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BRONCHOPNEUMONIA IN BUFFALO-CALVES IN ASSIUT GOVERNORATE: II- STUDIES ON CHANGES OF ACID-BASE BALANCE, ELECTROLYTES AND SOME ANTIOXIDANTS ASSOCIATED WITH THE DISEASE (With 3 Tables)

By

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الإلتهابات الشعبية الرئوية في العجول الجاموسى بمحافظة أسيوط ٢- دراسات عن التغيير التفي الإتزان الحمضى القاعدى -الشوارد (الكهرويات) وبعض مضادات الأكسدة المصاحبة لهذا المرض

على السباعي ، عبد الرحمن أحمد على ، على حسن صديق

أجريت در اسة حقلية على عدد (١٢٦) عجل جاموسي نامية من كلا الجنسين تر اوحت أعمار هم من ٢-٩ شهور في عدة مزارع بمحافظة اسيوط تم فحص هذه الحيوانات إكلينيكيا ومعملياً فكان من بينها (٣٦) عجلاً سليماً ظاهرياً والكلينيكيا ومعملياً واستخدمت كمجموعة ضابطة إأما باقى العجول وعددهم (٩٠) عجل فكانت تعانى من النز لات الشعبية الرئوية وشخصت بناء على العلامات المرضبة المميزة وقد استهدفت الدر اسة معرفة مدى تأثير النزلات الشعبية الرئوية على الإتزان الحمضي القاعدي وغازات الدم والكهرويات (الصوديوم - البوتاسيوم -الكلور ابد) ، بالإضافة إلى تأثير هذا المرض على مستويات الفيتامينات المضيادة للأكسدة (أ -بيتا كاروتين - ه - ج) في مصل الدم . وقد أظهر الفحص الأكلينيكي للعجول المصابة الكحة الطرية والإفرازات الأنفية الثقيلة مع صعوبة التنفس وفقدان الشهية للأكل والهزال والضعف العام إ أظهرت نتائج التحليل وجود نقص معنوى جدا في قيم الأس المهيدر وجيني ((oH) في الحيوانات المصابة بالنزلات الشعبية الرنوية عند مقارنتها بالمجموعة الضابطة مع وجود إرتفاع معنوى جدا في قيم الضغط الجزئي لثاني أكسيد الكربون (Pco₂) ، أما بالنسبة لقيم كلا من الصغط الجزيئي للأكسجين (Poz) والزيادة القاعديسة (B.E) والبيكربونات القياسية. (Hco2) فكانت الإختلافات غير جو هرية . أما بالنسبة لقيم الكهرويات في سيرم الدم فقد أظهرت التحليلات الإحصائية إنخفاض معنوى في مستوى الكلور ايد بينما ظهرت زيادة معنوية في قيم البوتاسيوم في سيرم دم العجول الجاموسي المريضة عند مقارنتها بمثيلاتها في Assiut Vet. Med. J. Vol. 46 No. 92, January 2002

الضوابط. أما عن نتائج التحليل بالنسبة للفيتامينات المضادة للأكسدة في سيرم الدم فقد أظهرت وجود إنخفاض معنوى جدا في مستويات كل من فيتامين أ - بيتا كاروتين - فيتامين هـ، وأيضا فيتامين ج في سيرم دم العجول الجاموسي المريضة بالنز لات الشعبية الرئوية. كذلك وقد خلصت الدراسة إلى أن الإلتهاب الشعبي الرئوى في العجول غالباً ما يصحبه إختلال في الإتزان الحمضي القاعدي وغازات الدم والكهرويات وأن لهذه التغيرات أهمية قصوى في التشخيص والعلاج . أظهرت الدراسة ان الإلتهابات الشعبية الرئوية في العجول غالباً ما انتشخاص في مستويات الفيتامينات المصادة للأكسدة ، والذي قد يعود إلى التأثيرات الضارة المشتقات الحرة المصاحبة للإلتهاب .

SUMMARY

This study was carried out on 126 growing buffalo-calves of both sexes. their ages varied from 3-9 months old belonged to some private farms in Assiut Governorate. These calves were subjected to careful clinical examinations. 36 calves were clinically healthy and served as control group, the remaining 90 calves were suffered from signs of bronchopneumonia. The study revealed that the most prominent clinical sings of bronchopneumonia in affected buffalo-calves included moist cough, nasal discharge which varied from mucoid to mucopurulant with harried respiration, anorexia, and depression. Some complicated cases were died. Blood gases analysis revealed significant decrease (P < 0.01) in blood pH, meanwhile carbon dioxide tension (Pco₂) in infected bufflo-calves revealed highly significant increase (P < 0.01). Oxygen tension (Po₂), base excess (B.E) and bicarbonate (Hco_3^-) levels in calves affected with bronchopneumonia were insignificantly affected and still within the normal accepted physiological levels. Significant decrease (P < 0.05) in chlorid levels with significant increase (P < 0.05) in potassium levels were detected in diseased buffalo-calves. Serum antioxidant vitamins revealed significant decrease (P < 0.01) in levels of vitamin A, B-carotene, E and C when compared with control calves. It could be concluded that bronchopneumonia in calves is usually associated with disorders in Acid-Base balance and electrolyte values and these changes was very helpful in the diagnosis and prognosis of such condition. The study also indicated deficiency of variable antioxidant vitamins (vit A, E and C) values, such variations could be attributed to increase in free radicals associating the infection.

Key words: Bronchopneumonia, Buffaloe, Aced-Base Balance.

INTRODUCTION

Diseases of respiratory tract are the most important diseases of feedlot. One of the most serious disease problems among calves is bronchopneumonia, this causes a great economic losses to producers from reduced live weight, inefficient feed conversion, delay marketing, deaths and costly preventive and therapeutic programs (*Jensen and Macky*, 1979). The causes of bronchopenumonia are multifactorial including stressors, viral and bacterial agent.

Environmental stresses including hunger, thirst, extreme hot an cold climatic temperature, fear and anxiety during transport, weaning, dehorning, dipping, castration and vaccination play a role in predisposing of outbreaks of respiratory diseases (Jensen and Macky, 1979).

Pasteurella spp., and corynebacterium pyogenes (Al-Allawy, et al. 1979), staph aureus, streptococci, E.coli, Haemophilus somnus as well as Klebsiella pneumoniae and salmonella spp., were involved and identified as the main causative bacterial agents of respiratory disorders in buffalo-calves (Al-Allawy et al., 1979; Elyas, 1982; Umlauft, et al., 1987; Ismail et al., 1993 and Mosier, 1997).

Viral causes which are incriminated in the incidence of bronchopneumonia include Para influenza type 3 (PI₃), infections bovine rhinotracheitis (IBR) respiratory syncytical virus (RSV) and rhinoviruses (Pire *et al.*, 1981 and El-Sebaie *et al.*, 1984).

Blood gas analysis is one of the most important aids in the diagnosis and prognosis of respiratory complication (El-Sebaie *et al.*, 1987 and Abd El-Raof and Hassan 1999). Acid-Base balance of the body fluids is important because the chemical reactions of the body being controlled by enzymes are very greatly influenced by the changes in pH (Tasker, 1969). Respiratory system play an important role in regulation of Acid-Base balance and blood gases through the elimination of carbon dioxide through the ventilation process (Breazile *et al.*, 1971; Fisher *et al.*;1980 ; Coles, 1986, El-Sebaie *et al.*, 1987 and Bouda and Jagos, 1991).

Respiratory acidosis occurs due to reduction in the elimination of Co_2 in the blood and carbonic acid concentration is increased which may associate with interference of gaseous exchange in bronchitis or pneumonia (Davenport, 1974; Coles, 1986; El-Sebaie *et al.*, 1987 and Carlson, 1997).

Electrolytes and Acid-Base balance are interrelated in the body in that the various anions and cations participate in physicochemical buffering of body fluids against sudden changes in blood pH (Simmons, 1962 and Gingerich, 1981). Evaluation of electrolyte levels and acidbase parameters of patient provide necessary information that will lead to greater understanding of the nature of disease process and guide line to the way of therapy (Brobest, 1975 and Robert *et al.*, 1990).

The majority of vitamins, particularly those with antioxidant properties, proved to increase the immune response of farm animals against a variety of antigens and accordingly to improve their resistance to infectious diseases. It seems from these current literatures (Beisel, 1982; Reddy *et al.*, 1985 and 1987; Cumminus and Brunner, 1989; Komy,1991 and Hoffmann, 1997) that it deals with feed supplementation of antioxidant vitamins more than monitoring their concentration in diseases affecting respiratory system of calves.

The aim of this investigation was to study the extent of changes in blood gases, Acid-Base balance and serum electrolyte values in association with bronchopneumonia in buffalo-calves. It was also aimed to study the possible changes could be occurred in serum blood levels of antioxidant vitamins (A, B.Carotine, C and E) under such circumstances

MATERIALS and METHODS

1. Animal:

A total number of 126 buffalo-calves of both sexes 3-9 months old were involved in this investigation. The calves were belonged to private farms at different villages in Assiut Governorate, observed clinical signs of bronchopneumonia were recorded in 90 calves. 36 healthy buffalo-calves under the same environmental, managemental and nutritional conditions were used as control.

2. Samples:

Two blood samples were collected from each animal:

a. The first one was used for blood gas analysis in which 2 ml of blood were collected anerobically into syringe whose dead space had previously filled with 1:1000 unit sodium heparin. The air bubbles were expelled from the syringe and the needle were inserted into a rubber stopper to prevent exposure of the blood to the air. After collection, the samples were immediately placed in ice bath to avoid metabolic changes in the blood.

The samples were analysed within 2 hours from collection using

CIBA Corning pH/blood gas analyzer model 238. The analyzer directly measures at 37°C. Blood pH, carbon dioxide tension (Pco₂ mm Hg), oxygen tension (Po₂ mm Hg). Bicarbonate (Hco_3^{-1} mmol/L. and base excess (B.E mmol/L.) were calculated automatically by the blood gas analyzer.

b. The second blood sample was taken in a dry, clean and sterile centrifuge tubes for obtaining clear sera .The obtained sera were used for the determination of serum electrolytes levels (sodium, potassium and chloride) and serum antioxidant vitamin levels (A, β -Carotene, E and C).

Blood serum sodium and potassium levels were determined using flame photometer (Corning Model 400). Serum chloride was determined by the use of chloride meter (Corning Model 925 England).

Blood Serum vitamin A and β -Carotene levels were estimated calorimetrically after the methods of (Carr and Price 1926). Vitamin E levels were estimated after the method of (Hawk, *et al.*, 1954). Blood serum levels of vitamin C were estimated after the method of (Lowery, *et al.* 1945). The obtained data were statistically analysed by *Microstat Computer Program* (SAS, 1986).

RESULTS

The mean values of blood pH, blood gases and Acid-Base parameters were illustrated in Table (1). Mean levels of blood serum electrolyte levels in both healthy and diseased buffalo-calves with bronchopneumonia were illustrated in Table (2).

Mean levels of blood serum antioxidant vitamins in both clinically healthy calves and those with bronchopneumonia were illustrated in Table (3).

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Clinical status	Number of calves	Mean ± S.D	РН	Pco ₂ mmHg	Po ₂ mm mmHg	Hco ₃ ⁻ m mol/L	B.E m mol/l	
Clinically healthy	36	x S.D	7.401	46.559	58.653	24.713	-0.967	
Buffalo calves(Control)	50	L	± 0.043	±1.842	± 5.225	±2.719	±0.052	
Buffalo calves with	90	x S.D	7.297**	68.426**	54.223	27.487	-1.071	
bronchopneumonia	90		± 0.021	± 2.418	± 3.678	± 2.619	±0.048	

Table 1: Values of blood Gases and Acid-Base balance

 in both clinically healthy and diseased buffalo-calves

Table 2: Mean values of serum electrolytes

 in both clinically healthy and diseased buffalo-calves

Clinical status	Number	Mean	Sodium	Potassium	Chloride		
	of calves	± S.D	m mol/L	m mol/L	m mol/L		
Clinically healthy	26	x	133.873	5.226	102.925		
buffalo calves(Control)	36	S.D	± 3.927	± 0.092	± 3.883		
Buffalo calves with	00	х	121.399	6.372*	88.648*		
bronchopneumonia	90	S.D	±5.320	± 0.087	± 2.677		

 Table 3: Mean values of Antioxidant vitamins in

 both clinically healthy and diseased buffalo-calves

Clinical status	Number of calves	1 .	lean S.D	Vit A µg/100ml	β- Carotene µg/100ml	Vit E µg/100ml	Vit C mg/100ml
Clinically healthy	36	x	S.D	48.926	139.611	229.274	2.458
buffalo calves(Control)				± 1.643	± 4.425	± 13.714	± 0.355
Buffalo calves with	90	x	S.D	23.816**	79.583**	122.819**	0.812**
bronchopneumonia	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,			± 0.733	± 2.762	± 8.615	± 0.112

DISCUSSION

The clinical inspection of diseased calves revealed various degrees of anorexia, nasal discharge, cough and lacrimation. The physical examination showed congested mucous membranes, harried respiration, progressive emaciation and lately recumbancy. Auscultation of affected animal lungs indicated different stages of inflammation, where harsh, exaggerated vesicualr sounds, and rales were heard at different areas of the lungs. These findings were similar to those recorded by Radostits *et al.* (1995) and Abd El-Raof and Hassan (1999). More information regarding clinical signs and aetiology were published in the first part of this study (Mottelib *et al.*, 2001).

In this investigation the mean values of Acid-Base balance and blood

gases in healthy buffalo-calves (Table 1) are in agreement with data previously published by (Singh and Kohli, 1980; Khamis, 1984; El-Sebaie *et al.*, 1987 and Abdel Raof and Hassan, 1999).

The role of respiratory system in the regulation of Acid-Base is by removal of carbon dioxide from the blood and reduce the concentration of cabonic acid in blood (Donawick and Baue, 1968 and Carlson, 1997).

Any disease affecting the lung directly affect reflect on the values of Acid-Base balance. Bronchopneunomia in buffalo-calves interfere with the main function of the lung mechanism and consequently increase in retention of Co_2 in blood which transformed into carbonic acid resulting in respiratory acidosis (Carlson, 1997 and Abd El-Raof and Hassan, 1999).

Regarding the results of blood pH, blood gases and Acid-Base status in buffalo-calves with bronchopneumonia (Table 1), there were significant decrease (P<0.01) in blood pH values when compared with those healthy ones. Carbon dioxide tension was (Pco₂) significantly increased (P<0.01). Oxygen tension (Po₂), base excess (B.E) and bicarbonate (Hco_3^-) values were insignificantly affected but still within the normal accepted physiological levels. These results were nearly agreeable with those reported by (Rounghton ,1964; Alpern, 1967; Brobest,1975; El- Sebaie *et al.*, 1987; Linden, *et al.*1995 and Abd El-Raof and Hassan, 1999).

The fall in blood pH in diseased buffalo-calves and increased Pco_2 could be attributed to decreased plumonary ventillation and retention of excess Co_2 in blood associated with bronchopneumonia in buffalo-calves (Coles, 1986; El-Sebaie et al, 1987 and Abdel Raof and Hassan, 1999).

Alpern (1967) and Brobest, (1975), reported that in pneumonia and bronchitis, there was a marked increase in Pco₂ values in blood associated with dropping of blood pH values. The authors declared that such alteration mainly due to interference in the gaseous exchange and retained carbon dioxide. Rounghton, 1964, added that the respiratory acidosis usually accompanied with partial compensation and consequently increase in blood Hco₃. Linden *et al.*, 1995, recorded a decrease of arterial oxygen tension with bronchopneumonia in calves, the authors attributed such decrease to disturbances of blood oxygenation process in bronchopneumonia as a result of respiratory diseases.

With regards to electrolytes values (Table 2), mean serum sodium values showed insignificant decrease and mean chloride values were significantly decreased (P<0.05), while serum potassium values were significantly increased (P<0.05). These results were nearly similar to those of Cohn and Alexkaplan (1971) and Abd El-Raof and Hassan (1999).

The fall in serum chloride levels might be due to increased blood bicarbonate Hco_3^- (Rounghton, 1964). The increase of the serum potassium levels might be related to the accumulation of acids (H⁺) in the blood, the H⁺ inters the cell and the intracellular K⁺ ions come out (Coles, 1986).

Concerning the antioxidant vitamins (Table 3), highly significant decrease (P<0.01) in mean levels of blood serum vitamin A, β -Carotene, vitamin E and vitamin C. Such reduction in blood serum vitamin A and β -carotene can be attributed either to deficiency of these vitamins in the ration or due to decreased its absorption and metabolism in diseased condition or due to bacterial toxins and free radicals which affect directly on the liver (site of storage) or to the degree of anorexia which is reflected upon the absorption of vitamin A (Atkins and Bodell, 1972; and Chew, 1987). Another explanation of such reduction in vitamin A and β -Carotene levels was offered by Radostits *et al.* (1995) who stated that the low level of vitamin C interfers with the absorption of vitamin A in blood.

Meanwhile the decrease in blood serum levels of vitamin E in diseased buffalo-calves could be explained on the bases that vitamin E is responsible for immuno-enhancement and it enhances humoral immmune response and its deficiency lead to decrease in such immunity with consequent stress on the animal (Bendich, 1987; Reddy *et al.*, 1985 and 1987). Another explanation for such reduction in vitamin E levels may be attributed to loss of appetite by the animal (Keneko and Cornelius, 1971).

Highly significant decrease in the blood serum levels of vitamin C in affected buffalo-calves, could be explained by Dobsinska, *et al.* (1981) and Hornig, *et al.* (1984) who reported that vitamin C inter in the defensive mechanism and has a detoxified action in the animal body. Another view may explain the fact that vitamin C is responsible for improving the immunocharging capacities especially as an antioxidant agent and enhance the ability to withstand infections (Anderson, 1982;

Panuch and Delafuents, 1985; Aderianne, 1987 and Bode, 1997). On the other hand Chatterjee *et al.* (1975), stated that any infections in the lungs cause a reduction in the ascorbic acid concentration in the blood plasma. Meanwhile Cumminus and Brunner(1989), also proved that calves with respiratory infections have lower plasma vitamin C concentration.

It could be concluded that bronchopnenomia in buffalo-calves has a major influence on blood pH and Pco_2 values resulting in respiratory acidosis. Further more, bronchopneumonia in buffalo-calves have a great reflection on the blood serum levels of antioxidant vitamins.

REFERENCES

- Abdel Raof, Y.M. and Hassan, H.Y. (1999): Ultrasonography and other aids for calf pneumonia diagnosis, 5th Sci. Cong. Egyptian Society for cattle diseases, 28-30 Nov.1999, Assiut, Egypt.
- Aderianne, B, (1987): The role of antioxidant vitamins on immune function tech. Syoposium, Daytond, Florida, U.S.A. 101-115.
- Al-Allawy, T.A.; Mottelib, A.A.; Nashid, S.M. and Salem, H. (1979): A study on pneumonia in buffalo calves in Egypt J.of Egyptian Vet.Med. Assoc., 39(2): 23-28.
- Alpern, D. (1967): Pathologic physiology. Pp:153-155. Mir Publichers, Moscow.
- Anderson, R. (1982): Ascorbic acid and immune functions : Mechanism of immunostimulation. In J.N. counseil and D.H. Hornig(eds), "Vitamin C" London : Applied science, pp. 249-272.
- Atkins, A and Bodell, P. (1972): New Eng. J., 286:27-31 Cited in Komy, S.R., 1991.
- Bendich, A. (1987): Role of antioxidant vitamins on immune function . in Symposium proceedings "The role of vitamins on animal performance and immune response". Technical Symposium, Animal Health and Nutrition. Hoffmann-La Roche Inc. Florida, pp.16-33.
- Besiel, W.R. (1982): Single nutrients and immunity Am.J.Clin. Nutr., 35 Supp : 417-468.
- Bode, A.M. (1997): Metabolism of vitamin C in health and disease. Adv.Pharmac., 38:21-47.
- Bouda, J. and Jagos, P. (1991): Disorders in the acid-base balance In. : Vrzgula, L., ed. Metabolic disorders and their prevention in farm animals. Amesterdam : El-sevier, pp.248-268.

- Breazile, J.E.; Beames, C.G.; Cardielhac, P.T. and New Comet, W.S. (1971): "Acid-base balance" In text book of Vet. Physiology. Lea and Febiger, Philadelphia.
- Brobest, D. (1975): Evaluation of clinical disorders of acid-base balance J.Am. Vet. Med.Ass., 166(4), 355-364.
- Carlson, G.P. (1997): Fluid, Electrolyte and Acid-base Balance. In: Kaneko, J.J; Harvey, J.W. and Bruss, M.L.eds. Clinical Biochemistry of Domestic Animals 5th Ed. San Diego : Academic Press, 485-516.
- Carr, F.H. and Price, E.A. (1926): Colourimetric determination of serum vit A and β -Carotene. Boichem. J.20: 498.
- Chatterjee, I.B.; Mayunder, A.K., Mondi, B.K. and Subraminian, N. (1975): Synthesis and some major functions of vitamin C in animals. Ann. N.Y. Acad. Sci.; 258 :24-47.
- *Chew*, *B.P.* (1987): Vitamin A and β-Carotene on host defense J.Dairy Sci. 70 : 2732-2743.
- Cohn, C.M.D. and Alexkaplan (1971): "Text book of clinical pathology, pp:252-254. Williams and Wilkins Co., Baltimore.
- Coles, E.H. (1986): A text book of veterinary clinical pathology, 4th Ed. W.B. Sauders Co., Philadelphia, London. Toronto. P.220-260
- Cumminus, K.A. and Brunner, C.J. (1989): Dietary ascorbic acid and immune responses in calves J.Dairy Science 72 : 129-134.
- Davenport, H.W. (1974): The ABC of acid-base Chemistry 6th ed. Chicago. The university of chicago press.
- Dobsinska, E.; Sova, Z.; Kopac, V. and Thron, M.(1981): Relationship between blood glucose and ascorbic acid and the body weight gain in calves in a large capacity calf house Veteinari Medicina, 26:203-212.
- Donawick, W.J. and Baue, A.E. (1968): Blood Gases, Acid-base balance and Alveolar-Arterial Oxygen Gradient in Calves. Am. J.Vet.Res. Vol.29No.3:561-567.
- El-Sebaie, A .H.; Ibrahim, A.H., Ibrahim, A.A., Hofmann, W. and Amer, A.A. (1984): Investigation on an outbreak of enzootic bronchopneumonia in fattening buffalo-calves in Assiut. A.R.E. 1-Clinical, microbiological and hematological studies, Assiut Vet. Med. J. Vol. 11, No.22, 163-167.
- El-Sebaie, A; Amer, A.:Nafie, E.; Abd El-All, Th. And Sadiek, A.H. (1987): Clinical, Haematological, and acid-base changes accompanying some respiratory and alimementary

manifestations among fattening buffals calves, Assiut Vet. Med. J. Vol.19, No.37, 1987.

- *Elyas, A.H. (1982):* Mycological and Bacteriological studies on the causes of pneumonia affecting buffalo-calves. Ph.D.Thesis, Fac. Vet.Med., Assiut University.
- Fisher, E.W.;Sibartie, D. and Grimshow, W.T.(1980): A Comparison of the pH, Pco₂, Po₂ and Tco₂ content in blood from brachial and caudal auricular arteries in normal cattle. Brit. Vet. J. 136(1) :496-499.
- Gingerich, D.A. (1981): Fluid, Shock and blood therapy. "In current veterinary Therapy", Food Animal Practice . pp.17-27. Edited by Howard, and W.C. McMullan. Saunders company. Pheladelphia. London Toronto.
- Hawk, P.B.; Oser, B.L. and Summerson, W.H. (1954): Determination of vit E. in practical physiological chemistry McGraw-Hill Book Company Jnc. NewYork. Toronto London, pp.1271-1275.
- Hoffmann, F. (1997): Vitamins and immune response of farm animals symposium proceedings. La Roche LTd. USA.
- Hornig, D.; Glatthaar, B and Moser, U. (1984): General aspects of ascorbic acid function and metabolism. In "Ascorbic Acid in Domestic Animals Ed. Wegger, I.; Tagwerker, F.J. and Moustgaare, J. The Royal Danish Agricultural Soc. Copenhagen.
- Ismail, M., Jakeen El-Jakee, S.A. Attia, Bagwaata and Soheir Shoukry (1993): Bacterial causes of respiratory disorders in buffalocalves in Egypt, Vet. Med. J. Giza. Vol.41, No.2, 95-99
- Jensen, R. and Macky, D.R. (1979): Diseases of feed lot cattle. 3rd Ed. Philadelphia Lea and Febiger. P. 312-355.
- Kaneko, I.J. and Cornelius, C.E. (1971): Clinical biochemistry of domestic animals , 2nd Ed. Vol. II, Academic press, New York. and London.
- Khamis, G.F.A(1984): Blood gases and acid-base bolance in healthy buffalo-calves. M.V.Sc. Theseis Fac.Vet. Med. Assiut University.
- Komy, S.R. (1991): Blood Biochemical values In Pneumonic suckling calves as indicators for diagnosis and treatment, M.V.Sc Thesis, Fac of Vet. Med. Dept. of Animal Medicine, Assiut University, Egypt.

- Linden, A.; Desmecht, D., Amory, H.; Daube, G.; Lecomte, S. and Lekeux, P.(1995): "Pulmonary ventilation, mechanics, gas exchange and haemodynamics in calves following intratracheal inoculation of pasteurella haemolytica" Zentralbl Veterinar Med. Assoc., 42:531-544.
- Lowery, O.H.; Lopez, J. A and Bessey, O.A. (1945): Determination of vit C. J.Biol. Chem., 60 : 609.
- Microstat computer program (SAS, 1986): User's Guide Statistics, SAS, Institute Cany, North Carolina.
- Mosier, D.A. (1997): Bacterial Pneumonia. Vet Clin. Of North America, Food Animal Practice, 483-493.
- Mottelib, A.A.; Ali, A.A., Sayed.A.S. and Abd El-Rahman, A.A. (2001): Bronchopn-eumoina in buffalo-calves in Assiut Governorate 1-Bacterial causes, clinical, haematological and biochemical changes associated with the disease, Assiut Vet. Med J. (In Press).
- Panuch, R.S. and Delafuents, J.C. (1985): Vitamins and immunocompetence. In G.H. Bourne(ed), "World Nutritional Determinants" Basel : Karger, pp.97-133.
- Pirie, H.M.; Pringle, C.R.; Allan, E.M. and Kennedy; G.J (1981): Acute fatal pneumonia in calves due to respiratory syncytica virus Vet. Rec., 104, 411-416.
- Radostits, O.M.; Blood, D.C. and Gay, C.C. (1995): "Veterinary Medicine" A text book of the diseases of cattle, sheep, pigs, goats, and horses. 18 Ed. Baillere Tindal, London, Great Briatain.
- Reddy, P.G.; Morrill, J.L.; Minocha, H.C.;; Morill, M.B.; Dayton, A.D. and Frey, R.A. (1985): Effect of supplemental vitamin E on the immune system of calves. J.Dairy Sci. 69:164-171.
- Reddy, P.G.; Morrill, J.L, Minocha, H.C. and Stevenson, J.S (1987): Vitamin E is immunostimulatory in calves . J. Dairy Sci 70:993-999.
- Robert, W.; Bunnett, Elizabethlee-Lewandrowski, and Ent Lewandrowski, (1990): Electrolyte, Acid-base balance, In textbook of clinical laboratory medicine. Edited by McClatchey, D.K.1st Ed., William and Wilkins, Baltimore, Philadelphia, Hong Kong, London, Munich, Sydney, Tokoyo. pp.331-354.

- Rounghton, F.J.W. (1964): "Transport of oxygen and carbon dioxide in Hand book of physiology. "Vol.1, respiration Sec.2pp:767-829. Edited by Fenn, W.O and Rahn, h., American Physiological Society, Washington.
- Simmons, D.H. (1962): Clinical disorders of fluid and electrolyte metabolism. Mcgraw-Hill book company, INC. New York, Toronto and London.
- Singh, J.I.T. and Kohli, R.N. (1980): Acid-base status and blood gasses in normal buffalo clave. Zbl. Vet. Med., A.27, 343-345.
- Tasker, J.B. (1969): Fluid, Electrolytes and acid-base abnormalities in cattle. J.A.V. Med. Ass., 155(12): 1906-1909.
- Umlauft, K.D.; Schulz,G.;Franle, F.; Schanfelder, U.; Pannwitz, S. Blohm, H.; Clans, D. and Liebig, F.(1987): Diagnosis of infections in calf pneumonia. Monatshefte fur Veterinarmedizin, 42(16): 583-588.