheat, 20% barley, 12% soy, 8.3 MJ of NE/kg, and 16.0% CP, as-fed basis), with 100 mg of vitamin E/kg, 0 mg of vitamin C/kg, 1.87 g of Mg/kg, and 1.65 g of Trp/kg, as-fed basis. Five supplements were tested in 4 analogous experiments: an herbal product, Mg, Trp, vitamin C, and vitamin E. The dose, duration, and route of administration of the supplementation are presented in Table 1.

In Exp. 1, 2 treatments were tested: 1) control treatment without supplementation, and 2) supplementation of an herbal product. The experimental design of Exp. 2 and 3 was the same as Exp. 1, but in Exp. 2 Mg was supplemented and in Exp. 3 Trp was supplemented. In Exp. 4, 3 treatments were tested: 1) control treatment without supplementation, 2) supplementation of vitamin C to feed, and 3) supplementation of vitamin E to feed.

Experiments 1, 2, and 3 were replicated 4 times, whereas Exp. 4 was replicated 6 times, at different outside temperatures (varying from 3 to 16.9°C). Per replicate of the first 3 experiments, 22 control pigs and 22 supplemented pigs were randomly chosen, of which 10 control pigs and 10 supplemented pigs were selected for blood sampling and meat quality measurements (see later). In Exp. 4, 11 control pigs, 11 pigs supplemented with vitamin C, and 11 pigs supplemented with vitamin E were randomly chosen, of which 7 pigs per treatment were selected for meat quality measurements (see later). Table 2 summarizes the treatments per experiment and the number of pigs used.

Supplements [i.e., L-Trp (98%, Ajinomoto Eurolysine, Orffa, Londerzeel, Belgium), vitamin E (α-tocopheryl acetate; Rovimix E-50 SD, DSM Nutrition, Deinze, Belgium), or vitamin C (l-ascorbic acid; Rovimix Stay-C 35, DSM Nutrition)] were mixed into the feed for 30 min in a feed mixer, just before the beginning of a new replicate. Once, a supplemented feed sample and a control feed sample were analyzed for the added supplement (Trp; HPLC; AFNOR XPV 18-114; Manz and Philipp, 1981), α-tocopheryl acetate (HPLC; Manz and Philipp, 1981), or ascorbic acid (titration with 2,6-dicholophenolindolphenol). During the period in which Trp, vitamin C, or vitamin E was supplemented, feed intake per pen for the control and supplemented pigs was measured.

The herbal product (Sedafit ES, Phytosynthèse, Saint-Bonnet de Rochefort, France) and Mg (Mg acetate; Magac, Verdugt, Tiel, The Netherlands) were dissolved in the water by means of a medicator unit (Mini Schippers, Bladel, The Netherlands), having a reservoir in which the products were concentrated 50 times. For practical reasons, 4 pens at 1 side of a compartment were supplied with supplemented tap water, whereas the pens at the opposite side of the compartment had access to tap water without a supplement.

**Slaughter Procedures and Measurements**

At the end of the feeding period, pigs were weighed and were fasted for 18 h. In the morning (at 0630), pigs of 2 pens were mixed, and pigs were transported (95 ± 16 min) to a commercial slaughterhouse over a distance of 110 km, with 4 (Exp. 1 to 3; 2 control, and 2 supplemented groups) or 3 (Exp. 4; 1 control, 1 vitamin C-supplemented, and 1 vitamin E-supplemented group) groups of 11 pigs (mix of 2 pens). From each group, 5 (Exp. 1 to 3) or 7 (Exp. 4) pigs were selected on the basis of their live BW (between 100 and 115 kg) and sex (same ratio in each group) for blood sampling and meat quality measurements. The pigs spent at least 1 h (85 ± 15 min) in lairage in the
same groups as on the trailer. The dimensions of the compartments on the trailer and during lairage were 2.43 m × 2.73 m and 2.00 m × 3.07 m, respectively.

Pigs were stunned electrically (240 V, 800 Hz for 2 s) and killed by exsanguination. At slaughter, blood samples from the selected pigs were collected in two 4-mL tubes (Venoject, Terumo, Haasrode, Belgium) containing 9.0 mg of NaF plus 9.0 mg of K$_2$Oxalate and 60 USP U of lithium heparin, respectively, and immediately stored on ice. Next, the samples were centrifuged for 15 min at 713 × g, and the plasma was frozen and stored at −20°C until analysis. Enzymatic, colorimetric methods were used to analyze these samples to determine the concentrations of glucose (GOD/POD Trinder’s method, Instrumentation Laboratory, Zaventem, Belgium) and lactate (NAD/LM, Sigma-Aldrich, Bornem, Belgium) in the plasma collected in the NaF-K$_2$Oxalate tubes, and creatine kinase (CK-NAC, Instrumentation Laboratory), NEFA (NEFA C, Wako Chemicals GmbH, Neuss, Germany), and cortisol (EIA, Active cortisol, Diagnostic Systems Laboratories, Veghel, The Netherlands) in the plasma collected in the heparin tubes.

In the slaughter line, skin lesions on shoulder, loin, and ham, indicative of fights (Faucitano, 2001) were visually assessed using a 4-point scale with 1 = no damage, 2 = slight skin damage, 3 = skin damage affecting quality, and 4 = extreme damage (Barton Gade et al., 1995), with a standard series of photographs as a template. A SKGII-device (Schlachtkörper Klassifizierungs Gerät, Tecpro GmbH, Willich, Germany) determined loin width. The pH and temperature of the LM between the 4th and 5th ribs was determined 45 min (pH$_1$) postmortem using a pH meter equipped with an insertion glass electrode and a temperature probe (PH/PT-STAR, R. Matthäus, Pöttmes, Germany). Similarly, the pH was measured in the m. biceps femoris (BF).

After chilling overnight at 2°C, the carcasses were commercially cut and transferred to a grocery store, where the meat quality of the loin was measured 48 h postmortem. Between the 4th and the 5th back ribs of the LM muscle, the ultimate pH (PH/PT-STAR), and electrical conductivity (Pork Quality Meter, Intek Klassifizierungs-technik, Aichach, Germany) were measured. The loin color in the transverse cut between the fourth and the fifth (back) ribs, was evaluated with the Japanese color scale (1 = pale gray to 6 = dark purple; Nakai et al., 1975) and the Comission Internationale de l’Eclairage (CIE, 1976) values (L*, a*, and b*) measured by a chromameter (CR300, Minolta, Osaka, Japan) with a D65 illuminant (diffuse illumination/0° viewing angle). On this cut, the water holding capacity (WHC) was determined according to the

### Table 1. Dose, duration, and route of administration of the supplemented products

<table>
<thead>
<tr>
<th>Product</th>
<th>Dose</th>
<th>Duration</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herbs$^1$</td>
<td>2.5 g/L</td>
<td>2 d</td>
<td>Drinking water</td>
</tr>
<tr>
<td>Magnesium$^2$</td>
<td>3 g/L</td>
<td>2 d</td>
<td>Drinking water</td>
</tr>
<tr>
<td>Tryptophan$^3$</td>
<td>6 g/kg$^4$</td>
<td>5 d</td>
<td>Feed</td>
</tr>
<tr>
<td>Vitamin E$^5$</td>
<td>150 mg/kg$^4$</td>
<td>21 d</td>
<td>Feed</td>
</tr>
<tr>
<td>Vitamin C$^6$</td>
<td>300 mg/kg$^4$</td>
<td>21 d</td>
<td>Feed</td>
</tr>
</tbody>
</table>

$^1$Commercial herbal product with Valerina officinalis L. and Passiflora incarnata L. as active components.
$^2$Mg acetate.
$^3$L-Tryptophan.
$^4$As-fed basis.
$^5$D,L-α-Tocopheryl acetate.
$^6$L-Ascorbic acid.

### Table 2. Treatments per experiment and total number of pigs tested per treatment

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Treatment</th>
<th>No. of pigs/T$^1$</th>
<th>No. of meat quality$^2$/T-R</th>
<th>No. of replicates</th>
<th>Total No. of pigs/T</th>
<th>Total No. of meat quality$^2$/T</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control$^3$</td>
<td>Herbs$^4$</td>
<td>22</td>
<td>10</td>
<td>4</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>Mg$^5$</td>
<td>22</td>
<td>10</td>
<td>4</td>
<td>88</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>Trp$^6$</td>
<td>22</td>
<td>10</td>
<td>4</td>
<td>88</td>
</tr>
<tr>
<td>4</td>
<td>Control</td>
<td>Vitamin C$^7$</td>
<td>11</td>
<td>7</td>
<td>6</td>
<td>66</td>
</tr>
</tbody>
</table>

$^1$T = treatment; R = replicate.
$^2$Number of pigs selected for meat quality measurements.
$^3$No supplementation.
$^4$Commercial herbal product with Valerina officinalis L. and Passiflora incarnata L. as active components.
$^5$Mg acetate.
$^6$L-Tryptophan.
$^7$Vitamin C = L-ascorbic acid.
$^8$Vitamin E = D,L-α-tocopheryl acetate.
filter paper method of Kauffman et al. (1986), where a decreasing weight of the applied filter paper indicated an increasing WHC.

**Statistical Analyses**

Data on meat quality variables and plasma constituents were analyzed with a mixed model (SAS Inst. Inc., Cary, NC), with treatment as the main variable and sex and loin width as covariates. The feed intake data were also analyzed by a mixed model, with treatment as the main variable and average slaughter weight of the pen as the covariable. For both models, replicate was used as a random factor. For the feed intake data, pen was experimental unit; for the other analyses, pig was the experimental unit.

Least mean squares were calculated, and differences between treatments or periods were separated using orthogonal contrasts. Errors were checked for normality and an independent distribution with constant variance. If necessary, a transformation (ln x) was performed (in the case of WHC, a* values, b* values, and cortisol, glucose, NEFA, and creatine kinase concentrations). The means of the transformed data were retransformed, and the SE were calculated using the delta method (Serfling, 1980).

Because of the low incidence of severe skin damage, pigs were regrouped into 2 groups with and without lesions on the shoulder, loin, or ham. These data were analyzed using the $\chi^2$ frequency analysis.

**RESULTS**

When the supplement was provided via the feed, in case of Trp, vitamin E, and vitamin C, feed samples were analyzed for these supplements. The amount of L-Trp in a nonsupplemented sample and a Trp-supplemented sample was 0.2 and 5.56 g of Trp/kg of feed, respectively. In a control sample and a vitamin E-supplemented sample, 81.4 and 250 mg of $\alpha$-D,L-trytophan and 0.2 and 5.56 g of Trp/kg of feed, respectively. For vitamin C, no L-ascorbic acid was found in a control sample and a vitamin E-supplemented sample, whereas the amount in a vitamin C-supplemented sample was 286 mg of L-ascorbic acid/kg of feed.

Analysis showed no differences between the feed intake of pigs supplemented with the control feed and pigs supplemented with the Trp feed (control: $1.63 \pm 0.09$ kg of feed/pig-d; Trp pigs: $1.62 \pm 0.09$ kg of feed/pig-d, $P = 0.89$). For Exp. 4, feed intake differences were found between treatments: the vitamin E-supplemented pigs had a lower feed intake ($1.84 \pm 0.08$ kg feed/pig-d) than the control ($2.15 \pm 0.08$ kg feed/pig-d, $P = 0.03$) and vitamin C-supplemented pigs ($2.13 \pm 0.08$ kg feed/pig-d, $P = 0.03$).

Cortisol analysis of plasma samples collected at slaughter showed no significant differences between the different treatments in any experiment (Table 3). Similarly, the plasma concentrations of glucose, lactate, NEFA, and creatine kinase at slaughter of pigs supplemented with herbs, magnesium, tryptophan, vitamin E, or vitamin C were analyzed using the least square means ($\pm$ SEM) of plasma cortisol, glucose, lactate, NEFA, and creatine kinase at slaughter of pigs supplemented with herbs, magnesium, tryptophan, vitamin E, or vitamin C

![Table 3](https://example.com/table3.png)

Within a row and experiment, means without a common superscript letter differ, $P < 0.05$.

1. For all experiments, control was without supplementation.
2. Commercial herbal product with Valeriana officinalis L. and Passiflora incarnata L. as active components at 1 g/L of drinking water for 2 d.
3. Mg acetate at 3 g/L of drinking water for 2 d.
4. L-Tryptophan at 6 g/kg of feed for 5 d, as-fed basis.
5. Vitamin E (D,L-$\alpha$-tocopheryl acetate) at 150 mg/kg of feed for 21 d, as-fed basis.
6. Vitamin C (L-ascorbic acid) at 300 mg/kg of feed for 21 d, as-fed basis.
7. Retransformed data, geometric means. CK = creatine kinase.
Table 4. Percentage of pigs with or without shoulder, loin, and ham skin lesions per treatment of pigs supplemented with herbs, magnesium, tryptophan, vitamin E, or vitamin C

<table>
<thead>
<tr>
<th>Item</th>
<th>Experiment/treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control¹</td>
<td>Herbs²</td>
<td>Control</td>
<td>Magnesium³</td>
</tr>
<tr>
<td>No. of carcasses</td>
<td></td>
<td>88</td>
<td>88</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>Shoulder lesions</td>
<td>No</td>
<td>74.6*</td>
<td>59.0*</td>
<td>68.9</td>
<td>67.8</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>25.4*</td>
<td>41.0*</td>
<td>31.1</td>
<td>32.2</td>
</tr>
<tr>
<td>Loin lesions</td>
<td>No</td>
<td>85.1*</td>
<td>69.2*</td>
<td>57.8*</td>
<td>76.7*</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>14.9*</td>
<td>30.8*</td>
<td>42.2*</td>
<td>23.3*</td>
</tr>
<tr>
<td>Ham lesions</td>
<td>No</td>
<td>89.5</td>
<td>88.5</td>
<td>92.2</td>
<td>92.2</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>10.5</td>
<td>11.5</td>
<td>7.8</td>
<td>7.8</td>
</tr>
</tbody>
</table>

¹For all experiments, control was without supplementation.
²Commercial herbal product with *Valeriana officinalis* L. and *Passiflora incarnata* L. as active components at 1 g/L of drinking water for 2 d.
³Mg acetate at 6 g/kg of feed for 5 d, as-fed basis.
⁴L-Tryptophan at 6 g/kg of feed for 5 d, as-fed basis.
⁵Vitamin E (D,L-α-tocopheryl acetate) at 150 mg/kg of feed for 21 d, as-fed basis.
⁶Vitamin C (l-ascorbic acid) at 300 mg/kg of feed for 21 d, as-fed basis.
*Within an experiment, the incidence of skin lesions was dependent on treatment, χ² statistic; P < 0.05.

tate, and creatine kinase did not differ between the supplemented and nonsupplemented group of an experiment. Pigs provided with Mg (P = 0.002) or Trp (P = 0.04) had lower plasma NEFA concentrations at slaughter than their control group, and the pigs supplemented with vitamin C had a greater plasma NEFA level compared with the control (P = 0.03) and vitamin E group (P = 0.01).

Table 4 shows that 25.4% of the control pigs in Exp. 1 had shoulder lesions, in contrast to 41.0% of pigs supplemented with herbs (P = 0.05). Also, a greater frequency of pigs with loin lesions was observed for this latter treatment (P = 0.03). The pigs supplemented with Mg had a lower incidence of loin lesions, compared with the control pigs (P = 0.01). Overall, the incidence of ham lesions was low (approximately 10%), and no differences between treatments were found.

The supplementation of herbs, Mg, Trp, vitamin E, or vitamin C did not result in a different pH of LM and BF muscles 45 min postmortem in comparison with the control group of each experiment (Table 5). Also, the temperature of LM did not differ between the control and the supplemented groups.

Measurement of the pH in the LM 48 h postmortem revealed no significant differences between the treatments of any experiment (Table 6). Similarly, the electrical conductivity was equal for the control and the supplemented groups. Considering the color of the investigated muscle, the same color characteristics were observed and measured for the experiments with herbs, Mg, and Trp. In Exp. 4, in which the supplementation of vitamin E and vitamin C was tested, color differences were found. Judging the color with the use of the Japanese color scale resulted in greater color scores (less pale meat) for loins of pigs supplemented with vitamin E (P = 0.0007) and vitamin C (P = 0.006) compared with the control loins. The L* value of LM of pigs treated with vitamin C was lower than the L* value of the muscle of control pigs (P = 0.04) and vitamin E pigs (P = 0.04). In this experiment, the a* and b* values of the muscles of the control pigs were the lowest. More precisely, the loin of the control group was less red than the loin of the vitamin E- (P = 0.03) and vitamin C- (P = 0.03) supplemented pigs, and the loin of the pigs fortified with vitamin E had a greater yellow component compared with the loin of the control pigs. The WHC was equal for the 2 or 3 treatments within an experiment.

**DISCUSSION**

Mixing a supplement in the feed only a few days before slaughter would only be practical in commercial circumstances in case the appropriate equipment is available. Moreover, it is recommended that pigs be fasted for at least 12 h preslaughter (Eikelenboom et al., 1991), so that effective results will be expected when providing the supplement via the drinking water until the beginning of pigs’ loading. A disadvantage is the more difficult way to control the supplement intake per pig. For the short-term supplies in the current study, only the herbs and Mg are added via the drinking water because the product had to be concentrated 50 times. Tryptophan was also supplemented for a short period but was not dissolvable at the required concentration, so it was added through the feed.

**Herbs**

The herbal product did not affect the plasma cortisol, glucose, lactate, NEFA, and creatine kinase concentra-
least square means (±SEM) of meat quality variables measured 45 min postmortem in the LM and biceps femoris of pigs supplemented with herbs, magnesium, tryptophan, vitamin E, or vitamin C.

<table>
<thead>
<tr>
<th>Experiment/treatment</th>
<th>Control</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Item/variable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. carcasses</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>pH1 LM</td>
<td>6.03 ± 0.09</td>
<td>6.06 ± 0.05</td>
<td>6.05 ± 0.04</td>
<td>6.06 ± 0.05</td>
<td>6.09 ± 0.04</td>
</tr>
<tr>
<td>pH2 BF</td>
<td>6.05 ± 0.09</td>
<td>6.06 ± 0.05</td>
<td>6.05 ± 0.04</td>
<td>6.06 ± 0.05</td>
<td>6.09 ± 0.04</td>
</tr>
</tbody>
</table>

1For all experiments, control was without supplementation.
2Commercial herbal product with Valeriana officinalis L. and Passiflora incarnata L. as active components at 1 g/L of drinking water for 2 d.
3Mg acetate at 3 g/L of drinking water for 2 d.
4L-Tryptophan at 6 g/kg of feed for 5 d, as-fed basis.
5Vitamin E (D,L-α-tocopheryl acetate) at 150 mg/kg of feed for 21 d, as-fed basis.
6Vitamin C (L-ascorbic acid) at 300 mg/kg of feed for 21 d, as-fed basis.
7pH measured 45 min postmortem in the LM.
8Temperature measured 45 min postmortem in the LM.
9pH measured 45 min postmortem in biceps femoris.
10For commercial herbal product with Valeriana officinalis L. and Passiflora incarnata L. as active components at 1 g/L of drinking water for 2 d.
11Mg acetate at 3 g/L of feed for 5 d, as-fed basis.
12Vitamin E (D,L-α-tocopheryl acetate) at 150 mg/kg of feed for 21 d, as-fed basis.
13Vitamin C (L-ascorbic acid) at 300 mg/kg of feed for 21 d, as-fed basis.

In the current study, Mg supplementation could not lower the plasma cortisol concentration at slaughter. Similarly, Apple et al. (2005) reported no effect of Mg inclusion (2.5% Mg mica) in the diets of finishing pigs on the serum cortisol concentration. Also the plasma glucose, lactate, and NEFA concentrations of the latter study were not influenced by Mg supplementation. Geesink et al. (2004) observed no difference in plasma glucose and lactate concentration between control pigs and pigs fortified with 6.8 g/kg of feed grade Mg acetate.
Table 6. Least square means (± SEM) of meat quality variables measured 48 h postmortem in the LM of pigs supplemented with herbs, magnesium, tryptophan, vitamin E, or vitamin C.

<table>
<thead>
<tr>
<th>Item/variable</th>
<th>Experiment/treatment</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Herbs</td>
<td>Control</td>
<td>Magnesium</td>
<td>Vitamin E</td>
</tr>
<tr>
<td>No. of carcasses</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>pH&lt;sup&gt;7&lt;/sup&gt;</td>
<td>5.55 ± 0.02</td>
<td>5.56 ± 0.02</td>
<td>5.54 ± 0.04</td>
<td>5.52 ± 0.04</td>
<td>5.55 ± 0.04</td>
</tr>
<tr>
<td>PQM,&lt;sup&gt;8&lt;/sup&gt; ms/cm</td>
<td>6.03 ± 0.46</td>
<td>6.17 ± 0.46</td>
<td>6.70 ± 0.21</td>
<td>7.20 ± 0.21</td>
<td>6.18 ± 0.52</td>
</tr>
<tr>
<td>Color</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japanese scale&lt;sup&gt;9&lt;/sup&gt;</td>
<td>2.57 ± 0.08</td>
<td>2.66 ± 0.08</td>
<td>2.70 ± 0.10</td>
<td>2.62 ± 0.10</td>
<td>2.48 ± 0.19</td>
</tr>
<tr>
<td>L* value&lt;sup&gt;10&lt;/sup&gt;</td>
<td>53.4 ± 0.6</td>
<td>54.8 ± 0.6</td>
<td>53.1 ± 0.6</td>
<td>53.6 ± 0.6</td>
<td>53.9 ± 0.9</td>
</tr>
<tr>
<td>a* value&lt;sup&gt;10,11&lt;/sup&gt;</td>
<td>6.51 ± 0.41</td>
<td>6.74 ± 0.43</td>
<td>6.49 ± 0.26</td>
<td>6.81 ± 0.28</td>
<td>6.33 ± 0.27</td>
</tr>
<tr>
<td>b* value&lt;sup&gt;10,11&lt;/sup&gt;</td>
<td>4.27 ± 0.26</td>
<td>4.64 ± 0.28</td>
<td>4.34 ± 0.21</td>
<td>4.43 ± 0.22</td>
<td>4.51 ± 0.23</td>
</tr>
<tr>
<td>WHC,&lt;sup&gt;11&lt;/sup&gt; mg</td>
<td>48.9 ± 3.4</td>
<td>56.3 ± 4.0</td>
<td>57.4 ± 9.3</td>
<td>68.1 ± 11.1</td>
<td>40.8 ± 5.5</td>
</tr>
</tbody>
</table>

<sup>1</sup>Within a row and experiment, means without a common superscript differ, P < 0.05.
<sup>2</sup>For all experiments, Control was without supplementation.
<sup>3</sup>Commercial herbal product with Valeriana officinalis L. and Passiflora incarnata L. as active components at 1 g/L of drinking water for 2 d.
<sup>4</sup>Mg acetate at 3 g/L of drinking water for 2 d.
<sup>5</sup>L-Tryptophan at 6 g/kg of feed for 5 d, as-fed basis.
<sup>6</sup>Vitamin E (D,L-α-tocopheryl acetate) at 150 mg/kg of feed for 21 d, as-fed basis.
<sup>7</sup>Vitamin C (L-ascorbic acid) at 300 mg/kg of feed for 21 d, as-fed basis.
<sup>8</sup>pH measured 48 h postmortem.
<sup>9</sup>PQM: electrical conductivity.
<sup>10</sup>Japanese color scale, from 1 = pale gray to 6 = dark purple.
<sup>11</sup>L*: lightness (larger number indicates a lighter color); a*: redness (larger number indicates a more intense red color); b*: yellowness (larger number indicates more yellow color).
<sup>zz</sup>Retransformed data, geometric means. WHC = water holding capacity.
for 5 d. Otten et al. (1995), on the contrary, demonstrated lowered plasma glucose and lactate concentrations at slaughter in pigs provided with Mg fumarate during the growing and finishing phase. Pigs fortified with Mg had fewer loin lesions than control pigs, which may be an indication that these pigs fought less and were more relaxed. Also in our previous experimental research in which pigs were vibrated after supplementation (Peeters et al., 2005), Mg pigs were visually calmer than control pigs. Similarly, Kuhn et al. (1981) reported visually calmer pigs after long-distance transportation due to Mg supplementation, and studies with normal Mg-sufficient rats, mice, and guinea pigs showed that intraperitoneal injections with Mg decreased motility and explorations (Kaemmerer and Kietzmann, 1984). Contrary, Caine et al. (2000) observed an overall greater frequency of aggression for pigs receiving dietary supplementation of Mg aspartate hydrochloride (40 mg/kg of BW for 7 d), which was more pronounced in homozygous halothane-negative pigs than in heterozygous halothane-positive pigs. In the current study, no differences in pH of the LM and BF muscle were found between the Mg-supplemented and control pigs. Several authors reported elevated pH of the LM after Mg supplementation from different sources and for different application periods (D’Souza et al., 1998; Caine et al., 2000; Lahucky et al., 2004), whereas in other studies no effect of Mg on the initial pH was found (D’Souza et al., 1999, 2000; Frederick et al., 2004; Apple et al., 2005). Considering the ultimate pH (24 h or 48 h), there was no influence of Mg in the majority of studies (Schaefer et al., 1993; D’Souza et al., 1999, 2000; Apple et al., 2000, 2002; Hamilton et al., 2003; Frederick et al., 2004), which was also the case for the current study. It may be that differences would be found using a greater sample size. Similar to Apple et al. (2005) and Frederick et al. (2004), no temperature differences were registered between the LM of the control and Mg pigs of the current study. However, in the studies of Schaefer et al. (1993) and Caine et al. (2000), the muscle temperature decreased after Mg supplementation of short and long duration, respectively. In the current study, the electrical conductivity did not differ between the 2 tested groups of carcasses, as found by Lahucky et al. (2004) after a supplementation with 3.6 g of MgO/(pig d) for 5 d. Considering the color of LM 2 d postmortem, no influence was expected based on the results of previous studies (Caine et al., 2000; Apple et al., 2002, 2005, Frederick et al., 2004, Lahucky et al., 2004), which is confirmed in the current study. Geesink et al. (2004) reported darker (based on L* values and Japanese color scale) and redder (based on a* values) meat after a Mg acetate supplementation of 5 d but only when pigs were slaughtered upon arrival at the abattoir and not allowed to rest for 2 h. It may thus be assumed that pigs have to experience a greater stress level to obtain effects on meat color due to Mg supplementation. Several authors (D’Souza et al., 1998, 1999, 2000; Hamilton et al., 2003; Lahucky et al., 2004) demonstrated drip loss reduction in the LM of pigs that received short-term Mg supplementation, whereas longer supplementation periods in the experiments of Apple et al. (2000, 2002, 2005) and Caine et al. (2000) seemed not to be effective. Other studies with shorter application time were without a reducing effect of Mg on drip loss (Frederick et al., 2004; Geesink et al., 2004). In the current study, no effect of Mg supplementation was found on the WHC. Apple et al. (2000) stated that the magnitude of the effect of Mg supplementation on meat quality might be related to the stress susceptibility or resistance of pigs used in the experiment.

Tryptophan

Peeters et al. (2005) demonstrated that a supplementation of 6 g of Trp/kg of feed for 5 d was able to increase the plasma Trp concentration and the ratio of the Trp concentration to the sum of the concentration of other large neutral amino acids (Tyr, Phe, Val, Leu, and Ile). Because of the sedative effect of a subsequent increased brain serotonin level, synthesized from Trp, a lowering effect on cortisol secretion might be expected (Henry et al., 1992). In the current study, the plasma cortisol level of the pigs fed the Trp-supplemented diet tended to be lower than that of the control pigs at slaughter, but the difference was not significant. Mormede and Dantzer (1979) demonstrated no effect of Trp (50 to 200 mg L-Trp/kg of BW) on the plasma cortisol level after a fear, avoidance, and frustration test, but a dose-dependent lowering of the cortisol concentration was found after introducing the pig into a new environment. Meunier-Salan et al. (1991) found no influences of dietary Trp (0.14, 0.23, or 0.32% for 3 wk) on plasma cortisol concentrations. In our previous studies, where pigs were vibrated to evoke a stress response (Peeters et al., 2004, 2005), neither salivary cortisol, plasma glucose, lactate, NEFA, nor creatine kinase differences were found between the groups with and without Trp supplementation. In the current study, Trp supplementation caused a lowering of the plasma NEFA concentration, which might be explained by the fact that Trp and NEFA have the same carrier, i.e., albumin (Davis et al., 2000). In a study of McIntyre and Edwards (2001), pigs fed diets with excess Trp (0.32%) were the least active and spent the most time sleeping compared with the pigs supplemented with 0.12% (deficient) and 0.22% (optimal) Trp. Also Peeters et al. (2004) observed that Trp-supplemented pigs lay down more during a vibration test. On the contrary, the effect of Trp on the exploration time and number of grunts in an open-field test were relatively minor in the study of Meunier-Salau¨ ne t al. (1991). If a reduced incidence of aggression due to Trp is assumed in pigs (Warner et al., 1998), a lowering of skin lesions caused by fights during transport and lairage was expected. Nevertheless, no effect of Trp
supplementation on the frequency of shoulder, loin, or ham lesions was observed. The supplementation of 6 g of Trp/kg of feed for 5 d before slaughter could also not influence the pH, carcass temperature, electrical conductivity, color, or WHC of LM. Similarly, Sève et al. (1991) found no differences in the meat quality of pigs supplemented with a deficient (0.70 g/16 g of N protein), adequate (1.15 g/16 g of N protein), or excess level (1.60 g/16 g of N protein) of Trp in their diet. Surprisingly, Henry et al. (1992) reported that the muscle pH (adductor femoris and semimembranosus muscle) 45 min and 24 h postmortem was increased after a Trp-deficient (0.09 vs. 0.13%) diet, especially in females compared with castrated males. Greater dietary Trp levels (0.12 vs. 0.16%) increased the pH 45 min and 24 h postmortem, especially in the LM (Henry et al., 1996). This could be explained by the pH pattern 24 h postmortem in relation to the Trp amount observed by Henry and Sève (1993), with the lowest pH values after feeding a Trp-adequate diet and a greater pH with both lower and greater Trp supplies. However, no effect of 5 g of Trp/kg of diet for 5 d on the pH or color of loin and ham was reported in slaughtered pigs by Adeola and Ball (1992), but they did find a lower frequency of severe PSE after Trp supplementation. This decreased incidence of PSE was not found by Warner et al. (1998) when the same application time and doses of Trp were used. Overall, the effect of Trp on meat quality was not frequently tested, and in most cases no effect or a relatively minor effect was demonstrated.

**Vitamin E**

In a previous study, we demonstrated that an extra 150 mg of α-tocopheryl acetate/kg of diet doubled the plasma vitamin E concentration after 3 wk of supplementation. Also no effect of vitamin E supplementation to the diet on the feed intake was found, whereas in the current experiment supplemented pigs ate less of their diet than the control pigs (and the vitamin C-supplemented pigs). Because in other studies no effect of vitamin E addition on feed intake was demonstrated, even with longer application times, greater vitamin E concentrations, or both (Asghar et al., 1991a; Hoving-Bolink et al., 1998; Onibi et al., 1998; Lauridsen et al., 1999a), it can be assumed that this lower feed intake is due to another unknown factor. Assuming that α-tocopherol preserves the integrity of muscle cell membranes by preventing the oxidation of membranal phospholipids (Asghar et al., 1991a), a reduced leakage of enzymes such as creatine kinase into the plasma may be expected (Duthie et al., 1989; Nockels et al., 1996; Peeters et al., 2005). Nevertheless, only a numerically lower creatine kinase concentration could be demonstrated, similar to Asghar et al. (1991b) and Lauridsen et al. (1999a) who did not find significant differences. Pigs fed additional vitamin E were more relaxed during a vibration test (Peeters et al., 2005). If pigs are more relaxed in a stress situation, fewer skin lesions on the carcasses of vitamin E pigs might be observed, but no significant effects were noticed. As in other studies with pH measurements (Cannon et al., 1996; Hoving-Bolink et al., 1998; Gebert et al., 1999; Lauridsen et al., 1999b; Hasty et al., 2002), the pH values, measured 45 min and 48 h postmortem, were not affected by a supplementation of vitamin E. Vitamin E has not been reported to have a direct effect on glycolytic potential or postmortem glycolysis; thus no dietary effect on muscle pH was expected (Hasty et al., 2002). The muscle temperature was also not influenced, which is in agreement with Lauridsen et al. (1999b), Hasty et al. (2002), and Rosenvold et al. (2002). The cuts of the LM were darker (measured by the Japanese color score), redder (a* value), and more yellow (b* value) from the pigs fed vitamin E compared with the control treatment. Considering the ability of vitamin E to retard the oxidation of myoglobin or oxymyoglobin, or both, to metmyoglobin (Faustman et al., 1989), a color improvement was expected. The redness values give more important information with respect to the sensory perception of meat color: consumers consider bright red colored meat as fresh and are averse to brown meat (Dirinck et al., 1996). The influence of vitamin E on meat redness was also reported by Asghar et al. (1991a) and Monahan et al. (1994); however, other authors did not find any influences of diets with an increased vitamin E level (Dirinck et al., 1996; Cannon et al., 1996; Hoving-Bolink et al., 1998; Kerth et al., 2001; Rosenvold et al., 2002). Nevertheless, in the majority of these latter studies, greater vitamin E levels and longer application times were used compared with the current study. Considering yellowness, only Hasty et al. (2002) reported a tendency for vitamin E to increase b* values linearly. Alpha-tocopherol could preserve the integrity of muscle cell membranes by preventing the oxidation of membrane phospholipids during storage and reduce the loss of sarcoplasmic fluid through the muscle cell membrane (Asghar et al., 1991a). However, no differences in drip loss between the diet groups were found, and this agrees with findings of Cannon et al. (1996), Gebert et al. (1999), and Hasty et al. (2002). The effect of vitamin E on drip loss and color stability beyond the 48 h could not be investigated because meat was used for commercial purposes. The stabilizing effect of vitamin E on heart rate variables resulting in a greater coping ability to a stress situation (Peeters et al., 2004, 2005) was not reflected in the meat quality.

**Vitamin C**

In the previous study (Peeters et al., 2005), no increases in plasma vitamin C concentration were observed after 3 wk of 300 mg of vitamin C/kg of supplementation, which may be due to the use of an unstable L-ascorbic acid source. In the current study, a more stable vitamin C source was used. Indeed, analysis of
a feed sample showed an additional amount of 286 mg of L-ascorbie acid/kg of feed, whereas in the previous study, only a difference of 193 mg of L-ascorbie acid/kg of diet was found after an addition of 300 mg/kg of feed. The addition of vitamin C to the feed did not affect the feed intake compared with the control group. No changes in cortisol concentrations were observed after vitamin C supplementation at slaughter, similarly to Pion et al. (2004) who supplemented a greater vitamin C concentration for a shorter application period (1,000 or 2,000 mg of vitamin C/L of drinking water for 48 h). As in broiler chickens (Lauridsen et al., 1997) and humans (Thompson et al., 2001; Dawson et al., 2002), vitamin C supplementation did not affect the creatin kinase concentration, explaining that vitamin C did not protect against oxidative stress or that the oxidative stress (resulting in the leakage of creatine kinase in plasma) was not strong enough. Vitamin C supplementation elevated the NEFA concentrations compared with the control and vitamin E group for an unknown reason. Remarkably, pigs supplemented with vitamin C also had the greatest concentration of NEFA of all treatments (Mg, Trp, vitamin C, vitamin E, carazolol, and control treatment) in our previous study (Peeters et al., 2005). In a study of Pion et al. (2004), no influence of a short-term vitamin C supplementation on pH, temperature, color, and fluid loss was observed. However, Mourot et al. (1992) did report a lowering of pH and a color improvement after a long-term vitamin C supplementation (250 mg of vitamin C/kg of feeds from 35 to 100 kg of BW). In the current study, only an effect on the meat color 48 h postmortem but not on the pH can be reported: the LM was less pale, as demonstrated by both the subjective (Japanese color scale) and the objective (L* value) color measurement methods, and redder (greater a* value) than the samples of the control group. These findings (no pH effect, but a color effect) may indicate that the role of vitamin C in the improvement of the meat quality is mainly as an antioxidant (Packer et al., 1979) and less or not as a glycolytic inhibitor (through its metabolite oxalic acid; Tonon et al., 1998) at the used doses.

The results of previous studies (Peeters et al., 2004, 2005) indicated that supplementation of Mg, Trp, vitamin C, and vitamin E improved the coping ability during transport simulation compared with a control treatment. Testing these supplements in the current study with slaughter pigs revealed that this increased stress coping ability was not reflected in an improvement of the meat quality. The amelioration of the meat color after vitamin C and vitamin E supplementation was possibly due to their antioxidative properties, rather than a lower stress response. Combining the results of the studies, a supplementation of vitamin E could be advised due to most effective action on the stress responses (Peeters et al., 2004, 2005) and improved meat quality. Also the relaxing effect of Mg causing fewer loin lesions is an advantage in view of welfare and economy.

LITERATURE CITED


