Yolk Sac Retention and Removal in the North Island Brown Kiwi (*Apteryx mantelli*).

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Abstract

Yolk sac retention or failure to reabsorb yolk is a common problem in several ratite species and has been recognized as a cause of early death in kiwi chicks in the North Island brown kiwi (*Apteryx mantelli*). We have performed 13 successful yolk sac removal operations on 15 kiwi chicks at Kiwi Encounter in Rotorua, and here we report on the predisposing factors and clinical signs of yolk sac retention, along with the surgical procedures and factors affecting chick survival.

Introduction

The egg yolk provides nourishment to the developing avian embryo during incubation, and is internalized into the abdomen of the chick just prior to hatching. Initially all chicks lose weight as they utilize this yolk. When they start to eat their weight will initially plateau and then begin to rise.

Yolk sac retention or failure to reabsorb yolk is a common problem in other ratite species such as ostrich (Jensen et al. 1992, Dzoma and Dorrestein 2001). Mushi et al. (2004) suggested that the presence of a yolk sac in an ostrich chick beyond day 13 should be considered as retained. Retained yolk sac in the North Island brown kiwi (*Apteryx mantelli*) has become a recognized cause of early death in kiwi chicks. Early detection of the retained yolk sac is essential to the survival of the affected chicks. This is dependant on good record keeping of the chicks' weight over the initial stages after hatching and monitoring the graphed results of those weights. Chicks with retained yolks have characteristic growth irregularities that differ to the average growth rate of normal captive reared kiwi chicks.

Along with the abnormal increases in weight, staff in the rearing facility should look for other signs such as inflamed umbilicus, abdominal distension, inability to stand, weight loss despite continuing to eat (Tully and Shane 1996). Recognition of this and close monitoring of chicks whose growth rate differs from the normal leads to much higher success in their survival leading up to, during and after surgery.

Materials and Methods

Birds identified as requiring surgical yolk sac removal were hatched artificially as part of the Bank of New Zealand 'Operation Nest Egg' at Kiwi Encounter, Rainbow Springs, Rotorua. The length of time individual eggs were incubated artificially was dependant on the age eggs were removed from nests in the wild. Kiwi eggs were incubated in either still air incubators (temperature 35.5°C, relative humidity 25-60%) or in a forced draft incubator (temperature 33.5°C, relative humidity 80%), with a total incubation length of 78 days. Relative humidity was adjusted as required to maintain an actual percentage weight loss of 14-18% (range 12-22%) depending on the

characteristics of each egg. Once the chick had externally pipped the shell, the egg was moved into a hatcher (temperature 33.5°C, relative humidity ~60%) until the chick was fully hatched. The chick remained in the hatcher for 48 hours and the temperature was gradually reduced over this period to 28°C. Once the chick was dry and active, it was moved into a large brooder (1.7 x 0.70 m, sleeping area 0.30 x 0.70 m) filled with damp peat moss (Yates®). The chicks were introduced to a standard artificial diet (containing minced ox heart, blended fruit and vegetables, banana, porridge, wheat-germ, Hills® science diet cat biscuits and kiwi vitamin pre-mix) around day 6-7 of age.

Chicks were weighed each morning and the amount of food consumed carefully recorded. The rate of weight decline in the first 7-12 days was carefully monitored as the chicks were becoming established onto the artificial food. Any chicks that experienced a difficult hatch requiring assistance, or that had some external yolk on hatching (manually internalized after hatching), potentially had a greater risk of the incidence of a retained yolk at a later stage, and were monitored even more closely. Chicks remained in the brooder room until they were well established on the artificial diet and had retained their hatch weight before they were placed into large outdoor runs, or released into a predator-free area back in the wild, at around 3 weeks of age.

Candidates for surgery manifested two or more of the clinical symptoms. Chicks with retained yolk sacs will tend to fluctuate between weight gain and weight instead of putting on weight in a linear pattern from day 8 onwards. They will also exhibit a substantial amount of weight loss (10-14 g) at 14-16 days of age. The second common indicator of a potential yolk sac problem is when chicks initially establish themselves on the artificial diet and start to gain weight normally, but then suddenly stop eating about one week later.

Other predisposing factors included: a prolonged or difficult hatch, an assisted hatch, some external yolk present at hatching, an external or 'protuberant' umbilicus (see Tully and Shane 1996). All these factors are considered when examining the chick and its' associated rate of growth and its predisposition to a retained yolk sac at a later stage.

Surgical and anaesthetic technique

All birds were anaesthetized using Isoflurane (VCA I.S.O.) delivered by mask from a Stephens Anesthetic Machine. It was necessary to maintain anaesthesia at higher than normally expected concentrations of anesthetic and relatively high flow rates (1-1.5 litres per minute). The head was maintained higher than the thorax to minimise reflux of proventricular fluid and the neck held straight to avoid obstruction of the airflow. Moderate elevation of the thorax also reduced the pressure which a large yolk sac may place on the abdominal and thoracic air sacs while the bird is lying on its back.

In early surgeries we intubated the chicks but found the endotracheal tubes blocked too quickly in small chicks and masks provided satisfactory anaesthesia with fewer complications. In the earlier surgeries we also gave a bolus of IV fluids (Lactated Ringers Solution) at 10ml/kg over a five to seven minute period at the completion of the surgery but subsequently we found oral fluids upon recovery were adequate.

The chicks were positioned in dorsal recumbency on a heat pad, with the legs pulled caudally. Abdominal feathers were plucked from the ventral region of the abdomen and this provided ample access to the area around the umbilicus. The area was prepared aseptically for surgery in the usual manner using Povidine Iodine and methylated spirits.

A skin incision was carefully made circumferentially around the umbilicus and extended laterally at the 3 and 9 o'clock positions to an appropriate length to allow for removal of the yolk sac without

rupturing it. An incision was then made through the remaining layers of body wall until the abdominal cavity was entered, following the lines of the previously made skin incision. Extreme care was taken not to rupture the yolk sac. Once the incision had been made the yolk sac was generally exteriorized by applying traction on the umbilicus with forceps while manipulating it from within the abdomen with sterile cotton buds soaked in sterile saline. Depending on the size of the yolk sac, internal bilateral pressure with two cotton buds may have been necessary to extract the yolk through the incision.

Once the yolk sac had been externalized, a hemostat was clamped around the yolk stalk as close as possible to the yolk sac. The vitelline blood vessels and yolk stalk between the duodenum and the yolk sac were ligated as close as possible to the intestine using 4/0 Catgut. The vessels and stalk were then cut between the ligature and hemostat.

The yolk sac was removed from the abdomen along with the excised abdominal wall, peritoneum and any other tissue linking the yolk sac to the external umbilicus. This allowed complete removal of the yolk sac, its connection to the umbilicus and the umbilicus itself. On some occasions, the yolk sac ruptured during surgery. This necessitated peritoneal lavage with warm sterile saline after the removal of the ruptured yolk sac and umbilicus.

Closure of the abdomen was two layered. The abdominal wall and peritoneum was closed using a simple continuous suture pattern using 3/0 Polygycolic acid braided suture (Trade name: Serofit). The skin was closed separately in a simple interrupted fashion using 3/0 nylon (Trade name: Supramid).

Recovery from anaesthesia was usually quite rapid and relatively smooth. In two instances where the chick was slow to recover from anaesthesia, pure oxygen was provided by mask for a few minutes. The bird was placed in a warm, dark padded box in sternal recumbency in a quiet environment. Within 5-10 minutes kiwi were capable of holding up their head and extending the neck vertically. The bird was then left for 1-2 hours to allow full recovery and co-ordination.

Where possible the yolk was submitted for bacterial culture. Amoxycillin/Clavulanic Acid (Trade name: Clavulox) were administered at 50 mg/kg twice daily post-operatively for 5-7 days, and Baytril was administered at 15 mg/kg once daily. If the yolk sac had ruptured during surgery or if there was evidence of inflammation or infection in the visceral cavity, the duration of antibiotics was extended to 10 days.

Oral administration of fluids was routinely carried out at 3-4 hourly intervals over the course of the day (3-5 mls fluid). Post-operative tube feeding was sometimes required using Hills a/d™ for those birds not probing and showing an interest in food day post-surgery. The a/d™ was given via crop tube twice daily as a suspension (three times per day if necessary). Once again we gave 3-5 mls depending on the size of the chick. The chicks always had access to the normal artificial diet during this time and as soon as they started to gain weight or showed an interest in the artificial diet, crop feeding ceased.

When the chick returned from surgery, it was confined to the small sleeping area of the brooder for 12-24 hours, with an additional heat source provided. The peatmoss was removed from the brooder, and the chick placed on non-slip matting until the sutures were removed after 7-10 days. Chick care continued as normal from this point on and recovery was usually rapid.

Statistical analysis

We compared the hatch weight and growth rates of chicks that had yolk sac removal operations to that of a random sample of chicks (experimental control) that were raised without complications at

Kiwi Encounter. The chicks in the random sample were chosen from the same seasons that yolk sac removals were performed, so that incubation and chick rearing protocols were the same for the two groups. For the two groups, we compared hatch weight, the weight and age in days at which chicks were placed in outside runs, and the weight and age in days chicks were released into the wild using one-way analysis of variance (ANOVA) in Genstat 8.1 (VSN Software Inc). Three of the chicks with yolk sac operations and 14 of the control chicks were not held on site after being removed from the brooder. Instead, they were placed in a fenced predator-proof enclosure until they reached the weight required for release into the wild. These chicks were excluded from the analyses comparing the weight and age of release of yolk sac and control birds.

Results

Of a total of 406 kiwi chicks raised as part of Operation Nest Egg at Kiwi Encounter between 1995 and 2006, 17 (4.2 %) have presented with retained or infected yolk sacs. These seventeen chicks were diagnosed with retained yolk sacs between the 1999/2000 and 2005/2006 kiwi breeding seasons, with 15 undergoing surgery. Of these 13 survived (86%) and were subsequently released back into the wild.

Chick D7 (Mahi) was the first chick to die of an undiagnosed retained yolk sac at 31 days of age in 1999. Mahi hatched with a small marble sized external yolk, weight gain was normal and the chick regained hatch weight at 23 days of age. Two days prior to his death, Mahi's appetite was reduced and he lost 18.5 g overnight. The following day he stopped eating completely and lost a further 27 g, before dying in the brooder the next day.

Gross findings of the autopsy indicated a yolk sac remnant mass (measuring 25 x 28 x 20 mm) adherent to the intestine by a thin stalk, containing brown-black friable material. Histopathology found the mass attached to the intestine to be composed of a large quantity of haemorrhagic necrotic material surrounded by a thick connective tissue capsule containing areas of congestion and haemorrhage. Around the periphery of this necrotic material and within the capsular wall were a large number of mononuclear inflammatory cells, many giant cells, some heterophils and many necrotic nuclei. There were many colonies of bacteria within the inflammatory zone and within the necrotic tissue. The chick was had good fat reserves and there were no significant findings in the proventriculus, gizzard, pancreas, liver or kidney. Microbiological analyses indicated moderate growth of heavily mixed organisms in the abdominal cavity, heavy growth of heavily mixed organisms including some anaerobes, and a small number of heavily mixed organisms present in the liver. The diagnosis was septicaemia due to retained and infected yolk sac and acute haemorrhagic myositis (Twentyman and Alley 1999).

The second chick to die of an undiagnosed retained yolk sac at 22 days of age was ED5 (Rocket). This egg was artificially incubated for 17 days, the chick had a normal hatch and a normal growth rate until it suddenly lost weight and then died.

The age that kiwi eggs were brought in from the wild ranged from 0-74 days and the majority of eggs were incubated artificially without incident. Both the number of viable eggs delivered to Kiwi Encounter and their subsequent hatching success was variable in the first 5 years (1995/96 – 1998/99, number of viable eggs: 1-18; average hatching success 67.9 %, range: 33-100 %). From 1999/00 – 2005/06 the number of viable eggs per year has increased from 17 to 120, and hatching success is consistently high (90+ %).

Overall there has been no increase in the incidence of retained yolk sacs in kiwi chicks but the early detection and subsequent surgical intervention has resulted in high chick survivorship. At surgery the chicks ranged in age from 9-25 days (average ± standard error = 14.9 ± 1.24 g, n = 15). The length of surgery times ranged from 8 - 40 minutes, and recovery from anaesthesia was usually quite rapid and relatively smooth (3-12 minutes until the chick was active and crouching). The

average weight and standard error of the chicks at surgery was 276 ± 2.89 g (range: 223 -319, n = 15). The average size (± standard error) of the yolk sac removed was 38.9 ± 6.6 g (range: 10 – 85, n = 15).

Of the two chicks that died after a retained yolk sac was identified, the DE5 (Hiriwa) egg was incubated for 9 days and hatched unassisted. Post hatch weight loss was normal, but the chick became weak and lethargic at 11 days old. The condition of the chick deteriorated further and surgery occurred the following day, where a 24 g yolk sac removed but the chick died 8 hours after surgery. The second egg BP.Di1 (Sam) was incubated for 61 days and the chick hatched normally, although it was noted to have an unusually large yolk filled abdomen on hatching. At 10 days of age, a potential retained yolk sac was determined as the abdomen was still grossly distended. Surgery occurred at 15 days of age and the chick had an 80 g yolk sac removed that ruptured on removal. The chick did not respond to post-surgery care and subsequently developed respiratory complications and was euthanasied 5 days post surgery. Both of those birds were recumbent on presentation for surgery, highlighting the need for early detection to increase the chances of survival.

In four instances, the yolk sac ruptured during surgery. Where possible yolk sacs were sent for microbial culture (n = 7), but there were only two instances where the yolk sac was actually infected. In retained yolk sacs the yolk and other areas of yolk sac membranes appeared normal and there was no microbial growth after 48 hours and no anaerobes isolated.
There was no significant difference in the proportion of viable eggs that were cracked on arrival at Kiwi Encounter between normal eggs and retained yolk sac eggs, with 23 % of normal eggs and 17 % of yolk sac removal eggs having some degree of cracking ($\chi^2 = 0.77$, df = 2, P > 0.05).

Table 1: The degree of cracking in the shells of normal eggs and those of chicks requiring yolk sac removal arriving at Kiwi Encounter between 1995 and 2006, and the survival rates of each category.

Egg damage category	Normal Eggs	Number of chicks released	Yolk sac Eggs	Number of chicks released
None	270	205	14	11
Small	38	25	1	1
Moderate	31	22		
Severe	13	12	2	2
Total	352	264	17	14

The hatch weight of yolk sac operation chicks and control birds were not significantly different, at approximately 339 g for both groups. Yolk sac removal chicks went into the outside runs at the similar weights to control birds, although they took significantly longer to reach this weight ($F_{1,86}$ = 18.95, P = 0.001; Table 2, Figure 1). Yolk sac and control chicks remained in the outside runs at Kiwi Encounter for the same length of time, although yolk sac chicks were significantly lighter than control birds when released ($F_{1,67}$ = 4.62, P = 0.035; Table 2).

Table 2: Hatch weight, weight and age in days to leaving brooder box, and weight and age in days at release to the wild for 17 chicks with yolk sac operations and 75 chicks with normal development.

Parameter	Yolk Sac average (± s.e)	Sample size	Control average (± s.e.)	Sample size	P
Hatch weight	339.0 ± 7.7	17	338.3 ± 3.2	75	0.921
Age outside	39.2 ± 2.4	17	30.1 ± 0.9	75	<0.001
Weight outside	439.8 ± 9.3	17	427.3 ± 5.2	75	0.291
Age release	131.8 ± 13.7	8	141.7 ± 4.1	61	0.316
Weight release	1068.1 ± 54.6	8	1144.4 ± 14.7	61	0.035

Discussion

The relative size of a yolk sac is often a good determinant of whether or not to perform surgery. The yolk takes up the majority of abdominal space in young chicks and causes typical abdominal distension. In kiwi chicks that have died at point of hatch, the internalized yolk sac can be around 120 g (S. Bassett, unpublished data). Prinzinger and Dietz (2002) determined that 48 % of the kiwi eggs initial yolk mass can be found as spare yolk in the young chick. By one week of age the normal yolk sac is practically depleted and does not cause a rounded protrusion of the abdomen. The weight record of the chick also may influence the decision for surgery. In general ratite chicks will normally begin to consume food between 3-7 days of life (Deeming 1995). While wild kiwi chicks begin to forage just outside the burrow at 4-6 days of age, chicks reared in captivity are not introduced to food until 6-7 days of age, and a corresponding weight increase begins at this time. If the chick is suffering from infection of the yolk sac, the chick will lose weight in the first 7-10 days after hatching as normal chicks would. However, instead of putting on weight in a linear pattern from day 8 onwards, these chicks will tend to fluctuate between weight gain and weight loss. At about 14-16 days the chick may lose a substantial amount of weight (10-14 g). At this stage some chicks still remain relatively bright, but some young chicks with undiagnosed yolk sac problems can die within 48 hours without surgery. These chicks generally have poor body condition and have had difficulties establishing onto the artificial diet. Another common indicator of a potential yolk sac problem is when chicks establish themselves on the artificial diet and start to gain weight normally, but then suddenly stop eating about one week later (when the chicks is around 2-3 weeks old). These chicks may then loose a small amount of weight (3-5 g), but the sudden loss of appetite and cessation of eating and general lethargy are an indication of potential yolk sac problems. Palpation of the abdomen at this time will sometimes reveal abnormal distention.

Once symptoms are apparent the only successful solution is yolk sac removal. The critical factor in ensuring a successful post-operative outcome is the timing of the surgery. Yolk sac retention cases should have surgery as soon as possible following the day of the dramatic weight loss (or when symptoms become apparent). At this stage the chicks are still relatively bright and can withstand the anaesthesia. However, if the surgery is delayed until the birds are obviously weak and depressed the prognosis for a successful surgical outcome is greatly diminished. The prognosis for survival following surgery is estimated to be approximately 80% for emus and 50% for ostriches (Tully 1996), and is currently 86% for kiwi post surgery.

Retained yolk sacs were originally thought to be a result of bacterial infection, with almost all retained yolk sacs classified as infected in one study in ostrich (Deeming 1995, 2005). This is in contrast to Dzoma & Dorrestein's (2001) findings, who concluded retained yolk sacs were common in ostrich, and levels of bacterial infection were 22 % for retained yolk sac chicks and 26 % of normal chicks. Similarly, we found infections in only two of our yolk sac removal chicks. We have had a high proportion of cracked eggs successfully hatch with the chicks having no subsequent yolk sac problems, and the proportion of cracked eggs has been similar for normal and yolk sac removal chicks at Kiwi Encounter. Therefore it appears that infection by environmental bacteria is not a primary cause of retained yolk sacs in kiwi.

Two retained yolk sacs were undiagnosed at Kiwi Encounter resulting in death, while one chick was miss diagnosed as having a retained yolk sac when it suffered from a compacted proventriculus. In this case the use of X-rays and Barium would have resulted in the correct diagnosis of this condition.

Palpation of the abdomen in a chick with a retained yolk sac normally revealed a firm distended abdomen with no identifiable organ structure (Jensen 1992). Ultrasound examination may be useful to identify the spherical, fluid-filled structure but some skill is needed in this discipline. X-rays may also be helpful, but the smaller retained yolk sacs are more difficult to see radiographically. The use of Barium to highlight the gut helped in some situations to aid in identifying the yolk sac. However, careful monitoring of feeding and weight gain patterns, along with the other behavioural indicators are the most effective ways to detect potential yolk sac retention.

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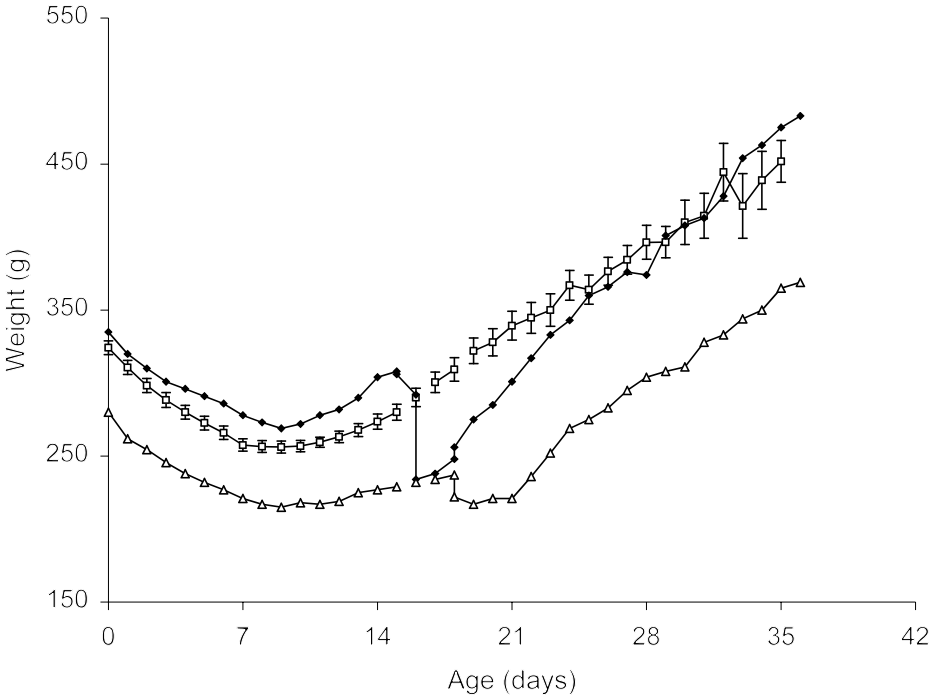
Acknowledgments

The authors would like to thank the staff of Kiwi Encounter, Rotorua, and the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, as well as all the Department of Conservation staff and other field workers who have delivered eggs to Kiwi Encounter as part of Operation Nest Egg.



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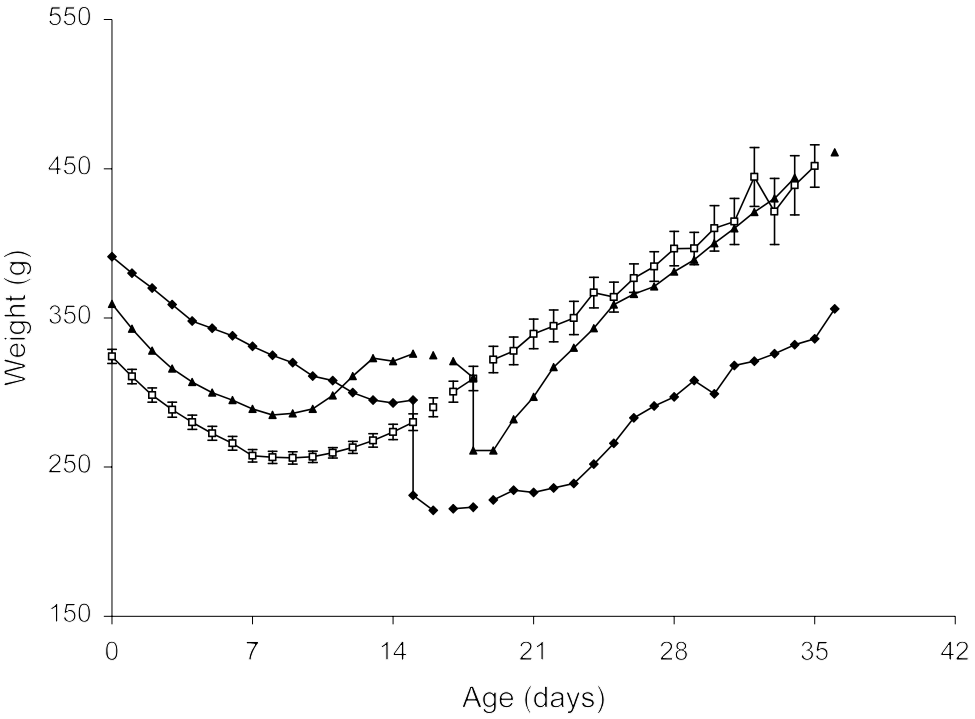

Figure1: The average rate of growth (± standard error) in normal kiwi chicks in captivity (open squares), and chicks that have undergone yolk sac surgery,: a) TK.Ai4 (open triangles) and W.TN9 (filled triangles, and b) M.Ru2 (filled triangle) and T.ST3 (filled diamonds).