

Research Notes

Hepatic and Cardiac Oxidative Stress and Other Metabolic Changes in Broilers with the Ascites Syndrome

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ABSTRACT The objective of this study was to evaluate the gluconeogenic response of *in vitro* stimulated hepatocytes from control broilers and broilers with clinical manifestations of the ascites syndrome. The basal rate of glucose synthesis from lactate was found to be threefold greater in sick birds than in the control group and stimulation obtained with epinephrine was found to be quantitatively similar in both groups. Under basal conditions, the hepatocytes from the sick broilers exhibited 60% more ammonium than the control birds.

In addition, the quantification of thiobarbituric acid reactive substances, as indicators of cellular lipoperoxidation, showed an increase of over 100% in heart and liver of sick broilers fowl. In conclusion, the complex integrated response of gluconeogenesis to epinephrine is maintained in broilers with ascites, although their hepatocytes present changes compatible with those observed in cases of oxidative stress. It is not known whether this stress is a cause or a consequence of the ascites syndrome.

(Key words: ascites, broiler, free radicals, gluconeogenesis, oxidative stress)

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INTRODUCTION

The ascites syndrome (AS) is an entity with constant epidemiologic, clinical, and anatomopathological characteristics (Paasch, 1991). It affects broilers and broiler breeders from the 3rd wk of age on, with maximum mortality rates at the 6th and 7th wk (Paasch, 1991; Suárez and Rubio, 1989). The clinical signs of AS are: raised feathers, apathy, cyanosis of the head and feet, growth of the abdomen, slow walking with legs spread, and dyspnea (Rodríguez and Rosiles, 1988; Paasch, 1991). The predisposing factors for ascites are genetics, nutrition, environment, care or management, and infection (Rodríguez and Rosiles, 1988; Dale and Villacres, 1988; Suárez and Rubio, 1989; Odom, 1993). Economic losses due to mortality during the growth period may reach 5% of the population (Arce *et al.*, 1987; López, 1991; Odom, 1993).

The pathology behind AS becomes apparent with the appearance of hypoxia caused by any of the predisposing factors, which causes erythrocyte deformation (Dale and Villacres, 1988; Mirsalimi and Julian, 1991), right heart hypertrophy, an increase in blood pressure at the level of the pulmonary artery (Dale and Villacres, 1988; Paasch 1991; Maxwell and Robertson, 1993), generalized

passive chronic heart congestion, hydropericardium, and ascites (Julian *et al.*, 1986). This cardiopulmonary condition causes repercussions in liver function (Julian *et al.*, 1986), including the following histopathological lesions: contraction of the liver cords, individualization of the hepatocytes, vacuolization of the liver cell cytoplasm, capsular fibrosis, and deposit of intracellular protein material (Maxwell *et al.*, 1986; Wilson *et al.*, 1988).

There is no information on the molecular changes that occur as a consequence of AS. This study intends to explore the liver capacity of broilers with AS, in comparison with a control group, in response to stimuli which, in the rat, normally causes the activation of three effectors: glucagon (Staddo and Hansford, 1989), epinephrine (Garrison and Haynes, 1973), or adenosine (Díaz *et al.*, 1991) in relation to the metabolism of carbohydrates. It is not known whether the responsiveness of the hepatocytes from birds with AS is affected. On the other hand, it has been hypothesized that the development of AS can be partially due to the generation of free radicals from inflammatory cell infiltration with the subsequent depletion of tissue antioxidants (Enkvetchakul *et al.*, 1993). The toxicity of those free radicals has been widely documented (Comporti, 1985; Aust *et al.*, 1993). The content of malondialdehyde in the hearts and livers of normal broilers and those with AS were then compared; malondialdehyde was measured as a thiobarbituric acid reactive substance (TBARS), which continues to be the most widely used

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indicator of lipoperoxidation caused by an excess of free radicals in tissues (Gutteridge and Halliwell, 1990).

MATERIALS AND METHODS

Six-week-old Arbor Acres broilers were used. Spontaneous AS developed in less than 1% of the total population. Selected animals, according to the criteria included below, were deprived of feed during the 24 h previous to the start of the experiment. Individual chickens with some clinical signs of AS (raised feathers, apathy, cyanosis of the head and feet, growth of the abdomen, slow walking with legs spread, and dyspnea) were initially included in the AS category. Definite inclusion in this category was made once the birds were killed and at least two gross pathological indicators of AS were observed: i.e., hydropericardium, ascites, right heart hypertrophy or general congestion. Animals from the same flock without a single clinical sign or pathological indicator of AS were included in the category of control birds.

Chickens were anesthetized with ether and hepatocytes from individual birds were prepared using the Berry and Friend (1969) technique modified by Guinzberg *et al.* (1987). Cell viability was quantified using the exclusion technique using 0.2% trypan blue. The cells were incubated in Krebs-Ringer bicarbonate at pH 7.4, containing 1% bovine serum albumin and 1.2 mM calcium under a saturated atmosphere of O₂/CO₂ (95/5%), continually stirred for 60 min at 37 C. In order to carry out this experiment, the incubation medium was supplemented with 5 mM ammonium carbonate and 10 mM of glucose for the determination of the ammonium and 10 mM lactate for the determination of glucose. Ammonium was added to assure the availability of this ion for different metabolic routes in hepatocytes enriched with 10 mM glucose. The method employed for ammonium measurement cannot distinguish between the endogenous substrate or the added salt; therefore, the reported results represent the clearance in total ammonium.

After incubation, the different samples were placed in an ice bath for 10 min and then centrifuged at a speed (50 × g) for 10 min and the aliquots from the supernatant were used to quantify ammonium, according to the method described by Gutman and Bergmeyer (1974). For glucose determination, a technique described by Fales (1963) was used. For the determination of TBARS, (Zentella *et al.*, 1993) a homogenate of liver and heart prepared with distilled water was used. The reagents were of high purity.² In the analysis of the results, control and AS populations were compared using the Student's *t* test within each treatment group. The *n* value in the figures refers to the number of independent

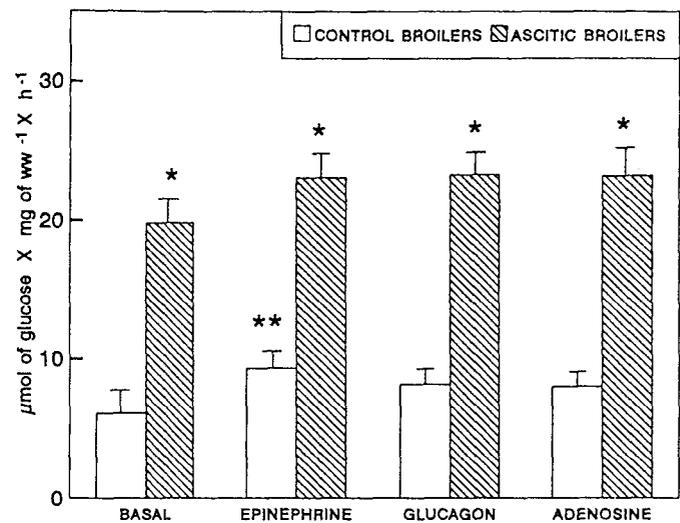


FIGURE 1. Glucose formed from lactate in broilers hepatocytes. The cells were incubated with 10 mM of lactate, in the presence of epinephrine, glucagon, and adenosine, each at [10⁻⁶ M]. The data are means ± SE and marked with one asterisk if significantly different from control values, and two asterisks if significantly different from basal values; *n* = 10 for the determinations in the control group and *n* = 11 for those birds with ascites.

experiments, each one performed with samples from a separate bird.

RESULTS AND DISCUSSION

Figure 1 shows the gluconeogenic activity of the hepatocytes of AS broilers and those of the control group under basal conditions and when exposed to epinephrine, glucagon, and adenosine. The hepatocytes from the control group show a moderate gluconeogenic response to the stimulators used, having statistical significance for epinephrine (*P* < 0.05). In birds with ascites, there was an increase in glucose observed 60 min after incubation by hepatocytes in the absence of stimulating gluconeogenesis effectors. The difference is statistically significant when compared to the control group. Maxwell *et al.* (1986) reported on the absence of liver glycogen granules in birds with AS. This fact, and that the birds were deprived of feed for 24 h, suggest that the greater concentration of glucose detected in broilers with AS comes from gluconeogenesis using lactate as the substrate and possibly other endogenous substrates such as amino acids derived from liver proteins. The stimulation caused by epinephrine, glucagon, and adenosine in the hepatocytes from animals with ascites is quantitatively similar to that observed in the hepatocytes from normal birds (close to 3 μmol/g wet weight per h); but was not significantly different when metabolic stimulators were added. The data suggest that, in the case of those birds with AS, the high rate of basal gluconeogenesis masks the effect of the hormones or adenosine.

With the objective of exploring the fate of ammonium, the experiment shown in Figure 2 was carried out.

²Obtained from Sigma Chemical Co., St. Louis, MO 63178-9916 and Baker de México, D. F. 75595-07301.

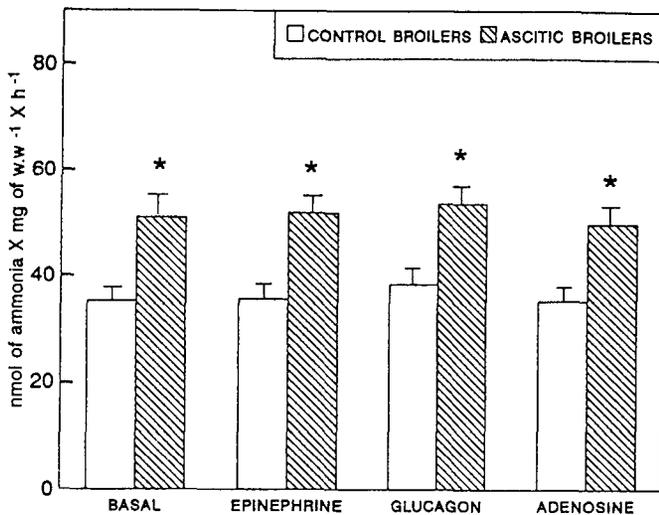


FIGURE 2. Ammonium detected in hepatocytes isolated from broilers. The cells were incubated with 5 mM $(\text{NH}_4)_2\text{CO}_3$, in the presence of epinephrine, glucagon, and adenosine, each at $[10^{-6} \text{ M}]$. The data are means \pm SE and marked with one asterisk if significantly different from control values. $n = 18$ for the determinations in the control group and $n = 16$ for those birds with ascites.

As indicated in the figure, the pool of ammonium is greater in the prepared hepatocytes of those broilers with AS than in controls. The effectors used did not modify the generation of ammonium in either group of birds. These data point to a greater protein catabolism in the livers of AS broilers than in controls, and suggest that deaminated amino acids might contribute to the high rate of gluconeogenesis found in hepatocytes from birds with AS (Figure 1). This result agrees with the

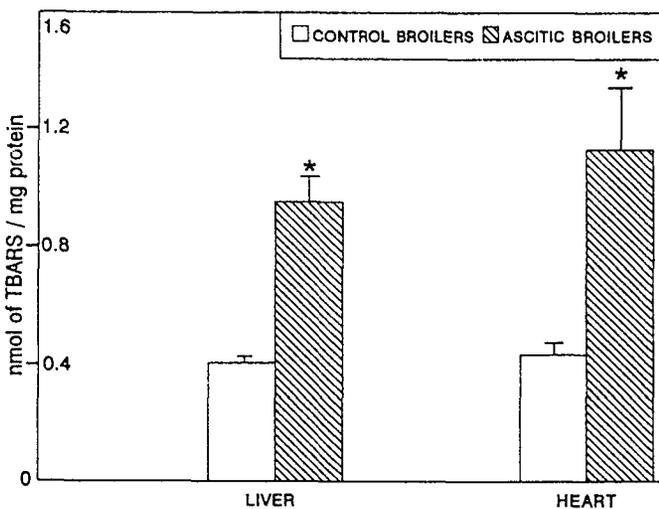


FIGURE 3. Levels of lipoperoxidation measured as thiobarbituric acid reactive substances (TBARS) in liver and heart of control group and broilers with ascites. The data are means \pm SE and marked with one asterisk if significantly different from control values. $n = 16$ for liver determinations in the control group and in birds with ascites and $n = 8$ for heart determinations in both groups.

decrease in serum albumin concentration levels reported by Yersin *et al.* (1992).

The data reported in Figure 3 show an increase in the concentration of TBARS in the liver and heart of broilers with ascites, indicating a high level of lipoperoxidation in these organs, a process that is started by an increase in the basal levels of oxygen reactive species (free radicals). The increase of lipoperoxidation and the increase in protein catabolism, which occurred in the cells obtained from birds with AS, are data observed in the "oxidative stress" scheme (Sies, 1993). Still to be integrated is the oxidative stress scheme in those cases of ascites and to define whether it is a consequence of other alternatives attributable to AS or if it is, in fact, the origin of the AS pathologic scheme.

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REFERENCES

- Arce, M. J., C. C. López, and P. C. Vázquez, 1987. Análisis de la incidencia del síndrome ascítico en el Valle de México. *Tec. Pec. Mex.* 25:338-345.
- Aust, S. D., C. F. Chignell, T. M. Bray, B. Kalyanaraman, and R. P. Mason, 1993. Contemporary issues in toxicology. Free radicals in toxicology. *Toxicol. Appl. Pharmacol.* 120: 168-178.
- Berry, M. N., and D. S. Friend, 1969. High yield preparation of isolated rat liver parenchymal cells. *J. Cell. Biol.* 43: 506-520.
- Comporti, M., 1985. Biology of diseases. Lipid peroxidation and cellular damage in toxic liver injury. *Lab. Invest.* 53: 599-623.
- Dale, N., and M. Villacres, 1988. Relationship of two-week body weight to the incidence of ascites in broilers. *Avian Dis.* 32:556-560.
- Díaz, A., R. Guinzberg, S. Uribe, and E. Piña, 1991. Metabolic responses of isolated hepatocytes to adenosine; dependence on external calcium. *Life Sci.* 49:505-510.
- Enkvetchakul, B., W. Bottje, N. Anthony, and R. Moore, 1993. Compromised antioxidant status associated with ascites in broilers. *Poultry Sci.* 72:2272-2280.
- Fales, F. W., 1963. Glucose (enzymatic). *Stand Methods Clin. Chem.* 4:101-112.
- Garrison, J. C., and R. C. Haynes, 1973. Hormonal control of glycogenolysis and gluconeogenesis in isolated rat liver cells. *J. Biol. Chem.* 248:5333-5343.
- Guinzberg, P. R., I. Laguna, A. Zentella, R. Guzmán, and E. Piña, 1987. Effect of adenosine and inosine on ureagenesis in hepatocytes. *Biochem J.* 245:371-374.
- Gutman, I., and H. U. Bergmeyer, 1974. Urea. Pages 1971-1974 in: *Methods of Enzymatic Analysis*. H. U. Bergmeyer, ed. Academic Press, New York, NY.
- Gutierrez, J.M.C., and B. Halliwell, 1990. The measurement and mechanism of lipid peroxidation in biological systems. *Trends Biochem. Sci.* 15:129-135.

- Julian, R. J., G. W. Friars, H. French, and M. Quinton, 1986. The relationship of right ventricular hypertrophy, right ventricular failure, and ascites to weight gain in broilers and rooster chickens. *Avian Dis.* 31:130-135.
- López, C. C., 1991. Investigaciones sobre el síndrome ascítico en pollos de engorda. Pages 13-14 *in*: *Ciencia Veterinaria*. Vol 5. R. Moreno-Chan, ed. Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México. México, D.F., México.
- Maxwell, M. H., G. W. Robertson, and S. Spence, 1986. Studies on an ascitic syndrome in young broilers. *Ultrastructure. Avian Pathol.* 15:525-538.
- Maxwell, M. H., and G. W. Robertson, 1993. Hipoxia a nivel del mar indicadores ultracitoquímicos en pollos de engorda en iniciación con síndrome ascítico. Pages 19-36 *in*: IX Ciclo de Conferencias Internacionales sobre Avicultura. Proceedings, México, D.F., México.
- Mirsalimi, S. M., and R. J. Julian, 1991. Reduced erythrocyte deformability as a possible contributing factor to pulmonary hypertension and ascites in broiler chickens. *Avian Dis.* 35:374-379.
- Odom, T. W., 1993. Relación entre la genética, la incubación y el ambiente después del nacimiento del pollo de engorda. Pages 167-175 *in*: XI Ciclo de Conferencias Internacionales sobre Avicultura, Proceedings, México, D.F., México.
- Paasch, M. L., 1991. Desarrollo de algunas investigaciones sobre el síndrome ascítico en México. Pages 1-11 *in*: *Ciencia Veterinaria*. Vol. 5., R. Moreno-Chan, ed. Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, México, D.F., México.
- Rodríguez, V. M., and M. R. Rosiles, 1988. Concentración de once elementos minerales catiónicos esenciales en pollos de engorda con y sin ascitis. *Vet. Mex.* 19:111-115.
- Sies, H., 1993. Strategies of antioxidant defense. *Eur. J. Biochem.* 215:213-219.
- Staddo, J. M., and G. R. Hansford, 1989. Evidence indicating that the glucagon-induced increase in cytoplasmic free Ca^{2+} concentration in hepatocytes is mediated by increase in cyclic AMP concentration. *Eur. J. Biochem.* 179:47-52.
- Suárez, O. M., and R. M. Rubio, 1989. Uso de la restricción alimenticia como control parcial del síndrome ascítico. *Vet. Mex.* 20:193-195.
- Wilson, J. B., R. J. Julian, and I. K. Barker, 1988. Lesions of heart failure and ascites in broiler chickens. *Avian Dis.* 32:246-261.
- Yersin, A. G., W. E. Huff, L. F. Kubena, M. H. Elissalde, R. B. Harvey, A. D. Witzel, and L. E. Giroir, 1992. Changes in hematological, blood gas and serum biochemical variables in broilers during exposure to stimulated high altitude. *Avian. Dis.* 36:189-196.
- Zentella, de P. M., Y. Saldaña-Balmori, A. Hernández-Tobias, and E. Piña, 1993. Nonsteroidal antiinflammatory drugs lower ethanol-mediated liver increase in lipids and thiobarbituric acid reactive substances. *Alcohol. Clin. Exp. Res.* 17:1228-1232.