Vet.Med.J.,Giza. Vol.49, No. 3. (2001): 401-411.

MICROBIAL CONTAMINATION AND OTHER HATCHING PROBLEMS CAUSING DEAD -IN SHELL IN OSTRICH (STRUTHIO CAMELUS) EGGS DURING ARTIFICIAL INCUBATION

A. A. R. KHAFAGY*; and A. M. KAMEL**

Departments of Microbiology* and Wildlife**
Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt
(with one table and 3 figures)

Received: 29. 11. 2000.
Accepted: 17. 03. 2001.

SUMMARY

Complete data of 451 ostrich eggs from commercial incubators and hatcheries in Egypt were collected. Hatchability rate was only 45 (10%). Fertile and infertile eggs were 266 and 185 (59% and 41%) respectively. Fertility varied between eggs collected from different farms. Embryonic mortalities were higher at the start and end of incubation period (42 days). Most of these mortalities took place at the late stage of incubation (10-14 days) and were found related to percentages of water loss which cause severe oedema and later mass deaths. In fertile eggs (266); broken egg shell, malposition, severe oedema and microbial contamination were the predominant causes of chick mortalities reported with 19, 72, 52 and 78 (7,1%, 27%, 19.5% and 29.3%) respectively. Malpositioning was related to incorrect egg setting or inadequate turning and oedema was found to be correlated with egg size and the amount of water lost from incubated eggs

Of 266 fertile eggs, 73 showed bacterial (27.4%) and 5 fungal contaminations (1.9%). Salmonella enteritidis, S. typhimurium, S. typhi and S. paratyphi, E. coli, Staphylococcus aureus and Pseudomonas aeruginosa were the main bacteria isolated from contaminated and poorly handled ostrich eggs; whereas, Aspergillus fumigatus and Mucor species were the main fungal (1.9%) species isolated.

In conclusion, application of hygienic measurements, adequate egg turning, sufficient incubator humidity, periodic weighing of eggs for water loss in the incubators and hatcheries and proper egg handling are recommended in ostrich farms to reduce bacterial contamination and obtain significant and successful hatching results.

INTRODUCTION

Artificial incubation of ostrich eggs is poorly understood when compared with poultry (Burger and Bertram, 1981; Mellett, 1993 and Deeming et al., 1993a). Problems regularly encountered include achieving the correct weight loss from ostrich eggs during incubation (Ley et al., 1986; Button, 1993; Deeming et al, 1993a; and Deeming, 1993) and poor understanding of the pattern of embryonic development especially during hatching (Deeming, 1993). Proper artificial incubation of ostrich eggs is considered as an essential aspect of any commercial operations. Limited success in hatchability (only 50%) was reported in South Africa (Burger and Bertram, 1981) and variable results from eggs imported into the UK from Namibia and Zimbabwe (Deeming et al., 1993a, Deeming and Ayres, 1995; and Deeming, 1995). A poor knowledge of the pattern of embryonic development, especially during hatching, is thought to contribute in part to such poor results (Deeming, 1993), although other factors such as infertility and egg contamination are significant problems (Deeming, 1995). Egg contamination due to bacterial diseases or other infectious agents are a major health concern of ostrich production. There are few studies reporting dead- in shell embryos, and any observations of ostrich eggs during candling other than an increase in dark shadows (Hallam, 1992, and Deeming et al., 1993a). More detailed observations are needed to achieve a better hatching process and to clarify the various disease problems that could be encountered in dead- in shell embryos. The aim of the present study was to investigate the cause of embryonic mortalities in ostrich eggs due to poor handling and contamination and to determine the causative microbial agents encountered in dead-in shell embryos.

MATERIAL AND METHODS

1. Ostrich Eggs:

451 ostrich eggs from commercial incubators and hatcheries in Egypt were collected. The eggs were incubated in a 100 egg capacity, manually controlled incubator at 36°C, and 37% relative humidity (RH; 25°C wet bulb temperature) for 39 days. Eggs were automatically turned every 6 hours and broken egg shells were observed. After 39 days; the eggs were transferred to a hatchery set at the same temperature and RH as the incubator. Eggs were candled 7 days after the start of incubation and every 7 days thereafter to determine the position of the chick relative to the air cell and whether the air cell was piped. During candling ; smelled rotten, broken eggs and dead embryos in the incubator were removed to prevent contamination of other eggs. Any infertile eggs, as determined by candling, were removed from the incubator on 7-10 days of incubation, opened and examined to confirm lack of embryonic development. Egg weight was determined in order to compare actual weight with those with 15% weight loss. The remaining fertile eggs were ex-

amined for embryonic development to hatching stage. Un-hatchery eggs were collected and broken for examination for any obvious deformities and oedema.

2. Shell thickness of eggs

Shell thickness of eggs was measured to 0.01 mm, with a micrometer both at the air cell pole of the egg and at the equator. The two measurements were averaged to obtain shell thickness. Sterile swabs of the yolk sac contents, shell membranes, and egg albumin were collected for bacteriological examination.

3. Microbial Examinations

4-10 sterile swabs were collected from dead embryos and inoculated into Tetrathionate broth for enrichment before seeding onto solid media (Reissbrodt et al., 1996). Solid media used were MacConkey's agar (Cruickshank et al., 1975) and Salmonella -Shigella agar (Koneman et al., 1992). The isolated salmonellae were subjected to serological test (slide agglutination test) for identification also phagetyping. Hemolytic Escherichia coli was isolated using blood agar (detection of hemolysis), MacConkey's agar and identified by biochemical reactions (MacFaddin, 1979) and serological identification using slide agglutination test. Also Staphylococcus and Streptococcus species were isolated using Manitol salt 2.5%, NACL broth. and identified by phage typing) obtained from American type culture collection (ATCC), 12301 Parkloam DR, Rochville, MD 20852 USA. The basic technique of phage typing was described by (Cruichshank et al., 1975). Strains showing lysis (ranging from more than 50 plaques to confluent lysis) were recorded. For fungal examination, samples collected from dead embryos and were cultured on Sabouraud's dextrose agar (Difco-Detroit, MI, USA) and incubated at 41°C and identified microscopically according to the methods of Raper and Fennell (1977)..

RESULTS

1. Infertility and Hatchability in Ostrich eggs.

Of 451 ostrich eggs (Fig.1) introduced into commercial incubator and hatchery near Giza, Egypt, hatchability rate was found to be (10%). Fertile and infertile eggs were 266 and 185 (59% and 41%) respectively.

Embryonic mortalities were high at the start and end of incubation period (42 days). Most of these mortalities took place at the late stage of incubation (10-14 days) and were related to percentages of water loss which caused severe oedema and later mass deaths. In fertile eggs (266); broken egg shell, malposition, severe oedema and microbial contamination were the predominant symptoms reported with (7,1%, 27%, 19.5% and 29.3%) respectively.

2.Ostrich Eggs weight and shell thickness:

Egg weight averaged 1260±160 gm (range = 840-1712 gm) and estimated initial mass 1433 ± 1689 (range = 1035-2044 gm). Mean shell thichness was 1.88 ±0.16 mm and ranged from 1.36 to 2.17 mm, lighter eggs having thinner shells (r= 0.445, P< 0.001).

3. Dead-in shell

The incidence and main cause of dead-in shell eggs varied from malpositioning , severe generalized oedema and microbial contamination were the predominant findings reported with rate of (27%, 19.5% and 29.3%) respectively.

a. Malposition

Embryos with malposition problem (27%) were noted in the present study in dead-in shell ostrich embryos.

b. Oedema

Sever generalized oedematous embryos which stuck tightly to the eggs in the form of clear, gelatinous subcutaneous fluid especially in the legs, thigh and abdomen of chicks were observed in 52 out of 266 eggs examined (19.5%) (Fig.2),

c. Microbial Findings

Of 266 fertile eggs, 73 showed bacterial (27.4%)

Table. 1. The main bacterial and fungal pathogens isolated from dead-in shell ostrich embryos.

Bacterial isolates	Phage typing	No.	%
Bacterial		73/266*	(27.4%)
Salmonella enteritidis	Phage type 4	7	9.6%
Salmonella typhimurium	Phage type 141	5	6.8%
Salmonella typhi	-	2	2.7%
Salmonella paratyphi B	-	*3	4.1%
E. coli	078	2	2.7%
E. coli	01	5	6.8%
E. coli	0128	2	2.7%
E. coli	0166	6	8.2%
Pseudomonas aeruginosa	0:9, 0:6, 0:3	3	4.1%
Stapylococcus aureus	29	5	6.8%
"	52 A susceptible to lysis	4	5.4%
"	3 A	5	6.8%
"	6 Not susceptible to lysis	5	6.8%
"	53	5	6.8%
"	75	4	5.4%
"	84 Not susceptible to lysis	3	3.8%
"	42 D	7	9.6%
Fungal		5/266*	1.9%
Aspergillus fumigatus	-	3	60%
Mucor sp.	-	2	40%*

* No of isolates/ total No of examined eggs

and 5 fungal contaminations (5/1.9%). Bacterial isolates were mainly Salmonella species, E. coli, Staphylococcus aureus and Pseudomonas aeruginosa. No gross lesions of internal organs were noted but severe oedematous embryos showed evidence of internal haemorrhage but the yolk was completely absorbed (Fig. 3). Salmonella species (enteritidis and typhimurium) were the predominant bacterial pathogens besides E coli, Staphylococcus aureus and Pseudomonas aeruginosa, isolated in dead embryos and contaminated eggs. Aspergillus fumigatus and Mucor species were the main fungal species isolated.

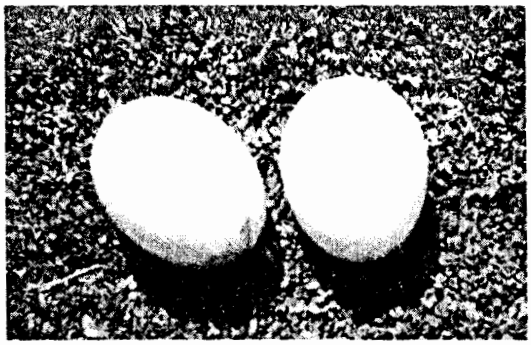

Fig. 1. Ostrich eggs from incubator.

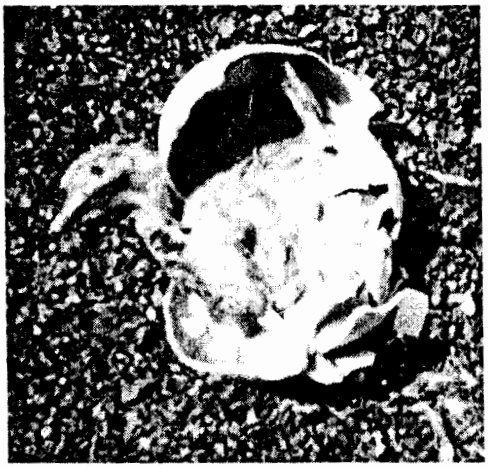

Fig. 2. Ostrich egg showing newly hatched odematous embryo from incubator.

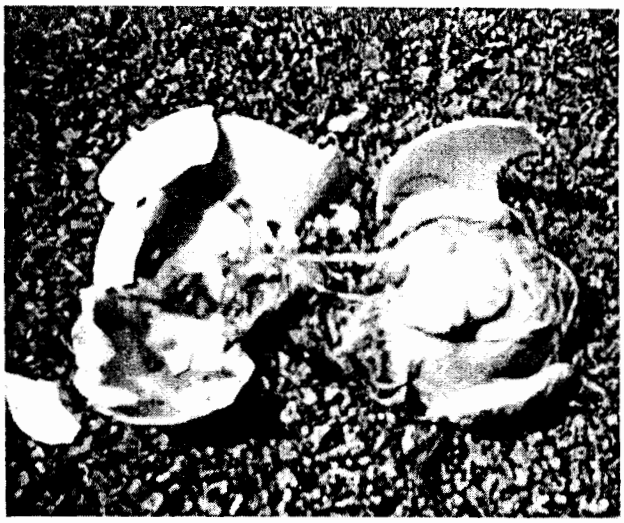

Fig.3. Ostrich egg showing severely odematous embryo in the thigh, legs and abdomen with unabsorbed yolk sac.

DISSCUSSION

Regular egg monitoring by candling is very important in ostrich production to provide early diagnosis of the development of microbial growth and ensured that contaminated eggs could be removed from the incubator before they contaminated other eggs, assessment of egg development and hatching process (Deeming, 1995).

Deeming (1993) mentioned that there are factors have significantly affect rate of egg hatchability during incubation and include temperature, humidity, respiratory gas exchange and egg turning.

The decreased hatchability rate (10%) observed in the present study was low and in accordance to that previously reported by Deeming (1995) who reported on 37.2 % hatchability from nine farms supplying eggs. Infertility (41%) mentioned in the present study was relatively higher than that reported by Mellett (1993) which revealed 30% and Deeming (1995) that reached 22.2%.

Embryonic mortalities were high at the start and end of incubation period (42 days). Most of this mortalities took place at the late stage of incubation (10-14 days) and were related to percentages of water loss which cause severe oedema and later mass deaths. In fertile eggs (266); broken egg shell, malposition, severe oedema and microbial contamination were the predominant lesions reported with 7,1%, 27%, 19.5% and 29.3%, respectively.

Ar and Rahn (1980) reported that 15% of the ini-

tial eggs was the average value for water loss for most bird eggs. The ostrich egg lies on the lower side of this average with mean weight loss of 13-14% being recorded (Burger and Bertram, 1981; Jarvis et al., 1985; and Swart et al., 1987).

The resulted egg weight (13%) with average weight loss was(1433 g) and similar to those previously measured for ostriches (1300-1500 g) by Leuthold, 1977; Keffen and Jarvis, 1985; Swart et al, 1987; Betram, 1992; Deeming 1993 and Brown et al, 1996). Shell thickness (1.36-2.17 mm) was similar to that previously reported by Keffen and Jarvis (1985) (1.79-1.83 mm) and Bowsher (1992) (1.48-2.77 mm) for eggs incubated in the USA. Egg weight averaged 1260 ±160 gm (range = 840-1712 gm) and estimated initial mass 1433 ± 1689 (range = 1035-2044 gm).

Malposition was the predominant lesion (27%) in dead -in shell ostrich embryos in the present study. This was similar to that reported by Philbey et al, (1991), who mentioned that malpositioning of embryos results in failure to hatch due to setting eggs upside down (air cell to the bottom) or horizontally. Other factors such as the inadequate turning of the eggs, insufficient water loss and possibly genetic factors (Tullet and Deeming, 1987; Wilson 1991a; and Jensen et al., 1992), as well as deficiency or excess of vitamin A in parent bird diets has been reported by (Angel, 1993). Brown et al (1996) mentioned that horizontal incubation for 2-3 weeks followed by vertical incubation has been shown to improve hatchability in comparison to eggs incubated either horizontally or vertically for the full incubation period.

Water loss from naturally incubated ostrich eggs was measured at 8.8-19.7% (Jarvis et al., 1985), 13.2% (Swart et al., 1987), and 15.6% (Burger and Bertram, (1981). Bowsher (1992) and Deeming (1993) reported that water loss below (10%) producing chicks that are oedematous, sluggish and frequently have unabsorbed yolks.

In the present study, oedema (19.5%) was observed as the cause of embryonic mortlaities during incubation procedures. Oedema in artificially incubated ostrich embryos is a common problem and has been reported by several authors (Phibey et al., 1991, Button, 1993; Terzicht and Vanhooser, 1993). Angel (1993) described that pantothenic acid deficiency in addition to insufficient water loss from the egg during incubation were considered as a cause of oedema. Rahn and Ar (1974) mentioned that during incubation, water is lost from eggs across pores in the eggshell and most bird eggs need to lose about 15% of their initial mass before piping takes place. Insufficient water loss results in poorly developed air cells, poor gas exchange and wet or water-logged oedematous

embryos, many of which die at or near time of hatch or soon after (Bowsher, 1992; Deeming et al., 1993 and Brown et al., 1996). Tullet and Deeming, 1982; Davis et al., 1988) mentioned that the proximal cause of death in oedematous embryos was hypoxia as a result of impaired oxygen diffusion across moist shell membranes. Successful hatching under artificial conditions revealed losses of 15.5% (Jarvis et al., 1985); 10-13% (Deeming et al., 1993) and 12% (Boshwer, 1992).

In the present investigation, bacterial contamination in fertile eggs reached (29.3%) and considered as one of the main causes of dead-in shell. The obtained findings showed that three serogroups of salmonellae were isolated from dead embryos that typed as S. enteritidis, S. typhimurium, and S. paratyphi. Similar were reported by Poppe et al (1991) and Orhan and Guler (1993). These organisms have been cultured from both the shell and interior of hatching eggs of domestic poultry and waterfowl (Board, 1969; Serviour et al., 1972; Bruce and Johnson, 1978, Mayes and Takeballi, 1983 and Burley and Vadhera, 1989). Bacterial contamination of ostrich eggs may be related to improper cleaning of eggs from faecal matters in the farms. This results is similar to that reported by Bruce and Drysdale (1991) who mentioned that the incidence of bacterial contamination is much greater when the egg is laid into an environment contaminated by feacal matters.

Salmonella species (enteritidis and typhimurium), E. coli, and Staphylococcus aureus were the main bacterial pathogens isolated in the present study in dead embryos (Table. 1). S. enteritidis phage type 4 was of high incidence (19.2%) and coincide with that reproted by (Barnhart et al, 1991 and Bernado and Machado (1990), relatively to other Salmonella species; S. typhimurium phage type 141 (6.4%), S. typhi (2.6%) and S. paratyphi (3.8%) are similar to that reported by (Poppe et al, 1991; and Orhan and Guler, 1993) and considered of zoonotic and of public health significance. Concerning the serological identification of the isolated E. coli, the obtained data showed that E. coli serovars were belonging to 078 (2.6%), 01 (6.4%), 0128 (2.6%) and 0166 (7.7%) respectively. The incidence of Staphylococcus aureus with different serological identification were belonging to phage types 84 (3.8%), 52A and 75 (5.1%), 29 and 3A (6.4%), 53 and 42D (8.9%) and 6 (10.2%) respectively. Some phages particularly 42D, 75 and 52A were appropriate than others since they lysed 34%, 23.3% and 22.3% of the isolates respectively. Susceptability to other phage (6& 84) was of lower orders (less than 2%). Pseudomonas aeruginosa (3.8%) isolated in the present study with serovar (0:9; 0: 6 and 0:3) was in accordance to that mentioned by Deeming (1995) and Brown et al, (1996). Aspergillus fumigatus and Mucor species were the main fungal species (1.9%) isolated in the present study. These findings are dif-

ferent from that reported by Deeming (1995, 1996); who mentioned that fungal contamination in ostrich eggs was relatively common at 49.3% of all contaminated eggs examined. However they accorded with that reported by Gulahan et al. (1999) who stated that the incidence of microbial spoilage was mainly due to bacterial contamination (including Aeromonas, E. coli, and Pseudomonas) and low fungal incidence (0.1-2.9%) (including Apsergillus and Mucor sp.)

In conclusion; ostrich eggs handling and collection should be carried out under hygienic measurements to avoid egg contamination, periodic egg weighting to avoid excessive water loss, egg broken should be discarded to reduce microbial penetration of the eggs and minimize losses in hatachability and significant commercial production.

REFERENCES

Angel, R. (1993). Age changes in digestibility of nutrients in ostriches and nutritional profiles of ostrich and emu eggs. In: Bryden, D. I (Ed.) Ostrich Odyssey: Proceeding of the Meeting of the Australian Ostrich Association Inc (Vic). No. 217: 135-142, (Sydney, University of Sydney).

Ar, A. and Rahn, H. (1980). Water in the avian egg: overall budget of incubation. Am. Zool, 20:373-384.

Barnhart, H. M.; Dreasen, D. W; Bastlen, R. and Pancorto, O. C. (1991). Prevalence of Salmonella enteritidis and other serovars in ovaries of layer hens at time of slaughter. J. Food. Protec., 54 (7), 488-491.

Bernado, F. M. A. and Machado, J. C. C. (1990). Prevalence of Salmonella in broiler carcases in Portugal. Epidemiological implication for man. Revista Portuguesa-de- Ciencias Veterinaries, 84, 31-45.

Betram, B. C. R (1992). The ostrich communal nesting system. (Princeton, Princeton University Press).

Board, R. G. (1969). The microbiology of the hen's egg. Advances in App. Micr,11:245-281.

Bowsher, M. W. (1992). Improvement of reproductive efficiency in the ostrich: characterization of late embryo mortality. Ph.D Thesis, Texas A&M University.

Brown, C. R; Peinke, D; and Loveridge, A. (1996). Mortality in near-term ostrich embryo during artificial incubation.

Bruce, J. and Drysdale, E. M. (1991). Egg hygiene: routes of infection, In: Tullett, S. G. (Ed.). Avian Incubation, PP: 257-267.

Bruce, J. and Johnson, A. L. (1978). The bacterial flora of unhatched eggs. Brit. Poul. Scie, 19: 681-689.

Burger, A. E. and Bertram, B. C. R. (1981). Ostrich eggs in artificial incubators: could their hatching success be improved? S. Afric. J. Sci 77: 188-189.

Burley, R. W. and Vadhera, D. V. (1989). The Avian egg chemistry and Biology (New York, John Wiley &Sons).

Button, C. (1993). Collaborative ostrich investigations.In: Bryden, D. I (Ed.) Ostrich Odyssey: Proceeding of the Meeting of the Australian Ostrich Association Inc (Vic). No. 217, PP. 1-66 (Sydney, University of Sydney).

Cruickshank, R.; Dugid, J. R.; Marmoin, B.P; and Swain, R. H. A (1975). Textebook of Medical Microbiology. 12th. Ed. Chirchill, Living stone, Edinburgh and New York.

Davis, T. A; Shen, S. S.; Ackerman, R. A. (1988). Embryonic osmoregulation: consequeences of high and low water loss during incubation of the chicken egg. J. Expt. Zoo., 245:144-156.

Deeming, D. C. (1995). The hatching sequence of ostrich (Struthio camelus) embryos with notes on development as observed by candling. Brit. Poult Sci, 36:67-78.

Deeming, D. C. and Ayres, L. (1995). Factors affecting the growth rate of ostrich (Struthio camelus) chicks in captivity. Vet. Rec. (In press).

Deeming, D. C. (1996). Microbial spoilage of Ostrich eggs. Brit. Poult Sci, 37: 3, 689-693.

Deeming, D. C. (1993). The incubation requirements of ostrich (Struthiocamelus) eggs and embryos. In: Bryden, D. 1 (Ed.) Ostrich Odyssey: Procedding of the Meeting of the Australian Ostrich Association Inc (Vic). No. 217, PP. 1-66 (Sydney, University of Sydney).

Deeming, D. C., Ayres, L., and Ayres, F. J. (1993a). Observation on the first commercial production of ostrich (Struthio camelus) eggs in the UK; Incubation. Vet. Rec, 132: 602-607.

Gulahan, D. B; Mehra, K. N; Chaturvedi, V. K. and Dhanesar, N. S. (1999). Bacterial and Fungal flora of dead in shell embryos. Indi. Vet. J. 76: 8, 750-751.

Hallam, M. G. (1992). TheTopaz Introduction to practical Ostrich Farming (Harare, The Ostrich Producers Association of Zimbabwe).

Jarvis, M. J. F.; Keffen, R. H. and Jarvis, C. (1985). Some physical requirements for ostrich egg incubation. Ostrich, 56: 42-51.

Jensen, J. M; Johnson, J. H and Weiner, S. T. (1992). Husbandry and Medical Management of ostriches, Emus and Rheas (Texas Wildlife and exotic Animal Teleconsultants).

Keffen, R. H. and Jarvis, M. J. F. (1985). Some measurements relating to ostrich eggs. Ostrich, 55:182-187.

Koneman, M. D; Allen, M. D; Janda, Ph. D; Schreckenberger, Ph. D. and Winn, J. R. M. D. (1992). J. B. Lippincott company, 227 East Washington Square, Philadelphia, Pennsylvania 19106.

Leuthold, W. (1977). Notes on the breeding biology of the ostrich, Struthio camelus, in Tsavo East National Park, Kenya. Ibis, 119: 541-544.

Ley, D. H., Morris, R. E. and Jarvis, C. (1986). Mortality of chicks and decreased fertility and hatchability of eggs from a captive breeding pair of ostriches. J, A.V.M.A, 189: 1124-1126.

MacFaddin, J. F. (1979). Biochemical test for identification of medical bacteria, Williams and Wickins Company. Baltimore. USA.

Mayes, F. J. and Takeballi, A. M. (1983). Microbial contamination of the hen;s egg: a review. J. Foo. Protec, 46: 1092-1098.

Mellett, F. D. (1993). Ostrich production and products. In: Maree,C. & Casey, N. H. (Eds) Livestock Production Systems, Principles and Practice, pp: 187-194.

Orhan, G and Guler, L. (1993). Bacteriological and serological identification of salmonella species in the orgaans and feaces of chickens, eggs, and feed. Veterinarium, 4: 2, 15-20; 43 ref.

Philbey, A. W; Button, C.; Gestier, A. W; Munro, B. E.; Glastonbury, J. R. W.; Hindmarsh, M. and Love, S. C. J. (1991). Anasarca and myopathy in ostrich chicks. Aust. Vet. J., 68: 237-240.

Poppe, C.; Jrwin, R. J.; Forsberg, C. M.; Clarke, R. C. and Ogyet, J. (1991). The prevalence of salmonella spp.

among Canadian registered commercial layer flocks. Epid. Tufec., 100 (2), PP: 259-270.

Rahn, H. and Ar, A. (1974). The avian egg: incubation time and water loss. Condor, 76:147-152

Raper, K. B. and Fennell, D. I. (1977). The genus Aspergillus (Huntingdon, NY, Robert E. Krieger Publishing Company).

Reissbrodt, R. (1996). Ferrioxamine, E. Supplemented- enrichment and enrichement media improve various isolation methods for Salmonella. Inte. J. Foo. Micr.

Seviour, E. M; Sykes, F. R. and Board, R. G. (1972). A Microbiological survey of the incubated eggs of chickens and waterfowl. Brit. Poul. Scie, 9: 110-120.

Swart, D; Rahn, H; and De Kock, J. (1987) Nest microclimate and incubation water loss of eggs of the African ostrich (Struthiocamelus var. domesticus). J. Expt. Zool. Suppl, 1: 239-246.

Terzicht, M. and Vanhooser, S. (1993). Postmortem findings of ostriches submitted to the Oklahoma Animal Disease Diagnostic Laboratory. Avian .Dis, 37: 1136-1141.

Tully, T. N. and Wade, J. (1994). Bacterial meningitis in an adult ostrich. Ostrich News, 8 (74), PP: 70-71.

Tullett, S. G. and Deeming, D. C. (1987). Failure to turn eggs during incubation: effects on embryo weight, development of the

chorioallantois and absorption of albumen. Briti. Poult. Sci, 28: 239-250.

Tullett, S. G. and Deeming, D. C. (1982). The relationship between eggshell porosity and oxygen consumption of the embryo in the domestic fowl. Comp. Bioch. and Phys, 72A: 529-533.

Wilson, H. R. (1991a). Physiological requirements of the developing embryo: temperature and turning. In: Tullett, S. G. (Ed.). Avian Incubation, pp. 145-156 (London, Butterworth-Heinemann).