

Management of Insect bite
hypersensitivity in the horse with a
supplement containing amino-acids,
polypeptides and vitamins
(Hippo-Ex-Cema®)

Drs. F. Driessen 0460877
Supervisor: Dr. M.M. Sloet van
Oldruitborgh-Oosterbaan

Index

Summary	3
Introduction	4
Material and methods	6
• Case selection	6
• Horses	6
• Supplement	7
• Experimental setting	8
• Questionnaire	8
• Weather conditions	8
• Statistical analysis	8
Results	9
• Horses	9
• Questionnaire	9
• Investigators	10
• Weather conditions	12
Discussion	12
Acknowledgements	14
References	15

Summary

Insect bite hypersensitivity (IBH) is mostly caused by *Culicoides spp.*, but other species are also thought to play a role in causing the disease. The disease has been described in literature over the world in many countries and prevalence up to 28% in some countries has been described. The only effective therapy at the moment is to prevent contact with *Culicoides spp.* This can be reached by accurately stabling the horses or covering the horses with insect blankets, so the *Culicoides spp.* can't come near the horses.

In this study, we are testing a new supplement, consisting of a powder and oil that should be mixed prior to use. The supplement contains several vitamins, polypeptides and amino-acids. Fifty horses participated in this study, 25 receiving placebo and 25 receiving Hippo-ex-cema® in an initial stage of 30 days. After this initial stage, all horses received approximately 30 days Hippo-ex-cema®. Each horse received, despite its body weight, the first 10 days 10 ml oil twice a day and 5 gram powder twice a day, followed by 20 days once a day 10 ml oil and once a day 5 gram powder. Owners were asked to fill in a questionnaire three times: one prior to the investigation, one after 30 days and the last after the final stage, to score the horses among others for their severity of IBH. The investigators scored the horses for their severity of IBH prior to the study and after 30 days.

A Chi-square test was used to process the results. The scores of the investigators were significantly different between 0 and 30 days. However, no significant difference was found in the scores from the owners, both not between 0 and 30 days and not between 30 and 60 days. No side-effects appeared during the investigation while using the supplement.

Samenvatting

Staat- en maneneczeem (SME) wordt voornamelijk veroorzaakt door *Culicoides spp.*, maar andere soorten spelen mogelijk ook een rol in de oorzaak van de aandoening. De aandoening wordt wereldwijd in diverse landen beschreven met prevalenties oplopend tot 28%. Op dit moment is de enige effectieve therapie het voorkomen van contact met *Culicoides spp.* Dit kan bereikt worden door consequent opstallen van paarden, zodat de *Culicoides spp.* niet in de nabijheid kunnen komen of door het gebruik van een eczeemdeken.

In het huidige onderzoek wordt een nieuw supplement getest, bestaande uit een olie en een poeder die voor de gift over het voer gemengd dienen te worden. Het supplement bevat diverse vitamines, polypeptiden en aminozuren. In het onderzoek zijn 50 paarden opgenomen, waarvan er 25 gedurende de eerste 30 dagen het echte supplement gevoerd kregen en 25 een placebo. Na deze eerste fase van 30 dagen, volgde een fase van 30 dagen waarbij alle paarden Hippo-ex-cema® toegediend kregen. Ieder paard kreeg, ongeacht het lichaamsgewicht, de eerste 10 dagen twee maal per dag 10 ml olie en twee maal per dag 5 gram poeder ('s ochtends en 's avonds), gevolgd door 20 dagen een keer per dag 10 ml olie en een keer per dag 5 gram poeder. De eigenaren werden verzocht om driemaal een enquête in te vullen, voor aanvang van de proef, na 30 en na 60 dagen, om de paarden o.a. op hun ernst van SME te scoren. De onderzoekers hebben de paarden gescoord op de ernst van de SME voor aanvang van de proef en na 30 dagen.

Een Chi-kwadraat test werd gebruikt om de resultaten te verwerken. De scores van de onderzoekers verschilden significant tussen 0 en 30 dagen van het onderzoek. In de scores van de eigenaren werd er echter geen significant verschil gevonden tussen zowel de aanvang van de proef en 30 dagen, als tussen 30 en 60 dagen. Er werden geen negatieve bijwerkingen gevonden van het supplement.

Introduction

Insect bite Hypersensitivity (IBH), or allergic dermatitis, is a common skin disorder in horses and is also known as *Culicoides* hypersensitivity, Queensland itch, sweet itch and summer eczema (Anderson et al 1988, Bjornsdottir et al 2006, Halldorsdottir et al 1991, Kleider et al 1984, Riek 1953). It is a chronic, recurrent seasonal allergic dermatitis, and clinical signs may be seen from April till October (Anderson et al 1988). Few severely affected horses show clinical signs throughout the year (Kleider et al 1984, Van den Boom et al 2008).

The causative agents to IBH are *Culicoides spp.*, but other species, like horsefly and deerfly are thought to have a part in the aetiology of IBH. They also provoked a positive skin reaction in IBH horses, just like *Culicoides spp.* However, the significance regarding reactivity on deerfly and horsefly antigens was not associated with a high sensitivity (26% and 33%) (Kolm-Stark et al 2002). Another research recorded that a few affected horses also responded to *Simulium spp.* (Fadok et al 1990). The studies of Baker et al (1978) and Quinn (1983) also mentioned that other insects than *Culicoides spp.* gave variable and generally weaker reactions, from which *Stomoxys spp.* gave the strongest reactions. In contrast with *Culicoides spp.*, *Stomoxys spp.* was not consistently significant between the affected and the normal horses.

Culicoides spp. are also known as 'biting midges' or 'midges'. They measure 1.5-5 mm long. They are bad fliers and only fly a few hundred metres from their larval habitats, but distances until 4.0 km have been reported. Only the females suck blood from different species, in order to feed the eggs (Braverman 1994). IBH is a hypersensitivity reaction to *Culicoides spp.*, and both immediate and delayed reactions take place in the horse (type I and IV hypersensitivity reactions) (Anderson et al 1991). Recent research showed that abundant proteins and potential allergens were not exclusively extracted from the salivary glands (Wilson et al 2008). IBH can be caused by various *Culicoides spp.*, dependently from country and region. In the Netherlands IBH is mostly caused by *Culicoides obsoletus* (94.1%) and *Culicoides pulicares* (5.81%) (De Raat et al 2008, Van der Rijt et al 2008).

Early symptoms of the disease include numerous papules/nodules and sensitization of the skin. Soon the horses will show one or more of the following symptoms: pruritus, scaling, hair loss, hyperkeratosis, crusting, open wounds, inflammation and complete baldness (Baker et al 1978, Kleider et al 1984, Anderson et al 1988). The research of Van den Boom et al (2008) showed that more than 40% of the affected horses show signs around the mane and tail, 15% around the tail only and approximately 20% of the horses also show symptoms on the ventral abdomen.

The disease has been described worldwide, with a varying incidence in different countries. Anderson et al (1988) describes a prevalence of 26% in British Columbia (Canada) and Steinman et al describes prevalence in Israel of 28%. No exact data are available for the Netherlands. The prevalence is also breed dependant and the breeds mostly affected in the Netherlands are Shetland ponies (8-9%) and Friesian horses (18%) (Schurink et al 2009, Van Grevenhof et al 2007). Other frequently affected breeds are Icelandic horses, with an incidence of 8,2% in Norway (Halldorsdottir et al 1991). The prevalence among imported Icelandic horses is even higher, around 34,5% in Germany, Denmark and Sweden and 26,9% in Norway (Bjornsdottir et al 2006, Halldorsdottir et al 1991). There are no data available for the prevalence among Icelandic horses in the Netherlands.

Unfortunately, no effective cure has been found for IBH until current time. Various treatments have been tested among the years but mostly were not successful. The only effective therapy until now are glucocorticosteroids, but they are not preferred for a

longer period of treatment because they may cause laminitis in the horse. The best way to control the symptoms of IBH remains avoidance of allergens. This can be reached by accurately stabling the horses, during sunset when *Culicoides spp.* are mostly active (Van der Rijt et al 2007). This is rather time-consuming and not for every horse owner a practical solution. The contact time between *Culicoides spp.* and horses can be further reduced by the use of repellents and fly rugs (Van den Boom et al 2008, Van der Rijt et al 2007). However in the study of De Raat et al (2008) no significant difference was found between the number of *Culicoides spp.*, the percentage of blood-fed *Culicoides obsoletus* and the total number of insects attracted to horses before and after treatment with a topical insecticide containing permethrin.

During previous research, some supplements were tested among horses with IBH. In the study of O'Neill et al (2002) 6 horses received flaxseed supplementation in a placebo controlled double blind trial. On day 42, there was a significant difference between the treatment and the placebo controlled group on the mean area of reaction to *Culicoides* by a skin test. In the double-blind placebo-controlled trial of Craig et al (1996) 33 horses with IBH received a fatty acid supplementation containing evening primrose and fish oil or hydrogenated coconut oil. No significant changes in clinical status could be found between test and placebo groups. Friberg et al (1999) tested the clinical efficacy of α -linolenic acid against corn oil (placebo) in 19 IBH horses in a double-blind crossover study during an eighteen week trial. They also did not find any significant difference between the test and placebo groups. Though, the owners stated that the horses improved during the test. In another research, sunflower oil and seal blubber oil were tested among horses with recurrent airway obstruction. There was a tendency to a lower neutrophil count in the pulmonary epithelial lining fluid, but this was not significant (Khol-Parisini et al 2007). In another study MicroLactin[®] (a patented dried milk protein concentrate from the milk of hyperimmunized cows, Duralactin Equine, Veterinary Products Laboratories, Phoenix, AZ) was fed to 13 horses with various forms of skin trauma. Four of them were seasonally affected by *Culicoides*-bite hypersensitivity and were protected by early supplementation with MicroLactin[®] before and during the insect season (Bello et al 2005). Stark et al (2001) measured zinc and copper plasma levels in Icelandic horse with IBH. The purpose of this study was to determine if there was a deficiency of zinc and copper so this could be supplemented if necessary. Zinc and copper plasma levels were not significantly different between affected horses and controls.

In man, vitamins are often used complementary for their anti-oxidant status. They are also tested among varying allergic diseases. For example vitamin E has been shown to be associated with a lower serum IgE concentration. However, diverse studies show contradictory results and therefore evidence is insufficient for a recommendation in case of an allergy (Laitinen et al 2005). In the study of Bielory et al (2005) varying vitamins and minerals are described for their function in asthmatic patients. Research has shown that Vitamin C, B6, B12, Magnesium, Zinc and Selenium are all deficient in asthmatic patients. Zinc has also been shown to have a low serum level in atopic dermatitis patients. The supplementation of Vitamin C, B6 and Magnesium showed contradictory results. The supplementation of Vitamin B12 and Zinc has not been tested yet, but could be beneficial. Patients who were given extra Selenium improved clinically, but no objective improvements in lung function were found.

There are only limited publications available about IBH horses which were supplemented with vitamins, polypeptides or amino-acids. In the study of Kolm et al (2007), lactoferrin (a glycoprotein) was tested in horses with IBH. Lactoferrin is known for its immunomodulatory and mast cell stabilising properties. mRNA levels at the site of intradermal challenges were measured before and after skin biopsy. No significant difference was found between mRNA expression pre- and post intradermal challenge.

In the current research, we are testing a new supplement, on the market in the Netherlands as Hippo-ex-cema® (W.S.H. Horse Health products, Biddinghuizen, The Netherlands). The manufacturer claims the product to improve IBH horses within 2 weeks: horses get calmer, itch takes off and lost hairs will grow back. Besides, there is no danger in overage of the supplement. On request of the manufacturer the product was tested in the early period of the *Culicoides spp.* season, starting in April 2009.

Material and methods

Case selection

An advertisement was placed in several horse magazines and more than 100 horse owners responded to be willing to participate in the project. From these reactions 50 horses were selected. The horses were suffering a moderate till severe form of IBH for at least 2 years, to be sure from a seasonal occurrence of IBH. Another criterion for inclusion was that the horses should be housed and managed exactly the same as the previous year. Horses that already received glucocorticosteroids this year were excluded from the study.

Horses

The mean age of the 50 participating horses was 11.8 ± 6.0 years (varying from 2-27 years), and 30 mares, 17 geldings and 3 stallions were included in the study. The participating horses were very diverse in races: 8 Shetland ponies, 10 Warmbloods, 18 pony's from different breeds (3 New Forest, 4 Welsh, 2 Connemara, 1 Dartmoor, 1 German pony and 7 crossbreeds), 12 cold bloods (4 Friesian horses, 1 Fjord horse, 3 Icelandic horses, 1 Merens, 1 Irish tinker horse, 2 Haflinger horses) and 2 other horses (a Lusitano crusado horse and an Arabian horse).

Gender	Placebo	%	Hippo-ex-cema®	%
Mare	16	64	14	56
Gelding	6	24	11	44
Stallion	3	12	0	0

Table 1: Gender of the horses in each group (calculation was made after the study, when data were available)

Breed	Placebo	%	Hippo-ex-cema®	%
Shetland pony	3	12	5	20
Other pony breeds	8	32	10	40
Cold bloods	9	36	3	12
Warm bloods	5	20	5	20
Other	0	0	2	8

Table 2: Breed of the horses in each group (calculation was made after the study, when data were available)

Age	Placebo	Hippo-ex-cema®
0-2 years	0	0
2-5 years	2	1
5-10 years	8	11
10-15 years	6	7
15-20 years	6	3
>20 years	3	3

Table 3: Ages of the horse in each group (calculation was made after the study, when data were available)

Mean age	11.8 ± 6.0
Placebo	12.4 ± 6.0
Hippo-ex-cema®	11.3 ± 6.1

Table 4: Mean age of the horses in each group (calculation was made after the study, when data were available)

Years affected by IBH	Placebo	Hippo-ex-cema®
0-2 years	0	0
2-5 years	7	13
5-10 years	16	7
10-15 years	1	5
15-20 years	1	0
> 20 years	0	0

Table 5: years affected by IBH in each group (calculation was made after the study, when data were available)

Supplement

A supplement, containing vitamins, polypeptides and amino-acids (Hippo-ex-Cema®, W.S.H. Horse Health products, Biddinghuizen, The Netherlands) was used in this experiment. The product contains an oil and a powder that should be mixed prior to use. The powder contains vitamins B1, B2, B3, B6, B9, B12, C & E, amino-acids, choline, magnesium and patented peptides. The oil is on basis of sunflower oil, and contains polypeptides, omega 3 fatty acids, omega 6 fatty acids and vitamins E & A (http://www.wshhhp.com/hippo-ex-cema_nl_bijsluiter.html). The product contains carriers, to transport the peptides across the gut barrier into the blood (Van den Windt, WSH Health products).

The placebo also contained an oil and a powder, but did not contain the added substances (amino-acids, polypeptides and vitamins). The oil used was the same as the Hippo-ex-cema® oil, namely sunflower oil and the powder consisted of wheat flower.

The horses received 10 ml oil and 5 gram powder twice a day during the first ten days. The remaining twenty days the horses received 10 ml oil and 5 gram powder once a day. The supplement and the placebo were mixed by the owner and added to the horse's food. If the horses hadn't shown any progress in symptoms of IBH, they had to start the following period again with a double dosage for the first ten days. Mares in heat should also receive a double dosage, because earlier research of the manufacturer showed that the action of the supplement was lower during this period.



Figure 1: The package used during the study, numbered from 1 to 50

Experimental setting

The supplement was tested during 60 days (April-June 2009) in a double-blind placebo controlled study. During the first month, 25 horses received placebo and the other 25 horses received the real supplement. The investigators visited all the competing horses and gave instruction to the owners how to use the supplement. All packaging were numbered from 1 to 50 by the manufacturer. Both investigators and owners did not know which horse received the placebo or real supplement. During the second month all horses received the real supplement. Before and after the first period, questionnaires were filled in by the owners. The investigators took pictures from the horses from 5 standard views: full-body photographs of each side and hind of the horse, close-up views from the head and manes from left and right and detailed photographs of individual lesions were taken. After visiting all the horses these photographs were evaluated and the horses were scored for their severity of IBH using these photographs. After the second round (all horses received the real product), the horse owners were contacted by phone and were asked to describe any alteration in IBH on the horse. They also had to give a score again for the severity of IBH on their horse. If a major improvement/worsening occurred, the horse owners were asked to take photographs and send them to the investigators.

Questionnaire

All owners were asked to fill in a questionnaire about their horses (Annex I). Firstly, some general information about the horse was asked (age, gender, breed, purpose of use etc). Then housing conditions and habitat were about to be described. This contained not only the amount of time stabled/on pasture, but also type of soil, type of area (wooded, open etc.) and the presence of non running water for 1 km around. Further the owners had to fill in what kind of food the horses received, and the amount they received of it a day. The next part of the questionnaire contained information about IBH. Owners were asked to fill in a score from 1 to 10 about the severity of IBH. A score of 1 is a slight form of IBH and a score of 10 is a very severe form of IBH. Next, the owners had to fill in scores from zero till ten about the following items (0: absent, 10: very severe): itch; broken hairs and scaling; bald patches/ hair loss/ pigmentation; complete baldness; crusts; open wounds; inflammations; thickened skin/ irritated skin/ rougae (ridged skin). The severity of IBH (equal/slighter/worse) in comparison to last year should also be filled in. At last, the owners had to fill in a few things about the treatment of IBH. They were asked about the use of repellents, a fly rug and previous treatments by themselves or a veterinarian. Any other medication the horses received should also be filled in.

Weather conditions

The weather is a factor that could influence the results of the test. *Culicoides spp.* are bad fliers (Braverman 1994) and therefore the weather conditions were reported as given by the Royal Dutch Meteorological Institute.

Statistical analysis

A Chi-square test was used to determine whether there was a significant difference between the placebo and the treatment group or not. SPSS 16.0 program was also used for reviewing the results and determining if the horses were grouped equally in the two groups (by gender, breed, age and years affected by IBH).

Results

Horses

Forty-six of the original 50 horses completed the study. In the first period two horses were taken out of the study by their owners. One horse (placebo) got extremely itchy, and the owner decided to sell the horse to a part of the country with a very low incidence of IBH. The other horse (placebo) was withdrawn because the owner decided to cover the horse with a fly rug for the first time in its life. In the second period another horse (real supplement) was withdrawn for the same reason and one other horse (placebo) was withdrawn because the owner failed to give the horse the supplement because the horse would not eat it. Out of 46 horses completing the study, 22 received the placebo and 24 the real supplement.

Questionnaire

Both the owners and investigators scored the horses for their severity of IBH. After the first period (30 days Hippo-ex-cema[®] or placebo) the owners in the placebo group mentioned 14 out of 23 horses showed a higher score for severity of IBH. In the real test group, the owners stated that 17 out of 25 horses increased in score. This means that those horses receiving the placebo in the first period of the study, 68.0% deteriorated and those receiving Hippo-ex-cema[®], 60.9% deteriorated. When using the Chi-square test, no significant difference was found between the groups (Chi-square is 0.2663 and p= 0.6058). No improvement, but also no aggravation in the severity of IBH occurred. (Table 6 and figure 1)

If the results on day 30 are compared with the results on day 60 (all Hippo-ex-cema[®]), the following was found: in the placebo/ Hippo-ex-cema[®] group, 12 out of 22 horses deteriorated (54.5%). In the Hippo-ex-cema[®] / Hippo-ex-cema[®] group 9 horses out of 24 deteriorated (37.5%). When using the Chi-square test no significant difference could be found between the placebo/Hippo-ex-cema[®] and the Hippo-ex-cema[®] / Hippo-ex-cema[®] group (Chi-square=1.3442 and p= 0.264). So also after the second period of the study, no statistical difference could be found between both groups. No side effects were mentioned from the use of the supplement (Table 7 and Figure 2).

Severity scored by the owners	Increased	%	Equal/Decreased	%	Total
Hippo-ex-cema [®]	17	68.0	8	32.0	25
Placebo	14	60.9	9	39.1	23
Total	31		17		48

Table 6: Severity scored by the owners after 30 days Hippo-ex-cema[®] or placebo

Severity scored by the owners	Increased	%	Equal/Decreased	%	Total
Hippo-ex-cema [®] /Hippo-ex-cema [®]	9	37.5	15	62.5	24
Placebo/Hippo-ex-cema [®]	12	54.5	10	45.5	22
Total	21		25		46

Table 7: severity scored by the owners after another 30 days Hippo-ex-cema[®]

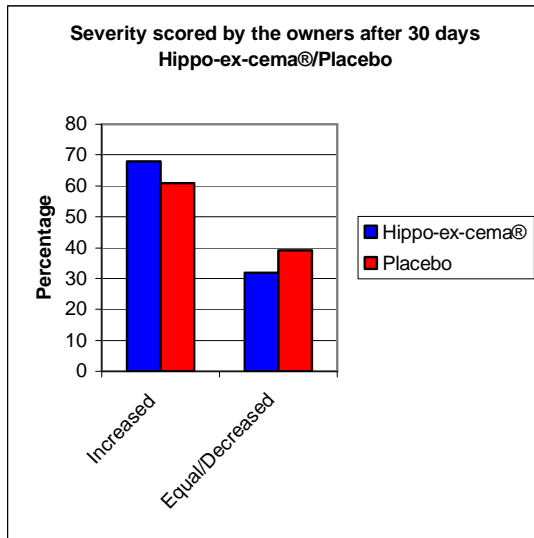


Figure 2: Change in score after 30 days of treatment according to the owners

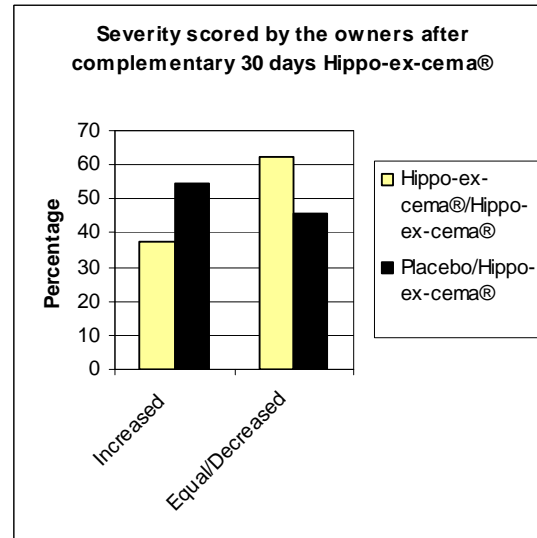


Figure 3: Change in score between 30 and 60 days of treatment according to the owners

Investigators

The investigators also scored the horses for their severity of IBH. Because the investigators only took photographs of the horses at the beginning of the study and after the first period, data are only available from this period.

According to the investigators, 15 out of 23 horses receiving placebo deteriorated and 8 of them improved or remained equal. This means that 65.2% of the horses deteriorated. From the horses receiving Hippo-ex-cema®, 9 out of 25 deteriorated and 16 improved or remained equal. This means that 36% of the horses deteriorated. When using the Chi-square test, a significant difference was found between the test and placebo group (Chi-square is 4.094 and $p = 0.0431$) (Table 8 and Figure 3, 5 till 12).

Severity scored by the investigators	Increased	%	Equal/Decreased	%	Total
Hippo-ex-cema®	9	36.0	16	64.0	25
Placebo	15	65.2	8	34.8	23
Total	24		24		48

Table 8: Severity scored by the investigators after 30 days Hippo-ex-cema® or placebo

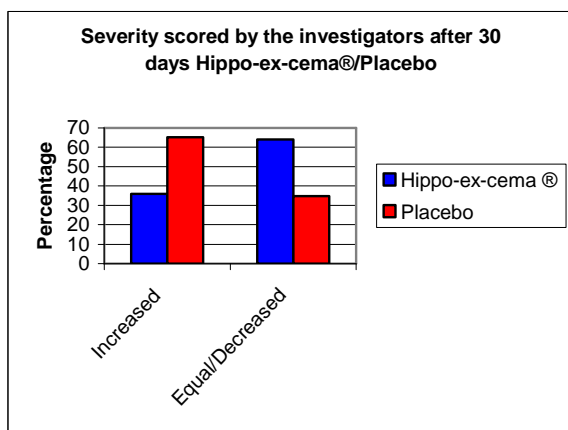


Figure 4: Change in score after 30 days of treatment according to the investigators



Figure 5 and 6: contestant number 21 (verum). Bald in the ventral neck (40 by 20 cm) on day 1 of the research, the second photograph shows that the spot was completely overgrown by hair after 30 days Hippo-ex-cema®.

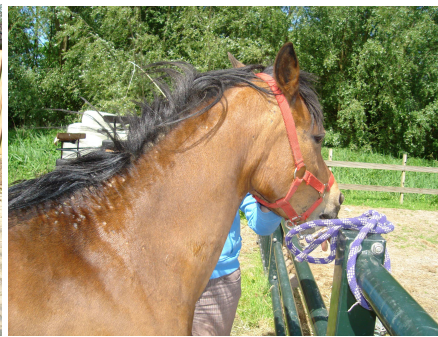


Figure 7 and 8: contestant number 10 (verum). This pony deteriorated during the first 30 days. The first photograph shows before, the second after 30 days. Hair loss because of scaling and bold spots can be seen.



Figure 9 and 10: contestant number 10 (verum). The first photograph is also after 30 days, the second photograph shows the same spot after another 30 days treatment with Hippo-ex-cema®.



Figure 11 and 12: contestant number 43 (placebo). This Shetland pony showed no signs of IBH during the first visit, unfortunately he deteriorated dramatically, even after the complementary 30 days Hippo-ex-cema®. This can be seen in the last photograph.

Weather conditions

The research was done during the months April-June 2009 and during all three months it was excellent weather for *Culicoides spp.* to survive. The ambient temperature is of great importance to the activity, longevity and survival to adulthood. Activity in *Culicoides spp.* is inhibited by ambient temperatures below a species-specific minimum (Van der Rijt, 2007). *Culicoides spp.* do not fly when it is windy or raining, or in direct sunlight. (Braverman, 1988; Van Grevenhof et al, 2007).

The weather conditions were reported by the Royal Dutch Meteorological Institute. During these three months mean temperatures were higher than long term averages. The mean temperature in April was 12.2 degrees Celsius (8.3 average), in May 13.9 degrees Celsius (12.7 average) and in June 15.6 degrees (15.2 average). Minimum temperatures were measured 2.4 in April; 2.4 in May and 4.5 degrees Celsius in June and maximum temperatures were 23.7; 25.3 and 26.7 degrees Celsius respectively. Total hours of sunshine were also higher than long term means, respectively 226 (162) in May, 248 (209) in May and 249 (192) in June. April and June were also rather dry months, less rain had fallen compared to previous years with 22 mm (44 mm) rain in April and 55 mm (71 mm) rain in June. In contrary to those two months, May was a relative wet month, with rain fall of 67 mm in stead of 57 mm measured over the past few years. These excellent weather conditions for *Culicoides* mean that it was expected that all patients (either in the verum or in the placebo groups) would deteriorate in signs of IBH.

Discussion

The severity of Insect bite hypersensitivity scored by the investigators showed a significant difference after 30 days. There were few horses that improved dramatically after receiving the supplement. Open wounds closed after a month and itch was reduced until almost absent. Some broken hairs were already growing again and bald spots were already overgrown. On the other hand, there were also some horses that did not respond to the supplement at all. The amount of itching and the severity of IBH remained almost the same in those horses. In the scores of severity of IBH, made by the investigators, also lots of horses got worse during the experiment. This was to be expected prior to the study, because we started early in the *Culicoides spp.* season around the period the first symptoms of IBH appeared. This automatically means that most horses scored very low the first time. For this reason we also considered an equal score as a positive result.

In contrary to the results of the investigators, both results (after 30 and 60 days) from the owners were not significantly different between the verum and the placebo groups. These results can be argued, because the owners had no measure for the amount of score they gave. They also had to make a score between 1 (slight) and 10 (severe) for the severity of IBH, but every owner had a different opinion for severity, so these scores are rather subjective. Some owners had never seen another IBH horse than their own, so if this horse had a slight form of IBH, they already considered it to be very worse. Besides, we noticed that the owners score didn't always match the appearance of the horse. They would rather go for a slight increase in score than an equal/decreased score even if the horse improved. The investigators scored the horses after visiting all 50 horses. They were able to compare the horses and give the same score to horses with the same injuries. Horses with open wounds scored automatically higher than horses which only lost (part of) manes and tail.

Compared to the last years study done with Hippo-ex-cema[®] (August/September 2008, De Raat et al 2008, report for research internship at Utrecht University) the time plan for this research was better: this study started in April, at the very beginning of the *Culicoides spp.* season. The previous investigation started in August, almost at the end of the season. This could be one of the reasons no significant difference was found between the two groups. The horses could be already suffering from heavy itch and severe lesions, so the supplement could not give any release. In this research, we started early in the *Culicoides spp.* season, so the supplement was already given when the first itch appeared. The supplement might have a better preventive then curative working.

The supplement was also tested for a longer period than in the 2008 project. In the study of De Raat (2008, report for research internship at Utrecht University) the supplement was tested for 21 days and that is rather short. In many research projects supplements are tested between 6 and 12 weeks (Craig et al 1997, Friberg et al 1999, Khol-Parisini 2007). In this study we started with 30 days Hippo-ex-cema[®] or placebo, followed by 30 days Hippo-ex-cema[®]. So, 24 of the original 50 horses received Hippo-ex-cema[®] for 60 days. Unfortunately, also after these 60 days no significant difference was found between the two groups considering the score of the owners, but the tendency of the results is better after 60 days than after 30 days. Maybe if the investigators also scored the horses after 60 days, better results would be received and a judgement could be made.

During the investigation we already mentioned that the owners were afraid to change their scores. If they had already given a moderate or severe score, they wouldn't give a slighter score effortless if improvement proceeded. On the other hand some owner's noticed improvement while the investigators did not notice any improvement. This may be contributed to the placebo-effect. The investigators scored the horses after visiting all 50 horses and so they could compare them to each other for equal scoring, having a better standard. The significant difference found in the scores from the investigators is for this reason thrust worthier than in case of the same result from the questionnaires filled in by the owners.

All horses competing in this experiment received the same amount of supplement. Every horse (from Shetland pony till warm blood) received a spoon with 5 grams of powder and 10 ml of oil a day. In other studies, supplements given are mostly weight dependant (Craig et al 1997, Khol-Parisini et al 2007, 'O'Neill et al 2002). If we consider this, the small ponies received the greatest amount of supplement per body weight, so they might better react to the supplement. The results of the test show contrary signs. There are both Shetland ponies and warm bloods that react positive to the supplement. But there are also Shetland ponies as well as warm bloods that didn't react at all to the supplement. It seems

that the amount of supplement given to the competing horses has no influence at all to the result of the study.

The horses all reacted very different to the supplement and some horses improved/remained equal while others deteriorated. This dispersal may be one of the reasons why the results from the owners were not significant and the scores from the investigators were nevertheless significant. Further investigation is necessary to make any conclusion why some horses react positive to the supplement and others do not.

Another weak point of this study contains the gift of the supplement. The horse owner itself gave the supplement to the horse. The investigators should have great trust in the owners, to know they gave the supplement strictly according to the schedule. Unfortunately, some misunderstanding took place in this study and horse owners didn't feed the exact dosage of the supplement. Further, mares should be given a double dosage when in heat. Many mares didn't show when they were in heat, so according to the manufacturer, many horses received an under dosage at least during a couple days of the study.

In future research with this supplement, the supplement can be tested for the entire season. It may give a better idea how horses react on the supplement over the entire summer, when symptoms of IBH are aggravating. If in future research horse owners again are asked to fill in a questionnaire, the questionnaire should be adjusted. The score the owners should give should be better categorized. The scoring should be more objective and all horses with same lesions should get the same score for severity of IBH. If scoring is more objective, results may also be thrust worthier.

In conclusion: The supplement Hippo-ex-cema[®] tested in this study, showed significant positive effect on the severity of IBH according to the investigators. No significant change was found for the severity of IBH according to the owners. More research has to be done to discover why some horses respond positively to the supplement and others did not.

Acknowledgements

We would like to thank all the horse owners for their co-operation in this study and for letting us use their horses. We would like to thank dr. Ruud Rulkens for advices and financial support.

Further we would like to thank Jan van den Broek for the statistical analysis and dr. Marianne M. Sloet van Oldruitenborgh-Oosterbaan for the help in organising the study and writing the report.

References

- Anderson GS, Belton P and Belton EM. A population study of *Culicoides Obsoletus* Meigen (diptera: Ceratopogonidae) and other *Culicoides* species in the fraser valley of British Colombia. *The Canadian Entomologist* 125: 439-447.
- Anderson GS, Belton P and Kleider N. *Culicoides obsoletus* (Diptera: Ceratopogonidae) as a causal agent of *Culicoides* hypersensitivity (sweet itch) in British Colombia. *Entomological society of America* 1991 (28) 5: 685-692.
- Anderson GS, Belton P and Kleider N. Hypersensitivity of horses in British Colombia to extracts of native and exotive species of *Culicoides* (Diptera: Ceratopogonidae). *Journal of Medical Entomological* 1993 (30) 4: 657-663.
- Anderson GS, Belton P and Kleider N. The hypersensitivity of horses to *Culicoides* bites in Britisch Colombia. *Canadian Vet J* 1988 (29): 718-723
- Baker KP and Quinn PJ. A report on clinical aspects and histopathology of sweet itch. *Equine veterinary journal* 1978 (10) 4: 243-248.
- Bello TR and Allen T. The use of MicroLactin for inflammatory conditions in equine veterinary practice. *Journal of Equine veterinary science* 2005 (25): 380-382.
- Bielory L, Chiamonte L, Ehrlich P and Field J. *Journal of asthma* 2003 (40): 47-53.
- Bjornsdottir S, Sigvaldadottir J, Brostrom H, Langvad B and Sigurdsson A. Summer eczema in exported Icelandic horses: influence of environmental and genetic factors. *Acta Veterinaria Scandinavica* 2006 (48) 3.
- Braverman Y. Nematocers (*Ceratopogonidae*, *Psychodidae*, *Simulidae* and *Culicidae*) and control methods. *Revue Scientifique et technique* 1994 (13) 4: 1175-1199.
- Craig JM, Lloyd DH and Jones RD. A double-blind placebo-controlled trial of an evening primrose and fish oil combination vs. hydrogenated coconut oil in the management of recurrent seasonal pruritis in horses. *Veterinary dermatology* 1997 (8): 177-182.
- De Raat IJ, Van den Boom R, Van Poppel M and Sloet van Oldruitenborgh-Oosterbaan MM. The effect of a topical insecticide containing permethrin on the number of *Culicoides* midges caught near horses with and without insect bite hypersensitivity in the Netherlands. *Tijdschrift voor diergeneeskunde* 2008 (133) 20 :838-842.
- Fadok V and Greiner EC. Equine insect hypersensitivity: skin test and biopsy results correlated with clinical data. *Equine veterinary journal* 1990 (22) 4: 236-240.
- Friberg CA and Logas D. Treatment of *Culicoides* hypersensitive horses with high-dose n-3 fatty acids: a double-blinded crossover study. *Veterinary dermatology* 1999 (10): 117-122.
- Halldorsdottir S and Larsen HJ. An epidemiological study of the summer eczema in Icelandic horses in Norway. *Equine Veterinary Journal* 1991 (23): 296-299.
- Khol-Parisini A, van den Hoven R, Leinker S, Hulan HW and Zentek J. Effects of feeding sunflower oil or seal blubber oil to horses with recurrent airway obstruction. *The Canadian journal of veterinary research* 2007 (71): 59-65.

Kleider N and Lees MJ. Culicoides hypersensitivity in the horse: 15 cases in Southwestern British Columbia. Canadian Veterinary Journal 1984 (25): 26-32.

Kolm G, Knapp E, Wagner R and Klein D. Lactoferrin, a glycoprotein with immunomodulatory and mast cell stabilising properties, in skin of horses suffering from Culicoides hypersensitivity. Veterinary science 2007 (83): 165-170.

Kolm-Stark G and Wagner R. Intradermal skin testing in Icelandic horses in Austria. Equine veterinary journal 2002 (34) 4: 405-410.

Laitinen K and Isolauri E. Management of food allergy: vitamins, fatty acids or probiotics? European journal of gastroenterology & hepatology 2005 (17): 1305-1311.

O'Neill W, McKee S and Clarke AF. Flaxseed (Linum usitatissimum) supplementation associated with reduced skin test lesional area in horses with Culicoides hypersensitivity. The Canadian Journal of veterinary research 2002 (66): 272-277.

Quinn PJ, Baker KP and Morrow AN. Sweet itch: responses of clinically normal and affected horses to intradermal challenge with extracts of biting insects. Equine veterinary journal 1983 (15) 3: 266-272.

Riek RF. Studies on allergic dermatitis (Queensland itch) of the horse: the aetiology of the disease. Australian journal of agricultural research 1954 (5): 109-129.

Schurink A, Van Grevenhof EM, Ducro BJ and Arendonk JAM. Heritability and repeatability of insect bite hypersensitivity in Dutch Shetland breeding mares. Journal of Animal science 2009 (87): 484-490.

Stark G, Schneider B and Gemeiner M. Zinc and copper plasma levels in Icelandic horses with Culicoides hypersensitivity. Equine veterinary journal 2001 (33) 5: 506-509.

Steinman A, Peer G and Klement E. Epidemiological study of Culicoides hypersensitivity in horses in Israel. Veterinary record 2003 (152): 748-751.

Van den Boom R, Ducro B and Sloet van Oldruitenborgh-Oosterbaan MM. Identification of factors associated with the development of insect bite hypersensitivity in horses in the Netherlands. Tijdschrift voor diergeneeskunde 2008 (133) 13: 554-559.

Van der Rijt R, Van den Boom R, Jongema Y and Oldruitenborgh-Oosterbaan MM. Culicoides species attracted to horses with and without insect hypersensitivity. Veterinary Journal 2008 (178): 91-97.

Van Grevenhof EM, Ducro B, Heuven HC and Bijma P. Identification of environmental factors affecting the prevalence of insect bite hypersensitivity in Shetland ponies and Friesian horses in the Netherlands. Equine Veterinary Journal 2007 (39): 69-73.

Wilson AD, Heesom KJ, Mawby WJ, Mellor PS and Russell CL. Identification of abundant proteins and potential allergens in Culicoides Nubeculoses salivary glands. Veterinary immunology and immunopathology 2008 (122): 94-103.

[Http://www.wshhnp.com/hippo-ex-cema_nl_bijsluiter.html](http://www.wshhnp.com/hippo-ex-cema_nl_bijsluiter.html)

Van den Windt, Arie. WSH Health products, inventor of Hippo-ex-cema®.