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Ascorbic acid effect on erythrocyte osmotic fragility, haematological parameters and performance of weaned rabbits at the end of rainy season in Makurdi, Nigeria

[1]Adenkola, A. Y., [2] Kaankuka, F. G., [3]Ikyume, T. T., [3]Ichaver, I. F and [2]Yaakugh, I. D. I

[1]Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State - Nigeria
[2]Department of Animal Nutrition, College of Animal Science, University of Agriculture, Makurdi, Benue State, Nigeria
[3]Department of Animal Production, College of Animal Science, University of Agriculture, Makurdi, Benue State, Nigeria.
Corresponding author: E-mail aadenkola@yahoo.com Tel +2348054977696
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1 SUMMARY

The experiment was conducted with the aim of determining the response of weaner rabbit to dietary ascorbic acid (AA) supplementation in hot environmental area of Makurdi, Nigeria. Twenty weaned rabbits of both sexes, aged between 6-8 weeks with an initial weight of 620 ± 42.95g in a 56 day trial. The animals were randomly allocated to two treatment groups with 10 rabbits per treatment in a completely randomized design. The experimental group was administered AA at the dose of 200 mg/kg, dissolved in 4 ml of sterile water orally, while the control group was administered with sterile water (4 ml) without AA *per os*. Both the experimental and control groups were given feed and water *ad libitum*. Records of feed intake and body weight were determined weekly for 8 weeks. At the end of the trial the animals were slaughtered by the throat-cut method and dressed to determine the dressing percentage and the relative cut-up parts. During the slaughtering 4 ml of blood sample was collected directly from each animal into Bijou bottles containing anticoagulant. Haematological parameters of packed cell volume was determined using micro-haematocrit method, haemoglobin concentration using cyanmethaemoglobin method, total erythrocyte count and total leukocyte count using the haematocytometric method while erythrocyte osmotic fragility was determined by the method of Faulkner and King (1970). The results indicated that the daily weight gain recorded in the experimental group was 8.93 ± 0.72 and significantly ($P < 0.05$) greater than 7.05 ± 0.51 obtained in the control animals. Also the feed to gain ratio in the control rabbits (6.07 ± 0.47) was significantly ($P < 0.05$) greater than the obtained value in the experimental rabbits (5.27 ± 0.60). Carcass weight obtained in the experimental animals was significantly ($P < 0.05$) higher than the recorded value in the control animal. All other haematological parameters results except neutrophil, eosinophil, basophil and monocyte obtained in the experimental animal was significantly ($P < 0.05$) higher than that of the control animal. The percentage haemolysis observed in the control animals was significantly ($P < 0.05$) greater than the observed value obtained in the experimental animals making the fragiligram of the control animals to be shifted towards right.

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2 INTRODUCTION

There has been interest in a possible nutritional role of ascorbic acid (AA) on the basis of the fact that endogenous synthesis may not be adequate to meet the full needs of animals at all times, and that requirements for ascorbic acid (AA) may be increased under stressful conditions (Celik and Ozturkcan, 2003; Whitehead and Keller, 2003). Many studies have demonstrated the efficacy of AA as antioxidant (Premkumar and Bowhns, 2004; Adenkola and Ayo, 2009; Adenkola *et al.,* 2009a). Currently, AA is the most widely used vitamin throughout the world (Naidu, 2003). AA is an effective antioxidant because it has an important metabolic role as reducing agent and function as an electron carrier (Rice, 2000). Adenkola and Ayo (2009) demonstrated that AA reduced the rectal temperature (RT) values in turkeys, especially during the hot hours of the day, and that it may be of value in combating adverse effects of heat stress in turkeys during the hot-dry season. AA has been found to reduce body temperature in pigs exposed to harmattan stress and, thus, alleviated the adverse effects of the season on health and productivity of pigs (Adenkola *et al.,* 2009b). Kutlu and Forbes (1993) reported that AA, at 250 mg/kg, ameliorated the heat-induced deterioration in performance and metabolism of broiler chicks while Adenkola and Anugwa (2007) demonstrated that AA supplementation improved weight gain and better feed utilization in piglets. Sahin *et al.* (2003) showed that dietary ascorbic acid and folic acid supplementation attenuated the decline in performance and antioxidant status caused by heat stress.

Rabbit production is being encouraged in Nigeria as a means of improving the daily protein intake of individuals (Taiwo *et al.*, 2005; Akinmutimi *et al.,* 2007; Abubakar *et al.*, 2009). Its high protein and low cholesterol content (Okonkwo *et al.*, 2008) gives its production enormous potential in alleviating the problem of animal protein supply in developing countries (Abubakar *et al.*, 2009; Akintola, 2009). There is paucity of information on performance characteristics and haematological parameters of weaned rabbit that are given dietary AA supplementation under hot humid condition. Haematological parameters have been demonstrated to be important indices of health, production and adaptability to prevailing environmental conditions in livestock (Adenkola and Ayo, 2009) and also as an indicator of stress in livestock (Adenkola and Durotoye, 2004; Togun and Oseni, 2005).

Therefore the objective of this study was to determine the response of rabbit to dietary AA supplementation in hot environmental area of Makurdi, Nigeria.

3 MATERIALS AND METHODS

3.1 Experimental site: The experiments was performed at the Federal Housing Estate Makurdi (07^0 N, 08^0 37/ E) in the Southern Guinea Savannah zone of Nigeria. Makurdi is situated along the River Benue, which is very warm, with the daily temperature ranging from 26.5 to 42^0 C. The area has an annual rainfall of 1,317 – 1,323 mm which spans 6 - 7 months (Ako, 2002). The experiment was conducted during the dry season when the meteorological condition could be stressful to the animals.

3.2 Experimental Animals and Management: Twenty weaned rabbits of both sexes, aged between 6-8 weeks with initial weight of 620.00g were used in a 56 day trial. The animals were randomly allocated to two treatment groups with 10 rabbit per treatment in a completely randomized design. The rabbits were housed individually in wire mesh cages located in an open-sided building for easy and effective cross ventilation. The animals were kept for two weeks prior to the commencement of the experiment, during this period they were screened for possible haemo and endo-parasites and accustomed to routine handling.

3.3 Experimental Design: On the experimental day, at 06:00h, the experimental group was administered ascorbic acid at the dose of 200 mg/kg, dissolved in 4 ml of sterile water orally, while the control group was administered with

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sterile water (4 ml) without AA *per os*. Both experimental and control groups were given feed and water *ad libitum*. Records of feed intake and body weight were determined weekly for 8 weeks which was the end of the experiment. At the end of the trial, the animals were slaughtered by the throat-cut method and dressed to determine the dressing percentage and the relative cut-up parts, according to the procedures of Oluyemi and Roberts (2000).During slaughtering of the animals 4 ml of blood sample was collected into Bijou bottles containing the anticoagulant, disodium salt of ethylene diaminetetra-acetic acid at the rate of 2 mg/ml of blood (Oyewale, 1991). After collection, the blood samples were transferred to the Physiology Laboratory, Department of Physiology and Pharmacology University of Agriculture, Makurdi, where haematological parameters; packed cell volume (PCV), haemoglobin concentration (Hb) total erythrocyte count and total leukocyte count were determined as described by Schalm *et al.* (1975).

3.4 Erythrocyte osmotic fragility determination: Erythrocyte osmotic fragility (EOF) test was carried out as described by Faulkner and King (1970) and Adenkola *et al.,* (2010). A Sodium chloride (NaCl) solution was prepared according to Faulkner and King (1970) in volume of 500 ml for each of the samples in concentrations ranging from 0.05 to 0.85 percent at pH 7.4. A set of 10 test tubes, each containing 10 ml of NaCl solution of concentrations, ranging from 0.05 to 0.85 percent, were arranged serially in a test tube rack. One set was used to analyze each sample. The test tubes were labeled with corresponding NaCl concentration. A one milliliter pipette was used to transfer exactly 0.02 ml of blood sample into each of the ten test tubes (Adenkola *et al.*, 2010). Mixing was performed by gently inverting the covered test tubes for about 5 times. The test tubes were allowed to stand at room temperature (26-27^{o} C) for 30 minutes. The contents of the test tubes were maintained at pH 7.4. Thereafter, the contents of the test tubes were re-mixed and centrifuged at 1,500 x g for 15 minutes. The supernatant of each test tube was transferred into a glass cuvette. The concentration of haemoglobin in the supernatant solution was measured using a spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone, UK) at 540 nanometer by reading the absorbance. The same procedure was repeated for every blood sample of each rabbit used for the study. The percent haemolysis was calculated using the formula (Faulkner and King, 1970).

$$\frac{\text{Optical density of test}}{\text{Optical density of distilled water}} \times 100 = \text{Percent haemolysis}$$

Erythrocyte osmotic fragility curve was obtained by plotting percent haemolysis against the saline concentrations.

3.5 Statistical analysis: All data obtained were subjected to Student's *t* test using Graph Pad Prism version 4.00 for Windows. Data were expressed as mean ± standard error of mean. Values of $P < 0.05$ were considered significant.

4 RESULTS

The composition of the feed given to the experimental animals is shown in Table 1 while table 2 showed the meteorological value obtained during the study period.

Table 1: Composition of Feed

Ingredients	Percentage Inclusion
Maize	39.24
Maize offal	15.00
Groundnut cake	42.26
Bone meal	3.00
Salt	0.25
Vitamin premix*	0.25
Total	100

Premix contained the following: (Univit 15 Roche) 1500 I.U. Vit A, 1500 I. U. Vit D, 3000 I. U. Vit E, 3.0g Vit K, 0.3gVit B_2, 8.0 g Vit B_6 , 0.3 g Vit B_{12} , 3.0g Nicotinic Acid, 5.0g Ca-Pantothenate, 10.00g Fe, 0.2g Al, 3.5g Cu, 0.15g Zn, 0.02g I, 0.01g Co, 0.01g Se

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Table 2: Meteorological Parameters during the Study Period

Meteorological Parameters	October	November
Ambient Temperature (^{0} C)	30.81 ± 0.30	33.40 ± 0.15
Rainfall (mm)	9.17 ± 2.34	0.400 ± 0.400
Sunshine (hr/day)	5.30 ± 0.50	8.23 ± 0.36

AA supplementation has no appreciable ($P > 0.05$) effect on feed intake (Figure 1). The daily weight gain recorded in the experimental group was 8.93 ± 0.72 which was significantly ($P < 0.05$) greater than 7.05 ± 0.51 obtained in the control animals, also the feed to gain ratio in the control rabbits (6.07 ± 0.47) was significantly ($P < 0.05$) greater than the obtained value in the experimental rabbits (5.27 ± 0.60) (Table 3). The amount of water consumed by experimental and control animals were shown in Figure 2. The highest water was consumed in the 8th week by experimental animal with a value of 2038. 29 ± 70.63 ml, while the lowest amount of water was consumed in the 1st week with a value of 1004.14 ± 74.17 ml. However there was a significant ($P < 0.05$) different in the value obtained for water consumed in the 2nd, 5th, 7th and 8th week of the experiment.

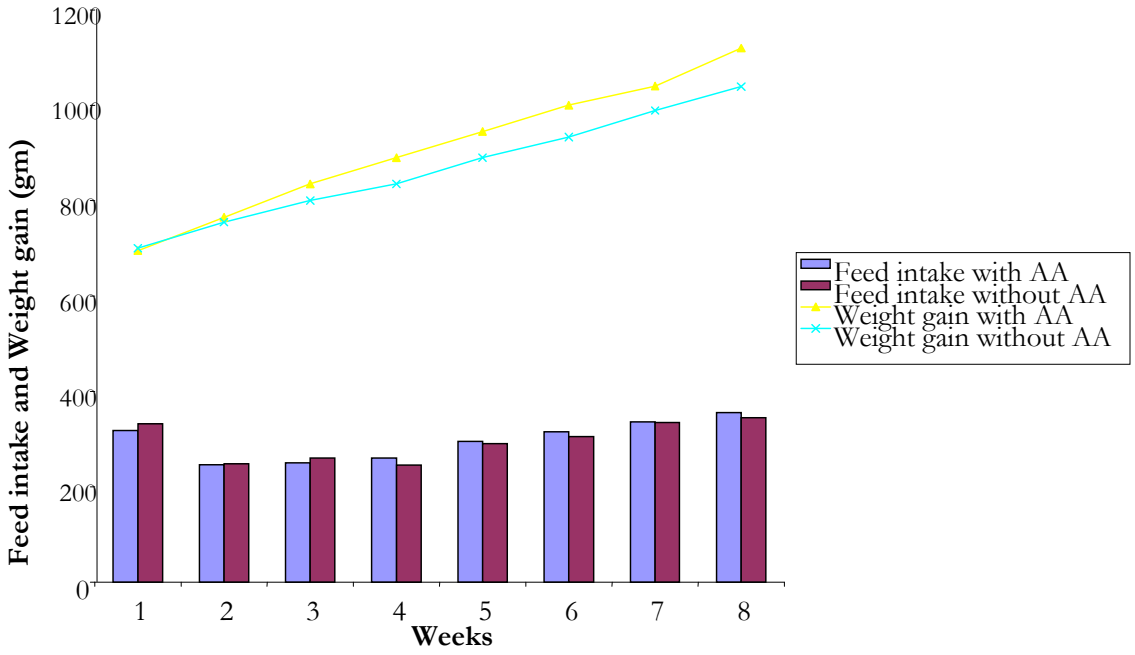

Figure 1: Effects of Ascorbic Acid on Feed Intake and Weight Gain of Weaned Rabbits at the End of Rainy Season

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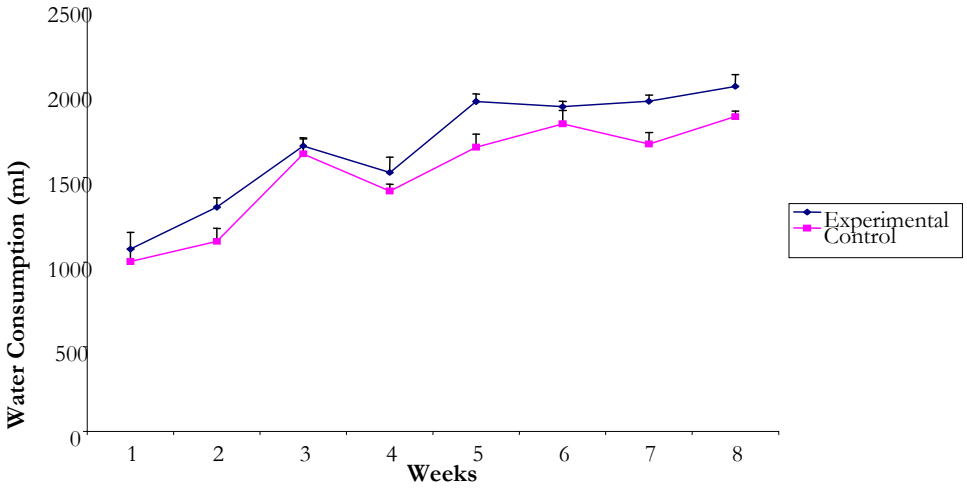

Figure 2: Effect of Ascorbic Acid on Water Consumption Rate of Weaned Rabbit at the End of Rainy Season

Table 3: Effect of Ascorbic acid Supplementation on the Performance of Weaned Rabbits

	Experimental	**Control**
Initial weight (g)	620 ± 42.95	620 ± 42.95
Final weight (g)	1120 ± 70.00[a]	1015 ± 55.80[b]
Average daily feed (g/day)	42.23 ± 1.61	41.72 ± 1.89
Average daily weight (g/day)	8.93± 0.72[a]	7.05 ± 0.51[b]
Feed/ Gain ratio	5.27 ± 0.60[a]	6.07± 0.47[b]

Table 4: Proportional Weight of Organs and Main Cuts of Rabbits Administered Ascorbic Acid at the End of Raining Season

Parts	**Control**	**Experiment**
Final weight	1130.00 ± 46.37[a]	1150.0 ± 52.44[b]
Dead weight	1100.00 ± 45.64[a]	1125.00 ± 25.00[b]
Carcass weight	141.3 ± 37.31[a]	149.3 ± 39.63[b]
Leg	208.6 ± 11.45[a]	226.8 ± 11.16[b]
Loin	101.6 ± 5.36	109.4 ± 6.77
Belly	56.08 ± 3.65	57.96 ± 2.79
Shoulder	199.1 ± 14.67	203.2 ± 7.72
Head	96.24 ± 5.81	99.00 ± 1.26
Weight of Stomach	76.42 ± 6.66	66.60 ± 9.86
Heart	2.74 ± 0.26	2.76 ± 0.20
Spleen	0.50 ± 0.12	0.56 ± 0.09
Kidney	7.58 ± 0.47	7.00 ± 0.12
Lungs	6.12 ± 0.64	6.72 ± 0.43
Liver	27.98 ± 1.18[a]	26.68 ± 1.73[b]
Length of small intestine	267.0 ± 14.11[a]	187.4 ± 22.51[b]
Length of large intestine	147.2 ± 15.59[a]	173.4 ± 21.25[b]
Weight of small intestine	42.10 ± 3.97[a]	44.20 ± 5.87[b]
Weight of large intestine	111.4 ± 9.82	97.68 ± 13.10
Bile	0.84 ± 0.29	11.58 ± 3.79
Fat	12.75 ± 6.14[a]	11.58 ± 3.79[b]

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The results of the carcass analysis showed that the control animal had final weight of 1015.00 ± 55.80 g which was significantly ($P < 0.05$) lower than the obtained value of 1120.00 ± 70.00 g in the experimental animal. The results of the dead weight and carcass weight obtained in the experimental was significantly ($P < 0.05$) higher than the recorded value in the control animal. The length of the small intestine value of 267.0 ± 14.11 cm obtained in the control animal was significantly ($P < 0.05$) longer than the recorded value of 187.4 ± 22.51cm recorded in the experimental animal, conversely the weight of small intestine in the experimental animal was significantly ($P < 0.05$) heavier than the value recorded in the control animal (Table 3). The obtained fat value of 12.75 ± 6.14 g was significantly ($P < 0.05$) higher than 11.58 ± 3.79 g recorded in the experimental animal. PCV value of 27.20 ± 2.89 % obtained in the control animal was significantly ($P < 0.05$) lower than the value of 31.20 ± 2.28 % recorded in the experimental animal. All other haematological parameters results except neutrophil, eosinophil, basophil and monocyte obtained in the experimental animal was significantly ($P < 0.05$) higher than that of the control animal (Table 3). The percentage haemolysis observed in the control animals was significantly ($P < 0.05$) greater than the observed value obtained in the experimental animals (Figure 3) at sodium chloride concentration of 0.85 %, 0.3 %, 0.2 %, and 0.1 % making the fragiligram of the control animals to be shifted towards right.

Table 5: Haematological Parameters of Weaner Rabbits during the Study Period

Haematological Parameters	Control	Experimental
Packed Cell Volume (%)	27.20 ± 2.89[a]	31.20 ± 2.28[b]
Haemolglobin Concentration (gm/%)	9.05 ± 0.95[a]	15.46 ± 4.39[b]
Total Red Blood Cell Count (× 10^6/µl)	6.48 ± 0.73[a]	9.11 ± 0.60[b]
Total White Blood Cell Count (× 10^3/µl)	3.20 ± 0.45[a]	1.84 ± 0.48[b]
Lymphocyte (× 10^3/µl)	1.87 ± 0.25[a]	1.36 ± 0.12[b]
Neutrophil (× 10^3/µl)	0.72 ± 0.16[a]	0.31 ± 0.07[a]
Monocyte (× 10^3/µl)	0.10 ± 0.03[a]	0.05 ± 0.01[a]
Basophil (× 10^3/µl)	0.08 ± 0.02[a]	0.07 ± 0.03[a]
Eosinophil (× 10^3/µl)	0.10 ± 0.04[a]	0.06 ± 0.02[a]

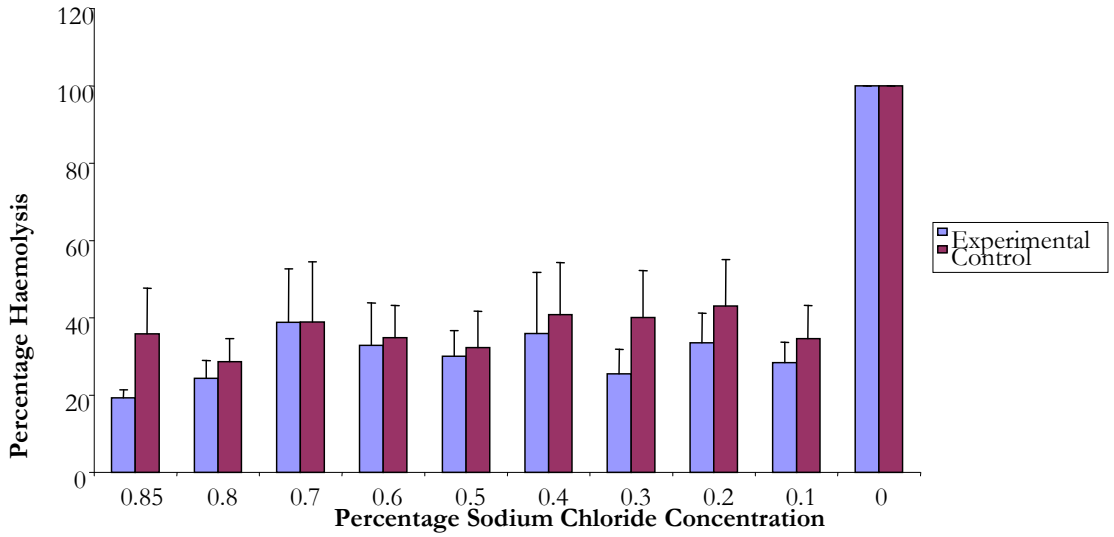

Figure 3: Effect of Ascorbic Acid on Erythrocyte Osmotic Fragility of Weaned Rabbits at the End of Rainy Season

5 DISCUSSION

The period preceding the rainy season in the zone was characterized by high ambient (30.81 ± 0.30 – 33.40 ± 0.15) temperatures throughout the day and long hours of sunshine with no rainfall, these meteorological parameters cause stress to livestock. The results of the present study agrees with previous work by Igono *et al.* (1982), Adenkola *et al.* (2009b) that the period preceding rainy season is thermally stressful to livestock. Donkoh (1989), Siegel (1995), Celik and Ozturkcan (2003) demonstrated that the stress of high ambient temperature may negatively influence the performance of broiler chickens by reducing feed intake, live weight gain and feed efficiency. AA is actively transported into tissues and its utilization increases during periods of stress. The animal ability to produce AA under stress may become inefficient leading to a reduction in plasma AA concentrations, thereby preventing tissue depletion. Levels of supplemental AA used in this experiment were sufficient to elevate plasma AA. This assumption was based on the findings of Chervyakov *et al.* (1977) who demonstrated that the dose regiment of AA in animals is between 100 – 500mg/kg. AA supplementation in this study improved daily weight gain as its supplementation significantly ($P < 0.01$) improved weekly weight gain, and the rabbit supplemented with AA had significantly heavier weights and better feed utilization than non-supplemented group this finding was in agreement with that of Balogun *et al* (1996) who demonstrated that AA supplementation improved weight gain and feed efficiency in broiler chickens during the hot-dry season in the Northern Guinea Savannah zone of Nigeria. The beneficial effect of AA supplementation under stressful conditions in the present study are in agreement with previous work by Njoku (1986), Kutlu and Forbes (1993), Sahin *et al.* (2002) and Sahin *et al.* (2003) that AA improves performance in birds. At high temperature, adrenocortical trophic hormone (ACTH) from the anterior pituitary gland triggers the production of corticosteroids from the adrenal cortex. Kutlu and Forbes (1993) reported that AA reduces the synthesis and secretion of corticosteroids, thus alleviating the negative effect of stress (McDowell, 1989) possibly through an inhibitory effect of AA on adrenal steroidogenesis. AA works as a co-enzyme playing an important role in the metabolism of amino acid (Kutle and Forbes, 1993; Kutlu, 2001) and could possibly decreases metabolic rate and energy expenditure by the body to cause increase in weight, or the increase in weight could be due to better feed utilization by AA supplemented group (Adenkola and Anugwa, 2007). The higher amount of water consumed by experimental group could be due to the fact that water is essential for metabolic activities. AA is effective antioxidant, and it plays and important role in metabolic activity (Rice, 2000). The heavier liver weight seen in the control rabbits could be due to compensatory ability of the liver to produce antioxidants required for scavenging the free radicals that must have been produced as a result of stressful environmental conditions as opposed to the experimental rabbits which were supplemented with an antioxidant (ascorbic acid). In rabbits, a lot of fermentation takes place in the hindgut, and this require a large surface area and this can account for longer length of large intestine recoded in the experimental group.

The observed increase in PCV in experimental group could be attributed to the effect of AA in protecting the membrane integrity of the erythrocyte as demonstrated by Candan *et al.* (2002) and Adenkola *et al.* (2010). This function directly affects the haemoglobin concentration and the total erythrocyte count in the experimental group being higher than the control group which was not supplemented with AA. However the lower value of total white blood cell count recorded in the experimental group could be due to the effect of AA in preventing the release of the white blood cells from their body pool due to its effect in inhibiting corticosteroids which are known to increase in animals under stress (Whitehead and Keller, 2003). The percentage haemolysis recorded was highest in the control animal and this could be due to the adverse effect of the harsh environmental stress on the erythrocytes, thereby rendering them more fragile and easily susceptible to haemolysis. The membrane of erythrocyte is rich in polyunsaturated fatty acids which are susceptible to lipid peroxidation which results in the loss of membrane fluidity and cellular lysis (Brzezinska-Slebodziiska, 2003). The result of this work is in agreement with the finding of Oyewale (1991) who demonstrated that the EOF increases with increase in medium temperature, and also with that of Oladele *et al.* (2003) that the highest EOF was obtained during the hot-dry season in poultry species in northern guinea savanna. The result also

agrees with that of Adenkola and Ayo (2009) and Adenkola *et al.* (2010) that AA protect membrane integrity of erythrocyte of livestock during stress.

6 CONCLUSION

It is thus concluded that supplementation of AA improved weight gain in weaned rabbits and this could enhance attainment of early market weight. It is thus recommended that AA be administered to weaned rabbit in order to attain market weight earlier, especially during the stressful season.

7 REFERENCES

Abubakar M, Gummi MB, Malami BS, Bashar YA. and Olorede BR: 2009. Supplemental value of Avizyme in diets of rabbits containing graded levels of wheat offal. *Journal of Animal Production Research Advances* 5(2): 94 – 97.

Adenkola AY. and Durotoye LA: 2004. Haematological study during prepartum and postpartum periods in brown savanna does in Zaria, Nigeria. *Proceedings of the 38th Annual Conference of the Agricultural Society of Nigeria*. pp 538-540.

Adenkola AY. and Anugwa FOI: 2007. Effects of dietary supplement of ascorbic acid on performance of piglets in Makurdi, Benue State of Nigeria. *Tropical Veterinarian* 25(1): 15 - 17.

Adenkola AY. and Ayo JO: 2009. Effect of Ascorbic Acid on Rectal Temperature Fluctuations in Indigenous Turkeys during the Hot-Dry Season. *International Journal of Poultry Science* 8(5): 457 – 461.

Adenkola AY, Ayo JO, Sackey AKB, Adelaiye AB. and Minka NS: 2009a. Excitability scores of pigs administered ascorbic acid and transported during the harmattan season. *Veterinarski. Arhiv,* 79 (5), 471-480.

Adenkola AY, Ayo JO. and Sackey AKB: 2009b. Ascorbic acid-induced modulation of rectal temperature in pigs during the harmattan season. *Journal Thermal Biology* 34 (13): 152 - 154.

Adenkola AY, Ayo JO, Sackey AKB. And Adelaiye AB: 2010. Erythrocyte osmotic fragility of pigs administered antioxidant and transported by road for short-term duration during the harmattan season. *African Journal of Biotechnology* 9(2): 226 – 233.

Akinmutimi AH, Absecon SF. And Osage CO: 2007. Growth performance of weaner rabbits fed sweet potato peels in place of maize base diet. *Proceedings of 32nd Annual Conference of Nigeria Society of Animal Production* 32: 7345 – 346.

Akinola LA: 2009. Economics of rabbit production in Gokana Local Government area of Rivers State, Nigeria. *Proceedings of 34thAnnual Conference of Nigeria Society of Animal Production* 34: 70 – 72.

Ako JE: 2000. Performance of sheep and goats under three feeding management systems. M. Sc. Thesis, University of Agriculture, Makurdi, Nigeria.

Balogun TF, Afolayan SB, Njoku PC. and Dafwang II: 1996. Effect of ascorbic acid (vitamin C) and acetysalycilic acid (aspirin) supplementation of broiler diets on performance during the hot season. *Samaru Journal of Agricultural Education* 5: 80 – 86.

Brzezinska-Slebodzinska, E. 2003. Species differences in the susceptibility of erythrocytes exposed to free radicals *in vivo*. *Veterinary Research Communication* 27: 211 – 217

Candan F, Gultekin F.and Candan F: 2002. Effect of vitamin C and zinc on fragility and lipid peroxidation in zinc-deficient haemodialysis patient. *Cell Biochemistry and Function* 20: 95 - 98.

Celik L. and Ozturkcan O: 2003. Effects of dietary supplemental L-carnitine and ascorbic acid on performance, carcass composition and plasma L-carnitine concentration of broiler chicks reared under different temperature. *Arch Tierenrnahrung*, 57: 27 – 38.

Chervyakov DK., Yevdokimov PD. And Vishker AS: 1977. *Drugs in Veterinary Medicine*. Kolos Publishing House, Moscow, 496pp (in Russian).

Donkoh A: 1989. Ambient temperature: a factor affecting performance and physiological responses of broiler chickens. *International Journal of Biometeorolology* 33: 259 – 265.

Faulkner WR. and King JW: 1970. *Manual of Clinical Laboratory Procedures*. Published by the

Chemical Rubber Company, Cleveland, Ohio, 354 pp.

Igono MO, Molokwu ECI and Aliu YO: 1982. Body temperature responses of Savanna Brown goat to the harmattan and hot-dry season. *International Journal Biometeorololgy* 26: 225 – 230.

Kutlu HR. and Forbes JM: 1993. Changes in growth and blood parameters in heat-stressed broiler chicks in response to dietary ascorbic acid. *Livestock Production Science* 36: 335 – 350.

Kutlu HR: 2001. Influences of wet feeding and supplementation with ascorbic acid on performance and carcass composition of broiler chicks exposed to a high ambient temperature. *Arch Tierenrnahrung*, 54: 127 - 139.

Naidu KA: 2003. Vitamin C in human health and disease is still a mystery ? An overview. *Nutrition Journal* 2: 1 - 10.

Njoku PC: 1986. Effects of dietary ascorbic acid (vitamin C) supplementation on the performance of broiler chickens in a tropical environment. *Animal Feed Science Technology* 16: 17 – 24.

McDowell LR: 1989. Vitamins in animal nutrition: vitamin C, folacin. McDowell, L. R. eds. Comparative Aspects to Human Nutrition. Academic Press,London, UK.

Okonkwo AC, Isaac LJ, Solomon IP. and Eyoh GD: 2008. Effects of dietary cassava leaf meal on growth performance of weaner rabbits. *Proceedings of 32nd Annual Conference of Nigeria Society of Animal Production* 32: 142 – 144.

Oladele SB, Ayo JO, Ogundipe SO. and Esievo KAN: 2003. Seasonal and species variations in erythrocytes osmotic fragility of indigenous poultry species in Zaria, Northern Guinea Savannah zone of Nigeria. *Bulletin of Animal Health and Production in Africa* 51: 204 - 214.

Oluyemi, J. A and F. A. Roberts, 2000. Poultry Production in Warm Wet Climates. 2nd Ed., Spectrum Books Ltd, Ibadan, PP. 244.

Oyewale JO. 1991. Osmotic fragility of erythrocyte of guinea-fowl at 21 and 156 weeks of age. *Veterinarski Arhiv,* 61(1) 49 - 56.

Premkumar K and Bowhns CL: 2004. Ascorbic acid does not increase the oxidative stress induced by dietary iron in C_3 mice. *Journal of Nutrition* 134: 435 - 438.

Rice ME: 2000. Ascorbate regulation and its neuroprotective role in the brain. *Trends in Neuroscience* 23: 209 - 216.

Sahin K., Sahin N. and Yaraloglu S: 2002. Effects of vitamin C and vitamin E on lipid peroxidation, blood serum metabolites and mineral concentration of laying hens reared at high ambient temperature. *Biolology of Trace Element Research* 85: 35 – 45.

Sahin K, Onderci M, Sahin N, Gursu MF. And Kucuk O: 2003. Dietary vitamin C and folic acid supplementation ameliorates the detrimental effects of heat stress in Japanese quail. *Journal of Nutrition* 133: 1882 – 1886.

Schalm OW, Jain NC. and Caroll EJ: 1975. *Textbook of Veterinary Haematology,* 2nd Edition, Published by Lea and Febiger, Philadelphia, Pp. 129 – 250.

Siegel HS: 1995. Stress, strains and resistance. *British Poultry Science* 36: 3 – 22.

Taiwo AA, Adejuyigbe AD, Adebowale AE, Oshotan JS and David OO: 2005. Performance and nutrient digestibility of weaned rabbits fed forages supplemented with concentrate. *Nigeria Journal of Animal Production* 32(1): 74 – 78.

Togun VA. and Oseni BSA. 2005. Effect of low level inclusion of Biscuit dust in broiler finisher diet on pre-pubertal growth and some haematological parameters of unsexed broilers. *Research Communications in Animal Science* 1(2): 10-14

Whitehead CC. and Keller T. 2003: An update on ascorbic acid in poultry (Reviews). *World's Poultry Science Association* 59: 161 – 184.