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

Johnson, N. C., Popoola, S. O.

Rivers State University, Department of Animal Science,
Nigeria, 234 909 462 4825
ntinya@alumni.uoguelph.ca

Rivers State University, Department of Animal Science,
Nigeria, 234 803 946 8955
dimeji222@yahoo.com

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) and 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_O (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 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., Carry, NC) according to the experimental model as: $Yij = \mu + Di + Eij$. Where Yij = the observation; μ = overall mean common to all treatments; Di = the effect of the i^{th} 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. Anti-oxidant levels of pigs fed different vitamins-based diets

Item	T_O	T_A	T_C	T_{AC}	T_{AE}	T_{CE}	SEM	P-value
AOP (U/mg)	0.65^a	0.78^b	0.78^b	0.93^c	0.95^d	0.98^c	0.001	0.001
CAT (IU/mg)	13^a	13.7^b	14^b	15.5^c	15.3^c	16.2^d	0.14	0.027
SOD (U/mg)	1.3^a	1.5^a	1.5^a	2^b	2^b	2^b	0.16	0.050
GSH-Px (nmol/dl)	1^a	1^a	1^a	1.8^b	2^b	2^b	0.07	0.044
GSH (mg/dl)	2^a	2^a	2^a	3^b	3^b	3^b	0.01	0.043

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 T_O group compared with other groups. Furthermore, animals in treatments T_A and T_C had similar ($P > 0.05$) values of AOP. T_{AC} animals had AOP values significantly ($P < 0.05$) higher than those of T_O, T_A and T_C. However, animals of T_{AE} had higher ($P < 0.05$) AOP values compared to animals of T_{AC} group. Overall, animals in the T_{CE} 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 T_O group compared to all treatment groups while animals in groups T_A and T_C had similar CAT levels. Similarly, animals in treatments T_{AC} and T_{AE} had similar CAT levels that were significantly ($P < 0.05$) higher than those in treatments T_A and T_C. Animals in T_{CE} group significantly ($P < 0.05$) had the highest level of CAT compared to animals in T_{AC} and T_{AE}. Superoxide dismutase (SOD) levels were similar ($P > 0.05$) in animals of T_O, T_A, and T_C that were significantly ($P < 0.05$) lower than animals in the T_{AC}, T_{AE} and T_{CE} 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

Item	T_0	T_A	T_C	T_{AC}	T_{AE}	T_{CE}	SEM	P-value
GSSH(umol/dl)	1^a	1^a	1^a	0.83^a	0.17^b	0.17^b	0.12	0.005
OX (umol/dl)	0.12^a	0.10^b	0.10^b	0.09^b	0.09^b	0.09^b	0.003	0.002
Cortisol(ng/ml)	1.97^a	1.19^b	1.18^b	1.15^b	1.16^b	1.07^c	0.02	0.048
MDA(nmol/ml)	0.17^a	0.16^b	0.16^b	0.14^c	0.11^d	0.11^d	0.001	0.001

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 T_O, T_A, T_C, T_{AC} except for T_{AE} and T_{CE} that also had similar concentrations of GSSH that were significantly ($P < 0.05$) lower than the levels found in T_O, T_A, T_C, and T_{AC} groups. For xanthine oxidase (OX) T_O group significantly ($P < 0.05$) had the highest concentration of OX compared to all other treatments with similar concentrations of OX. Similarly, T_O group significantly ($P < 0.05$) demonstrated the highest concentrations of cortisol compared to all other treatment groups. T_A, T_C, T_{AC} and T_{AE} groups had similar concentrations of cortisol that were significantly ($P < 0.05$) lower than that of T_O group while T_{CE} animals significantly ($P < 0.05$) showed the lowest concentrations of cortisol compared to all other treatment groups. Malondialdehyde (MDA) concentration was also significantly ($P < 0.05$) highest in T_O animals compared to all other treatments. T_A and T_C had similar concentrations of MDA that were significantly ($P < 0.05$) lower than that of T_O group but significantly ($P < 0.05$) higher than that of T_{AC} group. T_{AE} and T_{CE} groups had similar concentrations of MDA that were actually the lowest ($P < 0.05$) MDA concentrations compared to T_{AC} 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 worthy 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 xenobiotic, removal of hydro-peroxides 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_C 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_O 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

[1]. E. Bentter. 1982. Catalase in red cell metabolism, a manual of Biochemical Methods. Bentter, E. (Ed.) Grune and Straton, New: 105-106.

[2]. D. J. Buckley, P. A. Morrissey and J. I. Gray. 1995. Influence of dietary vitamin E on the oxidative stability and quality of pig meat. J. Anim. Sci. 73:3122-3130.

[3]. E. Devrim, H. Ozbek and I. Durak. 2007. Effects of high-cholesterol diet and antioxidant vitamins combination on oxidant/antioxidant status in heart, kidney, liver and testis tissues from rats. J. Food Lipids 14: 386-395.

[4]. P. H. Hemsworth, J. L. Barnett and G. J. Coleman. 1993. The human-animal relationship in agriculture and its consequences for the animal. Anim. Welfare 2: 33-51.

[5]. P. Gatellier, Y. Mercier, E. Rock and M. Renerre. 2000. Influence of dietary fat and vitamin E supplementation on free radical production and on lipid and protein oxidation in turkey muscle extracts. J. Agric. Food Chem. 48: 1227-1433.

[6]. Q. Guo, B. T. Richert, J. R. Burgess, D. M. Webel, D. E. Orr, M. Blair, G. E. Fitzner, D. D. Hall, A. L. Grant and D. E. Gerrard. 2006. Effects of dietary vitamin E and fat supplementation on pork quality. J. Anim. Sci. 84:3089-3099.

[7]. W. U. Habig, M. J. Pubst and W. B. Jacoby. 1974. Glutathione s-transferes: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249:7130-7139.

[8]. N. C. Johnson, S. O. Popoola, and O. J. Owen. 2019a. Effects of single and combined antioxidant vitamins on growing pig performance and pork quality. Inter. J. Advanc. Res. Public. 3 (8):86-89.

[9]. N. C. Johnson, M. Diri, and O. J. Owen. 2019b. Physiological responses of rabbits to oral vitamin E supplementation during oxidative stress induced by short term hypothermia. Inter. J. Advance Res. Public. 3 (9):174-179.

[10]. A. Aslan and I. Meral. 2007. Effect of oral vitamin E supplementation on oxidative stress in guinea pigs with short term hypothermia. Cell Biochem. Funct 25: 771-715.

[11]. H. Misra and I. Fridorich. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247 (10): 3170-3175

[12]. NRC, (2012). Nutrient Requirements of Swine. 11th Ed. Natl. Acad. Press, Washington, DC.

[13]. S. S. Ovuru, N. A. Berepubo and M. B. Nodu. 2003. Biochemical blood parameters in semi adult rabbits experimentally fed crude oil contaminated diets. Afr. J. Biotech. 3:343-345.

[14]. P. F. Surai. 2002. Antioxidant protection in the intestine: a good beginning is half the battle, In: Nutritional Biotechnology in the Feed and Food Industries. Proceedings of Alltech's 18th Annual Symposium (T. P. Lyons and K. A. Jacques, Eds.), Nottingham University Press, Nottingham, UK, pp. 301-321.

[15]. F. Ursini. 2000. The world of glutathione peroxidases. J. Trace Elem. Med. Biol. 14:116 – 122.