The effect of a supplement containing sunflower oil, vitamins, amino acids, and peptides on the severity of symptoms in horses suffering insect bite hypersensitivity

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SUMMARY

Insect bite hypersensitivity (IBH) is most often caused by *Culicoides spp.*, although other insect species are also thought to play a role in causing this disease. The disease has been reported to occur in most countries, with a prevalence of up to 28% in some countries in some breeds. The only truly effective therapy currently available is to prevent horses from coming into contact with *Culicoides* midges, which can be achieved by stabling horses during certain times of the day or by covering them with insect blankets.

The effect of a new dietary supplement containing vitamins, polypeptides, and amino acids on IBH severity was tested at the start of the *Culicoides* season (April 2009). Fifty horses participated in this placebo-controlled, double-blinded study: 25 horses received the supplement and 25 received placebo for 30 days; thereafter all horses received the supplement for a further 30 days. The investigators scored the severity of IBH in all horses, based on clinical evaluation and digital photographs taken before and after the first 30 days of the trial. The owners were asked to assess the severity of the signs prior to the investigation and after the first and second 30-day periods. For each period, IBH clinical symptoms were classified as increased, decreased, or the same.

Results showed that investigator-assessed symptoms became worse in more horses receiving placebo than in horses receiving supplement, indicating a positive effect of the supplement. However, there were no treatment-group differences when symptom severity was scored by the horse owners. No side-effects were observed.

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SAMENVATTING

Het effect van een supplement met zonnebloemolie, vitaminen, aminozuren en peptiden op de ernst van symptomen van paarden met staart- en maneneczeem

Staart- en maneneczeem (SME) wordt voornamelijk veroorzaakt door Culicoides spp. *(knutten), maar andere insectensoorten spelen mogelijk ook een rol bij het ontstaan van deze aandoening. De aandoening wordt wereldwijd in diverse landen beschreven met prevalenties oplopend tot 28 procent. Op dit moment is de enige effectieve therapie het voorkomen van contact van paarden met* Culicoides spp. *Dit kan bereikt worden door het consequent opstallen van paarden of door het gebruik van een eczeemdeken. Aan het begin van het knuttenseizoen (april 2009) werd 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 vitaminen, 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 waarin alle paarden het echte supplement toegediend kregen. De onderzoekers hebben aan de hand van klinische evaluatie en foto's de paarden gescoord op de ernst van de SME voor aanvang van de proef en na 30 dagen. De eigenaren werden verzocht om driemaal de ernst van SME te scoren: voor aanvang van de proef, na 30 en na 60 dagen. Voor iedere fase van de studie werd nagegaan of de ernst van de symptomen toenam, afnam of hetzelfde bleef.*

De scores van de onderzoekers lieten zien dat bij paarden in de placebogroep significant vaker een verslechtering van SME symptomen optrad, hetgeen wijst op een positief effect van het supplement. Er werden geen negatieve bijwerkingen gevonden van het supplement. In de beleving van de eigenaar was er geen verschil tussen paarden behandeld met het supplement en de placebo.

INTRODUCTION

Insect bite hypersensitivity (IBH), or sweet itch, is a common skin disorder in horses and is the most common cause of summer season pruritus. It is a chronic, seasonally recurrent, allergic dermatitis, and clinical signs are usually present between April and October, when the *Culicoides* species are most active (1, 17). The first symptoms include papules/nodules and sensitization of the skin, followed by pruritus, scaling, hair loss, hyperkerato-

sis, crusting, open wounds, inflammation, and/or alopecia (1, 2, 10). More than 40% of affected horses have signs around the mane and tail, 15% around the tail only, and about 20% have signs on the ventral abdomen (17).

The disease is reported to occur worldwide, with the incidence varying between countries. Anderson et al (1) found a prevalence of 26% in British Colombia (Canada) and Steinman et al (16) reported a prevalence of 28% in Israel. No exact data are available for the Netherlands, but a recent study estimated the IBH prevalence to be about 6.8% in horses and 8.4% in ponies (Schoot et al, submitted for publication). The prevalence is also breed dependent and an incidence of 8-9% and 18% was reported for Shetland pony and Friesian mares, respectively, in the Netherlands (14, 19). Even within a country or region the occurrence of IBH may vary considerably, depending on the suitability of the surroundings for *Culicoides*. In the Netherlands, IBH is most prevalent in the east (Twente, Achterhoek) and south (Brabant, Limburg).

The insects responsible for causing IBH are usually *Culicoides* spp., but other species, including Simulium, Tabanus and Stomoxys spp, may also cause allergic dermatitis. Many different *Culicoides* spp. exist, and the species causing IBH seems to vary between countries, with varying cross-reactivity between different *Culicoides* spp. Recent studies have identified *Culicoides obsoletus* (94.1%) and *Culicoides pulicares* (5.81%) as being the most common species found in the vicinity of horses in the Netherlands (7, 18), and intradermal testing has confirmed that these two species also cause IBH in horses in the Netherlands (15). No effective cure has been found for IBH, and the best way to control symptoms remains the avoidance of specific allergen contact. This can be achieved by stabling horses continuously, or at least during sunrise and sunset, when *Culicoides* spp. are most active (18), or by the use of insect blankets and/or the application of insecticides and repellents.

Previous research has produced conflicting results regarding the efficacy of feed supplements in reducing IBH symptoms in horses. O'Neill et al (12) reported a reduced mean skin test response to *Culicoides* in 6 horses after 6 weeks of flaxseed supplementation in a placebo-controlled, double-blind trial, while Craig et al (6) found no difference in clinical status between horses receiving hydrogenated coconut oil or a fatty acid supplement containing evening primrose and fish oil. Friberg et al (8) compared the clinical efficacy of α-linolenic acid and corn oil (placebo) in 19 horses with IBH in a double-blind cross-over study lasting 18 weeks. The authors found no significant difference between the test and placebo groups, although owners stated that the horses had improved during the test period. Four horses suffering from IBH appeared to be less severely affected when fed a supplement based on the milk of hyperimmunized cows before and during the insect season (3), although the study did not include a control group.

Dietary nicotinamide supplementation in high doses is reported to inhibit the antigen-induced release of histamine in vitro and in vivo (20), and this may prove helpful in the management of IBH in horses. In humans, vitamins are often used because of their antioxidant properties and their value in various allergic diseases has been investigated. For example, vitamin E has been shown to be associated with lower serum IgE concentrations, but contradictory results mean there is insufficient evidence to support its use in allergic disease (11). A study by Bielory et al (4) revealed that serum vitamin C, vitamin B6, and magnesium levels were low in patients with asthma, but the results obtained with supplementation were ambiguous.

The aim of the present study was to investigate the effect of a feed supplement[a] based on sunflower oil and containing vitamins, polypeptides, and amino acids on the severity of clinical signs of IBH in horses.

MATERIAL AND METHODS

Case selection

Fifty horses that had had moderate-to-severe IBH for at least the previous 2 years were recruited to the study (Table 1). The second inclusion criterion was that the horses should be housed and managed in exactly the same way as in previous year(s). Horses that had been treated with glucocorticoids in the year of the study were excluded.

Supplement

The experimental supplement[a] contained sunflower oil and polypeptides, omega-3 fatty acids, omega-6 fatty acids, and vitamins E and A (http://www.wshhhp.com/hippo-excema_nl_bijsluiter.html), and was mixed, prior to administration, with a powder containing vitamins B1, B2, B3, B6, B9, B12, C, and E, amino acids, choline, magnesium, and patented peptides. The effect of the supplement was compared with that of placebo, which consisted of the same oil with wheat flour without added amino acids, polypeptides, or vitamins.

Experimental procedure

The supplement was administered for 60 days (April-June 2009) in a double-blind, placebo-controlled study. During the first 30 days, 25 horses (supplement group) received the supplement and the other 25 horses (placebo group) received the placebo. Horses in the supplement group received the oil (10 ml) and powder (5 g) mixture twice a day during the first 10 days and once daily for the remaining 20 days, as advised by the manufacturer. The supplement and the placebo were mixed by the owner and added to the horse's feed. All horses received the supplement during the second period of 30 days. The investigators visited all participating horses at the start of the trial and instructed the owners on how to administer the supplement (and placebo). Both investigators and owners were blinded as to whether horses received the placebo or the supplement.

a Hippo-Ex-Cema® (W.S.H. Horse Health products, Biddinghuizen, the Netherlands).

Before and after the first treatment period, the investigators evaluated all horses clinically and made digital photographs using 5 standardized views: full-body photographs from both sides and from behind, and close-up views of the head and neck from the left and right sides. In addition, detailed photographs were taken of any lesions that were present. All horses were visited twice (before and after 30 days of treatment), and a clinical IBH score was calculated (0 to 10) on the basis of the clinical features and the photographs, with scores being awarded for the number of skin lesions, and evidence of skin inflammation, alopecia, scaling, crusting, and hair loss. For each horse it was determined whether the IBH score had increased, decreased, or remained the same. A score that remained the same or that had decreased (the horse had fewer lesions) was considered to be 'positive' as it was expected that all horses would have more symptoms and lesions during the *Culicoides* season. If the score increased

horse	breed	age	gender	verum or placebo	author score 1	author score 2	authors	owner score 1	owner score 2	owner score 3
1	KWPN	8	gelding	verum	2	1	p	5	2	2
2	Fjord	17	mare	placebo	2	2	p	2	4	3
3	New Forest	6	mare	verum	1	3	n	3	6	2
4	Ijslandic	19	mare	placebo	4	5	n	7	8	7
5	Ijslandic	18	mare	placebo	3	4	n	6	7	7
6	Welsh	18	mare	verum	3	4	n	2	6	7
7	KWPN	23	mare	placebo	1	1	p	3	3	5
8	Welsh cross	6	mare	verum	2	2	p	1	2	5
9	Welsh cross	3	mare	placebo	1	2	n	1	3	4
10	Welsh x New F.	7	mare	verum	1	7	n	1	6	3
11	Friesian	9	mare	placebo	1	3	n	2	2	1
12	Merens	15	mare	verum	2	2	p	1	2	3
13	KWPN	7	gelding	placebo	1	2	n	1	3	3
14	Friesian	7	gelding	verum	1	1	p	1	2	2
15	pony	6	gelding	placebo	1	2	n	3	2	8
16	Shetland	10	mare	verum	2	2	p	5	5	lost
17	Welsh	6	gelding	verum	2	2	p	1	2	2
18	Welsh cross	18	mare	placebo	2	3	n	3	6	7
19	KWPN	4	gelding	verum	1	2	n	3	6	3
20	KWPN	2	stallion	placebo	2	2	p	2	2	4
21	Shetland	9	mare	verum	5	1	p	8	5	4
22	Haflinger	18	mare	placebo	2	2	p	5	3	8
23	NRPS	13	gelding	verum	2	4	n	1	6	8
24	Friesian	8	gelding	placebo	2	2	p	3	4	8
25	pony cross	10	gelding	verum	2	2	p	1	4	6
26	Shetland	11	mare	placebo	2	lost		2	lost	lost
27	Ijslandic	12	mare	verum	2	2	p	3	5	8
28	Arab cross	24	gelding	verum	1	1	p	2	6	6
29	Connemare	16	mare	placebo	1	lost		1	lost	lost
30	Connemare	12	mare	placebo	3	3	p	2	1	2
31	Shetland	8	mare	verum	3	4	n	6	6	4
32	Shetland	12	mare	verum	2	2	p	3	3	3
33	Shetl. x Welsh	6	stallion	placebo	3	7	n	1	5	2
34	warmblood	23	gelding	placebo	2	2	p	2	2	1
35	KWPN	8	mare	verum	2	2	p	3	3	2
36	Welsh	10	mare	placebo	1	2	n	3	3	3
37	Shetlander	27	gelding	verum	3	2	p	2	1	2
38	Tinker	9	gelding	placebo	1	2	n	1	6	7
39	Dartmoor	15	mare	verum	2	3	n	3	6	4
40	Welsh	5	gelding	verum	2	5	n	2	6	4
41	Haflinger	9	mare	placebo	3	4	n	2	6	2
42	Fjord cross	6	gelding	verum	2	3	n	2	8	9
43	Shetland	14	stallion	placebo	4	9	n	4	8	9
44	KWPN	22	mare	verum	3	2	p	4	4	3
45	Shetland	8	mare	placebo	2	2	p	2	5	7
46	New Forest	11	mare	verum	2	2	p	1	2	3
47	KWPN cross	10	mare	placebo	1	2	n	2	1	1
48	New Forest	13	mare	placebo	1	2	n	2	4	8
49	Lusitano	13	gelding	verum	4	4	p	1	2	2
50	Friesian	19	mare	placebo	3	4	n	6	7	lost

lost
positive result
negative result

Table 1: Breed, gender, and age of the 50 horses included in the study, and the IBH scores of investigators and owners, before (score 1), after 30 days (score 2), and after 60 days (score 3); p = positive (score the same or lower) and n = negative (score higher).

(more lesions), the response to the supplement was considered to be 'negative'.

Additionally, owners were asked to score the severity of IBH before treatment and after the first and second treatment periods, using a scoring system based on the presence of pruritus, broken hairs and scaling, bald patches/hair loss/pigmentation, crusting, open wounds, inflammation, and thickened/irritated skin. These scores were compared to determine whether IBH severity had increased, decreased, or remained the same. A positive or negative result was determined as described above.

Weather conditions

Prevalent weather conditions affect *Culicoides* activity (5, 17) and may have influenced this trial. Therefore, the weather conditions for the duration of the study, as provided by the Royal Dutch Meteorological Institute, were recorded.

Statistical analysis

Statistical analysis was performed using the SPSS software package (version 16). Differences between the treatment and placebo groups were investigated using a Chi-square test. A P-value < 0.05 was considered to be statistically significant.

RESULTS

Horses

The mean age of the 50 participating horses (30 mares, 17 geldings, 3 stallions) was 11.8 years (range 2-27 years): 11.3 years in the treatment group and 12.4 years in the placebo group (n.s.). The horses were of different breeds (Table 1). Of the 50 horses, 48 completed the first treatment period and 46 completed both treatment periods (22 receiving placebo; 24 receiving supplement). During the first treatment period, two horses were withdrawn by their owners. One horse (placebo) developed extreme pruritus, and the owner decided to sell the horse to someone living in an area with a very low IBH incidence. The other horse (placebo) was withdrawn because the owner decided to use an insect blanket for the first time. During the second treatment period, one horse (supplement) was also withdrawn because the owner used a blanket and one horse (placebo) was withdrawn because the horse refused to eat the supplement.

Symptom severity

The results obtained by the investigators are shown in table 1 and figure 1. A significantly (P=0.043) larger proportion of horses in the placebo group (65.2%) than in the supplement group (36%) had more severe symptoms after the first 30-day treatment period. Some horses in the supplement group showed a significant improvement in symptoms after receiving the supplement (open wounds closed and pruritus almost completely disappeared). There were also horses in which the pruritus and the severity of IBH remained the same or even became worse. Some horses improved in the second treatment period when

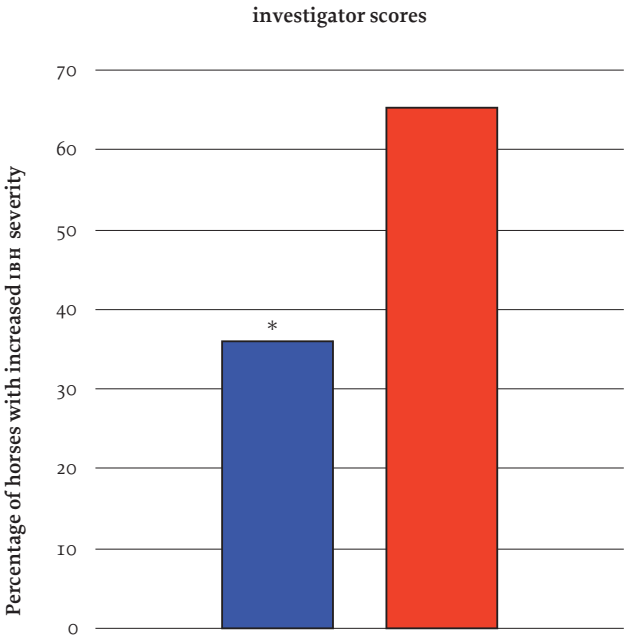

Figure 1. The percentage of horses in which IBH severity, as assessed by the investigators, increased during the first 30 days of the trial (blue= supplement, red = placebo).
* P<0.05

they received the supplement after a period of placebo (figures 2a and 2b).

The results of the owner IBH scores are also presented in table 1. After the first treatment period, the IBH severity score increased in 68% of horses in the supplement group and in 61% of horses in the placebo group. After the second treatment period, the IBH severity score increased in 38% of horses in the supplement group and in 55% of horses in the placebo group. Neither of these differences was statistically significant. Owners did not report any (negative) side-effects after use of the supplement.

Weather conditions

During the 3 months of the study, the mean temperature (reported by the Royal Dutch Meteorological Institute) was higher than the long-term average. The mean temperature was 12.2° Celsius (8.3°C average) in April, 13.9° C (12.7°C average) in May, and 15.6°C (15.2°C average) in June. Minimum recorded temperatures were 2.4°C in April, 2.4°C in May, and 4.5°C in June, and maximum temperatures were 23.7°C, 25.3°C, and 26.7°C, respectively. The total number of hours of sunshine was also higher than the long-term average, with 226 (162 average), 248 (209) and 249 (192) hours in April, May, and June, respectively. April and June were rather dry months, with less rain (22 and 55 mm) than in previous years (44 and 71 mm). May was a relatively wet month, with 67 mm of rain compared to the average of 57 mm measured over the previous few years. These results indicate that it was a 'good' year for *Culicoides* and that worsening of IBH symptoms was to be expected during the study.

Figure 2a-2b. Horse number 10 after one month placebo (a) and after 1 month treatment with the supplement tested (b).

DISCUSSION

The study started in April, around the time when the first symptoms of IBH normally appear, and thus the severity of IBH symptoms would be expected to increase thereafter, as the population of *Culicoides* midges increased. Weather conditions during the study period suited *Culicoides* activity and so we expected that symptoms would become worse during the study. We therefore considered stable or fewer symptoms to be a positive result.

Contrary to the results reported by the investigators, the results reported by the owners after the first treatment period showed no difference in symptom severity between the supplement and placebo groups. The absolute scores of different owners are difficult to compare because most owners only had one horse suffering from IBH and no other horses that could serve as a reference. Further, the owners' score for the severity of symptoms before and after the first 30-day treatment period was probably less objective than that of the investigators, who also used photographs to score the lesions. In an attempt to overcome this problem, owners were asked to compare IBH severity before and after the first and second treatment periods. However, it was obvious that some owners' scores did not match the appearance of the horse at all. The lack of experience of owners in using a semi-quantitative scoring system is the most likely explanation for their inconsistent scoring. The investigators scored the horses, using their clinical experience and the photographs, and this facilitated consistent scoring of IBH symptoms and reduced the risk of a (unconscious) change in the rigour of the scoring method.

All horses received the amount of supplement (10 ml of oil and 5 g of powder daily) recommended by the manufacturer, regardless of the horse's weight. In other supplement studies, the amount of product given was usually adjusted to the horse's weight (6, 9, 12). However, the manufacturer of the supplement used in this study set the dose to be used, so that small ponies received a relatively greater dose of supplement than horses. If the effect were dose dependent, small ponies might have been expected to respond better to the supplement; however, there was no difference between Shetland ponies and Warmbloods.

Interestingly, individual horses reacted very differently to the test supplement: in some horses the IBH symptoms improved or remained the same, while in others the symptoms became worse. Further research is necessary to find out why individual horses reacted so differently to the supplement. Of course, many factors influence the severity of symptoms of IBH, including the number of *Culicoides*, weather conditions, and management factors. However, in the present study the use of a randomized double-blind approach should allow a reliable assessment of the effects of the supplement.

A potential weak point of the study is the fact that the supplement was administered by the horses' owners. However, all owners volunteered to take part in the trial, and the owners of horses with IBH tend to be very motivated to do anything they can to alleviate the symptoms of their horse(s). It is possible that owners might have adhered less strictly to the dosage regimen if they were under the impression that their horse was receiving the placebo product. However, the study was blinded (for the investigators and owners) and the authors did not have the impression that there was a lack of owner compliance in administering the supplement.

To optimize the investigation of a new supplement, it may be better to test the supplement during the entire *Culicoides* season and to make more regular veterinary assessments of the horses. This may provide more accurate information on how horses react to the supplement, as there are often short-term variations in IBH severity throughout the season and these fluctuations will have a greater impact on a trial of shorter duration. However, this was not possible in the present study because of time and financial constraints. It might also be worth investigating the possible preventive effect of the supplement by starting supplementation even earlier than in the present study, well before any symptoms are observed. In future studies it may also be better to standardize the scoring system used by owners by providing a more objective score system, such as that used in atopic humans or dogs (13). However, these score systems were too complicated to use in the present study.

Although the supplement appeared to stabilize or

reduce the severity of IBH symptoms compared with placebo, its mode of action is unknown. It also remains to be determined which ingredient, or combination of ingredients, is responsible for the observed effect. It is possible that the supplement somehow modulates the reaction of the immune system and thus the hypersensitivity reaction occurring in IBH. However, it cannot be excluded that consumption of the supplement somehow makes horses less attractive to *Culicoides* midges. This possibility could be investigated by trapping insects attracted to, and feeding on, horses treated with the supplement or placebo (7, 18).

In conclusion, the supplement tested in this study had a significant positive effect on the severity of IBH symptoms, as rated by the investigators, and no side-effects were observed. No significant change in IBH severity was reported by the horse owners. More research needs to be performed to identify factors determining why some horses responded and others did not.

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