

# Egg and egg shell quality during experimental infectious bronchitis virus infection in laying hens

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## Introduction

Infectious bronchitis virus (IBV) is a viral disease of poultry and one of the factors responsible for deterioration in egg production and quality. The disease is potentially a major threat to the egg industry as egg quality problems currently cost millions of dollars a year. In Australia, IBV was first reported by Cumming (Cumming, 1962). Australian strains of IBV are thought by some to be a cause of deterioration of egg and egg shell quality but evidence one way or the other for this is lacking. The effects of Australian strains of IBV on the oviduct of laying hens have received little research attention. The present trial was conducted to study the effects of two Australian strains of IBV on egg and egg shell quality. The strains used in this trial have been placed in to two different genetic groups (Sapats, *et al.*, 1996). The two strains can induce pathology in the oviduct of Leghorn hens (Chousalkar *et al.*, 2007) and albumen-forming regions of the oviduct of Isa Brown hens (Chousalkar and Roberts, 2007).

## Materials and Methods

Day-old Isa Brown hens (150) were reared under strict isolation and biosecurity and divided into three groups at 25 weeks of age. At 30 weeks of age, birds were exposed to one of two strains of IBV: T or N1/88 strains and one group was left unchallenged as a control. All eggs were collected at 3 and 2 weeks prior to challenge and then daily during the week immediately before infection to determine any inherent differences among the groups. Eggs were collected and analysed daily up to 5 weeks post infection (p.i.) and again at weekly intervals 6, 7, 8, 9 and 10 weeks p.i. All eggs were analysed for the internal quality parameters albumen height, Haugh units and yolk colour score. Egg shell quality was measured as reflectivity, egg weight, deformation, breaking strength, shell weight, shell thickness and percentage shell.

During the first trial, the eggs in both the infected groups appeared to be more elongated than the control eggs. The length and breadth of eggs was measured between 6 and 10 weeks post infection and confirmed that the eggs from the challenge groups were more elongated. Therefore, a follow-up trial was conducted to study the changes in length and breadth of eggs laid by hens from infected hens. A total of 36 Isa Brown laying hens at the age of 35 weeks were divided into three groups. 15 hens per challenge treatment and 6 hens as a control. Eggs were collected daily for 3 weeks post infection. Length and breadth were measured using a digital vernier caliper and values were used to calculate the shape index (breadth x 100/ length).

All hens were bled at 28 weeks of age (2 weeks before infection), 35 weeks (5 weeks post infection) and 40 weeks (10 weeks post infection). Three mL blood samples were collected in heparinised syringes and stored on ice. Ionised calcium was measured using an AVL 983 Electrolyte Analyser (with ion selective electrodes) and results were presented in mmol/L. Data were analysed by ANOVA and Fisher's protected LSD was used to distinguish differences between means. Significance was assumed at  $P < 0.05$ .

## Results

### Clinical findings

There were no significant effects of challenge or time in relation to challenge on egg production, although one hen from the N1/88-infected group and two hens from the T-infected group stopped laying from the second week p.i. Visual loss of shell colour was noticed in both infected groups from 4 to 8 days p.i. Some hens from both T and N1/88-infected groups showed coughing and sneezing from 3 to 9 days p.i. Feed intake did not vary significantly over the weeks after challenge or amongst the groups and there were no significant interactions. However, feed intake tended to be lower in the T-infected group from 2 to 4 weeks p.i. (Fig. 1). There were no visible deformities in egg shells in the N1/88 infected group, but occasional occurrence of black-spotted egg shells was noted in the T-infected group. Eggs with yolks that separated from the albumen during egg breakout and with meat spots were observed mostly between the 10<sup>th</sup> and 16<sup>th</sup> days p.i. in the N1/88 and T-infected groups.

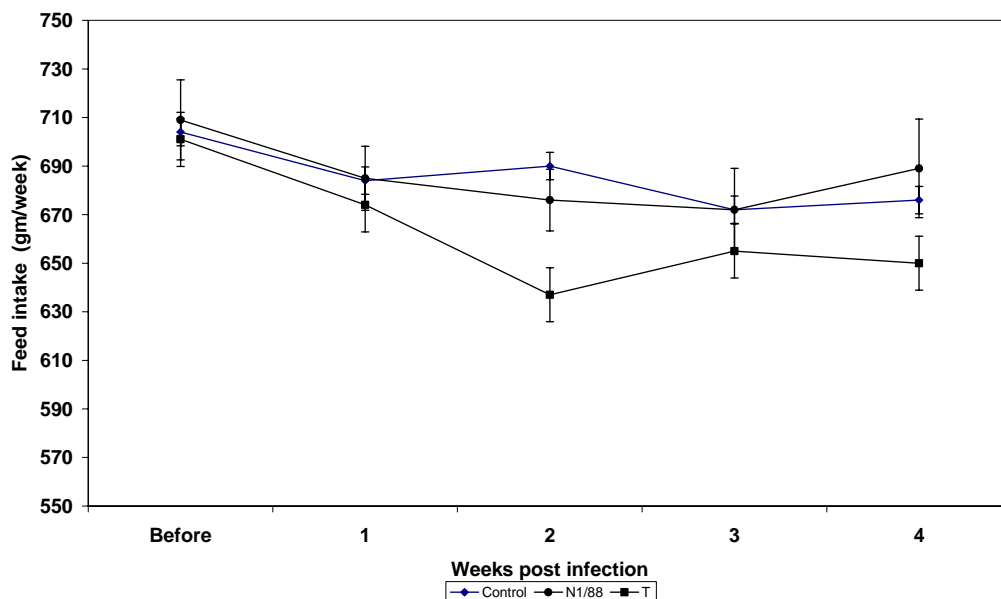


Fig 1: Feed intake before infection and for 10 weeks post infection for the three IBV treatment groups. Bar represents mean standard error.

### Egg quality

Data are presented for egg quality measurements only where there was a significant interaction between IBV treatment group and time post-infection. In eggs laid over the 3 weeks before challenge, egg weight, deformation and percent shell varied significantly over the weeks. There was a significant main effect of treatment group and week of experiment on breaking strength. However, there was no interaction between treatment group and time post-challenge. The same differences between groups and time in relation to challenge were recorded post-challenge. Overall, shell weight and shell thickness increased significantly over the 10 weeks p.i. Such differences were not recorded before challenge. However, no significant difference was recorded among treatment groups. Over ten weeks p.i., there were significant main effects and significant interactions between treatment group and weeks p.i. on albumen height and Haugh units (Fig 2).

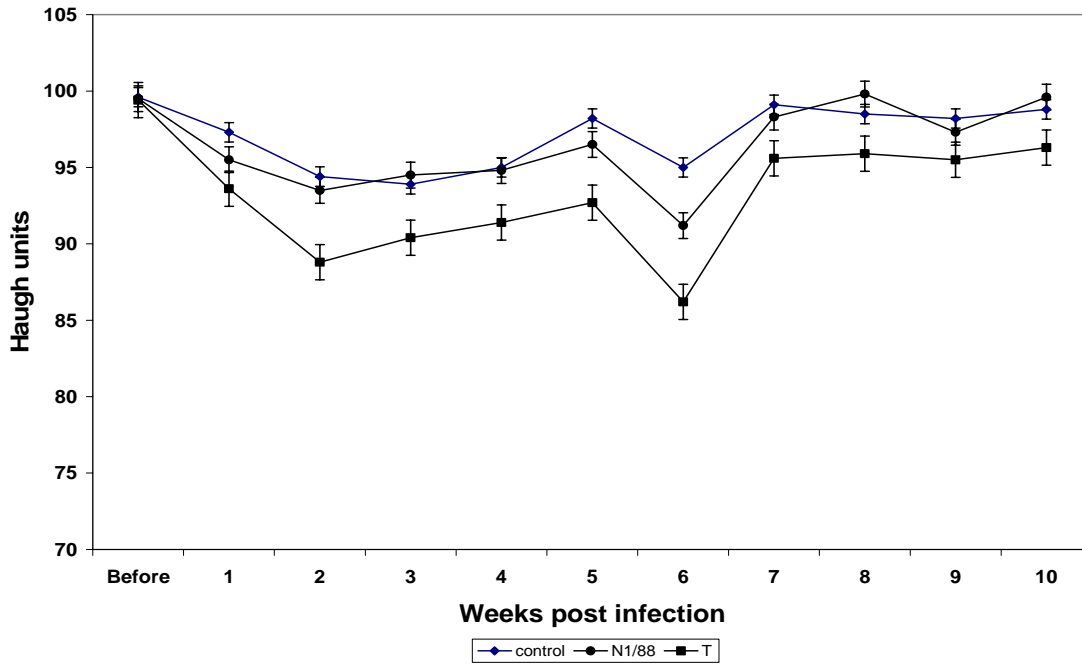


Fig 2: Haugh units before infection and for 10 weeks post infection for the three IBV treatment groups. Bar represents mean standard error.

Such differences were not recorded within these parameters before challenge. Compared to control hens, the albumen height in N1/88 group was significantly lower than the control at 2, 5 and 6 weeks p.i. and Haugh Units were significantly lower at 1, 5 and 6 weeks p.i. Except for the 8<sup>th</sup> week p.i., albumen height and Haugh Units were significantly lower from the first week p.i. until the end of the experiment in the T-infected group. Overall, there were no significant differences amongst the infected groups for yolk colour score but there was a significant variation over the weeks in relation to challenge and a significant interaction between group and week of experiment. Yolk colour score was significantly lower in T-infected hens from 2 to 4 weeks p.i. (Fig 3).

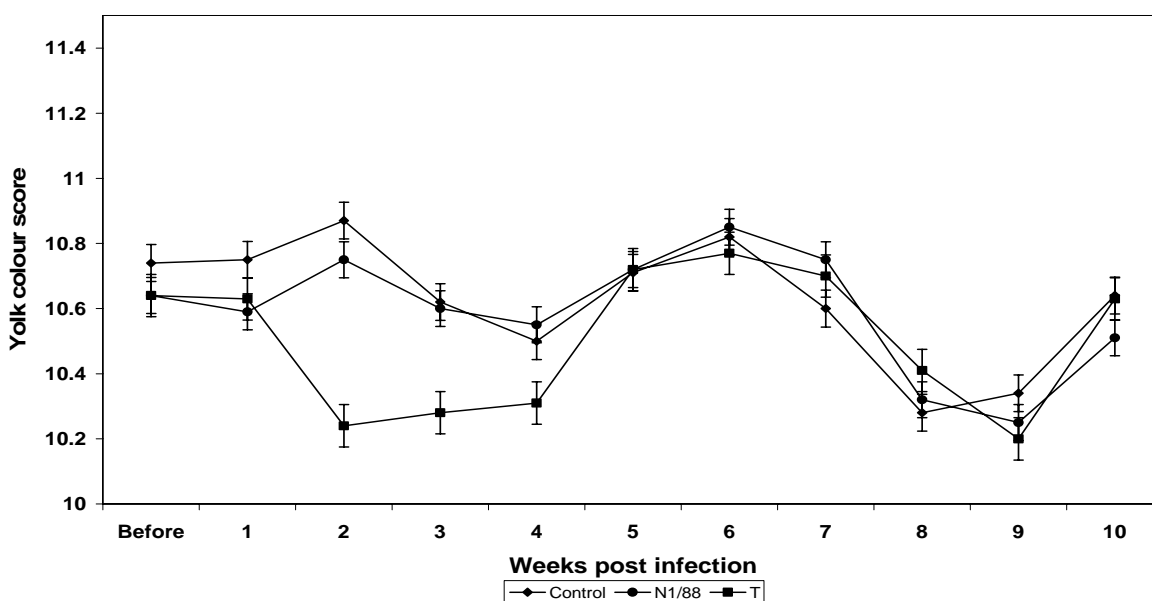


Fig 3: Yolk score before infection and for 10 weeks post infection for the three IBV treatment groups. Bar represents mean standard error.

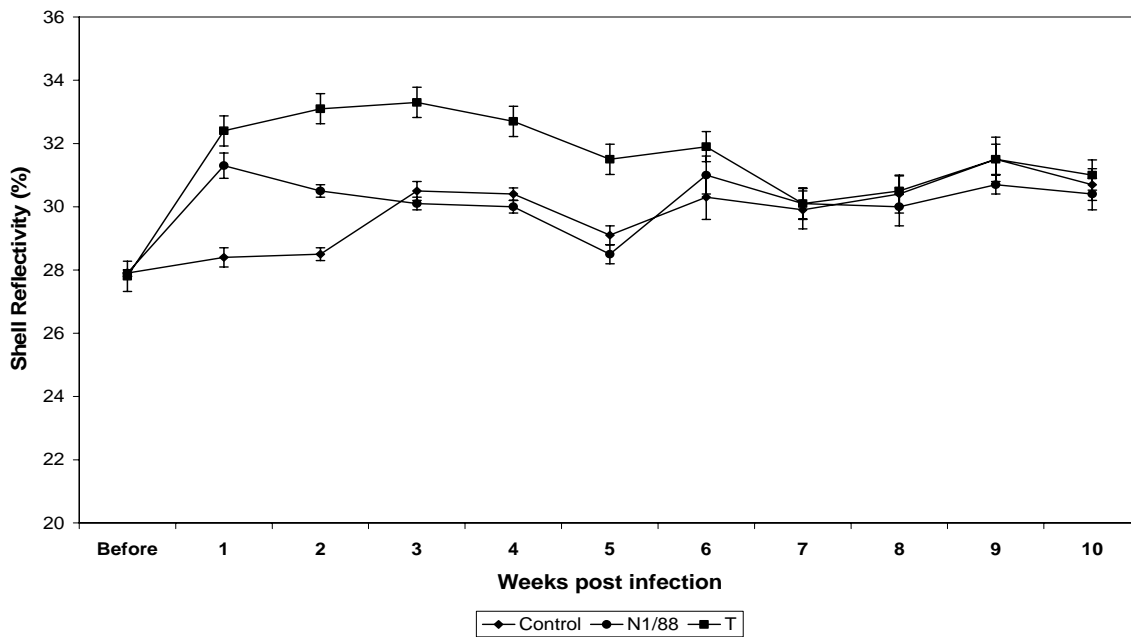


Fig 4:

Shell reflectivity before infection and for 10 weeks post infection for the three IBV treatment groups. Bar represents mean standard error.

Over the 10 weeks after challenge, there were significant main effects of treatment, time in relation to challenge and significant interaction between treatment group and time on shell reflectivity (Fig 4). As compared to the control group, shell reflectivity was significantly higher in the N1/88 group in the 2<sup>nd</sup> week p.i. whereas, in the T-infected hens, reflectivity was significantly higher from 1 to 5 weeks p.i.

When shape index was measured from 6 to 10 weeks p.i., compared to control hens, it was significantly lower in the eggs laid by hens of the T-infected and N1/88-infected groups only at 8 weeks p.i (Figure 5)

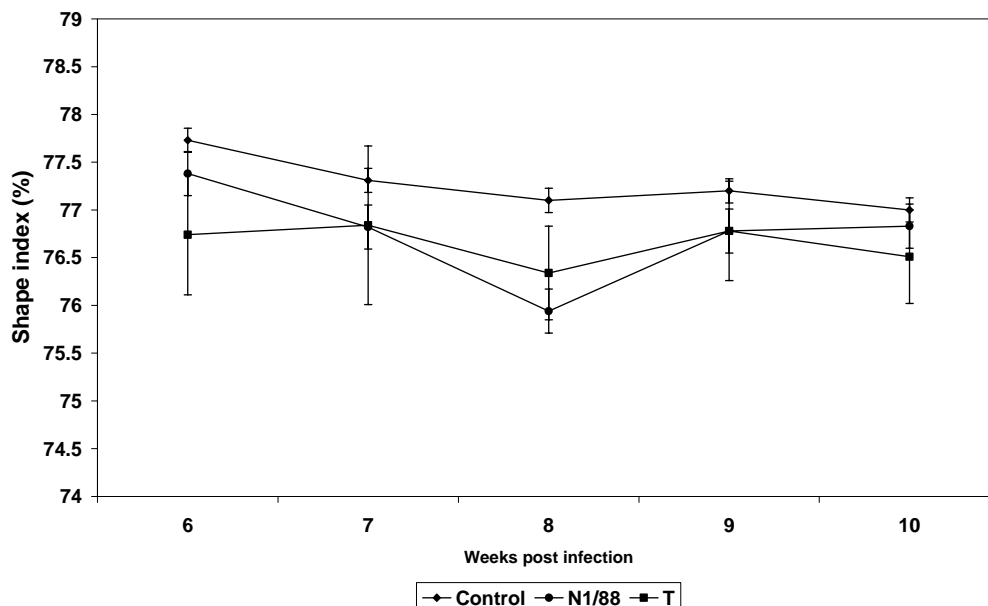


Fig. 5: Egg shape index across 6 to 10 weeks post infection for the three IBV treatment groups. Bar represents mean standard error.

The eggs collected daily from infected hens for 3 weeks p.i., during the follow-up trial, showed the time course of change in shape index. Eggs laid by infected groups were more elongated (lower shape index) as compared to the control. The shape index varied significantly among the groups ( $P= 0.0009$ ), but not over the weeks p.i. and there was no interaction between groups and weeks p.i.

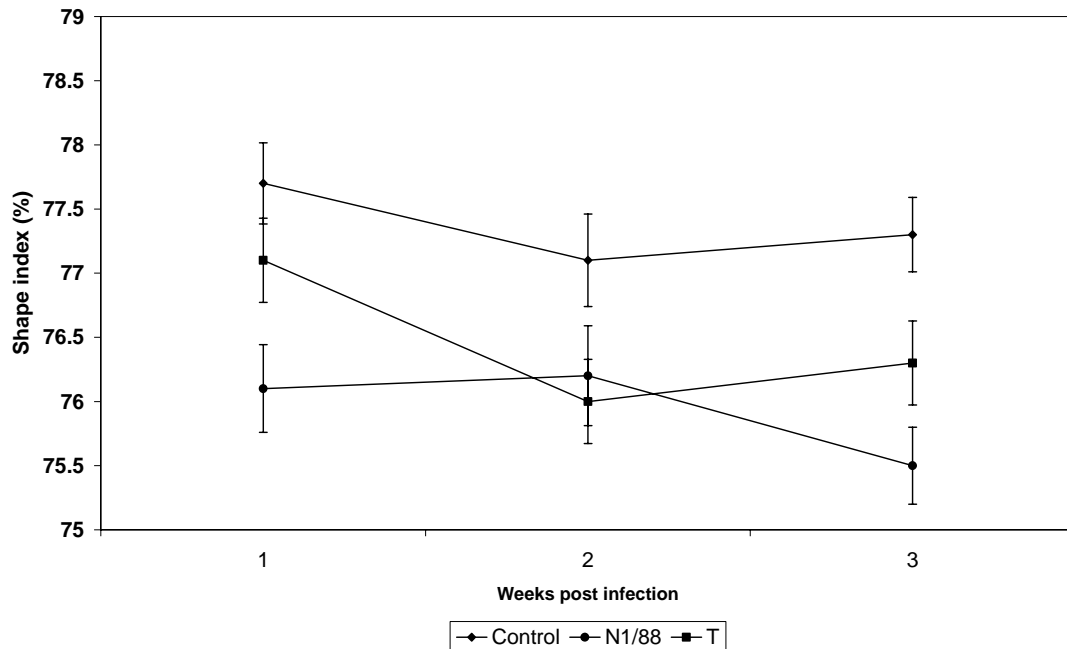


Fig. 6: Egg shape index from 1 to 3 weeks post infection for the three IBV treatment groups during the follow-up trial. Bar represents mean standard error. In N1/88-infected hens, shape index was significantly lower from the 1<sup>st</sup> to 3<sup>rd</sup> weeks p.i., whereas, in T infected hens, egg shape index was significantly lower during the 2<sup>nd</sup> and 3<sup>rd</sup> weeks p.i. (Figure 6). Overall, egg shape index was lower in the T-strain and even lower in N1/88-infected hens as compared to the control.

Over all, there was no significant effect of challenge on ionised calcium, although ionised calcium levels were significantly lower in T and N1/88-infected hens at 5 weeks p.i. (Fig 7). There was no significant interaction between challenge and time in relation to challenge but there was a significant effect of time in relation to challenge.

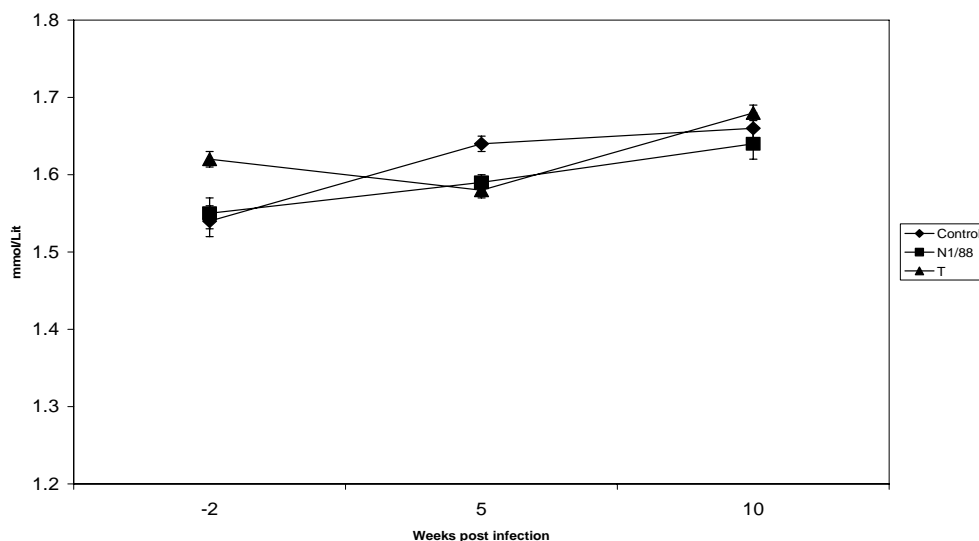


Fig 7: Effects on ionised calcium levels before and after IBV infection over 10 weeks p.i.

## Discussion

There were no significant effects of IBV infection on egg production although mean production over the 10 weeks p.i. was highest in the control group (95.0%) and lower in the T (91.6%) and N1/88 (92.0%) groups. It may be that the relatively small number of birds in each treatment group made detection of production effects of IBV more difficult. IBV clearly has major effects on albumen height and Haugh Units and these parameters in the T-infected group were significantly lower than controls from the first week p.i. until the end of experiment, indicating prolonged effects of IBV. The fluctuation in the N1/88 group could be due to selective removal of hens showing low albumen height for other studies. This finding is consistent with our results regarding ultrastructural changes in the magnum during T and N1/88-infection and thus indicates that both of these Australian strains of IBV have tropism for the upper reproductive tract. Our findings support the view that IBV is associated with thinning of albumen (Sevoian and Levine, 1957). The significantly lower yolk score in T-infected hens from 2 to 4 weeks p.i. could be attributed to the transient decrease in feed intake observed in the T group. However, feed intake did not vary significantly within the treatment groups or over the weeks p.i. Similar findings regarding feed intake were recorded by Roberts (2005). The significant increase in shell reflectivity in the T-infected group was reported earlier by Jolly *et al*, (2005), although it was in vaccinated HyLine Brown and HyLine Grey hens with no effect on vaccinated Isa Brown hens.

Temporary loss of shell colour from N1/88 infection has not been reported previously. Reduction in shell colour during IBV infection with other strains has been reported in the past (Cook and Huggins, 1986). In the present experiment, reflectivity was measured using a sensitive shell reflectivity meter and pale shells were visibly recorded only between 4 and 8 days p.i. However, paler egg shells may not be regarded well by Australian consumers. Other measures of egg shell quality did not vary significantly. The trend towards the hens in the N1/88-infected group having lower egg weight and higher breaking strength may be due to inherent differences among hens that were present before challenge, although there was no significant variation in egg weight within the groups. Deformation did not alter within the group, a finding that is in accordance with the study of Roberts (2005). Increase in shell weight and shell thickness towards end of the experiment could be due to increase in egg weight and such findings were reported earlier in ISA brown hens by Leary (1999). There was no obvious pattern in shell percentage. Thus in the present study, when the hens were challenged with two strains of IBV (T and N1/88), there was a deterioration in albumen quality which was for a short period in N1/88 infected hens but more prolonged in T strain infected hens. This finding explains the uterotropism of Australian strains of IBV for the fully functional oviduct. Egg shell quality was affected only by changes in shell colour. Reduction in plasma calcium has not been reported before and needs further investigation.

A decrease in egg shape index with progressive increase in hen age was reported earlier by Leary (1999). However, in the present study, the hens were in their early lay period. The change in shape index in infected groups observed in the present study could be attributed to alterations in oviduct movements during IBV infection or to the changes in albumen quality. However, very little attention has been paid to establishing the cause of change in shape index during IBV infection and further studies are necessary to clarify this effect of IBV.

## Acknowledgements

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