Tropical Agricultural Research Vol. 14: 80-86 (2002)

Characterization of Ascitic Fluid in Broiler Chicken in Mid Country of Sri Lanka

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ABSTRACT. *A knowledge of the nature and constituents of ascitic fluid (AF) will facilitate the understanding of the pathophysiological events leading to ascitic syndrome (AS), a metabolic disorder seen in fast growing broiler chicken characterised by excess accumulation of non-inflammatory transudate in the peritoneal cavity. A study was conducted to determine the nature and constituents of AF. Twenty-six, 35-41 days old broiler carcasses with distension and fluid accumulation in the abdominal cavity which is suggestive of AS, were collected from a processing plant, whereas 23 strain and age matched broiler carcasses with no measurable amount of fluid in the abdominal cavity, served as the control group. Detailed post-mortem examinations were conducted and AF was cultured on blood and nutrient agar. No bacterial growths were observed in most samples. The AF was clear, yellow to straw with or without fibrin clots. AF volume varied from 2 - 210 ml (60.6±13 ml) in AS affected carcasses while no measurable amount of AF was present in carcasses of the control group. The pH of the supernatant varied from 6.2 - 7.2 (6.73±0.08). The colour varied from 3 - 100 icteric index units (13.4±4.6 icteric index units). The transparency ranged from 13 - 89% (61.7±4.9%). The absolute viscosity was between 5 - 7.5 cP (5.75±0.5 cP). The electrical conductivity was from 9.8 - 14.8 mS (12.2±0.76 mS). The total protein content estimated by the refractometer and biurate methods were 1.2 - 4.0 g/dl (2.2±0.17 g/dl) and 1.38 - 4.29 g/dl (3.08±0.16 g/dl), respectively. The estimated albumin content of the supernatant varied from 0.46 - 2.07 g/dl (1.06±0.14 g/dl). Erythrocytes (58.64±10.83%) and mesothelial cells (33.69±10.44%) were the major cell types present in the sediment. A transudate with low protein content when compared to plasma and a non-inflammatory cellular composition is characteristic for AF.*

INTRODUCTION

Ascites syndrome (AS) in broiler chicken is characterised by accumulation of excess non-inflammatory transudate in various spaces of the peritoneal cavity, marked distention of the abdomen with the fluid accumulation, dark congested musculate and small body size. This is a metabolic disorder, associated with cardio-pulmonary insufficiency in compensating the high oxygen demand, and is commonly seen in fast growing broilers, mostly male birds. This condition has also been reported in meat ducks (Julian, 1988), guinea fowl (Cowen *et al.*, 1988) and turkey pullets (Julian, 1993). Ascites syndrome is one

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of the major sources of economic loss to the broiler industry throughout the world. It causes more than 1% mortality in many flocks (Julian, 1993) and about 0.35% carcass condemnation at processing (Maxwell *et al.*, 1986; Riddell, 1998). Annual death losses from AS are estimated to cost the world poultry industry in excess of US $ 500 million whilst the estimated annual production loss is around US $ 100 million (Maxwell and Robertson, 1997).

Ascites syndrome may be caused by several mechanisms such as obstruction of lymphatic drainage, decreased plasma osmotic pressure, increased vascular permeability and most importantly by increased hydrostatic pressure in vena cava and the portal system as a result of right ventricular failure (Julian, 1993). In general, avian transudate fluids are characterized by a low cellularity when compared to plasma (Zinkl, 1986), a total protein content less than 3 g/dl (Campbell, 1988), clear to yellow colour and a non-inflammatory cellular composition. An increase in cellularity and protein of transudate could occur content due to changes in hydrostatic pressure and long-term retention (Campbell, 1988).

Although, extensive research has been conducted on the aetiology, pathogenesis and pathology of AS (Huchzermeyer and De Ruyck, 1986; Julian and Wilson, 1986; Maxwell *et al.*, 1986; Huchzermeyer *et al.*, 1987; Julian, 1987; Julian *et al.*, 1989; Julian and Goryo, 1990; Julian, 1993; Deceypere *et al.*, 2000) very little information is available on AF, which is the main manifestation of AS. Hulan *et al.* (1984), Julian (1985), Maxwell *et al.* (1986) and Campbell (1988) have described the physical nature of AF and the types of cells present in it. Bezuidenhout (1988) described the anatomy of the peritoneal spaces and the compartmentalization of AF in various peritoneal spaces. A better understanding of the properties of AF will help to describe the pathogenesis of AS. Therefore, the objectives of this study was to identify the physical, chemical and biological properties of AF in broiler chicken in mid country of Sri Lanka.

MATERIALS AND METHODS

Sample collection

Twenty six, 35 - 41 days old commercial broiler carcasses (19 male and seven female) from a broiler breeder strain, with a marked distention and fluid accumulation in the abdomen suggestive of ascites were collected prior to evisceration from a large scale processing plant at Athgala, Gampola. Two hundred and thirty three strain and age-matched carcasses (125 males and 108 females) with no distention of the abdomen and fluid accumulation served as the control group. These carcasses were from broiler chicken reared in large scale "buy back" operations with flock size of 2000 - 5000 birds under similar management systems in Nawalapitiya, Dolosbage, Pupurassa, Wattegama and Polgolla. The birds were maintained in a single house or several pens, and fed a standard commercial broiler diet. Food and water were provided *ad libitum*. The birds were vaccinated three times against infectious bursal disease. Artificial light was provided during the night and the airflow in the poultry houses was satisfactorily maintained.

Analysis of ascitic fluid

Bacteriology

Soon after collection, the carcasses were transported in ice to the laboratory. The ventral abdominal skin of 26 birds with AS was sterilized with 70% ethyl alcohol and heated spatula. The AF was collected from the right ventral hepato-peritoneal cavity using a 23 gauge needle inserted along the mid ventral line, just distal to the sternum (Campbell, 1988) and cultured in blood and nutrient agar. They were incubated at 37°C for 24 h and observed for the growth of bacterial colonies. Peritoneal swabs from 23 carcasses from the control group were also cultured.

Volume

Detailed postmortem examinations were conducted in all carcasses. Ascitic fluid was aspirated using a 50 cc disposable syringe and dispelled to a measuring cylinder, and the volume was measured. All the carcasses in the control group were observed for the presence of any measurable volume of AF. The AF was centrifuged at 3000 rpm for 10 min to minimize the variability among samples and colts were removed if present. The supernatant was subjected to physical and chemical analysis and the sediment for cellular composition.

Colour

The colour of the supernatant was determined by matching against Potassium dichromate dilutions and measured in icteric index (II) units (Zinkl, 1986). One gram of Potassium dichromate diluted in 100 ml distilled water provided 100 II units (Jain, 1986).

Transparency, conductivity, viscosity and pH

Transparency of AF was measured against distilled water using a colorimeter (Ciba-Corning, UK) whereas the electrical conductivity and viscosity were measured using a calibrated digital conductivity metre (Milk Checker, Eisai Co., Japan) and a rotary viscometer (Tokimec BL, Tokimec Inc., Japan), respectively. The hydrogen ion concentration of the supernatant of AF was measured using a pH metre (Kent Eli 7015, UK).

Total proteins

The total protein (TP) content of the supernatant was analyzed by hand refractometer and biurate method. The refractometer is a more practicable and simple method for rough estimation of TP content (Jain, 1986). A drop of supernatant was placed on the refractometer (Bellingham and Stanley, UK) and the value was obtained by direct reading the scale. The biurate method is a more accurate method in estimation of TP content. A commercially available diagnostic kit (Randox, Randox Laboratories, UK) was

used and the measurement of the TP content was obtained by measuring the colour absorbency at 456 nm with a colorimeter (Ciba-Corning, UK). In this method, cupric ions in alkaline medium interacts with peptide bonds and forms a coloured complex and the colour absorbency thus measured is directly proportional to the TP content.

Albumin

Quantitative determination of albumin content in the supernatant was done using a commercially available diagnostic kit (Randox, Randox Laboratories, UK). The measurement of albumin is based on its quantitative binding to an indicator, Bromocresol Green, and the colour absorbency recorded at 578 nm with a colorimeter (Ciba-Corning, UK). The absorbency measured value is directly proportional to albumin concentration in the supernatant.

Cytology

The sediment of the AF was centrifuged at 1500 rpm for five minutes (Cytospin, Shandon, UK); cells were collected to a glass slide and fixed with absolute alcohol. The slides were stained with Geimsa and examined under 100×magnification of a light microscope for different type of cells.

RESULTS AND DISCUSSION

Physical properties

Ascitic fluid was present in large amounts in right and left ventral hepato-pertoneal cavities and to a lesser extent in left and right dorsal hepato-peritoneal cavities. No fluid was present in the intestinal peritoneal cavity. A clean, yellow to straw coloured fluid in measurable levels in the peritoneal cavity is an indication of ascites. The volume of AF varied from 2 - 210 ml (60.6±13 ml). In all carcasses of the control group no measurable levels of AF was detected. The volume, colour, absolute viscosity, electrical conductivity and pH of analysed AF samples are given in Table 1. The compartmentalization of AF in various peritoneal spaces observed in this study agrees with findings of Bezuidenhout (1988). The AF accumulates in peritoneal spaces surrounding the liver and the presence of fibrinous adhesions over its surface indicates that the fluid has leaked out from the liver. The variation in mean AF volume among samples in this study is in accordance with the findings of Maxwell *et al.*, 1986. The mean pH of the supernatant in this study (6.73±0.08) was lower than reported value (7.01) by Hulan *et al.*, 1984.

Chemical and biological properties

The TP content measured by refractometer and biurate method, and albumin content are given in Table 1. There was no growth of bacterial colonies in 17 AF samples cultured. In seven samples *Bacillus subtilis* and *Proteus mirabilis* colonies were observed. These were considered as environmental contaminants based on this irregular growth

pattern. No growth of bacterial colonies was observed in and of the peritoneal swabs cultured from the control group. The TP content of AF estimated by Biurate method was similar to the values obtained by Hulan *et al.* (1984). The TP content less than 3 g/dl, which is characteristic for AF, was observed in 12 samples. The TP content greater than 3 g/dl was observed in 14 samples may be due to long-term retention. As described by Campbell (1988), the absence of bacterial growth in most of the samples cultured indicates that the AF is free of bacteria. Ascitic fluid sediment consisted of large amounts of erythrocytes and mesothelial cells. Fewer numbers of lymphocytes, macrophages, heterophils, eosinophils, basophiles and monocytes were present. The cellular composition of the analyzed samples is also given in Table 1.

Table 1. Physical, chemical and cytological properties of ascitic fluid.

Factor (Unit)	Mean ± SE (Range)
Physical properties	
Volume (ml)	60.6 ± 13 (2-210)
Colour (II units)	13.4 ± 4.6 (3-100)
Absolute viscosity (cP)	5.75 ± 0.5 (5-7.5)
Electrical conductivity (mS)	12.2 ± 0.76 (9.8-14.8)
pH	6.73 ± 0.08 (6.2-7.2)
Chemical properties	
Total proteins	
Refractometer (g/dl)	2.2 ± 0.17 (1.2-4.0)
Biurate method (g/dl)	3.08 ± 0.16 (1.38-4.29)
Albumin (g/dl)	1.06 ± 0.14 (0.46-2.07)
Cell composition (%)	
Erythrocytes	58.46 ± 10.73 (2-100)
Heterophils	2.08 ± 0.60 (0-4)
Lymphocytes	2.85 ± 0.87 (0-7)
Monocytes	0.38 ± 0.23 (0-3)
Eosinophils	0.31 ± 0.17 (0-2)
Basophils	0.15 ± 0.10 (0-2)
Macrophages	2.62 ± 1.01 (1-12)
Mesothelial cells	33.69 ± 10.44 (1-100)

cP - centi Poise mS - milli Seimens

The AF sediment contained mostly erythrocytes and mesothelial cells. The cellular content of AF analyzed by a direct smear method by Maxwell *et al.* (1986) mostly contained lymphocytes, erythrocytes and macrophages. According to Campbell (1988) mononeuclear leukocytes, granulocytes and mesothelial cells appear in increasing numbers in modified thansudates.

CONCLUSIONS

The physical, chemical and cellular composition variation among samples may be due to changes in hydrostatic pressure and long-term retention. A transudate with low protein content and a non-inflammatory cellular composition free of malignant cells in the analyzed ascitic fluid samples may indicates a circulatory pathophysiological process involved in the development of ascites syndrome.

ACKNOWLEDGEMENTS

The authors wish to thank the managing director, M. Imitiaz, and the staff of the processing plant of Crystal Springs Pvt. Ltd., Athgala, Gampola for their collaboration. The technical assistance given by H.W.B.A.B. Wanigesinghe and R.M.M.L.B. Wawegama is also appreciated.

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