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AMMONIA COMPROMISES THE DYNAMICS OF SOME BLOOD PARAMETERS IN EXPOSED TO STRESS RABBITS

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Abstract

The aim of the present study was to evaluate the effect of ammonia on stress-induced alterations of hematocrit, erythrocyte count, leukocyte count and free fatty acids. Twenty male rabbits of the New Zealand White breed at the age of 4 months were randomly allocated into two groups: control - reared under low air ammonia levels (1.4-14.6 ppm) and experimental – reared under higher ammonia levels (28-57 ppm). The rabbits of both groups were exposed to forced running stress for 15 min on day 37 of the trial and two weeks later they received i.m. injection of 0.1 mg synthetic adrenocorticotropin (ACTH) per rabbit. Ammonia prevented ACTH-induced decline of leukocyte count at 2 h after the injection. Hematocrit values in the control rabbits increased at 20 ($P<0.01$) and 60 min ($P<0.01$) following exposure to forced running without any change in erythrocyte count, whereas in the experimental rabbits hematocrit was significantly higher at 2 h following ACTH injection relative to basal level ($P<0.01$). Plasma free fatty acids in the control rabbits declined in response to forced running ($P<0.01$) and ACTH injection ($P<0.01$). Similar but not significant decline was observed in the experimental rabbits.

Key words: rabbit, stress, hematocrit, erythrocytes, leukocytes, free fatty acids

Introduction

Ammonia is a common environmental contaminant which affects peripheral macrophages and spleen lymphocyte production of cytokines constituting a potent inflammatory response (Murata and Hornito, 1999).

Atmospheric ammonia in confinement facilities is a major harmful pollutant which affects recovery rate of heterophils (Murata and Hornito, 1999). Exposure of nursery pigs to 35 ppm of ammonia has been shown to double lymphocytes and monocytes count (Mitloehner, 2004).

Most of the ammonia-related investigations are focused on the deleterious effect of ammonia and the defense mechanisms of the respiratory tract against pathogens (Drummond et al., 1981; Al-Mashhadani, 1984; Gustin et al., 1994).

Thus, the aim of the present study was to evaluate the effect of ammonia on stress-induced alterations of hematocrit, erythrocyte count, leukocyte count and free fatty acids.

Materials and Methods

Twenty New Zealand male rabbits (Oryctolagus cuniculus) at the age of 4 months and average weight of 3 kg were randomly allocated into two groups (control and experimental) – 10 rabbits in each group. Rabbits were reared individually in wire-floor cages, provided with feeders and nipple drinkers – feed and drinking water were supplied *ad libitum*. Air temperature, relative humidity and CO_2 levels were within the following limits - 16-24 °C; 40-70% and 480-1260 ppm. The rabbits of both groups were reared together from birth until the start of the experiment in a room with natural ventilation and relatively high air ammonia levels (15-21 ppm). During the 51 d long experiment the rabbits of the control group were reared under low air ammonia levels (1.4 – 14.6 ppm), whereas the experimental rabbits were kept under higher levels of naturally occurring ammonia (28-57 ppm) via window closing. Thirty seven days after starting of the experiment all rabbits

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were exposed to 15 min forced running. Blood samples were collected by ear venipuncture before (basal level) and after termination of the forced running session (at 20 and 60 min). Two weeks later the rabbits of both groups received (i.m.) 0.1 mg tetracosactide (Adrenocorticotropin, 1-24) in 0.5 ml of saline. Blood was taken before and following adrenocorticotropin (ACTH) injection (at 1 and 2 h). The acute effect of air ammonia on adrenal function was assessed by spreading liquid ammonia on the floor, immediately after the end of forced running session and ACTH injection which resulted in an increase in air ammonia level as high as 158 ppm. Plasma free fatty acids were measured by Rabbit free fatty acids ELISA kit manufactured by Cusabio Biotech. CO., LTD. Total erythrocyte and leukocyte counts were determined by manual haemacytometer chamber count. Hematocrit was measured by the microhaematocrit method. Air ammonia was recorded via AeroQual S200 Monitor, equipped with ammonia sensor head (0-100±0.1 ppm). The results of one factor statistical analysis are expressed as means ± SEM.

Results and Discussion

Hematocrit level increased in the control rabbits at 20 min and 1 h after termination of the forced running session (Figure 1). The increase of hematocrit level was most probably due to the exercise-induced shift of plasma water into the extracellular space (Mairbäurl, 2013). Increased hematocrit value was reported in dog following 20 min trotting (Piccione et al., 2012). Exposure of the experimental rabbits to forced running did not have significant effect on hematocrit level relative to basal value. This could be due to increased deformability of the red blood cells, since ammonia stimulates NO production (Monfort et al., 2002) which on its turn increases red blood cells deformability (Starzyk et al., 1999; Bateman et al., 2001; Bor-Kucukatay et al., 2003; Kleinbongard et al., 2003) thus affecting the rheological properties of blood and the amount of oxygen that can be carried per volume of blood (Mairbäurl, 2013). Erythrocyte count in the control rabbits was not changed after termination of the forced running (Figure 2) and supports our interpretation of the observed increase of hematocrit. Adrenocorticotropin administration did not influence hematocrit levels in both groups at 1 h following the administration. However, it caused significant increase of hematocrit value at 2 h after ACTH administration in the experimental group (Figure 1). Adrenocorticotropin has been reported to increase hematocrit in rats (Kenyon et al., 2007). Also, adrenocorticotropin was found to decrease erythropoietin level and to increase the release of red blood cells from storage sites thus increasing hematocrit and blood hemoglobin (Zhang et al., 2004). The observed increase of hematocrit value in the experimental rabbits was not due to increased erythrocyte count since erythrocyte count was not changed following ACTH administration (Figure 2). We assume that hematocrit elevation in the experimental rabbits after ACTH administration was due either to increase of erythrocyte volume or to reduction of plasma volume because of ammonia-induced change in acid-base balance (Roberts, 2001). Exposure to

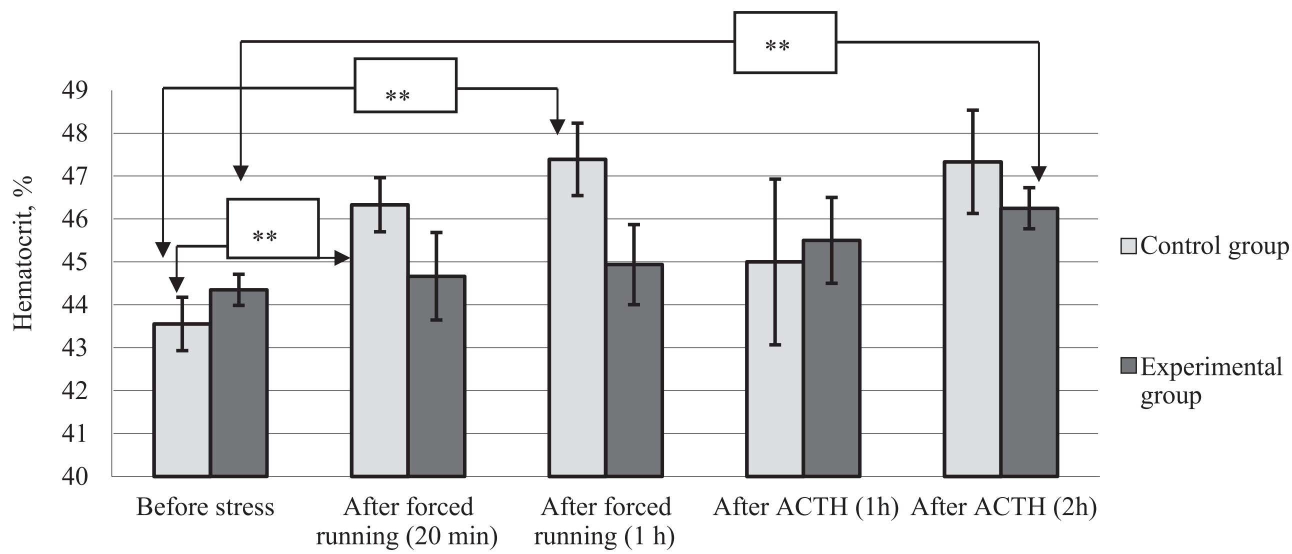

Fig. 1. Effect of ammonia on hematocrit levels in rabbits exposed to forced running and ACTH injection ** P<0.01

both forced running and ACTH caused significant decline in white blood cell (WBC) count except for the experimental rabbits at 2 h following ACTH administration (Figure 3.). These data are not consistent with the reported increase in WBC count following exercise in man (Natale et al., 2003). The authors found that each type of exercise (peak aerobic exercise, prolonged endurance exercise and a standard circuit of resistance exercises) was related with increase of different leukocyte subpopulations. In our earlier experiment we found unchanged WBC count in rabbits exposed to psychological stress (Dyavolova et al., 2013). Ammonia prevented leukocyte count decline at 2 h following ACTH ad-

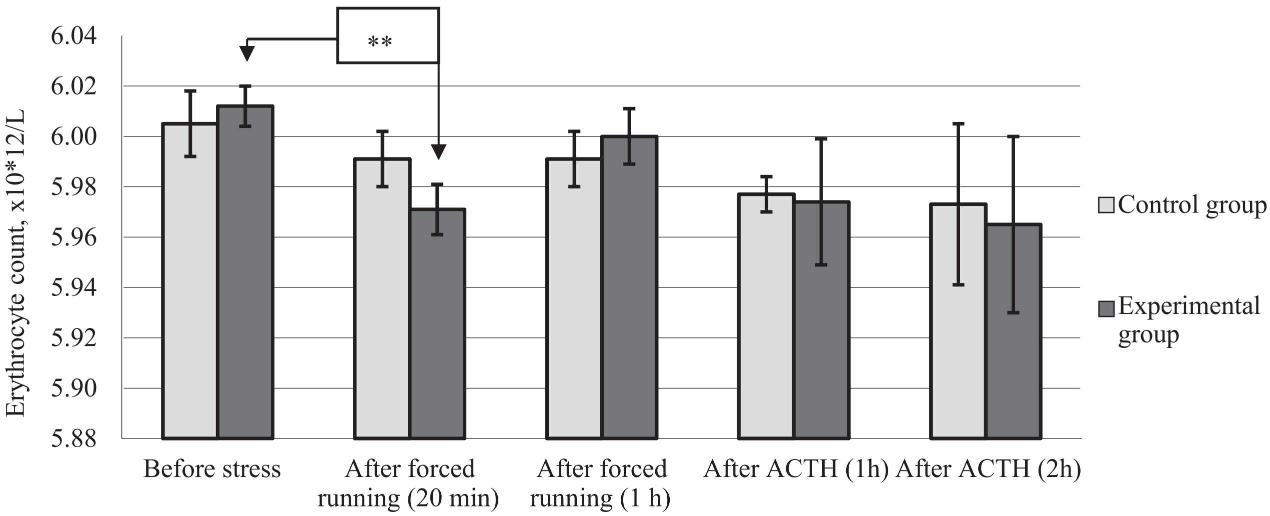

Fig. 2. Effect of ammonia on erythrocyte count in rabbits exposed to forced running and ACTH injection ** P<0.01

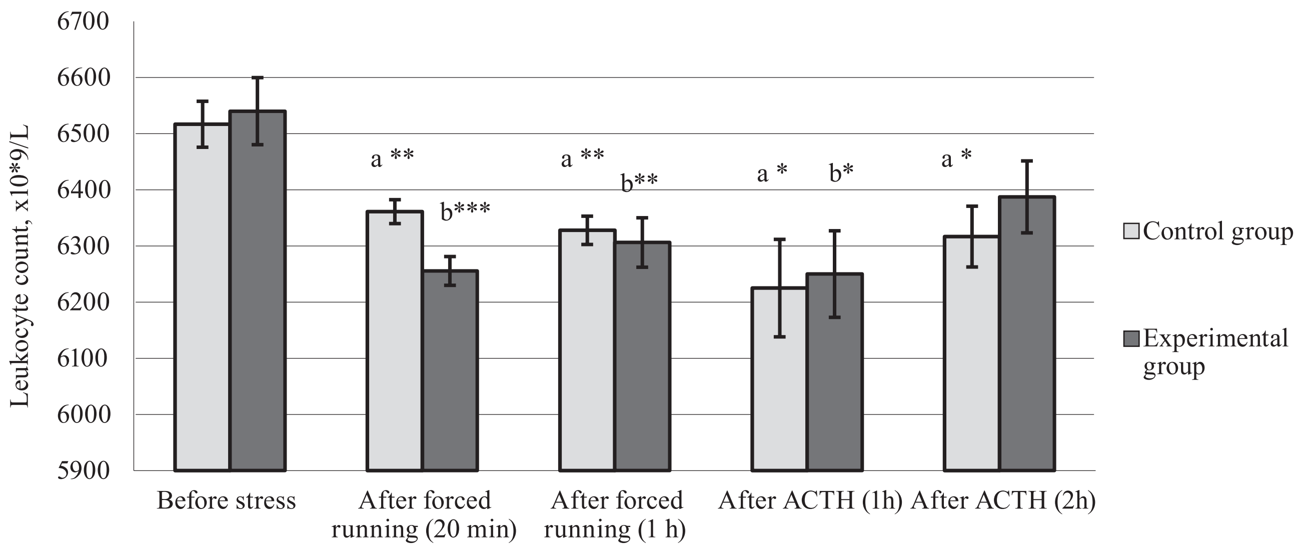

Fig. 3. Effect of ammonia on total leukocyte count in rabbits exposed to forced running and ACTH injection

* P<0.05 ** P<0.01 *** P<0.001

a – mean value is significantly different from the basal value of the control group

b – mean value is significantly different from the basal value of the experimental group

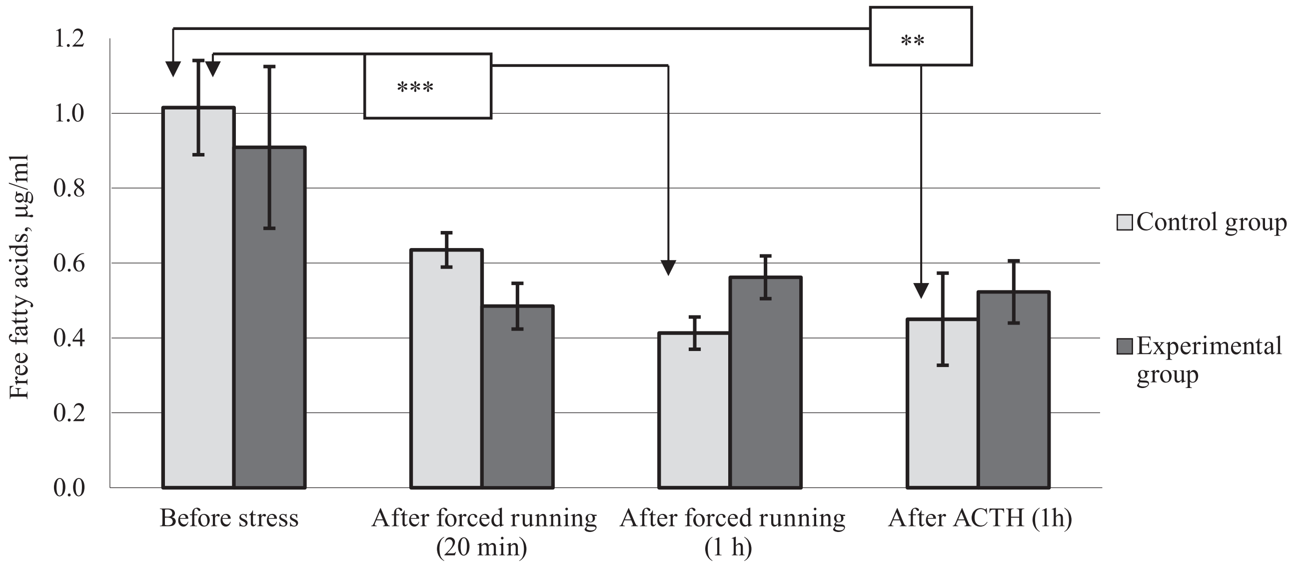

Fig. 4. Effect of ammonia on plasma levels of free fatty acids in rabbits, exposed to forced running and ACTH injection ** $P<0.01$ *** $P<0.001$

ministration. Chronic exposure to high ammonia levels did not influence basal plasma free fatty acids (FFA). Plasma FFA declined insignificantly in both groups at 20 min and 1 h following exposure to forced running except for the control rabbits at 1 h where the rate of decline reached level of significance (Figure 4). Free fatty acids have been reported to decline in healthy young men following 10 min heavy work (Jeukendrup et al., 1998). Martin et al. (1993) found decreased FFA turnover and oxidation during 90 to 100 min bout of submaximal exercise, which they attributed to a slower rate of FFA release from adipose tissue (Martin et al., 1993). On the contrary, psychological stress was reported to increase plasma FFA level (Apple et al., 1995). Adrenocorticotropin administration caused significant decline of plasma FFA relative to basal level in the control rabbits ($P<0.01$). It is widely recognized that stress causes insulin resistance, decreased intake of glucose by tissues and gluconeogenesis. Adrenocorticotropin is known to exert direct effect on lipid metabolism, independent of glucocorticoid action (Skoog, 2007). Spirovski et al. (1975) reported increased sensitivity of adipose tissue to ACTH in adrenalectomized rats concomitant with increased glucose utilization by adipocytes which resulted in enhanced reesterification and reduced release of FFA by the tissue. This finding could explain at least partly the observed decline of FFA. Plasma FFA in the experimental rabbits declined in response to ACTH but did not reach level of significance. This could be due to the lipolytic effect of ammonia which was reported to be independent from the nerve-controlled secretion of adrenal medullary hormones (Wiechetek et al., 1989). Besides, the higher ammonia levels may have influenced the rate of lipolysis through ammonia-induced decrease in plasma T_3 and T_4 levels (Brüngger et al., 1997; Fidanci et al., 2010), which in their turn stimulated the expression of genes involved in energy metabolism, lipolysis and lipogenesis (Oppenheimer et al., 1991).

Conclusion

Ammonia changed hematocrit and erythrocyte values following exposure to forced running. Adrenocorticotropin-induced dynamics in hematocrit, leukocyte and free fatty acids values were compromised by ammonia.

References

Al-Mashhadani, E. H., 1984. Respiratory tract damage, heat loss patters and performance of poultry exposed to atmospheric ammonia. *Dissertation/Thesis*, The University of Nebraska – Lincoln, United States.

Apple, J. K., M. E. Dikeman, J. E. Minton, R. M. McMurphy, M. R. Fedde, D. E. Leith and J. A. Unruh, 1995. Effects of restraint and isolation stress and epidural blockade on endocrine and blood metabolite status, muscle glycogen metabolism, and incidence of dark-cutting longissimus muscle of sheep. *J Anim. Sci.*, **73**(8): 2295-2307.

Bateman, R. M., J. E. Jagger, M. D. Sharpe, M. L. Ellsworth, S. Mehta and C. G. Ellis, 2001. Erythrocyte deformability is a nitric oxide-mediated factor in decreased capillary density during sepsis. *Am. J. Physiol. Heart. Circ. Physiol*., **280**: H2848–H2856.

Bor-Kucukatay, M., R. B. Wenby, H. J. Meiselman and O. K. Baskurt, 2003. Effects of nitric oxide on red blood cell deformability. *Am. J. Physiol Heart Circ. Physiol*., **284**: H1577–H1584.

Brüngger M., H. N. Hulter and R. Krapf, 1997. Effect of chronic metabolic acidosis on thyroid hormone homeostasis in humans. *Am. J. Physiol*., **272** (5) (Pt 2): F648-653.

Drummond, J. G., S. E. Curtis, J. Simon and H. W. Norton, 1981. Effects of atmospheric ammonia on young pigs experimentally infected with Ascaris suum. *Am. J. Vet. Res*., 42 (6): 969-974.

Dyavolova, M., I. Yanchev, D. Gudev and P. Moneva, 2013. Effect of high ammonia level on stress-induced hematological changes in rabbits: preventive effect of pyridoxine. *Bulg. J. Agric. Sci*., **19**: 828-834.

Fidanci, U. R., H. Yavuz, C. Kum, F. Kiral, M. Ozdemir, S. Sekkin and A. Filazi, 2010. Effects of Ammonia and Nitrite-Nitrate Concentrations on Thyroid Hormones and Variables Parameters of Broilers in Poorly Ventilated Poultry Houses**.** *Journal of Animal and Veterinary Advances*, **9**(2): 346-353.

Gustin, P., B. Urban, J. F. Prouvost and M. Ansay, 1994. Effects of atmospheric ammonia on pulmonary hemodynamics and vascular permeability in pigs: interaction with endotoxins. *Toxicol. Appl. Pharmacol*., 125 (1): 17-26.

Jeukendrup, A. E., W. H. Saris and A. J. Wagenmakers, 1998. Fat metabolism during exercise: A review. *Int J Sports Med*., **19** (4): 231-244.

Kenyon, L. M. C, D. Dunbar, J. Mullins and M. Bailey, 2007. Renal effects of ACTH: functional and microarray studies in the mouse. *Endocrine Abstracts*, **13:** 191.

Kleinbongard, P., A. Dejam, T. Lauer, T. Rassaf, A. Schindler, O. Picker, T. Scheeren, A. Gödecke, J. Schrader, R. Schulz, G. Heusch, G. A. Schaub, N. S. Bryan, M. Feelisch and M. Kelm, 2003 Plasma nitrite reflects constitutive nitric oxide synthase activity in mammals. *Free Radic Biol Med*., 1, **35**(7): 790-6.

Mairbäurl, H., 2013. Red blood cells in sports: effects of exercise and training on oxygen supply by red blood cell. *Front Physiol*., **12** (4): 332.

Martin, W. H. 3rd, G. P. Dalsky, B. F. Hurley, D. E. Matthews, D. M. Bier, J. M. Hagberg, M. A. Rogers, D. S. King and J. O. Holloszy, 1993. Effect of endurance exercise on plasma free fatty acids turn over and oxidation during exercise. *Am. J. Physiol*., **265**(5) (Pt 1): E708-14.

Mitloehner, F. M., 2004. Acute and chronic effects of ammonia on inflammation, immunology, endocrine function, performance and behavior of nursery pigs. Research Report. *Animal welfare,* NPB #03-159, 1-22. http://www.pork.org/FileLibrary/ResearchDocuments/03-159-MITLOEHNER.8-27-04.pdf

Monfort, P., E. Kosenko, S. Erceg, J. J. Canales and V. Fellipo, 2002. Molecular mechanism of acute ammonia toxicity: role of NMDA receptors. *Neurochem. Int*., **41**(2-3): 95-102.

Murata, H. and R. Horino, 1999. Effects of in vitro atmospheric ammonia exposure on recovery rate and luminol-dependent chemiluminescence of bovine neutrophils and bronchoalveolar macrophages. *J. Vet. Med. Sci*., **61**(3): 279-281.

Natale, V. M., I. K. Brenner, A. I. Moldoveanu, P. Vasiliou, P. Shek and R. J. Shephard, 2003. Effects of three different types of exercise on blood leukocyte count during and following exercise. *Sao Paulo Med J*., **121**(1): 9-14.

Oppenheimer, J. H., H. L. Schwartz, J. T. Lane and M. P. Thompson, 1991. Functional relationship of thyroid hormone-induced lipogenesis, lipolysis and thermogenesis in rat. *J. Clin. Invest*, **87**(1): 125-132.

Piccione, G., S. Casella, M. Panzera, C. Giannetto and F. Fazio, 2012. Effect of moderate treadmill exercise on some physiological parameters in untrained Beagle dogs. *Exp Anim.,* **61**(5):511-515

Roberts, K. E., 2001. Fluid and electrolyte regulation. Chapter 11, 369-392, In *Critical Care Nursing of Infants and Children*, Martha A.Q. Curley and Patricia A. Moloney-Harmon(Editors), (Philadelphia: W.B. Saunders Co., 2001), **1**,128 pages.

Skoog, M., 2007. Mechanisms behind the lipid lowering effects of ACTH. Lund University, Faculty of Medicine Doctoral Dissertation Series, 2007:142 Printed by Media-Tryck, Lund, Sweden.

Spirovski, M. Z., V. P. Kovacev, M. Spasovska and S. S. Chernick, 1975. Effect of ACTH on lipolysis in adipose tissue of normal and adrenalectomized rats in vivo. *Am. J. Physiol*., **228(**2): 382-385.

Starzyk, D., R. Korbut and R. J. Gryglewski, 1999. Effects of nitric oxide and prostacyclin on deformability and aggregability of red blood cells of rats ex vivo and in vitro. *J. Physiol. Pharmacol*., **40**: 629-637.

Wiechetek, M., P. Podgurniak, R. Zabielski and M. Podgurniak, 1989. The effect of adrenal denervation on the metabolic effects of hyperammonemia in sheep. *Can. J. Physiol. Pharmacol*., **67**(9): 1062-1066.

Zhang, Y., M.C. Andrews, C. G. Schyvens, K. U. McKenzie and J. A. Whitworth, 2004. Adrenocorticotropic hormone, blood pressure, and serum erythropoietin concentrations in the rat. *Am J Hypertens*., **17**(5 Pt 1): 457- 461.