The Effects Of The Ingestion Of Single And Combined Dietary Antioxidant Vitamins On Blood And Electrolyte Parameters Of Growing Pigs

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Abstract: Haematological and electrolyte responses of growing pigs to ingestion of single and combined antioxidant vitamins were investigated. 36 pigs of average body weight (BW) of 6 ± 0.79 were randomly divided into one of six experimental groups. The animals received similar commercial diet for one week after which they were offered their experimental diets at 5% of their BW. Animals ingested 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) and T_CE (100mg of vitamin C + 100mg of vitamin E)/kg of diet, respectively for 4 weeks. Packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) and white blood cell (WBC) levels of animals on the combined vitamin diets were significantly (P < 0.05) better than the control and single vitamin diets. Concentrations of electrolytes were similar (P > 0.05). It was concluded that combined dietary antioxidant vitamins at 200mg/kg of diet better improved haematological parameters but had no effects on serum electrolytes.

Key words: Antioxidant Vitamins, Haematology, Electrolytes and Pig.

1 INTRODUCTION
Vitamin deficiencies normally occur with chronic consumption of diets that are deficient in vitamins leading to vitamin deficiency symptoms that can result in the susceptibility of such animals to pathogenic invasion [2]. Despite the fact that most animals are capable of endogenously synthesize some of the essential vitamins, such as vitamin C, dietary sources still remain the major means by which animal vitamins needs and their requirements can be met [8 - 9]. To this point therefore, lack of vitamins can often results in pathological conditions that affect blood vessel that can also lead to death of the animal [5]. Different studies have shown that adequate intakes of vitamins via their diets reduced the damage due to oxidation in meat [3]. [13] demonstrated that intake of pharmaceutical level of vitamin E in the diet of chicken resulted in lesser lipid peroxidation in chicken semen. Vitamins A, C and E exhibit antioxidant potentials in varying degrees. Thus, there may be some degree of synergy when these essential vitamins are combined in swine diets as it relates to the overall health and productivity of the pig. For instance, it has been shown that vitamin C helps in the restoration of vitamin E. Therefore, it is possible that a combination of vitamin C and E as with other antioxidant vitamins would be of high advantage in this regard [8; 10; 12]. It may also help in maintaining better serum levels of electrolytes. To this end, the objectives of this study are to investigate the haematological and electrolyte parameters responses of the growing pig to single or combined dietary effects of vitamins A, C and their combinations with vitamin E.

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_0 (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 C 100mg + vitamin E 100mg/kg of diet), respectively.

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, blood samples were collected from all animal groups into both ethylene diamine tetracetic and non-ethylene diamine tetracetic acid treated tubes for blood and electrolyte parameters, respectively and were immediately snap frozen for later analyses. For blood parameters PCV, RBC, Hb, WBC and their differentials (neutrophils, lymphocytes, eosinophils, monocytes and basophils) were analysed for. For blood electrolytes, sodium (Na’), potassium (K’) and chloride (Cl’) were analysed for. Blood parameters were analysed by haematology auto-analyser machine (BC-2300) while electrolytes were analysed for using the flame photometric.
and spectrophotometric methods according to [1]. Briefly, final electrolyte values were obtained by dividing results obtained by optical density of test samples by the optical density of standards x concentration of standards.

Statistical Analysis
Data obtained were subjected to ANOVA using Proc. GLM of SAS (SAS Inst., Carry, NC) according to the experimental model as: $Y_{ij} = \mu + D_i + E_{ij}$

Where $Y_{ij}$ = the observation; $\mu$ = overall mean common to all treatments; $D_i$ = the effect of the $i^{th}$ diet and $E_{ij}$ = the error term. Means were compared using Tukey’s test and $\alpha$-level of 0.05 was used for all statistical comparisons to represent significance.

3 RESULTS AND DISCUSSION
The results of the mean values of PCV, Hb, RBC and their standard errors are shown in Table 1.

<table>
<thead>
<tr>
<th>Item</th>
<th>$T_0$</th>
<th>$T_A$</th>
<th>$T_C$</th>
<th>$T_{AC}$</th>
<th>$T_{AE}$</th>
<th>$T_{CE}$</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>28.00b</td>
<td>28.33a</td>
<td>28.00a</td>
<td>30.17b</td>
<td>30.83a</td>
<td>34.00b</td>
<td>0.45</td>
<td>0.023</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>8.50a</td>
<td>8.83a</td>
<td>9.33a</td>
<td>10.83b</td>
<td>10.83b</td>
<td>13.17a</td>
<td>0.32</td>
<td>0.025</td>
</tr>
<tr>
<td>RBC (ul⁻¹)</td>
<td>6.17a</td>
<td>6.33a</td>
<td>6.33b</td>
<td>6.67b</td>
<td>7.00a</td>
<td>7.67a</td>
<td>0.19</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Means with similar superscripts are not significantly ($P > 0.05$) different.

The PCV of treatments $T_O$, $T_A$ and $T_C$ are similar meaning that they were not significantly ($P > 0.05$) different. Treatments $T_{AC}$ and $T_{AE}$ had similar percentage levels of PCV but significantly higher ($P < 0.05$) than those of $T_O$, $T_A$ and $T_C$, respectively. However, treatment $T_{CE}$ had the highest percentage ($P < 0.05$) levels of PCV compared to all treatments. Similarly, the Hb levels of animals in treatments $T_O$, $T_A$ and $T_C$ were similar as they were not significantly ($P > 0.05$) different. Again, the Hb levels of $T_{AC}$ and $T_{AE}$ were similar but significantly ($P < 0.05$) higher compared to treatments $T_O$, $T_A$ and $T_C$. Treatment $T_{CE}$ had the highest ($P < 0.05$) level of Hb compared to all other treatments. Furthermore, $T_O$ and $T_A$ groups of animals had similar ($P > 0.05$) RBC levels that were also similar to $T_C$ group that were also similar to the $T_{AC}$ group that was also not significantly ($P > 0.05$) different from the $T_{AE}$ group. $T_{CE}$ group of animals had the highest concentration ($P < 0.05$) of RBC compared with all other groups. The results of white blood cells (WBC) and their differentials are shown in Table 2.

<table>
<thead>
<tr>
<th>Item</th>
<th>$T_0$</th>
<th>$T_A$</th>
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<th>$T_{CE}$</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (ul⁻¹)</td>
<td>7.50a</td>
<td>7.50a</td>
<td>7.50a</td>
<td>8.81b</td>
<td>8.81b</td>
<td>11.33a</td>
<td>0.27</td>
<td>0.036</td>
</tr>
<tr>
<td>Neu (%)</td>
<td>46.33a</td>
<td>38.67b</td>
<td>39.17b</td>
<td>42.50b</td>
<td>40.17b</td>
<td>36.84b</td>
<td>1.76</td>
<td>0.017</td>
</tr>
<tr>
<td>Lym (%)</td>
<td>38.67b</td>
<td>47.00b</td>
<td>47.83b</td>
<td>47.00b</td>
<td>46.67b</td>
<td>46.33b</td>
<td>1.36</td>
<td>0.019</td>
</tr>
<tr>
<td>Eon (%)</td>
<td>13.00</td>
<td>12.67</td>
<td>11.33</td>
<td>9.50</td>
<td>11.00</td>
<td>14.33</td>
<td>1.65</td>
<td>1.00</td>
</tr>
<tr>
<td>Mono (%)</td>
<td>2.00</td>
<td>1.67</td>
<td>1.67</td>
<td>1.00</td>
<td>2.17</td>
<td>2.50</td>
<td>0.52</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Means with different superscripts are significantly ($P < 0.05$) different. Legend: Neu = neutrophil; Lym: Lymphocyte; Eon: Eosinophil; Mono: Monocyte and Bas: Basophil.

The WBC of animals in treatments $T_O$, $T_A$ and $T_C$ were similar as they were not significantly ($P > 0.05$) different. However, the WBC of animals in treatments $T_{AC}$ and those of $T_{AE}$ were similar but significantly ($P < 0.05$) higher compared with those of $T_O$, $T_A$ and $T_C$. Animals in treatment $T_{CE}$ had the highest level ($P < 0.05$) of WBC compared to all treatment groups. The neutrophil levels of treatment $T_O$ was significantly ($P < 0.05$) higher compared to those of $T_A$, $T_C$, $T_{AC}$ and $T_{AE}$ that were similar. The neutrophil levels of $T_{CE}$ were similar to those of $T_{AE}$ but significantly ($P < 0.05$) lower compared to all other treatments. For lymphocytes, their levels were lowest ($P < 0.05$) in $T_O$ group compared with all other treatment groups that had similar levels. Eosinophils and monocytes levels were similar ($P > 0.05$) for all treatment groups. The results of the electrolytes are shown in Table 3.

<table>
<thead>
<tr>
<th>Item</th>
<th>$T_0$</th>
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<th>$T_C$</th>
<th>$T_{AC}$</th>
<th>$T_{AE}$</th>
<th>$T_{CE}$</th>
<th>SEM</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Na^+$ (mmol/l)</td>
<td>136.14</td>
<td>135.89</td>
<td>135.76</td>
<td>137.12</td>
<td>138.12</td>
<td>137.68</td>
<td>0.12</td>
<td>1.63</td>
</tr>
<tr>
<td>$K^+$ (mmol/l)</td>
<td>4.26</td>
<td>4.25</td>
<td>4.25</td>
<td>4.31</td>
<td>4.24</td>
<td>4.24</td>
<td>0.11</td>
<td>0.83</td>
</tr>
<tr>
<td>$Cl^-$ (mmol/l)</td>
<td>95.23</td>
<td>95.19</td>
<td>95.21</td>
<td>95.19</td>
<td>95.17</td>
<td>95.22</td>
<td>0.10</td>
<td>0.98</td>
</tr>
</tbody>
</table>

This study showed that pigs that received combined vitamins in their diets had significant preponderance of PCV, Hb and RBC. WBC and LYMP compared to the control and the single vitamins diets. This is a clear indication that antioxidant vitamins is capable of reducing environmental stressor loads on the growing animal and thus better support their growing process, especially when combined and more so with the combination of vitamins C and E [7; 12]. However, the control diet demonstrated significant higher value for NEU compared to the single and combined vitamins diets. This observation in this study further supports the fact that antioxidant vitamins can improve the quality of lives of porcine. Furthermore, these results demonstrated that antioxidant vitamins can stimulate the immune system of the animal as evidenced by the significant enhanced levels of LYMP in the animals that received pharmaceutical levels of the antioxidant vitamins compared with the control; by increasing the ratio of LYMP to NEU compared to the control. This is an indication that antioxidant vitamins could stimulate a protective immune response as previously eluded to that can be sufficient to enhance resistance to microbial pathogens and other environmental stressors. This finding agrees with those of previous studies [4; 6]. This finding may also explain in part the roles of antioxidant vitamins in activating the natural killer cells; a function more associated with T-lymphocytes [11]. Nevertheless, single or cobined dietary vitamins had no effects on all the three electrolytes, namely $Na^+$, $K^+$ and $Cl^-$ investigated in this study.

4 CONCLUSIONS
It was concluded that dietary antioxidant vitamins particularly the combination of vitamins C and E can improve haematological status of the growing pig and also
stimulate the immune system of the animal and thereby better support the health-status of the animal. This can also culminate in the support of growth process of the growing animal. This is true because the stimulated immune system is capable of protecting the growing animals against environmental stressors. However, the vitamins had no effects on the serum electrolytes studied.

REFERENCES


