Comparative investigations of infectious runting and stunting syndrome in vaccinated breeder chicks by inactivated reovirus and chicks from non-vaccinated breeders

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Summary

Reoviruses are important pathogens responsible for poor growth performance and silent losses in the poultry industry. They are associated with many disease and syndromes such as malabsorption (runting and stunting syndrome), respiratory diseases and immunosuppression. Broiler birds are most susceptible to viral infections during the early post hatching period. Therefore, the transfer of maternal immunity to embryonated eggs is proved to be a primary means of protection from viral infections. In the present investigation, growth performance and pathology in breeder vaccinated and non-vaccinated chicks were studied after a challenge with the homologous malabsorption strain of the reovirus. Improvements in growth performance (mean live body weight, feed conversion ratio, broiler performance efficiency index, and protein efficiency index) in breeder vaccinated chicks were compared with non-vaccinated breeder chicks. The non vaccinated chicks showed various signs and lesions indicative of the reoviral malabsorption syndrome (MAS), whereas the vaccinated chicks showed very minimal alterations, demonstrating that the vaccination of breeders with homologous strains of the reovirus is profitable, and can help to increase the performance of broiler birds.

Key words: Breeder, Malabsorption, Reovirus, Stunting

Introduction

Infectious runting and stunting syndrome is also recognized as the malabsorption syndrome (MAS) as it affects gastrointestinal tracts of young broiler birds and causes enteritis, malabsorption of nutrients, growth reduction and stunting (Giambrone *et al*., 2007). It is a complex disease with multiple etiologies and involves different enteric viruses including reovirus, rotavirus, enterovirus, parvovirus, calicivirus and others (Goodwin *et al*., 1993; Nili *et al*., 2007). Although MAS has several etiological factors, a number of reports indicate that isolated reoviruses are capable of causing varying degrees of enteritis and reductions in growth performances (Rosenberger *et al*., 1989). Despite, the fact that reovirus is considered one of the most important pathogens seen in the enterocytes of MAS affected birds, certain studies indicate that it may play a secondary role (Montgomery *et al*., 1997). Reovirus infection is endemic in many poultry farms without causing direct mortality and has been frequently associated with a variety of disease conditions in chickens including viral arthritis/tenosynovitis, stunting syndrome, respiratory disease, enteric disease, immuno-suppression and MAS (Heide, 2000).

Malabsorption syndrome is distributed worldwide in 1 to 2 week old broiler chickens and continues to cause decreased body weight gain, increased mortality, downgraded carcass quality and secondary diseases, leading to significant economic loss. Malabsorption syndrome is characterised by stunting and uneven growth in a flock with a high culling rate, diarrhea with undigested feed resulting in wet litter, retarded feathering, pigment loss and bone abnormalities, all occurring in the first three weeks of the chicks' life (Rebel *et al*., 2006).

To increase the profitability of broiler farming, it is necessary to avoid such silent infections in farms. The solutions may include better managing practices, an all in all out strategy, better biosecurity and effective vaccination. Maternal immunity derived from breeder pullet vaccination is considered the first line of defense against virus infections in early age (Giambrone *et al*., 1992; Cookson *et al*., 2005). The present study aimed to

use a reovirus vaccine (inactivated) against MAS by applying a challenge model with the homologous strain of the reovirus, and to find its effect on the overall health and performance of vaccinated and unvaccinated broiler breeders.

Materials and Methods

Before the study started, all necessary permissions were obtained from the Institutional Bio-Safety Committee and the Institutional Animal Ethics Committee.

Broiler birds

A total number of 50 straight-run, one day old and healthy "Vencobb" broiler chicks were obtained from M/s. Venkateshwara Hatcheries Ltd., Pune. They were equally divided into groups A (25 chicks from vaccinated breeders with inactivated homologous strain of reovirus), and B (25 chicks from non-vaccinated breeders with inactivated reovirus). The birds were reared under a deep litter system following standard and uniform management practices.

Vaccines and virus

All the vaccines, namely, LaSota, IBD, IB and inactivated IBH vaccine as well as the virulent reovirus were obtained from M/s. Ventri Biologicals, Pune. Required medicines including vitamin B complex, coccidiostat, antibiotics and supplements were purchased from the local market.

Broiler feed

Broiler feed (starter and finisher) was obtained from M/s. Huma Hatcheries and Breeding Farms, Udgir.

Experimental study

Fifty one day old susceptible chicks were equally divided into two groups; all challenged intramuscularly with 0.1 ml of 10 $TCID_{50}$ (per bird) MAS strain of reovirus on the third day. The chicks were observed daily for 24 days afterwards, and no mortality was recorded. Blood samples were collected at the end of the experiment before birds were humanely sacrificed by dislocation of neck.

Growth performance

Birds from each group were weighed individually on days 3, 14, 21, 28 (groups A, and B) and their mean live body weight (g/b) was computed. A measured quantity of feed (g/b) was offered to the birds of each group and the left over feed was recorded after completion of the experiment. The difference between the feed offered and the left over feed was recorded as actual feed intake. The feed conversion ratio (FCR) and the broiler performance efficiency index (BPEI) for each group was calculated using standard formulas (Mervat *et al*., 1999). Protein efficiency was calculated based on the consumed unit protein to the unit body weight gain ratio in each group (Persia *et al*., 2003).

Hematological findings

At the end of the trial, blood samples (n=6) were randomly collected into sterilized and heparinized glass containers for hematological studies. The samples were analyzed for total erythrocyte count (TEC), total leukocyte count (TLC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte count (DLC).

Serum biochemical findings

At the end of trial, blood was collected in non-heparinized centrifuge tubes and centrifuged for 15 min (4000 rpm) for serum collection. Serum samples were collected in sterilized screw cap serum collection vials and stored in a deep freezer at -20°C for further biochemical analysis. Total serum protein, serum albumin, total serum cholesterol, serum creatinine, serum iron, serum calcium and phosphorous were estimated following standard protocols using biochemical kits obtained from M/s. Coral Diagnostics, Mumbai. Serum globulin concentration was calculated as the difference between total protein and albumin.

Signs and gross pathological findings

During the experiment, the birds of both groups were keenly observed for any altered clinical manifestation. At the end of the experiment, birds from both groups were humanely sacrificed by neck dislocation and subjected to necropsy. Group-wise gross pathological findings were then recorded.

Histopathology

Tissue samples were collected from the mid-duodenal segment, jejunum, kidney, liver, bursa of Fabricious, thymus, spleen and brain, washed in a normal saline solution (pH = 7.2) and preserved in 10% neutral buffered formalin. Samples were then processed and stained with haematoxaline and eosin stains. Stained slides were then examined under a microscope and lesions were noted.

Virus neutralizing antibody titers in serum

Serum samples of individual birds from both groups were collected on the 3rd day and subjected to quantification of the neutralizing antibody titers using a serum neutralization test in a primary chicken embryo fibroblast cell (CEF) culture in a microtiter system (Nyoman, 2006). Serial 10-fold dilutions of CEF adapted malabsorption strain reovirus were mixed in equal amounts of serum, incubated at 37°C for 1 h on a shaker. Next, 0.2 ml of the solution was inoculated into each well of the CEF monolayer (5 wells for each dilution). The virus dilution without serum was treated in the same manner; however, only 0.1 ml was inoculated to each well of the CEF monolayer (5 wells for each dilution). After 5 days, the cultures were observed for any cytopathic effects (CPE).

Statistical analysis

The statistical analysis and interpretation of data was carried out using SPSS software version 16.0 (2007). T-tests were run to analyze the data. Difference in mean values were considered significant at the P<0.01 and P<0.05 levels.

Results

Growth performances in the trial indicated that body weight (Fig. 7), feed intake, FCR, PE and BPEI improved in chicks whose parents were vaccinated with inactivated reovirus vaccine (group A) as compared to those of unvaccinated parents (group B). However, better serum NDV (New castle disease virus) titers were recorded in group B as compared to group A up to their first 27 days (Table 1).

Once being challenged with the homologous strain of MAS reovirus, live weight was found to be significantly lower in 15-day-old chicks of unvaccinated parents compared with those of vaccinated parents (P<0.00001). Moreover, growth rate and live weight of birds was uneven in this group after the challenge. Growth was found to be uniform in chicks hatched from vaccinated parents, with an increasing trend in body weight even after the challenge with reovirus.

Significant reduction (P<0.05) was observed in erythrocytic counts (P=0.012) and haematocrit (P=0.0118). The heterophils (P=0.0050) showed significant reduction (P<0.01). However, in experiments after the challenge, significant increase (P<0.05) in MCV (P=0.0343) and significant increase (P<0.01) in lymphocytes (P=0.002) were recorded in chicks hatched from unvaccinated parents as compared to those hatched from vaccinated parents. The differences between TLC (P=0.558) values were not significant between the two groups (Table 2); nevertheless, total serum protein (P=0.0202), albumin (P=0.0302), serum iron (P=0.018), were found to be significantly lower (P<0.05), respectively, after the challenge in chicks hatched from unvaccinated parents. Similarly, significantly lower (P<0.01) globulin (P=0.0063) and calcium (P=0.0034) were reported. Non-significant values of serum phosphorous, cholesterol and creatinine were recorded within the groups (Table 3).

Signs and lesions

During the observation period, no specific signs and lesions developed in chicks hatched from vaccinated parents. However, those from unvaccinated breeders showed a variety of signs including abnormal feathering patterns (Fig. 1), depigmentation of beaks and shanks (Fig. 2), pale mucous membranes, lameness, initial mild diarrhea and undigested feed particles in droppings (Fig. 3). A systemic necropsy examination of the affected birds from group B revealed carcasses with quite prominent keel bones, atrophy of subcutaneous muscles and fatty tissues, brittle skeletons, undigested feed in thin walled swollen intestines (Figs. 4 and 5), catarrhal enteritis, thick proventriculus with raised glands, gizzard erosion, pale muscles, focal necrotic lesions on liver, distended gall bladder (Fig. 6), diffusely swollen and mildly congested kidneys and bursa and thymus mild atrophy.

Table 1: Performance of broiler birds with mean values throughout the experiment

			After 27 days of rearing					
Sr. No.	Group	No. of birds	Body weight (kg)	Feed intake (kg)	FCR	BPEI	PE	Serum NDV titers (GMT)
1	A	25	46.25	77.50	1.68	110.12	0.3204	40.32
2	B	25	27.50	67.50	2.44	45.09	0.4728	90.51
P-value			<0.00001	0.000046	<0.00001	<0.00001	<0.00001	
Significance			P≤0.01	P≤0.01	P≤0.01	P≤0.01	P≤0.01	

Table 2: Haematological parameters at the end of experiment

Particulars	TEC*	Hb*	PCV	MCV*	MCH	MCHC	TLC	DLC				
								L %#	H %#	E %	B %	M %
Group A	3.07[a]	17.75[a]	33.67	10.98[b]	58.07	52.86[a]	19.14	48.8[b]	30.5[a]	9.17[a]	1.33	10.2
SEM	0.09	0.36	0.67	0.09	2.17	1.73	0.65	0.47	0.57	0.55	0.33	0.47
Group B	2.80[b]	14.97[b]	33.57	12.00[a]	53.50	44.98[b]	20.15	56.5[a]	25.7[b]	7.17[b]	1.5	9.17
SEM	0.02	0.51	0.97	0.36	1.70	2.72	1.04	0.77	1.12	0.61	0.42	0.30
P-value	0.01196	0.01184	0.936541	0.0343	0.21644	0.08915	0.55805	0.00021	0.00503	0.08442	0.61088	0.20311
Significance	P<0.05	P<0.05	NS	P<0.05	NS	NS	NS	P<0.01	P<0.01	NS	NS	NS

NS: Non-significant, and [a, b] Significant difference at P<0.05* and P<0.01#

Table 3: Serum biochemical findings of broiler chicken at the end of experiment

Particulars	TP*	Albumin*	Globulin#	Cholesterol	Creatinine	SI*	SC#	SP
Group A	6.08[a]	1.53[a]	4.72[a]	136.33	0.25	434.2[a]	11.8[a]	8.67
SEM	0.47	0.04	0.33	5.14	0.02	40.82	0.64	1.40
Group B	4.48[b]	1.25[b]	3.23[b]	133.33	0.22	283.5[b]	7.92[b]	10.00
SEM	0.12	0.07	0.09	4.82	0.02	16.03	0.49	1.07
P-value	0.0202	0.03024	0.006353	0.74082	0.17469	0.018	0.00342	0.43271
Significance	P≤0.05	P≤0.05	P≤0.01	NS	NS	P≤0.05	P≤0.01	NS

NS: Non-significant, TP: Total proteins, SI: Serum iron, SC: Serum calcium, SP: Serum phosphorus. [a, b] Significant difference at P<0.05* and P<0.01#

Relative organ weights

After the challenge, the relative weights of kidney, brain and cecal tonsils significantly increased while that of thymus and bursa significantly decreased in group B compared to group A. However, no significant difference was found in the relative weights of proventriculus, gizzard, liver, heart, pancreas, small intestine, large intestine and cecum of birds hatched from vaccinated and unvaccinated parents.

Fig. 1: Birds from group B showing uneven growth and deviated feathering pattern

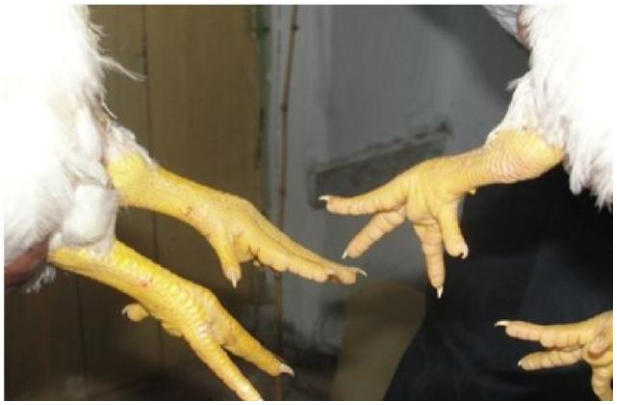

Fig. 2: Normally pigmented shanks (left) of birds from group A and depigmented shanks (right) birds from group B

Fig. 3: Faeces with undigested feed material indicated by coarse particles in birds from group B

Histopathological findings

During the observation period, no specific microscopic lesions were developed in chicks hatched from vaccinated parents; however, few birds showed mild cellular degenerations in their liver and kidneys.

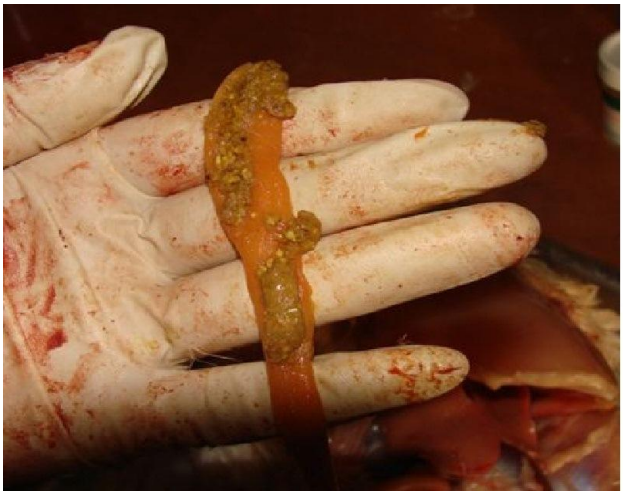

Fig. 4: Intestinal content with undigested feed material in birds from group B

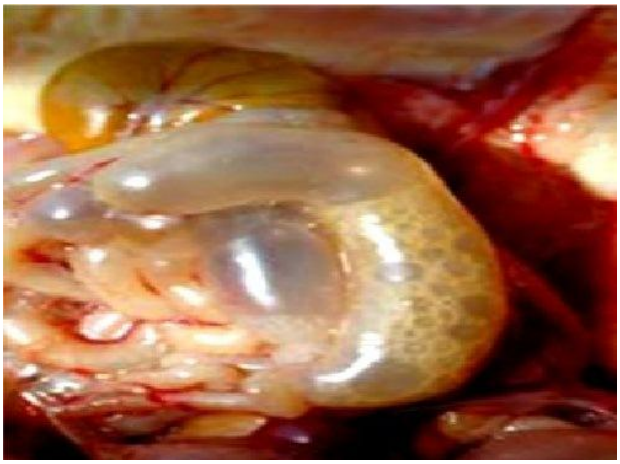

Fig. 5: Showing thin, gas filled intestines in birds from group B

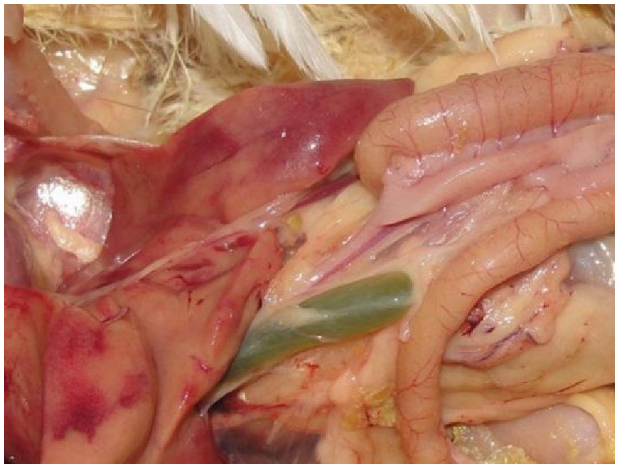

Fig. 6: Liver showing degenerative changes with enlargement of gall bladder in birds from group B

The chicks hatched from unvaccinated parents reflected changes corresponding to signs and gross lesions. In the liver of the affected birds, cloudy swelling and fatty changes were consistent findings (Fig. 8). There was also evidence of mild acute multifocal necrotic lesions. In a few birds, the kidneys showed desquamation of tubular epithelium into lumen and multifocal hemorrhages (Fig. 9). Proventriculus showed inflammatory changes along with the dilatation of glands (Fig. 10). There was severe atrophy of duodenal villi with cystic dilatation of crypts (Fig. 11). Heterophilic infiltration in the lamina propria and villi were also a consistent finding. Lymphoid organs such as the thymus, bursa of Fabricious and spleen revealed a reduction in the population of lymphoid elements.

Serum neutralizing titers against reovirus

The reovirus specific CPE was observed during serum neutralization tests. These CPEs include syncytia formation, cell detachment, giant cell formation and dead cells floating in the medium. The absence of serum neutralizing titers against malabsorption strains of reovirus in 3-day-old chicks of unvaccinated parents suggests that the parents were free from reovirus infections. Nevertheless, chicks hatched from eggs of vaccinated parents showed serum neutralizing titers of 8445 units per ml (GMT-42.22) against malabsorption strains of reovirus at their 3rd day. This suggests the passive transfer of humoral immunity from vaccinated parents to their offspring.

Fig. 7: Reduced body growth in birds from group B (right) and normal body growth in birds from group A (left)

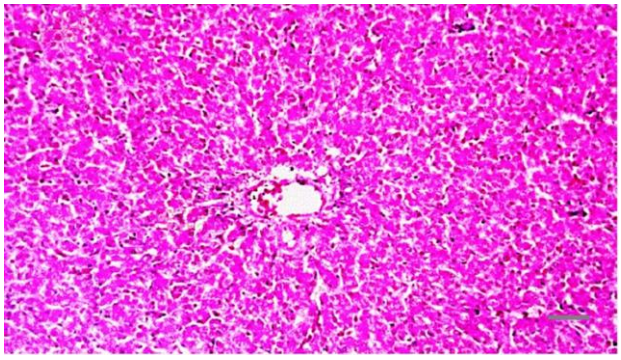

Fig. 8: Photomicrographs of hematoxylin and eosin stained broiler liver showing vacuolar degenerative changes from birds of group B (scale bar, 50 µm)

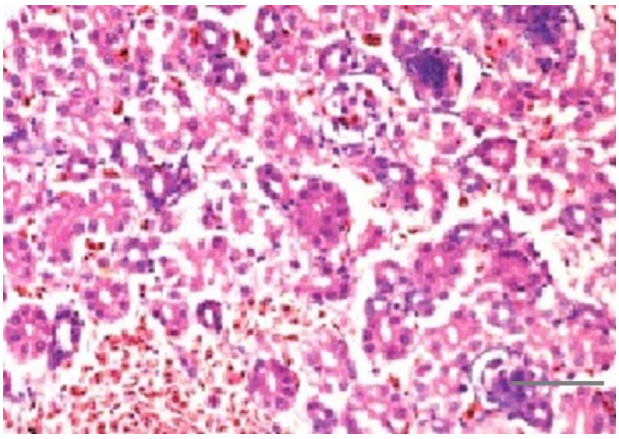

Fig. 9: Photomicrographs of hematoxylin and eosin stained broiler kidney showing degenerative changes and severe haemorrhages in the parenchyma from birds of group B (scale bar, 50 µm)

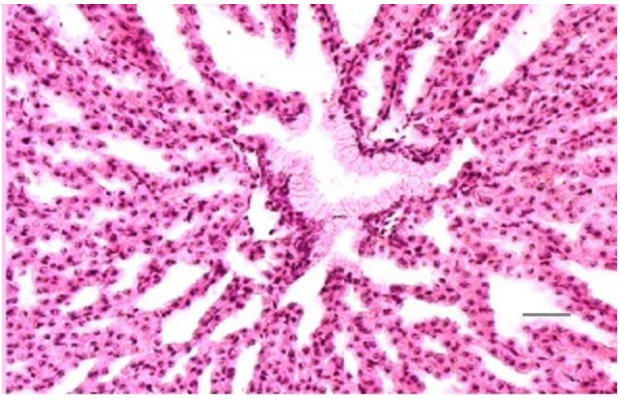

Fig. 10: Photomicrographs of hematoxylin and eosin stained broiler proventriculus showing dilatation of the proventricular gland along with infiltration of leucocytes from birds of group B (scale bar, 50 µm)

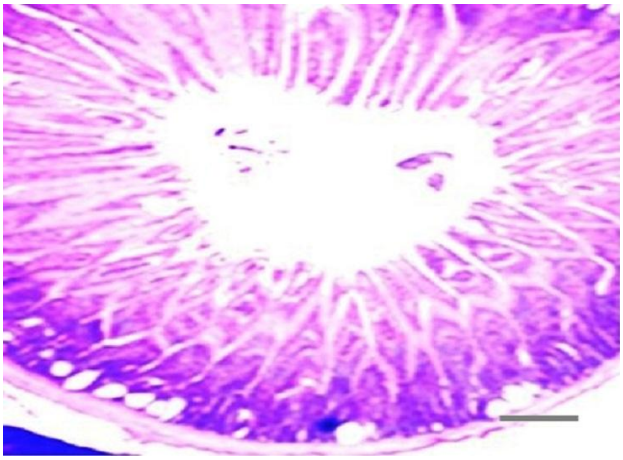

Fig. 11: Photomicrographs of hematoxylin and eosin stained broiler intestine showing atrophy and fusion of villi as well as cystic dilatation of crypt from birds of group B (scale bar, 100 µm)

Discussion

Poultry production is an important component of India's agriculture economy, providing employment for farmers as a subsidiary agricultural business. However, recent intensive developments in the poultry industry have been slowed down by constraints such as viral diseases which pose great economic threats to the business. Reoviruses are endemic in many farms. Most of the times, they do not directly cause mortality in birds; however, they are responsible for reduced production by dropping weight gain, increasing feed conversation ratio and immunosuppression. In the present study, pathology and growth performances are investigated after a challenge with a malabsorption homologous strain of the reovirus in breeder vaccinated chicks and those from non-vaccinated breeders with an inactivated homologus strain of reovirus.

Significant improvements ($P<0.01$) in body weight ($P<0.0001$), feed intake ($P=0.000046$), FCR ($P<0.0001$), PE ($P<0.00001$), and BPEI ($P<0.0001$) were observed in chicks obtained from breeders vaccinated with in-activated reovirus (group A) as compared to those obtained from unvaccinated breeders (group B). The two groups were also significantly different in terms of live weight; birds from non-vaccinated breeders showed uneven patterns of growth and live weight compared to the other group. Findings of the present study are similar to the earlier findings of Awandkar *et al*. (2012) and Kang *et al*. (2012) who attributed their results to the impairment of enzymatic digestion and absorption of nutrients from the gastrointestinal tract as the virus targets the pancreas and intestinal tract (Rebel *et al*., 2006). Chicks from non-vaccinated breeders had better serum NDV titres as compared to the other group. This could be explained by the existence of maternal derived reovirus antibodies in breeder vaccinated chicks which interfered with active immunization against other infections (Adriaan *et al*., 2003). Malabsorption syndrome has a direct effect on the health of affected birds and is characterized by the excretion of essential ingredients in the faeces. This causes significant decrease in essential serum hematological parameters such as protein, albumin, globulin, iron and calcium.

In the present study typically stunted birds had pale shanks, beaks, combs, wattles and mucous membranes. In addition, the droppings of the affected birds had undigested feed material. These observations agree with the pathogenesis of paleness described by Khan *et al*. (1995) and Rebel *et al*. (2006) who found that affected birds failed to absorb dietary carotenoid pigments, vitamin E and other essential nutrients necessary for normal body growth and skin coloration. Disturbances in enzymatic digestion also prevent the release of plant pigments from maize and cause paleness. This is known as "Pale bird syndrome" and "malabsorption syndrome", in which there is feathering retardation and splitting of primary wings and tail feathers, resulting in alopecia and abnormal feathering pattern (Kouwenhoven *et al*., 1992).

During the systematic necropsy examination it was observed that affected birds were severely emaciated and there keel bones were prominent. There was atrophy of subcutaneous fat and muscle tissue. Tang *et al*. (1987) observed that stunted birds exhibited severe weakness and had pale breast muscles. In the present study, the birds had fragile and brittle skeletons, assumed to be caused by the decrease in vitamin D absorption and exacerbated by the likelihood of intestinal calcium being chelated to lipid and lost in the faeces (Khan *et al*., 1995). Lymphoid organs also showed mild atrophy with the reduction of lymphoid elements, but these changes in may be caused by nutritional deficiencies rather than being the primary effect of viral infections. Also in the present study gastrointestinal tracts were distended with poorly digested feed and gases. Intestines were paler in color and catarrhal enteritis was a common lesion amongst the affected birds, similar to Page *et al*. (1980) and Giambrone (2007) findings. Microscopically, the villi of the duodenal region were atrophied, the epithelium at their tips was necrosed and some were sloughed off. There was cystic dilatation of the crypt along with edema and mononuclear cells infiltration in affected intestines. These histopathological findings were also observed by Rebel *et al*. (2006). Other studies have also reported the induction of a mild form of intestinal lesions in SPF chickens with enteric reovirus infection (Songserm *et al*., 2003).

In almost all the affected birds, the spleen was pale to brownish in coloration, and was atrophied as observed by Hieronymus *et al*. (1982). Another characteristic change noted during the present study was the reduced number of lymphoid follicles with a smaller number of lymphocytes per microscopic field in various lymphoid organs. This is a likely indication of imununosuppression as described by Nili *et al*. (2007). Khan *et al*. (1995) suggested that changes in lymphoid organs could be associated with poor nutrient utilization. Another change in the majority of the affected birds was that the kidneys were inflamed and congested. These changes coincide with the observations of Elmubarak *et al*. (1990). Moreover, the leukocytic infiltration in the interstitial tissues was quite evident microscopically. The blood vessels were dilated and hemorrhages in affected kidneys were also noticed. These histopathologic observations were previously reported by Elmubarak *et al*. (1990).

Findings of the present study indicate that breeder vaccination with inactivated reoviruses can increase the health of their progeny. Chickens are most susceptible to avian reoviruses in the post-hatching period and become increasingly resistant to infection with age (Rosenberger and Olson, 1997). Therefore, vaccination programs should aimed at immunizing breeders with inactivated vaccines to transfer the so-called passive immunity to progeny. Vertically transmitted maternal immunity is based on antibodies that are transferred passively to the egg. Reovirus-specific antibodies can provide protective immunity using different mechanisms which result in the improvement of growth performance and profit. Hence, the vaccination of breeders with inactivated homologous strains of reovirus can increase the growth performance

of their offspring in areas where reovirus infection is endemic.

Conflict of interest

Authors declare no conflict of interest.

References

Adriaan, AW; van Loon, M; Willeke, K; Hanneke, I; Suzanne, VR; Marijke, F; Virgil, EJ and Schijns, C (2003). The contribution of humoral immunity to the control of avian reoviral infection in chickens after vaccination with live reovirus vaccine (strain 2177) at an early age. Avian Pathol., 32: 15-23. doi: 10.1080/ 0307945021000070679.

Awandkar, SP; Manwar, SJ; Badukale, DM and Kulkarni, MB (2012). Growth performance of broilers in experimental reovirus infections. Vet. World. 5: 685-689. doi: 10. 5455/vetworld.2012.685-689.

Cookson, KC; Giambrone, JJ and Rodenberg, JH (2005). A reovirus progeny challenge study comparing breeder flocks on two different IBDV/reovirus programs. Poult. Sci., 84 (Suppl. 1): 112 (abst.).

Elmubarak, AK; Kheir, SAM and Abuelgasim, AI (1990). Occurrence of ranting and stunting syndrome in broiler chickens in Sudan. Revue Elev. Med. Vet. Pays. Trop., 43: 317-322.

Giambrone, JJ; Dormitorio, T; Cookson, K and Burns, K (2007). Monitoring the immune status of broilers against reoviruses using challenge and serologic data. J. Appl. Poult. Res., 16: 187-191.

Giambrone, JJ; Dormitorio, T and Lockaby, SB (1992). Coarse-spray immunization of one-day-old broilers against enteric reovirus infections. Avian Dis., 36: 364-368.

Goodwin, MA; Davis, JF and Player, EC (1993). Reovirus associated enteritis in Georgia broiler chicks. Avian Dis., 37: 229-233.

Hieronymus, RK; Pedrovillegas, D and Kleven, SH (1982). Identification and serological differentiation of several reovirus strains isolated from chickens with suspected Malabsorption syndrome. Avian Dis., 26: 246-254.

Kang, K; Mohamed, E; Holly, SS; Fernanda, D; Susan, MW; Taejoong, K and Stephen, CEM (2012). Investigation into the aetiology of runting and stunting syndrome in chickens. Avian Path., 41: 41-50. doi: 10. 1080/03079457.2011.632402.

Khan, SA; Mustafa, G; Chaudhary, RA; Iqbal, M and Khan, MI (1995). Infectious stunting syndrome of broiler chicks, clinical signs and pathological lesions. Asian Australas. J. Anim. Sci., 8: 1-6.

Kouwenhoven, B; Dwars, RM and Smeets, JFM (1992). Wet litter and high feed conversions, a new problem in broilers. In: McNulty, MS and McFerran, JB (Eds.), *New and evolving virus diseases of poultry*. Community Research and Technological Development Programme in the Field of Agriculture and Agro-Industry, Including Fisheries (AIR) Brussels, Belgium. PP: 243-251.

Montgomery, RD; Boyle, CR; Maslin, WR and Magee, DL (1997). Attempts to reproduce a runting/stunting type syndrome using infectious agents isolated from affected Mississippi broilers. Avian Dis., 41: 80-92.

Nili, H; Jahantigh, M and Nazifi, S (2007). Clinical observation, pathology, and serum biochemical changes in infectious stunting syndrome of broiler chickens. Comp. Clin. Pathol., 16: 161-166.

Nyoman, MA (2006). Neutralization of avian reovirus 1091 in chick kidney and vero cell cultures by monoclonal antibodies against μB/μBC and σC proteins. J. Ked. Hewan. 22: 68-73.

Page, RK; Fletcher, OJ; Rowland, GN; Gaudry, D and Villegas, P (1980). Malabsorption syndrome in broiler chickens. Avian Dis., 26: 618-624.

Persia, ME; Persia, CM; Schang, M and Azcona, J (2003). Nutritional evaluation of dried tomato seeds. Poultry Sci., 82: 141-146.

Rebel, JMJ; Balk, FRM; Post, J; van Hemert, S; Zekarias, B and Stockhofe, N (2006). Malabsorption syndrome in broilers. World Poult. Sci. Jr., 62: 17-30.

Rosenberger, JS; Sterner, FJ; Botts, S; Lee, KP and Margolin, A (1989). *In vitro* and *in vivo* characterisation of avian reoviruses. I. Pathogenicity and antigenic relatedness of several avian reovirus isolates. Avian Dis., 33: 535-544.

Songserm, T; Van Roozelaar, D; Kant, A; Pol, J; Pijpers, A and TerHuurne, A (2003). Enteropathogenicity of Dutch and German avian reoviruses in SPF white leghorn chickens and broilers. Vet. Res., 34: 285-295.

Tang, KN; Fletcher, OJ and Villegas, P (1987). Comparative study on the pathogenicity of avian reoviruses. Avian Dis., 31: 577-583.

Van der Heide, L (2000). The history of avian reovirus. Avian Dis., 44: 638-641.