

# *Effect of Na-Perborate on litter in reducing or healing of foot pad lesions in broiler chickens*

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## **Abstract**

**Foot pad lesions are a growing problem in the broiler industry. Footpads are being evaluated in slaughterhouses and serve as a welfare indicator. In this research the effect of Na-Perborate on litter in reducing ulceration of foot pads and the effect on healing of Foot Pad Dermatitis (FPD) in broiler chickens was investigated. Results were used to evaluate the hypothesis that Na-perborate, a H<sub>2</sub>O<sub>2</sub>-generating substance, inhibits anaerobic bacteria that could possibly play a role in the etiology of FPD. Two experiments were carried out on a broiler farm in Giessenburg, the Netherlands. In the first experiment 203 chicks were housed in 4 testpens whereof the litter of 2 testpens was treated with Na-perborate supplemented with sand and the litter of the other two, control, testpens was not treated. A significant reduction in the development of ulceration was found in treated groups. In the second experiment the same experimental design was used with 200 chicks except for the control testpens which were treated with sand this time. A significant increase was found in the healing of foot pad lesions in treated groups, but no significant reduction of ulceration was found. With the results of these experiments the hypothesis that anaerobic bacteria could possibly play a role in the etiology of FPD cannot be adopted nor rejected. Further investigation on this role and the possible use of Na-perborate as a remedy against FPD is desirable.**

## **Introduction**

Foot Pad Dermatitis (FPD), also called pododermatitis or footpad lesion, is a condition of inflammation and necrotic lesions on the plantar surface of the bird's feet.<sup>1</sup> FPD is a growing problem in the broiler industry. A prevalence study in the Netherlands performed by de Jong et. al showed that on average, 35.5% of the broilers had no lesions, whereas 26.1% and 38.4% had mild or severe lesions, respectively. Footpads are being scored in slaughterhouses and serve as a welfare indicator.<sup>2</sup> De Jong et al. (2011)<sup>3</sup> describe a scoring system for footpad lesions, '0' no lesion, '1' mild lesion and '2' severe lesion (with ulceration).

A strong link exists between litter quality (material, moisture) and the presence of FPD on broiler farms.<sup>2</sup> Litter quality is influenced by management factors as ventilation and drinker design. There also seems to be a seasonal effect (higher incidence of FPD in winter because of

high relative humidity). Mayne et. al (2005) suggested that wet litter contributed to the development of foot pad lesions because the continuous wetness would soften the footpad and make it more prone to mechanical damage.<sup>4</sup> They also suggested that wet litter and feces could form a hard crust on the footpads that could be an irritating factor by increasing pressure on certain areas of the footpad.<sup>4</sup>

Other causative factors for FPD investigated are nutritional deficiencies (especially biotin) and genetic variation.<sup>5</sup>

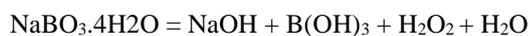
Michel et. al (2012) described the development of foot pad lesions not only macroscopically but also microscopically. The development of foot pad lesions starts with an enlargement of the scales with epidermal hyperplasia and hyperkeratosis. The lesion progresses to an ulcer with loss of the epidermis that is replaced by a crust of necrosuppurative material and

inflammation in the dermis. After healing all loss of scale aspect is visible.<sup>6</sup>

Literature about pododermatitis in ruminants mentions *Fusobacterium necroforum* and *Bacteriodes nodosus* as important agents in the etiology.<sup>7</sup> We suspect that anaerobic bacteria could play an important role in the etiology of FPD in broiler chickens as well. The bacteria that are important in bovine and ovine pododermatitis are obligate anaerobes and are sensitive for attack by free radicals. An example of a oxygen radical used in human medicine is hydrogen peroxide ( $H_2O_2$ ).  $H_2O_2$ -generating substances can be found in mouth washes and in ointments free obtainable in a 5% and 10% version.

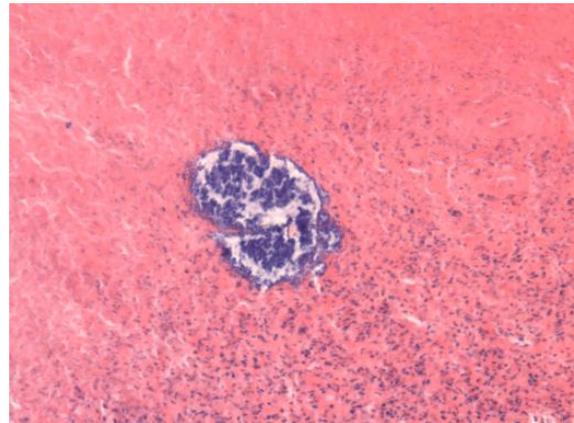
Researcher of this experiment (Marius Dwars) has already treated dozens of chickens on petting zoos with footpad problems with a topical application of a 3%  $H_2O_2$ -solution, which in most cases led to healing within 4 weeks. Researcher also provoked cleaves in the skin of the fingertips of his own left hand and healed these within 2-4 days by daily topical application of a 3%  $H_2O_2$ -solution. This treatment is not painful for human.

Natriumperborate is an example of a  $H_2O_2$ -generating substance. This substance dissociates by temperatures above  $60^\circ C$  (quickly) and under wet circumstances by lower temperatures (less quickly) by the following reaction:



Histological analysis performed by supervisor of the author at GD Deventer of foot pad lesions shows little nests of coccus shaped bacteria in the crust of necrotic material. The underlying dermis seems not to be invaded but a clear inflammatory response is visible. The lesion resembles an infection with coagulation necrosis caused by keratolytic bacteria that are present in dead material.

The aim of this research was to investigate the effect of Na-Perborate on litter in reducing ulceration of foot pads in broiler chickens. Results were used to evaluate the hypothesis that Na-perborate, a  $H_2O_2$ -generating substance, inhibits anaerobic bacteria that could possibly play a role in the etiology of FPD. After performing the experiment also and the effect of Na-Perborate on healing of Foot Pad Dermatitis (FPD) was used to evaluate above hypothesis.



**Fig. 1, Nest of coccus shaped bacteria in the crust of a broiler foot pad lesion (from database GD Deventer)**

## Materials and Methods

### *Broilers*

Experiments took place between October 2013 and February 2014 on the broiler farm of Arie Slingerland, Giessenburg, the Netherlands. Two flocks were examined. The first flock arrived on October 31th of 2013. This flock consisted of byproduct broilers. On the 19<sup>th</sup> of December 2013 the next flock arrived on the farm. This flock consisted of commercial Ross 308 chicks.

### *Housing and nutrition*

Selected chicks were placed randomized into 4 test pens. The test pens were built of wood and placed into the stable. Each testpen had a surface of  $4,95 m^2$  with 2 feeding pans (350 mm  $\varnothing$ ) and 6-7 nipple drinkers in it. Testpens were named A1, B1, A2 and B2.

### ***Treatment***

On the age of 20 days the first experiment started and 320 chicks were selected and placed randomized into the 4 test pens. In the first experiment the test pens of the treated groups, B1 and B2, 240 gram/m<sup>2</sup> product with 15% Na-perborate was scattered over the litter divided in four gifts of 40 gram/m<sup>2</sup> at a time and 80 gram/m<sup>2</sup> in the last gift. This equates 3 gram/m<sup>2</sup> Na-perborate at a time and 18 gram/m<sup>2</sup> Na-perborate throughout the whole experiment with a stocking density of 16 chicks/m<sup>2</sup>. The product existed of 15% Na-perborate supplemented with sand. Portions were made individually. Na-Perborate was grinded with pestle and mortar. For each proportion 15 gram grinded Na-perborate was weighed supplemented with 185 gram sand. Groups A1 and A2 were control groups. Product was scattered over the litter on day 1, 3, 7, 10 and 14 of the experiment. On the age of 22 days the second experiment was started and again 320 chicks were selected. Testpens were placed in the same stable on the same place and again named A1, B1, A2 and B2. This time the groups A1 and A2 were treated. We also decided to treat the control groups with the supplement product, sand, to exclude the effects of it. Product was scattered over the litter in groups A1 and A2 in 5 gifts of 40 gram/m<sup>2</sup> at a time on day 1, 5, 8, 12 and 15. Sand was scattered over the litter in groups B1 and B2 also in 5 gifts of 40 gram/m<sup>2</sup> on the same days.

### ***Quantitative assessment of the condition of foot pads***

Chicks were selected on footpadscore. To evaluate the condition of the footpads the scoring system of de Jong et. al (2011)<sup>3</sup> was used as reference. Chicks with score 1 'mild lesion' were selected with the presumption that footpad lesions of these chicks would aggravate by continuation of living in these conditions to score 2 'severe lesion'. On day 16 of the first experiment, the experiment was ended and

the footpad lesions of 203 chicks were scored with de Jong et. al (2011)<sup>3</sup> as reference. The dimension of the stable forced the researchers to build testpens with a surface of 4,95 m<sup>2</sup>. To achieve a stocking density comparable to the rest of the stable 80 chicks needed to be placed in each test pen. However time management did not allow enough time to evaluate all 320 chicks on one day, so the foot pads of only 203 chicks were evaluated. The foot pads of these individual chicks were evaluated after cleaning them with water and a bristle. Their lesions were photographed. Their footpadscores and the largest diameter of the lesion in mm were listed. On day 19 of the second experiment, the experiment was ended and the foot pads of 200 chicks were evaluated the same way. Again their lesions were photographed. Their footpadscores and the largest diameter of the lesion in mm were listed.

### ***Statistical analysis***

To analyse the results several Chi-square tests were executed (using IBM SPSS Statistics 20©). Results of the footpad score system were therefore converted to binary variables. ("0" = no ulceration, "1" = ulceration and "0" = not healed, "1" = healed) In the first analysis in which 'ulceration' and 'no ulceration' were compared chicks with score 0 and 1 were placed in 'no ulceration' whereas chicks with score 2 were placed in 'ulceration'. From each chick the two paws were reviewed and the highest score was used for statistical analysis as advised in the article of the Jong et. al (2011)<sup>3</sup>. This was advised because systematic differences were found between the left and right paws in which Footpad Lesion Score on the left paws was usually higher.<sup>3</sup> To prevent an undervaluation of the problem by only using right paws the highest score per chick was used for statistical analysis. In the second analysis, in which 'healed' and 'not healed' were compared, chicks with at

least one score 0 (on the right or left paw) were considered 'healed'.

Also the distribution of the different foot pad scores in the different groups were reviewed.

To analyse the difference in diameters of the lesions between the left and right paws and between treated and control groups several t-tests were executed.

The results of the two experiments were not analysed together but as separate experiments because there was a different treatment of the control group. (No treatment vs. treatment with sand).

## **Results**

### Experiment 1

In experiment 1 ulceration on the foot pads was found on 18,4% of the chicks in the treated groups. (103 chicks) Ulceration on the foot pads was found on 39,0% of the chicks in the control groups. (100 chicks) The risk for ulceration was significantly reduced in treated chicks compared to control chicks (OR = 0,35,  $P < 0.05$ ). The difference in proportions of ulceration between treated and control groups is 0,21 which equals a reduction of 52,8% of ulceration on the footpads in treated groups. The 95% Confidence Interval (CI) for the difference in proportions is 0.13-0.28 which corresponds with a reduction of 33,3%-71,8%.

Healing of foot pad lesions was found on 13,6% of the chicks in the treated groups. In the control groups healing was found on 18,0% of the chicks. This would mean that the odds ratio for healing of treated chicks would be 0,72 times as big (and thus smaller) as the odds ratio for healing on control chicks. However, statistical analysis showed that this association was not significant. ( $P > 0.05$ )

No significant differences were found between scores in testpen A1 and A2 respectively B1 and B2.

No significant difference in diameter of the lesions were found between right and left paws and between treated and control groups.

### Experiment 2

In experiment 2 ulceration on the foot pads was found in 70,0% of the chicks in the treated groups. (100 chicks) Ulceration of the foot pads was found on 80,0% of the chicks in the control groups. (100 chicks) This would mean that the odds ratio for ulceration on treated chicks would be 0,58 times as big (and thus smaller) as the odds ratio for ulceration on control chicks. However, statistical analysis showed that this association was not significant. ( $P > 0.05$ )

Healing of foot pad lesions was found in 26,0% of the chicks in the treated groups. In the control groups healing was found in 13,0% of the chicks. The odds ratio for healing on treated chickens is 2,35 times as big (and thus bigger) as the odds ratio for healing on control chickens (treated with sand). This association between treatment and healing is significant. ( $P < 0.05$ ) The difference in proportions is 0,13 which equals an increase of 50% of healing of the foot pad lesions in treated groups. The 95% Confidence Interval (CI) for the difference in proportions is 0.02-0.24 which corresponds with an increase of 13,3%-64,9%.

No significant difference in diameter of the lesions were found between right and left paws. A significant smaller average diameter of lesions was found in the control groups compared to the treated groups.

No significant differences were found between scores in testpen A1 and A2 respectively B1 and B2.

## Experiment 1:

Treatment		Footpad Lesion Score		Total
		<i>No ulceration</i>	<i>Ulceration</i>	
<i>NaP+sand</i>	<i>B1</i>	43 (82,7%)	9 (17,3%)	<b>52 (100,0%)</b>
	<i>B2</i>	41 (80,4%)	10 (19,6%)	<b>51 (100,0%)</b>
	<i>Total</i>	84 (81,6%)	19 (18,4%)	<b>103 (100,0%)</b>
<i>Control</i>	<i>A1</i>	32 (65,3%)	17 (34,7%)	<b>100 (100,0%)</b>
	<i>A2</i>	29 (56,9%)	22 (43,1%)	<b>49 (100,0%)</b>
	<i>Total</i>	61 (61,0%)	39 (39,0%)	<b>51 (100,0%)</b>
<b>Total</b>		<b>145 (71,4%)</b>	<b>58 (28,6%)</b>	<b>203 (100,0%)</b>

**Fig. 2 Treatment\*Ulceration Crosstabulation**

OR = (19\*61)/(84\*39) = 0,35, Pearson Chi-Square = 10.503, p=0.001

Treatment		Footpad Lesion Score		Total
		<i>Not healed</i>	<i>Healed</i>	
<i>NaP+sand</i>	<i>B1</i>	44 (84,6%)	8 (15,4%)	<b>52 (100,0%)</b>
	<i>B2</i>	45 (88,2%)	6 (11,8%)	<b>51 (100,0%)</b>
	<i>Total</i>	89 (86,4%)	14 (13,6%)	<b>103 (100,0%)</b>
<i>Control</i>	<i>A1</i>	42 (85,7%)	7 (14,3%)	<b>49 (100,0%)</b>
	<i>A2</i>	40 (78,4%)	11 (21,6%)	<b>51 (100,0%)</b>
	<i>Total</i>	82 (82,0%)	18 (18,0%)	<b>100 (100,0%)</b>
<b>Total</b>		<b>171 (84,2%)</b>	<b>32 (15,8%)</b>	<b>203 (100,0%)</b>

**Fig. 3 Treatment\*Healing Crosstabulation**

OR = (14\*82)/(89\*18) = 0,72, Pearson Chi-Square = 0.742, p=0.389

## Experiment 2:

Treatment		Footpad Lesion Score		Total
		<i>No ulceration</i>	<i>Ulceration</i>	
<i>NaP+sand</i>	<i>A1</i>	12 (24,0%)	38 (76,0%)	<b>50 (100,0%)</b>
	<i>A2</i>	18 (36,0%)	32 (64,0%)	<b>50 (100,0%)</b>
	<i>Total</i>	30 (30,0%)	70 (70,0%)	<b>100 (100,0%)</b>
<i>Sand</i>	<i>B1</i>	11 (22,0%)	39 (78,0%)	<b>50 (100,0%)</b>
	<i>B2</i>	9 (18,0%)	41 (82,0%)	<b>50 (100,0%)</b>
	<i>Total</i>	20 (20,0%)	80 (80,0%)	<b>100 (100,0%)</b>
<b>Total</b>		<b>50 (25,0%)</b>	<b>150 (75,0%)</b>	<b>200 (100,0%)</b>

**Fig. 4 Treatment\*Ulceration Crosstabulation**

OR = (70\*20)/(30\*80) = 0,58, Pearson Chi-Square = 2.667, p=0.102

Treatment		Footpad Lesion Score		Total
		<i>Not healed</i>	<i>Healed</i>	
<i>NaP+sand</i>	<i>A1</i>	41 (82,0%)	9 (18,0%)	<b>50 (100,0%)</b>
	<i>A2</i>	33 (66,0%)	17 (34,0%)	<b>50 (100,0%)</b>
	<i>Total</i>	74 (74,0%)	26 (26,0%)	<b>100 (100,0%)</b>
<i>Sand</i>	<i>B1</i>	42 (84,0%)	8 (16,0%)	<b>50 (100,0%)</b>
	<i>B2</i>	45 (90,0%)	5 (10,0%)	<b>50 (100,0%)</b>
	<i>Total</i>	87 (87,0%)	13 (13,0%)	<b>100 (100,0%)</b>
<b>Total</b>		<b>161 (80,5%)</b>	<b>39 (19,5%)</b>	<b>200 (100,0%)</b>

**Fig. 5 Treatment\*Healing Crosstabulation**

OR = (26\*87)/(74\*13) = 2,35, Pearson Chi-Square = 5.383, p = 0.020



Fig. 6 Photograph of a 0,1 and 2 Footpad Lesion Score respectively. (Note the loss of scale aspect on the 0 lesion resulting from healing of FPD<sup>6</sup>)

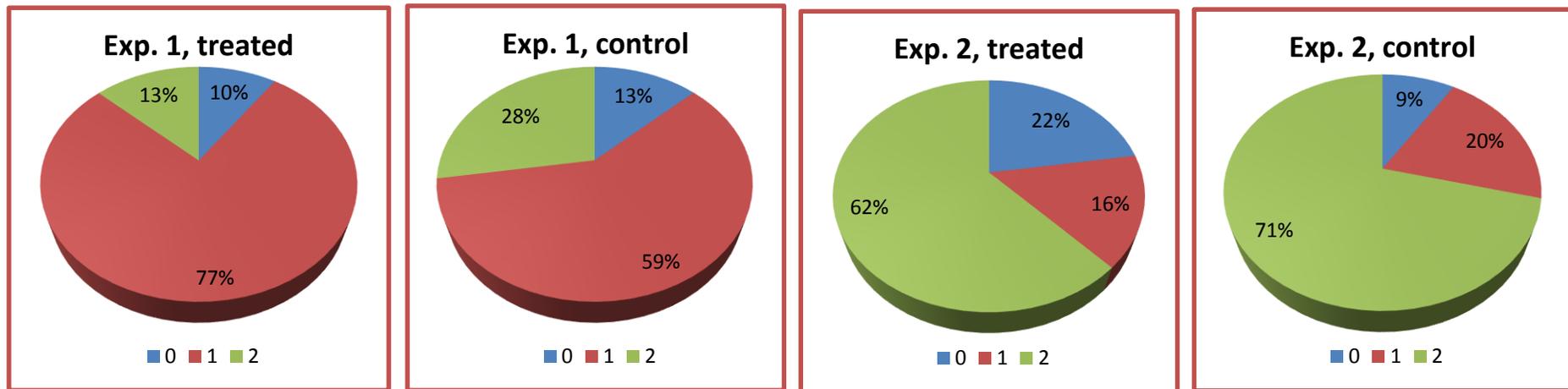


Fig. 7 Distribution of Foot Pad Lesion Scores on all individual paws in treated and control groups in experiment 1 and 2

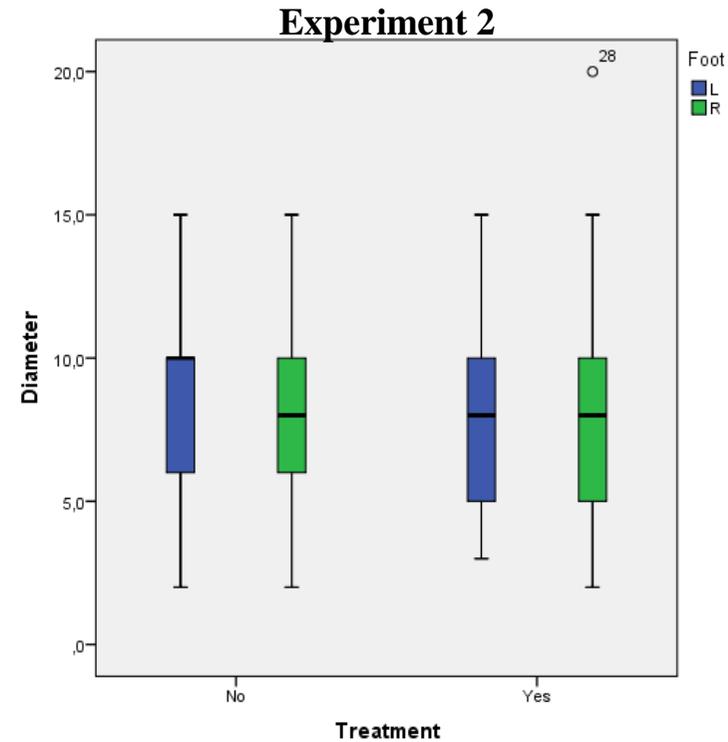
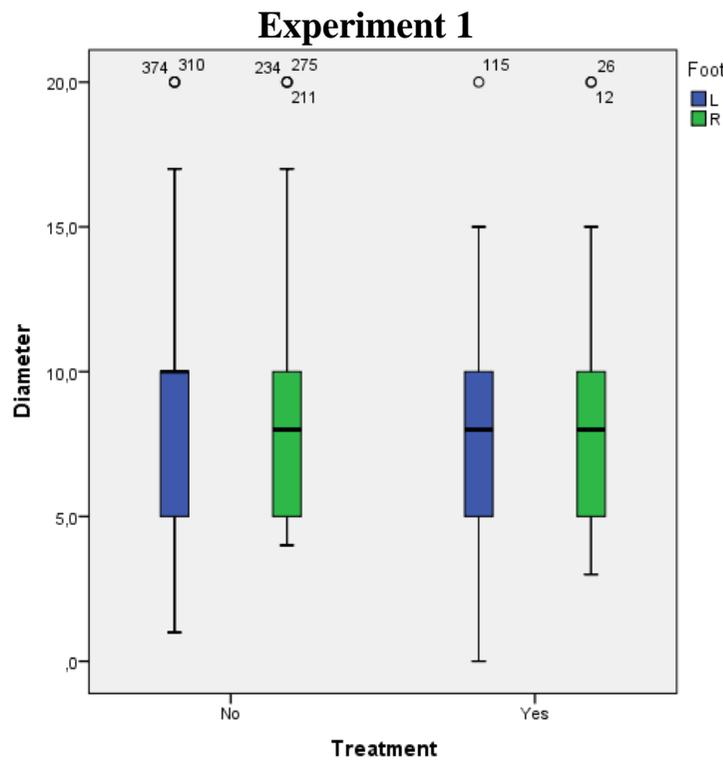
## Diameters of Foot Pad Lesions:

		Exp.1	Exp.2
<b>Treated</b>	R	8,584 mm	7,975 mm
	L	8,350 mm	7,573 mm
	T	8,466 mm	7,742 mm
<b>Control</b>	R	8,737 mm	8,351 mm
	L	9,091 mm	8,165 mm
	T	8,914 mm	8,516 mm

Fig. 8 Average diameters (R=right, L=left, T=total)

				Significant difference in diameter?
<b>Exp.1</b>	Treated R	vs.	Treated L	No (p=0.601)
	Control R	vs.	Control L	No (p=0.494)
	Treated	vs.	Control	No (p=0.190)
<b>Exp.2</b>	Treated R	vs.	Treated L	No (p=0.376)
	Control R	vs.	Control L	No (p=0,630)
	Treated	vs.	Control	Yes (p=0.017)

Fig. 9 Results of t-tests to evaluate the difference between right and left paws and between treated and control groups in both experiments



## Discussion

Data collected during this project, consisting of 2 experiments, focused on the role of Na-perborate on litter in reducing foot pad lesions in broiler chickens. The assumption that anaerobic bacteria could play a role in the process of the development of FPD (from healthy foot pads to hyperkeratosis to ulceration and eventually possible healing) was made based on histological findings and similarity to pododermatitis in ruminants. Analysis of the effect on Na-perborate on litter on the process of ulceration led to different findings in experiment 1 and 2 respectively. Significant result was found in experiment 1 in which the association between the treatment of litter with Na-perborate and the development of ulceration (Foot Pad Score 2) was tested. In experiment 2, in which the same association was tested, no significant result was found. Also the incidence of ulceration (Foot Pad Score 2) was much higher than in the first experiment. As can be seen in figure 9, the distribution of the Foot Pad Scores was much different between experiment 1 and 2. In experiment 2 however a significant result was found testing the association between the treatment of litter with Na-perborate and healing of foot pad lesions. This association was not found significant in experiment 1.

Differences between the experiments that possibly could partly declare the different results are discussed below. To begin with, the age of the chicks at which the experiments were started differ. In the first flock the experiment was started at the age of 20 days. In the second flock the experiment started at the age of 22 days. Lesions on the footpads of chicks of experiment 2 could have been developed further, not visible macroscopically, while starting the experiment. Beside experiment 2 lasted longer giving the foot pads more time to develop ulceration and eventually healing. Experiment 1 started at

the age of 20 days and lasted 16 days, on the day of scoring the chicks had reached the age of 36 days. Experiment 2 started at the age of 22 days and lasted 19 days, reaching an age of 41 days on the day of scoring.

As mentioned in the introduction genetic variation is also a factor in the development of FPD<sup>5</sup>. There was a genetic difference between the broilers used in experiment 1 and 2 respectively. In experiment 1 the flock consisted of byproduct broilers. In experiment 2 the flock consisted of commercial Ross 308 broilers. As a result, there also was a difference in the ratio of male/female broilers between the two experiments. Bilgili et. al (2006) showed that male broilers showed higher incidence and severity of FPD than females<sup>8</sup>. This higher incidence and severity could be related to body size since male broilers are usually heavier and more weight is placed on their footpads. An higher surface area of the footpads is in contact with the litter which could possibly lead to higher incidence and severity.<sup>5</sup> The male/female ratio however was higher in the first experiment.

Another difference between the two experiments is the treatment of the controls. In experiment 1 the controls were not treated, in experiment 2 sand was scattered over the litter in the control test pens. Sand has the ability to retain fluid thanks to capillary action. However it is questionable whether the quantity of sand that was used in this experiment is able to achieve this capillary action, making the litter less wet and reducing the difference in development of ulceration between treated and control groups. Research on sand as litter for rearing broilers showed no significant differences in foot pad lesions between chicks housed on pine shavings and chicks housed on sand. However chicks reared on sand consistently showed a lower incidence of foot pad lesions.<sup>9</sup>

Minimal to no difference in influence of weather has to be noted. Both experiments took place in winter. Experiment 1 took place in November with an average temperature of 6,7°C and 98 mm rainfall. The second experiment took place in January with an average temperature of 5,7°C and 65 mm rainfall.

With these results in mind the possible role of anaerobic bacteria in the etiology of FPD remains unclear. A hypothesis embracing the results of and the difference in results between the two experiments is that the anaerobic bacteria nested between the hyperkeratotic skinmaterial and in the crust disturb the cell division of the underlying cells of the skin. The first step in the development of FPD is probably caused by external irritation of the footpads by moist litter leading to hyperkeratosis. In the second step of the development of FPD, the one from hyperkeratosis to ulceration, the anaerobic bacteria could nest between the hyperkeratotic skinmaterial and could possibly disturb the cell division of the underlying skin cells withholding them from covering the underlying dermis and lead to ulceration. Beside they could act keratolytic. In the final step of the development of FPD, the one from ulceration to healing, the anaerobic bacteria could possibly again disturb the cell division of the underlying skin cells withholding them from forming a new epidermis by cell division of surrounding epithelial cells. The reduction of the cell-disturbing influence of the anaerobic bacteria by killing them with a H<sub>2</sub>O<sub>2</sub>-generating substance is displayed in experiment 1, in which ulceration in treated groups is significantly lower than in control groups. In experiment 2 reduction of the cell-disturbing influence of the anaerobic bacteria is displayed by a quicker healing of FPD, giving surrounding epithelial cells the change to divide and form a new epidermis. In experiment 2 Na perborate was unable to

reduce the cell-disturbing influence in the second step (from hyperkeratosis to ulceration), probably because the experiment was started later.

From these experiments may be concluded that this treatment, Na Perborate supplemented with sand, does have an effect on the development of Foot Pad Dermatitis. However the hypothesis that anaerobic bacteria could possibly play a role in the etiology of FPD cannot be adopted nor rejected.

In possible follow-up studies the foot pad lesions could be followed more longitudinal to further investigate above mentioned hypothesis. Scoring and photographing of the foot pad lesions could be done on more moments during the experiment giving a clearer vision of the development of the lesions and the influence of anaerobic bacteria on this development. Also treatment could start earlier before beginning lesions are visible. Other options for possible follow-up studies are to combine the experiments with histological analysis and try to cultivate anaerobic bacteria from foot pad lesions. Beside this it is very important to further investigate the role of sand in the effects we measured. Possible successes in follow-ups studies can be extended to other contact dermatoses in different animal species and probably even human.

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