

# **MANGANESE DEFICIENCY ASSOCIATED LEG DEFORMITIES IN RED-NECKED OSTRICHES (*Struthio camelus camelus*)**

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## **SUMMARY**

Red-necked ostriches (*Struthio camelus camelus*) are kept in Al Wabra Wildlife Preservation (AWWP) since 1998. The first chicks hatched in 2007. Two out of three chicks in 2007 and one out of four chicks in 2008 showed signs of leg deformities, especially slipped tendons. Manganese deficiency was suspected. A blood analysis was performed and manganese, zinc, vitamin E, iron, copper, selenium and calcium were measured. The three chicks were euthanized and examined. Histopathological findings revealed poor bone mineralisation and signs of a metabolic problem possibly associated with manganese deficiency. Factors which are known to cause leg deformities are manganese, calcium, copper or zinc deficiency in chicks or high selenium levels in breeding adults could be a cause of these leg deformities. In this study low manganese, calcium and copper and high selenium levels were detected and are most likely involved in these cases.

## **INTRODUCTION**

Red-necked ostriches (*Struthio camelus camelus*) are threatened by extinction and are registered in the Red Data Book. Populations under threat live in north-west to central Africa (COOPER, 2009).

Leg deformities are a major problem in ratites but they are also known in other bird species and in poultry (RITCHIE et al., 1994, JULIAN, 1984). MORE (1996) reported leg deformities as the main cause of death in farmed ostrich chicks accounting for 36% of all chick deaths. Different types of leg deformities are summarized under the term perosis, which is characterized by shortening of the leg bones, enlargement of the hock, twisting or bending of the distal end of the tibia and proximal end of the metatarsus, and slipping of the gastrocnemius tendon from its condyles (RIDDELL, 1981). Different factors including deficiencies in manganese, zinc, choline, biotin, folic acid, niacin and pyridoxine have been identified as cause of leg deformities. These deficiencies are known from poultry and are likely to be involved in leg deformities of ratites (RITCHIE et al., 1994). In addition other factors like genetics, nutrition and excessive growth rates due to overfeeding with high-protein diet and inadequate exercise and substrate are also implicated (REECE and BUTLER, 1984, BRUNING and DOLENSENK, 1986).

Red-necked ostriches have been kept at Al Wabra Wildlife Preservation (AWWP) since 1998. Three chicks hatched for the first time in early summer 2007. In 2008 four chicks hatched. They were kept in an indoor enclosure for around ten days, later in an outdoor enclosure until they were moved to a large outdoor enclosure at the age of around fifty days. Before spring 2009 the chicks received a wide variety of foods including fruits and vegetables but also Mazuri ostrich starter pellets (*Ostrich starter* (code 852188). The adult birds received alfalfa, fruits and pellets from Mazuri (*Ostrich grower/maintenance* (code 852187) by Mazuri Zoo Foods, *aleckwa Tiernahrung, Am Sandzug 2, D-67122 Altrip, Germany*) alternating with pellets from the local market (*Ostrich pellets* (16/18), *Aljabor grains and feed co., C ring road doha (Zip : 565), Doha, Qatar*).

This report describes the clinical and pathological findings of three ostrich chicks suffering from leg deformity as well as blood chemistry evaluations of six chicks and three adult ostriches.

## **METHODS**

In three chicks (two from 2007, one from 2008) leg deformities were reported between three to seven days after hatching. They were treated because of the leg deformity and euthanized after unsuccessful treatment at 1.5 to 3 months of age. Pathological examination was performed in the AWWP laboratory and histopathological examination of formalin fixed tissues was done at the Dutch Research Institute for Birds and Exotic Animals (NOIVBD). A blood evaluation of the whole group was performed, based on the hypothesis of slipped tendon and the suspicion of manganese deficiency. Vitamin E, iron, copper, manganese, zinc, selenium and calcium were measured by Vet Med Labor (Vet Med Labor, Division of IDEXX Laboratories, Mörikestrasse 28/3, D-71636 Ludwigsburg,

Germany). Methods of measuring were HPLC (high performance liquid chromatography) for vitamin E and ICP-AES (inductively coupled plasma atomic emission spectrometry) for zinc, manganese, selenium, and copper. Calcium measurements were performed by Spotchem SP4410 (Eppendorf-Netheier\_Hinz GmbH, Hamburg, GERMANY) in the AWWP laboratory.

Reference levels for parameters were obtained directly or calculated (conversion factors taken from *The Clinician's Ultimate Reference GlobalRPh.com*) from different sources (calcium, iron: MONIELLO et al., (2006), selenium: MUSHI et al. (1998b), vitamin E: ISIS (2002), copper: MUSHI et al. (1998a), manganese: BEZUIDENHOUT et al. (1994), zinc: Vet Med Labor). Manganese and calcium blood values were compared between birds with and without leg deformities by t-test (PASW 18.0; SPSS Inc., Chicago, IL).

## CASE REPORT

**Case 1:** Chick 7209 (male) was hatched on the 17<sup>th</sup> of June in 2007. It was reported with a splaying right leg four days after hatching but was not treated at this time. At the age of almost three months it suffered from a hip dislocation on the left leg. The dislocation was replaced and the bird was treated with homeopathics and vitamins and was kept in the hospital for four days. A slipped tendon leading to a valgus deformity of the left tarsometatarsus was observed some days later. Since shoe application and medical treatment with anti-inflammatory drugs, painkillers, vitamins and homeopathics did not lead to success the chick was euthanized at the age of three months. In histopathology, a metabolic bone problem resulting in abnormal bone growth was suspected. The blood analysis at two months of age from this chick revealed a very low manganese level, low zinc, copper and calcium levels, as well as elevated selenium and vitamin E levels and a normal iron level (table 1).

**Case 2:** Chick 7214 (male) was hatched on the 18<sup>th</sup> of June in 2007. It was treated in the hospital for 6 weeks directly after hatching for weakness and inadequate weight gain. A rolled toe syndrome involving the 4<sup>th</sup> (outer) toe of the left leg was diagnosed three days after hatching and subsequently treated successfully using bandaging techniques described in literature (RITCHIE et al., 1994). As there was no improvement in weight gain despite prolonged assisted feeding, the chick was euthanized at the age of 1.5 months. In gross pathology an asymmetric rib-cage, deformed ribs and a sternum malformation were observed. Histopathology revealed poor mineralization of the sternum and other bone parts. The muscles (skeletal and myocardial) were atrophic. The organs (spleen, kidneys and lungs) showed still juvenile aspects. A severe manganese deficiency was detected in the blood analysis at two months of age as well as severe calcium -, a moderate copper - and a slight iron deficiency. The zinc level was normal. Selenium and vitamin E levels were elevated (table 1).

**Case 3:** Chick 7791 (male) hatched on the 13<sup>th</sup> of May in 2008. A slipped tendon possibly leading to the observed severe valgus deformity of the left tarsometatarsus was hypothesized one week after hatching. As treatment, the left leg was fixed using hobbles to the right leg to stop splaying (STIÉVENART, 2008). Two months later, the chick was again reported with the same problem accompanied by an inflamed painful stifle joint on the same leg. It was treated with a bandage and hobbles application and antibiotics, anti-inflammatory drugs, painkiller and different vitamins. Ten days after the cast removal the chick was found with the right tibia fractured and was euthanized at the age of 2.5 months. At histopathology a hypothyroidism and a hyperparathyroidism was diagnosed. The latter is suggestive for a metabolic bone problem with low calcium blood levels. Manganese deficiency was suggested as a possible cause for the slipped tendon. The blood of this bird was examined twice, once at the age of 8 days and later at the age of 16 days. Both times, it was low in manganese, very low in calcium, low in copper and low in iron. Zinc levels were normal. Selenium levels were elevated. Vitamin E was only measured the second time and was elevated (table 1).

Blood evaluations were performed on most of the ostriches (3 adults and 6 chicks/juveniles). All except one adult was deficient in manganese and the birds with leg deformities were even lower in Mn than the others. The difference tended towards significance ( $p=0.081$ ). The results suggest that the groups did differ in their blood values but that sample size was too small for the differences to be statistically significant. However, it should be noted that three birds from the healthy group had blood levels in the range of the leg-deformed group, which might suggest that other factors, such as individual predisposition, played a role in the development of clinical signs.

Calcium levels were low in most of the ostriches (seven out of nine) and even lower in the ones with leg deformities than in the healthy birds (table 1). Again, this difference only tended towards significance ( $p=0.065$ ). Only one individual without leg deformities had a calcium blood level in the range of the leg deformed group.

All birds had elevated selenium blood levels, and all except one elevated copper levels.



Fig 1: Ostrich chick with a laterally rotated leg (photograph by AWWP)

Number	Manganese μmol/l	Zinc μg/l	Calcium mmol/l	Selenium μg/l	Copper μg/dl	Vitamin E mg/l	Iron μmol/l
Reference	0.3672	500-2000	2.79	31.5	68	0.17	9.22
AWWP mean	0.13° (± 0.09)	774 (± 220)	2.3° (± 0.35)	182* (± 45.17)	18.67° (± 6.06)	6.56* (± 6.14)	18.9* (± 14.17)
602	0.07°	770	2.46°	230*	15°	0.807*	11.28
615	0.45	1100	3.03	209*	18°	1.53*	44.77*
616	0.198°	1190	2.86	247*	21°	1.57*	45.13*
<b>7209 (y)</b>	<b>0.06°</b>	<b>346°</b>	<b>2.11°</b>	<b>105*</b>	<b>35°</b>	<b>11.7*</b>	<b>10.2</b>
7213 (y)	0.22°	1110	2.64	312*	21°	0.487°	44.77*
7213 (y)	0.02°	829	2.51°	151*	8°	3°	12.71*
<b>7214 (y)</b>	<b>0.083°</b>	<b>623</b>	<b>1.83°</b>	<b>154*</b>	<b>34°</b>	<b>9°</b>	<b>6.44°</b>
7896 (y)	0.24°	316°	1.64°	117*	16°	0.8*	4.2°
7790 (y)	0.05°	726	2.42°	177*	12°	4.01*	25.96*
7790 (y)	0.1°	768	—	183*	14°	26.2*	15.3*
<b>7791 (y)</b>	<b>0.056°</b>	<b>966</b>	<b>1.95°</b>	<b>131*</b>	<b>15°</b>	—	<b>1.9°</b>
<b>7791 (y)</b>	<b>0.068°</b>	<b>544</b>	<b>2.05°</b>	<b>168*</b>	<b>15°</b>	<b>13.1*</b>	<b>4.17°</b>

Tab. 1: Blood results of the group. The chicks are indicated with (y), \* stands for "higher than reference", ° means "lower than reference". The bold numbers indicate the birds mentioned in the report. Some birds have been tested twice at different dates.

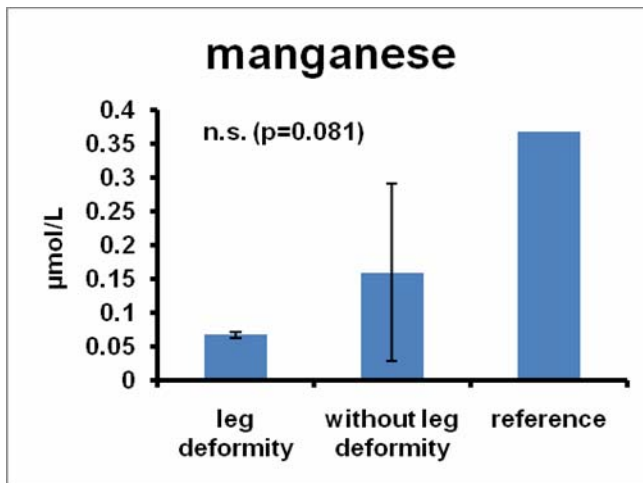


Fig 2: Manganese measurements from the leg deformity group and the healthy group compared with the reference range are shown. All birds have a manganese deficiency, and there is a difference tending towards significance ( $p=0.081$ ) between leg deformity birds and healthy birds.

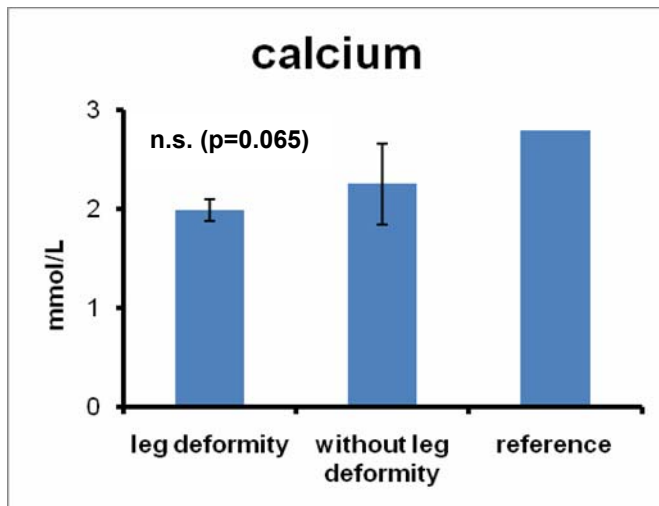


Fig 3: Calcium measurements from the leg deformity group and the healthy group compared with the reference range are shown. All birds have a calcium deficiency and there is a difference tending towards significance ( $p=0.065$ ) between leg deformity birds and healthy birds.

## DISCUSSION

Chick 1 and 3 showed slipped tendons. The limb deformity in case 2 is more likely a rolled toe than a slipped tendon. Due to the fact that some of the chicks hatched in 2009 also showed rolled toes but no slipped tendons it can be suggested that poor ground material followed by inadequate footing was the cause of this problem as described by RITCHIE et al. (1994).

Leg deformities were detected between three and seven days after hatching and were not present at the time of hatching as described by STIÉVENART (2008), even though MUSHI et al. (1999) found the highest prevalence of limb deformities first at the age of two to three weeks. In the study by LETERRIER et al. (1992) valgus angulation in chicken appeared progressively between two and seven weeks of age.

Some deficiencies in vitamins and minerals are reported to cause leg deformities and slipped tendons especially manganese, zinc, choline, biotin, folic acid, niacin, pyridoxine and calcium (RITCHIE et al., 1994, AGANGA et al., 2003, KISTNER et al., 2002). Manganese levels were low in the whole group but even lower in the affected birds. This finding agrees with cases of leg deformity and especially slipped tendon described by different authors (RITCHIE et al., 1994, KISTNER et al., 2002, AGANGA et al., 2003, KLASING, 1998). Based on the manganese deficiency and the compatible histopathology lesions, the diagnosis of slipped tendon was made for chicks 1 and 3. The histopathology of case 1 revealed a

metabolic problem resulting in abnormal bone growth what could be caused by manganese deficiency too (DORRESTEIN, 2010, pers. comm.). In the histopathology of case 2 a poor mineralization was described which could indicate calcium-/phosphorus-/vitamin D3- problems or also be a sign of manganese deficiency (DORRESTEIN, 2010, pers. comm.). In case 3 a hyperparathyroidism as a reaction on a calcium-/phosphorus-/vitamin D3- problem was found in the histopathology. These results stand in contrast with those reported by MUSHI et al. (1999) who found higher manganese levels in chicks with leg deformities than in healthy chicks. This discrepancy emphasises that leg deformities can be caused by a variety of factors, and that cases need to be carefully evaluated in each facility.

In 2008 the feeding management of the ostrich chicks was changed due to the suspicion of slipped tendon caused by manganese deficiency. The daily requirement of manganese for chicks, juveniles and breeding adults is 150mg, 120mg and 200mg, respectively (KISTNER et al., 2002). Before the change in spring 2009, chicks received a wide variety of foods including fruits and vegetables but also pellets. This composition (as they often ate more fruits than pellets) is high in energy and low in minerals. Since spring 2009, the chicks only received pellets. A diet with adequate manganese content, 151mg/kg and 141mg/kg in Ostrich starter and grower/maintenance, respectively, was chosen (*Ostrich starter (code 852188) and Ostrich grower/maintenance (code 852187) by Mazuri Zoo Foods, aleckwa Tiernahrung, Am Sandzug 2, D-67122 Altrip, Germany*). These pellets are mixed 1:1 with locally bought pellets which are much cheaper than imported Mazuri pellets. The adult birds receive alfalfa, fruits and pellets from Mazuri alternating with pellets from the local market. The composition of Mazuri pellets (*Ostrich grower/maintenance (code 852187) by Mazuri Zoo Foods, aleckwa Tiernahrung, Am Sandzug 2, D-67122 Altrip, Germany*) is correctly balanced for energy and minerals, but this might not be the case for locally bought pellets (*Ostrich pellets (16/18), Aljabor grains and feed co., c ring road doha (Zip : 565), Doha, Qatar*) as the ingredients of them are not known and can vary. The change of chick feeding seems to be successful since none of the chicks hatched in 2009 did show any signs of leg deformities except rolled toe that is suggested to be caused by inadequate footing due to poor ground material (RITCHIE et al., 1994).

Copper levels of all tested ostriches were lower than the reference range but there appears to be a large variation in reference ranges for serum copper values in ratites, with published ranges ranging from 18 µg/dl to 1200 µg/dl (CHANG REISSIG et al., 2002, BEZUIDENHOUT et al., 1994, AHMED et al., 2006). This is understandable given that serum copper values tend to be highly correlated with dietary copper, which again are influenced by soil copper levels, especially in grazing adults (KLASING, 1998). Four birds (among them one affected chick 7791) were deficient compared with the lowest reference range found in literature (table 1). Copper deficiency is associated with a failure in the cross-linking of collagen in bone, particularly in the growth plate resulting in bone and joint abnormalities such as enlargement of the hooks, a condition known as perosis (KLASING, 1998). It is known that the soil in deserts, e.g. in Qatar, is low in copper and therefore some animals in AWWP (e.g. gazelles) get mineral licks with additional copper (HAMMER, 2010 pers comm.). Whether copper deficiency contributes to the observed deformities in the ostrich chicks is unknown.

Also low calcium levels are known to cause limb deformities but more affecting the bone structures (AGANGA et al., 2003, JENSEN et al., 1992). The average level of calcium were low in the birds of this study. The reason for such low calcium level could be consequent to the presence of more calcium measurements of chicks, which seem to have lower calcium levels in general than adults. (one-day-old chicks reference: 2.15- 2.38 mmol/L, SIMPRAGA et al., 2004). There was a difference between affected ( $2.0 \pm 0.1$  mmol/l) and healthy animals ( $2.25 \pm 0.4$  mmol/l) which tends towards significance ( $p=0.065$ ) and only one bird from the healthy group had a calcium level in the range of the leg deformity group. Lower calcium levels in birds with leg deformities than in healthy birds were found in a report from BEZUIDENHOUT et al. (1994) too. Based on this result it can be suggested that calcium deficiency may be an additional factor in this leg deformity problem. One possible reason for a low calcium intake could be an ad libitum offer of fruits (typically low in calcium) so that the animals did not have to ingest pelleted feeds and alfalfa (both with adequate calcium contents).

High selenium levels in breeding ostriches are problematic because of the embryonic toxicity what caused malformations due to disturbances in the normal formation of bones and cartilage (KLASING, 1998). Since in the entire group including the breeding adults very high levels of selenium have been registered a possible contribution to leg deformity can't be excluded even though reference values were taken from ostriches with myopathy because normal ostrich reference values are lacking (MUSHI et al., 1998b). The cause of these high selenium levels remains unknown and they were not taken into account when the new feeding management was established.

Even though vitamin E levels in the three ostrich chicks with leg deformities were around a hundred times higher than the reference value they did not cause clinical symptoms and are irrelevant for this study. Zinc and iron levels were approximately in the reference range and the concerning birds did not show any clinical signs concerning these minerals.

The leg deformities of the ostriches seem to be a multifactorial problem. Manganese deficiency can be suggested to be a potential cause but also the elevated selenium levels as well as copper and calcium deficiencies should be taken under consideration. A balanced diet in adults and hatched ostriches can play an important role as a prevention tool. It is expected that leg deformities disappear or can be reduced with this feeding change by reducing fruit consumption and a good choice of pellets. Further data about the effects following one year of changed diet will be collected through the blood analysis which will be performed in February 2010.

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