Dietary Effects Of Single And Combined Antioxidant Vitamins On Antioxidant Enzymes And Oxidants Status Of Growing Pigs

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Abstract: The effects of single and combined dietary antioxidant vitamins on antioxidants: antioxidant power (AOP), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione (GSH) as well as oxidants, such as oxidized glutathione (GSSH), xanthine oxidase (OX), cortisol and malondialdehyde (MDA) were studied in growing pigs. Pigs received their dietary antioxidant vitamins as: To (control diet – contained vitamins at their basal levels); T_A (200mg of vitamin A); T_C (200mg of vitamin C); T_AC (100mg of vitamin A + 100mg of vitamin C); T_AE (100mg of vitamin A + 100mg of vitamin E); T_CE (100mg of vitamin C + 100mg of vitamin E)/kg of diet, respectively for 28 days. AOP and CAT levels, particularly with the combined vitamins were significantly (P < 0.05) higher compared with the control. SOD, GSH-Px and GSH concentrations were significantly (P < 0.05) higher with the combined vitamins compared with the control and single vitamin diets. GSSH concentrations were similar (P > 0.05) for T_AE and T_CE diets and significantly (P < 0.05) lower than those of control, T_A, T_C and T_AC diets. OX, cortisol and MDA concentrations were significantly (P < 0.05) lower in the vitamins diets compared with the control. It was concluded that antioxidant vitamins combinations, especially that of T_CE most improved antioxidant statuses while simultaneously reduced oxidant statuses in the growing pig.

Key words: Antioxidant vitamins, Antioxidant enzymes, Oxidants and Pig.

1 Introduction
Growing pigs are very fast growing animal species. The fast growing process usually results in the ‘sudden death syndrome’ often observed in growing pigs in the commercial setting [12]. The attendant effect of this syndrome leads to the reduction of the hog farmer profit margin and thus calls for the search for strategies in dealing with the situation. It is a known fact that animal health depends on many factors and it has come to the fore that diets play critical functions in the maintenance of animal health and prevention of various diseases [13], including the sudden death syndrome in the growing pig [12]. Nutrition therefore, remains the fundamental key in prevention-modulation reflecting a special emphasis on diet as an important strategy in the maintenance of animal health [14]. To this point, nutritional science has to move towards the development of recommendations for optimal dietary ingredients, especially the micro-nutrients such as vitamins for the maintenance of good health status of the growing animal for optimal productivity such as the growing pig in commercial conditions with specific emphasis on the health of the animal [12]. Presently, it has become clearer that antioxidant nutrient requirements for the protection of the pig need to be properly established as it relates to their synergies in terms of growth and the health of the animal [8], [12]. Among dietary factors as stated above antioxidants have a special role as they are the major players in health-related conditions of the animal [8]. This is highly correlated with how antioxidants modulate the animal protection system particularly the glutathione system. Vitamins A, C and E are known as antioxidant vitamins that regulate the glutathione defense system of the animal, especially the fast growing species such as the growing pig [12]. However, there is paucity of information about their antioxidant potentials especially when combined in the growing pig. Therefore, the objectives of this study are: to investigate the effects of single and combined effects of antioxidant vitamins A, C and E on antioxidant enzymes as well as their effects on oxidants status, respectively of the growing pig.

2 Materials and Methods

Animals and Housing
Thirty-Six (36) growing landrace pigs of average BW of 6 ± 0.79 (mean ± SD) kg were acquired and used for the experiment. The animals on arrival at the Animal Wing of the Department of Animal Science, Rivers State University were weighed to obtain their initial BW and randomly assigned to pens. Six pigs were assigned to each dietary treatment and fed at 5% of BW (as-fed basis) twice daily at 09:00h (half of the daily meal) and 16:00h, respectively. Animals received their assigned diets for four weeks (4 weeks). They also have unlimited access to water. Animal pens were cleaned regularly.

Experimental Diets
Six corn/soybean-based diets that are isocaloric and isonitrogenous were used in the study. However, vitamins A and C, as well as their combinations with vitamin E dietary contents were different as: To (control diet), T_A (vitamin A diet), T_C (vitamin C diet), T_AC (Vitamins A and C diet), T_AE (vitamins A and E diet) and T_CE (vitamins C and E diet).

That is, T_A (contain basal vitamin levels only); T_A (vitamin A 200mg/kg of diet); T_C (vitamin C 200mg/kg of diet); T_AC (vitamin A 100mg + vitamin C 100mg/kg of diet); T_AE (vitamin A 100mg + vitamin E 100mg/kg of diet); T_CE (vitamin A 100mg + vitamin C 100mg/kg of diet); T_CE (vitamin A 100mg + vitamin C 100mg/kg of diet); T_CE
Experimental Design
The experiment was designed and carried out as a completely randomized designed (CRD) with 6 pigs per treatment.

Blood Sample Collections and Analyses
At the end of study period, blood samples were collected from all animal groups into ethylene diamine tetracetic acid treated tubes for later analyses for antioxidant enzymes as well as oxidants, respectively, and were immediately snap frozen. Antioxidants analysed for were AOP, CAT, SOD, GSH-Px and GSH whereas oxidants analysed for were GSSH, OX, cortisol and MDA, respectively. AOP, GSH, GSH-Px, GSSH, OX and MDA were analysed according to the methods of [7]. CAT was analysed for according to the method of [1]. SOD was analyzed by the method of [11].

Statistical Analysis
Data obtained were subjected to ANOVA using Proc. GLM of SAS (SAS Inst., Cary, NC) according to the experimental model as: Yij = μ + Di + Eij. Where Yij = the observation; μ = overall mean common to all treatments; Di = the effect of the ith diet and Eij = the error term. Means were compared using Tukey's test and α-level of 0.05 was used for all statistical comparisons to represent significance.

3 Results and Discussion
The results of AOP, CAT, SOD, GSH-Px and GSH are shown in Table 1.

Table 1. Oxidant levels of pigs fed different vitamins-based diets

<table>
<thead>
<tr>
<th>Item</th>
<th>T₀</th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
<th>T₄</th>
<th>T₅</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOP (U/mg)</td>
<td>0.6</td>
<td>0.78</td>
<td>0.7</td>
<td>0.9</td>
<td>0.9</td>
<td>0.9</td>
<td>0.98</td>
<td>0.001</td>
</tr>
<tr>
<td>CAT (U/mg)</td>
<td>13</td>
<td>13.7</td>
<td>14</td>
<td>15.5</td>
<td>16.2</td>
<td>0.14</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td>SOD (U/mg)</td>
<td>1.3</td>
<td>1.5</td>
<td>1.5</td>
<td>3</td>
<td>3</td>
<td>0.16</td>
<td>0.050</td>
<td></td>
</tr>
<tr>
<td>GSH-Px (nmol/dl)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>0.07</td>
<td>0.044</td>
</tr>
<tr>
<td>GSH (mg/dl)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>0.01</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Means with the same superscripts within the same row are not significantly (P > 0.05) different
The antioxidant potential (AOP) value was significantly (P < 0.05) lower in animals of T₀ group compared with other groups. Furthermore, animals in treatments T₁ and T₂ had similar (P > 0.05) values of AOP. T₄ animals had AOP values significantly (P < 0.05) higher than those of T₀, T₁ and T₂. However, animals of T₅ had higher (P < 0.05) AOP values compared to animals of T₄ group. Overall, animals in the T₅ group had the highest (P < 0.05) AOP value compared to all animal groups. The level of catalase (CAT) was also lowest (P < 0.05) in animals of T₀ group compared to all treatment groups while animals in groups T₄ and T₅ had similar CAT levels. Similarly, animals in treatments T₄ and T₅ had similar CAT levels that were significantly (P < 0.05) higher than those in treatments T₀ and T₁. Animals in T₅ group significantly (P < 0.05) had the highest level of CAT compared to animals in T₄ and T₅. Peroxide dismutase (SOD) levels were similar (P > 0.05) in animals of T₀, T₁, and T₂ that were significantly (P < 0.05) lower than animals in the T₄, T₅ and T₆ that had similar SOD levels. The trend observed with SOD for the different treatments were mirrored in the levels or concentrations of GSH-Px and GSH (Table 1). The results of the oxidants are shown in Table 2.

Table 2. Oxidant levels of pigs fed different vitamins-based diets

<table>
<thead>
<tr>
<th>Item</th>
<th>T₀</th>
<th>T₁</th>
<th>T₂</th>
<th>T₃</th>
<th>T₄</th>
<th>T₅</th>
<th>T₆</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSSH (umol/dl)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.83</td>
<td>0.17</td>
<td>0.12</td>
<td>0.00</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>OX (umol/dl)</td>
<td>0.1</td>
<td>0.10</td>
<td>0.10</td>
<td>0.09</td>
<td>0.09</td>
<td>0.09</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
</tr>
<tr>
<td>Cortisol (ng/ml)</td>
<td>1.5</td>
<td>1.19</td>
<td>1.18</td>
<td>1.15</td>
<td>1.16</td>
<td>1.07</td>
<td>0.02</td>
<td>0.04</td>
<td>0.8</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>0.1</td>
<td>0.16</td>
<td>0.16</td>
<td>0.14</td>
<td>0.11</td>
<td>0.11</td>
<td>0.00</td>
<td>0.00</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Means with different superscripts within the same row are significantly (P < 0.05) different
The concentrations of oxidized glutathione (GSSH) were similar (P > 0.05) for T₀, T₁, T₂, and T₃ except for T₄ and T₅ that also had similar concentrations of GSSH that were significantly (P < 0.05) lower than the levels found in T₀, T₁, T₂, and T₃ groups. For xanthine oxidase (OX) T₀ group significantly (P < 0.05) had the highest concentration of OX compared to all other treatments with similar concentrations of OX. Similarly, T₀ group significantly (P < 0.05) demonstrated the highest concentrations of cortisol compared to all other treatment groups. T₁, T₂, T₃, and T₄ groups had similar concentrations of cortisol that were significantly (P < 0.05) lower than that of T₅ group while T₆ animals significantly (P < 0.05) showed the lowest concentrations of cortisol compared to all other treatment groups. Malondialdehyde (MDA) concentration was also significantly (P < 0.05) higher in T₀ animals compared to all other treatments. T₁ and T₂ had similar concentrations of MDA that were significantly (P < 0.05) lower than that of T₅ group but significantly (P < 0.05) higher than that of T₄ group. T₅ and T₆ groups had similar concentrations of MDA that were actually the lowest (P < 0.05) MDA concentrations compared to T₄ and other treatment groups, respectively. Antioxidants protect cells, tissues and eventually animals’ organs by reacting with free radicals [12]. Some of these antioxidants are produced in the body such as GSH and its cohorts in the GSH defense system or delivered with feed, thereby making them dietary essentials. However, worthy of note is the fact that the dietary sources of antioxidants are mostly responsible in stimulating the antioxidants that can be synthesized endogenously by the body, such as vitamins A and E on GSH production [12]. Nevertheless, it is worth to note that antioxidants synergistically work together in the animals’ body to achieve their defense functions [15], particularly the GSH defense system of the animal. To this extent therefore, one antioxidant member synergizes with another team member for the system to be efficient. This thus indicates that synergies amongst antioxidants require that diets should...
properly be fortified with adequate dietary antioxidant nutrients. In the ‘modus operandi’ of antioxidants functions SOD is widely perceived as the first level of antioxidant defense since superoxide radical is the major radical produced in physiological conditions in the cell [14]. The activity of superoxide dismutase (the family of enzymes) is mainly responsible for converting superoxide radicals into hydrogen peroxide and oxygen. The next stages of this defense process are related to the activities of GSH-Px and CAT which are mainly responsible for the conversion of the hydrogen peroxide to water. GSH is required largely as a coenzyme by a variety of enzymes, such as GSH-Px and also largely responsible for a variety of crucial life processes, such as detoxification of xenobiotics, removal of hydroperoxides and other free radicals [14]. Therefore, from nutrition standpoint the nutritionists and feed formulators need to recognize the importance of antioxidants dietary needs and its economic importance for efficient animal production. In this study, antioxidants vitamins significantly increased CAT, SOD, GSH-Px, GSH levels, including the antioxidant potential (AOP) values of the pigs that received them in their diets, particularly in combined form. These findings agreed with the studies of [3], [10] that found increase of these antioxidants in the heart, kidney, liver and testis tissues of rats with dietary antioxidant vitamins beyond the basal level. Inclusion of antioxidant vitamins at dietary pharmaceutical levels improved performance and quality of meats by incorporating antioxidants in cell membranes thereby stabilizing tissues with high levels of unsaturated fatty acids [5]. Conversely, while antioxidants values were significantly enhanced those of oxidants, namely: GSSH, OX, cortisol and MDA were significantly reduced by dietary antioxidant vitamins, especially when combined. This clearly demonstrates that the rate of oxidative stress was significantly reduced in animals that ingested the antioxidant vitamins diets [8] – [9]. GSSH is the oxidized form of GSH [12]. Therefore, the reduced level of GSSH in the plasma of animals, particularly those of T_AE and T_CE is an indication of high levels of GSH in the system of those animals. This also corroborates the fact that the sustained high levels of GSH in these animal groups mean that animals of these groups would have experienced lesser activities of oxidative stress compared to the control and some of the single dietary vitamin diet groups, such as those of the T_A and T_CE groups, respectively. Similarly, OX is a pro-oxidant enzyme thus its increased levels would lead to oxidative stress activities [3]. To this point therefore, the reduced levels of OX in plasma of animals with vitamins treated diets indicated reduced oxidative stress in those animals. This was further supported by significant low levels of cortisol. Cortisol is a stress hormone. It usually negatively affects animal growth and performance [12]. The reductions of their concentrations in this study in the antioxidant vitamins diet groups therefore is one of the positive ways in which pig production can be enhanced [4] as this is one of the strategies to positively approach animal production. This is again confirmed by significant low levels in animals that received antioxidant vitamins. MDA is the major by-product of oxidative stress [2], [6]. Their lowered levels here indicated that animals on vitamin treated diets experienced lesser activities of oxidative stress compared with the T_D animals group. These findings are in agreement with data found in the literature [2], [6]. The significant increased concentrations of antioxidants and significant reduced concentrations of oxidants further explain in part the significantly improved ADG and FE observed in the study of [8] with the antioxidants fortified diets compared with their control counterparts.

4 Conclusion
It was concluded that antioxidant vitamins up-regulated the defense system of the animals and as such are capable of maintaining and thus better support animal health to enhance their growth. However, this view is more in support of when the vitamins were combined, especially the combination of vitamins C and E (T_CE) which was observed to most improved antioxidant status while simultaneously reduced oxidant status in the growing pig.

5 References


