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SCIENTIFIC NEWS
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THIS STUDY OPENS THE PERSPECTIVE OF THE USE OF AN ADAPTED FOOD SUPPLEMENT IN THE MANAGEMENT OF STOMACH ULCERS IN HORSES, BOTH FOR PREVENTING THEIR OCCURRENCE AS WELL AS FOR AVOIDING THEIR RECURRENCE.

Dr Emmanuelle van Erck



HPH

HIGH PERFORMANCE HORSES

SCIENTIFIC NEWS

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TWYDIL®

Editor: Pavesco AG – TWYDIL®
Elisabethenstrasse 54
CH-4010 Basel

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Photographers: T. Jones, Worlington, England
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Contents	Page
A MESSAGE FROM THE PRESIDENT	3
WHAT'S NEW IN THE PREVENTION OF GASTRIC ULCERS ?	4
AUSTRALIA, THE LAND OF OPPORTUNITY	10
NEW RESEARCH & DEVELOPMENT DIRECTOR	14
NEW RANGE OF HIGH QUALITY DIAGNOSTIC TOOLS	17
TWYDIL® ANTI-DOPING PRECAUTIONS FURTHER REINFORCED	22
OXIDATIVE STRESS AND ERYTHROCYTE MEMBRANE FLUIDITY IN EVENTING HORSES	24
DO THE LABELS COMPLY WITH WHAT IS REALLY IN A PRODUCT ?	30
OPTIMISATION OF HAEMATOLOGICAL PARAMETERS IN HEALTHY SPORT HORSES	34
QUESTIONS TO PROFESSOR PIERRE LEKEUX	37
SAUDI ARABIA: REMARKABLE ACHIEVEMENTS IN A SHORT PERIOD OF TIME	38
VITAMINS AND THEIR BIOAVAILABILITY ORALLY AND BY INJECTION	42
MICROARRAY (BIO-CHIP) : A FUTURE TOOL IN COMPETITION HORSES MONITORING ?	46
TWYDIL® PRODUCTS	50

A MESSAGE FROM THE PRESIDENT



Four new products scientifically introduced.

Existing formulations continually improved.

*Each product now submitted to three tests to ensure negative doping effect
before release onto the market.*

Important investments in applied research.

*Joint venture with a top class laboratory.
Personnel increased by extremely well-qualified staff.*

Interest expressed in future scientific developments such as the genome.

TWYDIL® reinforces its position as incontestable market leader.

Happy reading!

*Valère Henry
President*

WHAT'S NEW IN THE PREVENTION OF GASTRIC ULCERS ?

Dr Emmanuelle van Erck-Westergren, DMV, PhD



the middle of a competition when the pain became too acute.

Are ulcers a frequent problem in horses ?

Several studies carried out in different populations of horses agreed in demonstrating that the presence of ulcers in horses is far more frequent than one could imagine and that it is intimately linked to their way of living and their level of work. Horses that live out in pasture are at the lower end of the ulcer scale: less than 5% of grazing horses do suffer from ulcers. However the more intense their work becomes, the higher their chances are of having gastric ulcers (Figure 1). In endurance horses, for which feeding management is a key to ensuring competitiveness, the proportion of horses suffering from ulcers exceeds 65%. A study carried out in a population of young thoroughbreds in training revealed that the incidence of gastric ulcers could reach as high as 99% when they entered racing competition!

Can horses suffer from gastric ulcers ?

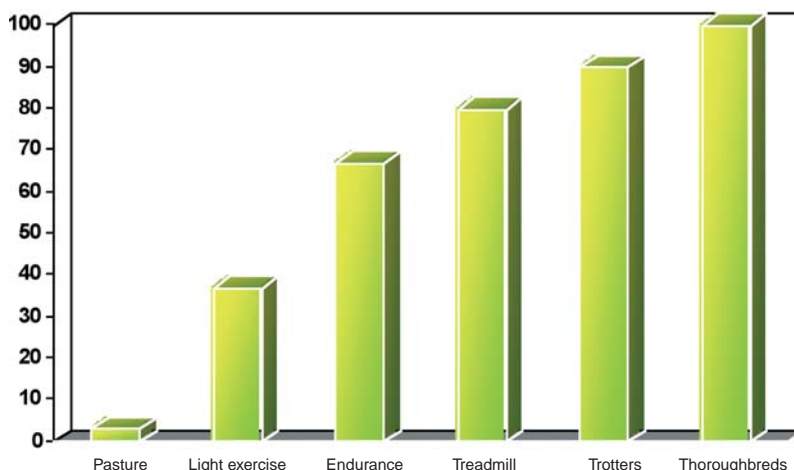
With the development of gastric endoscopy in the equine species, veterinarians have discovered that many horses suffered from gastric ulcers. In some cases, the presence of ulcers could not have been initially suspected, as some horses hardly expressed signs of discomfort or pain. The nature of clinical symptoms in a horse suffering from ulcers can be very variable and sometimes subtle, ranging from yawning during meals to clear colic in the most sensitive horse. Horses with ulcers can also grind their teeth or crib bite, others will frequently interrupt eating, leave grain in their manger or drink more than they used to.

Obviously gastric ulcers can have a detrimental impact on the performances of sport horses, mainly because of the pain they are likely to trigger during work but also during meals. The horses suffering from ulcers can loose state, they can show dull hair, weight loss, and lack in

muscular development although their work load is sustained. They can also come short of energy during work or even intolerant to exercise. Some horses have been known to stop in



Figure 1: Prevalence of gastric ulcers in horses



Why do horses develop gastric ulcers ?

Despite their size, horses have a proportionally small stomach. In their natural environment, horses spend their time eating grass, which transits rapidly through the stomach. Unlike other species whose stomach produces acid only during meal times, the equine stomach produces acid continuously. If a horse is fed only twice a day, and moreover if its diet is mainly composed of concentrates, food will be quickly digested and the horse will go on an empty, acid-producing stomach. Without food to digest, the acid can

irritate or even attack the walls of the stomach, eventually resulting in the occurrence of ulcers. Providing sufficient fibre source is an essential element in a horse's diet, the more so if the animal is kept in a box on a non-edible bedding (wood shavings or cardboard for instance). As fibres are digested more slowly, the stomach stays fuller for a longer period of time.

Several studies have shown that the incidence of ulcers is proportional to the level of work. Stress clearly can be a major promoting factor as it interferes with physiological mechanisms of mucosal protec-

tion and acid regulation. But stress is not the only likely cause. Physical activity brings the most acid-sensitive portion of the stomach's mucosa into prolonged contact with the gastric acid content. In a scientific study, horses were intubated with a catheter placed inside the stomach while exercising on a treadmill. The study showed that the acidity (determined by the measurement of intra-gastric pH) in the most sensitive portion of the stomach increased with increasing pace.

In humans, gastric ulcers are mainly caused by an infection with a bacteria named "Helicobacter pylori". Thus patients can be efficiently treated simply by taking antibiotics. These particular bacteria have rarely been isolated in horses, at least not in their active, pathogenic form.

What are the solutions?

Gastric ulcers seldom heal spontaneously in horses. To initiate a healing process, the horse's living and working conditions have to be completely reassessed. The most efficient measures are also the most radical: a prolonged stay out at pasture can allow an ulcer-affected horse to fully recover, as well as reducing significantly its workload, the frequency of competitions and transportations. Other simple measures can also contribute to relieve the horse and avoid a worsening of the ulcers like, for instance, dividing the horse's daily ration into smaller but more frequent meals and to ensure a permanent availability of forages. But how can owners or trainers apply such measures to their sport horses when the competition season is in full course?

There are some efficient medical solutions represented by anti-acid substances. These substances block totally or partially the acid production inside the stomach. Amongst these are found the H2-receptor inhibitors, like ranitidine or cimetidine, or the proton pump inhibitors like omeprazole. Omeprazole has become a standard treatment of gastric ulcers in foals and in horses suffering from extensive and/or deep mucosal ulcerations. The price of these substances can be a limiting factor in their use, as well as the fact that they cannot avoid the recurrence of ulcers, which are unfortunately quite fre-



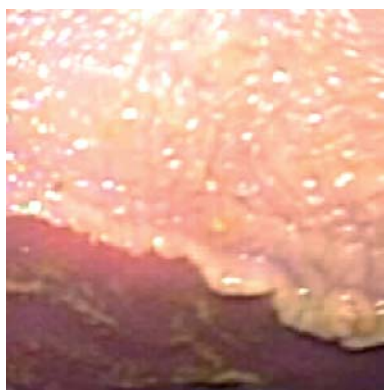


Photo 1 : Gastrosopic image at the level of the stomach margin (*margo plicatus*) in a healthy horse. The non glandular mucosa is located in the upper part of the picture (light pink), and the glandular portion in the lower part (dark red).



Photo 2 : Grade 2 ulcers (on a scale of 4) at the level of the stomach margin in a horse.

quent. The use of these substances is also under strict regulation for most competitions.

TWYDIL® has recently developed and tested two products to provide relief against low grade to moderate gastric ulcers in horses. The originality of these products is to provide a feed supplement to help solve the problem of ulcers in horses, thereby allowing them to continue competition. To validate the efficiency of these products, the study was undertaken in a population of horses at a high risk – working sport horses. Supplementation

1 contained fatty acids specifically formulated and supplementation 2* long chain fatty acids coupled with phospholipids on a soluble fibre base type chitosan glucosamine.

Thirty horses were selected for this study; these horses were either sport horses in training referred because they manifested one or several clinical signs suggestive of gastric ulcers, or race horses undergoing routine endoscopic examination (with or without clinical signs suggestive of gastric ulcers). In all horses, the diagnosis of ulcers was

confirmed by a thorough endoscopic exploration of the entire stomach (i.e. gastroscopy), from its entrance represented by the cardia, to the pylorus, which is the stomach's exit. A score was attributed to the severity of the lesions, according to a standardised international scoring system, with a scale ranging from 1 to 4. The TWYDIL® STOMACARE was only tested in horses suffering from ulcers with scores of 1 to 3. Score 4 ulcers being the most severe, a medical treatment was a prerequisite.

Horses underwent a first endoscopic examination and were then re-examined after 15 days and 1 month of supplementation. The horses were divided into 2 groups in a double-blind study : group A received 15 days of supplementation 1 then 15 days of supplementation 2*; group B received 15 days of supplementation 2* then 15 days of supplementation 1. The gastroscopy videos were subsequently and independently examined by two veterinarians unaware of either horses or supplementation.

Out of the 30 horses examined, 74% presented ulcerative lesions in the stomach; amongst those 57% had clinical signs suggestive of gastric ulcers (erratic appetite, lack of energy during work, dull coat, teeth grinding, etc...). On the other hand, 43% of these horses did not manifest any evident clinical signs, even though some had relatively high ulcer scores.

The stomach is composed of two parts: a non-glandular part that serves as a stocking area, and a glandular part that produces acid. In agreement with previous publications, it was noted that the most vulnerable area of the stomach was the non-glandular mucosa lining the glandular region. This danger zone, called the stomach's margin (*margo plicatus*), is the area the most likely to be exposed to the acid content of the stomach. All the horses examined in this study had lesions in that border area, sometimes associated with lesions in other portions of the stomach.

After 15 days of supplementation, a significant reduction of the severity of the lesions was observed. A decrease of 54 and 76% in ulcer scores was noted depending on the supplementation used (Figure 2).

TABLE 1. STUDY LAYOUT

Day 0 (J)	Clinical exam Initial gastroscopy	
	Group A	Group B
	Supplementation 1	Supplementation 2*
Day J+ 15 days (J15)	2 nd Gastroscopy	
	Supplementation 2*	Supplementation 1
Day J + 30 days (J30)	3 rd Gastroscopy	

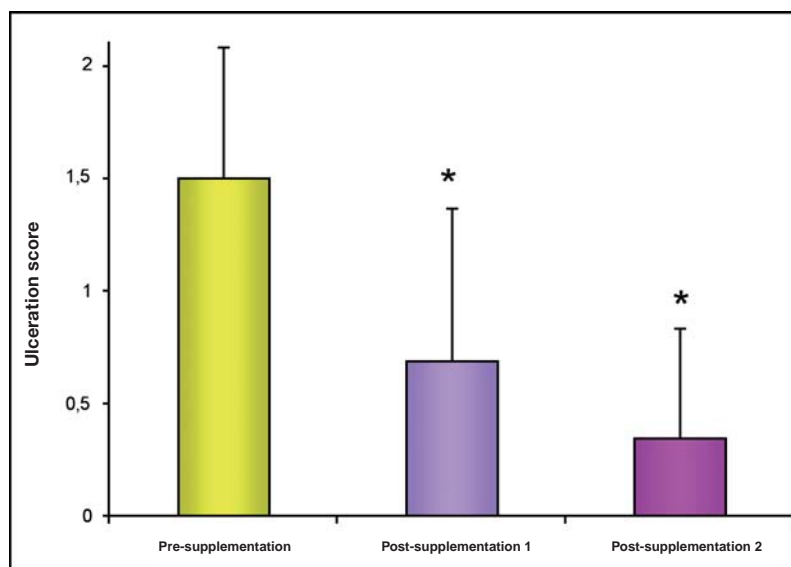


Figure 2 : Global effects of supplementation 1 and 2* on ulceration scores (presented as mean values and standard deviations). * signs indicate a statistical difference compared to pre-supplementation scores ($p<0,001$).

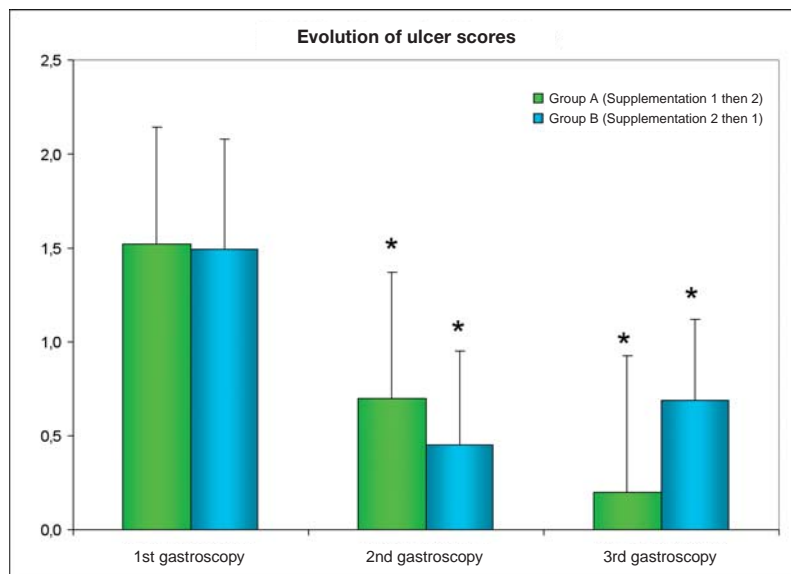


Figure 3 : Evolution of ulceration scores in groups A and B at each endoscopic examination. Group A received 15 days of supplementation 1 then 15 days of supplementation 2* ; Group B had 15 days of supplementation 2* then 15 days of supplementation 1. Data is expressed as means and standard deviations. * signs indicate a statistical difference compared to pre-supplementation scores ($p<0,001$).

After a month of supplementation, improvement was confirmed in all horses without exception, with a complete healing of the ulcers in 43% of the cases. Both supplementations showed a statistically significant efficiency in improving moderate ulcerative lesions of the equine

stomach, with a more manifest effect with the supplementation 2* in comparison to supplementation 1 (Figure 3). In two horses suffering from ulcers in the glandular portion of the stomach, a complete recovery was obtained as well.

When performing the gastroscopic



Photo 3 : Pathological hyperplastic reaction of the non glandular mucosa : the wall is thick and sloughs.

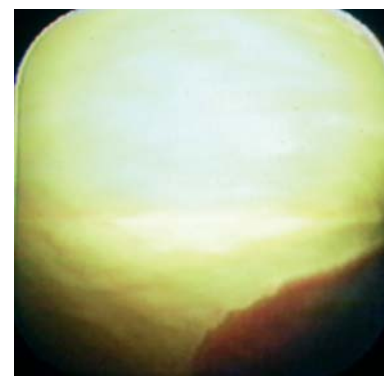


Photo 4 : Healing of the non glandular mucosa after a supplementation with TWYDIL® STOMACARE : the mucosa is uniformly regenerated.

recordings, a complete and homogeneous regeneration of the non-glandular mucosa was observed, promoting ulcer healing (Photos 3 and 4). The renewal of the non-glandular mucosa helped to improve the lesions of the stomach margin, that area of the stomach most exposed to the occurrence of ulcers. This regeneration phenomenon could be due to the high content in fatty acids of both supplementations tested. Fatty acids are constituents of the cellular membranes as well as of factors that are involved in mucosal protection and regulation of gastric acidity, such as several prostaglandins.

A high degree of satisfaction was also obtained from questioning the owners after one month of supplementation: 80% declared themselves satisfied with the supplementations undertaken. In 50% of the cases, they noted a marked improvement of spe-



cific clinical complaints (disappearance of colic signs during meals, increase in appetite, weight gain, etc...).

CONCLUSIONS

This study allowed to demonstrate the efficiency of supplementations 1 and 2* in the improvement, and in some cases the complete healing, of moderate gastric ulcer lesions in sport horses – a population particu-

larly at risk. The results obtained with supplementation 2* were superior to those obtained with treatment supplementation 1.

This study opens the perspective of the use of an adapted* food supplement in the management of stomach ulcers in horses, both for preventing their occurrence as well as for avoiding their recurrence.

Due to their controlled composition, these supplements can be given without risk up to the day of the competition.



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* Supplementation 2 = TWYDIL® STOMACARE



Dr Emmanuelle van Erck – Westergren, DVM, PhD

Dr Emmanuelle van Erck is a senior consulting veterinarian at the Equine Sports Medicine Centre (CEMESPO) at the University of Liège in Belgium. Her area of specialisation is athletic evaluation and investigation of poor performance in sport horses. Dr van Erck is also responsible of the consultations held at the Walloon race-track (Ghlin) where the CEMESPO has recently opened an outpatient clinic. The study has been lead in collaboration with Dr Brieuc de Moffarts, DVM, Ph. D.

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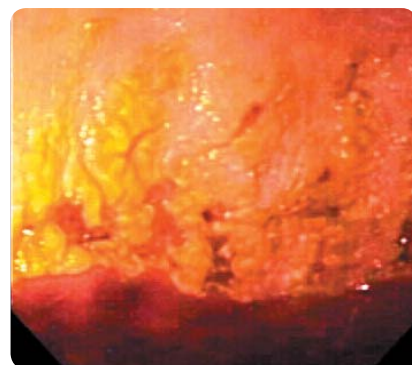
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AUSTRALIA, THE LAND OF OPPORTUNITY

Clive Harris



When asked to write this article about Horse Racing in Australia I felt privileged and a little inadequate to do it justice.

I have never held any position of authority within the industry and, for that matter up until now, ever been employed in the industry. My association has been predominately as a spectator and member of a local race club.

I am however a father, and have two children employed within the racing world.

My daughter married a young jockey from Kangaroo Island, Dwayne Dunn.

Dwayne, is a respected South Australian jockey who has not only ridden in the very biggest races Australia has to offer, but the young man has also been fortunate enough to ride in many parts of the world.

My eldest son, Brenton, became a fulltime trainer in recent times but, although he is yet to make his mark, I have every confidence that he will, given his enthusiasm, dedication and ability.

Australia prides itself on rewarding those with ambition, and if the truth be known anyone with a bit of guts and determination can achieve success in almost any field within Australia.

Right throughout our racing industry you will find stories of the

Aussie Battler making good from a small start. It may be a person who picked up a bargain from a horse sale and went on to win a fortune from racing.

In fact our most recent two times winner of the country's prestigious Melbourne Cup was a horse that was unsold at an auction.

The owner decided to race the horse instead, Tony Santic named her "Makybe Diva" and she has gone on to win in excess of ten million dollars.

There is the story of a young female New Zealand trainer Sheila Laxton who brought her horse "Ethereal" across the Tasman and won both the Caulfield Cup and the Melbourne Cups, before eventually calling Australia home, and who could forget the efforts of the great Irish Trainer Dermot Weld, a huge supporter of Australian racing who was rewarded with Melbourne Cup success by the efforts of "Vintage Crop" in 1993.

Australia is a very big country which many overseas visitors have difficulty comprehending.

If you started driving in Sydney and followed the coast until you arrived back in the home of the 2000 Olympics you will have travelled in excess of 12900 kilometers.

You may not see a lot of people except around the major towns, but

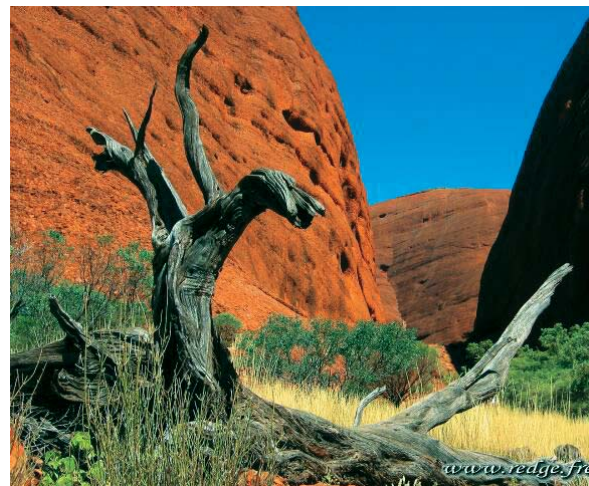
you will see the odd kangaroo and a lot more horses grazing in paddocks.

We have a population of a little over 20 million and nearly 10% of them love their racing and would visit a race track at least once a year.

Each of our states have public holidays for major race days, and there is none larger than the Melbourne Cup.

Telecast around the World, it is billed as the race that stops the Nation!

In Australia we have 454 race clubs and each one of them holds an





annual Cup Day where the locals flock in the thousands. We even have a race meeting held in a tiny town in the outback of Australia called Birdsville. What is unique about this event is that the majority of the spectators arrive by small aircraft.

Another "unique" race meeting is the Oakbank Easter Carnival, when over 100,000 people converge on a sleepy Hills town for a two-day event that promotes itself as the "biggest" picnic race meeting in the world.

These 454 race clubs hold over 22000 races per year. That is 60 races per day every day. The industry directly and indirectly employs 240,000 people serving 5 million customers excluding the people employed in the betting companies. There are currently 3 public companies running betting agencies in shops pubs and clubs. No one is too far from a Tote to put on a bet and if by chance you were too far you could always put your bet on by telephone or in true Aussie style have a bet with your mate.

Here are some more statistics to consider on racing in Australia. The industry is worth \$7.74 billion dollars to the Australian economy and, for a small nation, that is significant. It is expected to have an impact of \$41.1 billion dollars over 5 years.

In size, Australian Racing is third in the world behind USA and Japan.

Per capita, Australians are the world leading country in ownership of thoroughbred racehorses.

There are approximately 32000 registered racehorses and 34000 registered broodmares. These figures do not take into account all the pleasure horses. It is not difficult for a child in Australia to have a pony if they have a parent willing to oblige.

It is also very easy for a person who has a love of racing to become involved either as an outright owner or as part of a syndicate.

Just ask one of the 5500 trainers and they will make it happen.

There are many opportunities with a racehorse to participate with so many races being run all over Australia on everyday of the week.

Even if your horse is not a champion, but has some ability, then there will be a suitable race somewhere that it will have a chance of winning.

If you own a champion horse then it is easy to transport the horse to a State where the bigger races are on offer as there are no quarantine restrictions or travel problems between States of Australia.

Each State tries to coordinate their race carnivals so that they do not clash with each other.

In South Australia (www.racingsa.com.au), we have just finished our Adelaide Cup Carnival which was last held in the first half of May.

Adelaide is one of the smaller capital cities with a population of

approximately 1 million people, so our carnival is not anywhere near as big as Melbourne or Sydney, but we were delighted to have attracted well in excess of 30,000 racing fans.

That figure is expected to grow when from 2006, The Adelaide Cup is run in March and followed just days later by the Magic Millions race day.

There were 124 trainers including 65 from interstate and New Zealand. A total of 331 horses ran in the 25 races of the carnival including 142 from interstate and New Zealand. 52 jockeys rode over the three days including 30 from interstate. You can see from this example how a trainer with a good horse can seek out a suitable race if the trainer is prepared to travel and they do travel all over the country.

In some cases the trainer also has the regular jockey fly in for the race as well.

Races are broadcast each day on Sky TV and shown either on home pay TV or you can go to the nearest Hotel or Club and watch the event or listen to the radio broadcasts.

34000 broodmares are spread out all over the country. We have the large Studs but there is an opportunity for anybody to own a broodmare and we have many small breeders all enjoying the participation in racing and the excitement it brings.





Australian Racing also has an international participation with trainers of some of the best racehorses in the world being invited to take part in some of our better feature races. Many trainers also have overseas clients who own and race horses here in Australia and do so very successfully.

Australian racing has been able to make its mark on the world stage. Over a decade ago "Better Loosen Up" tasted success in the Japan Cup, more recently however it was "Choisir" that took the U.K by storm, and "Elvstroem" that did the same in Dubai.

Horse Racing and Thoroughbred Sales go hand in hand.

A company called Magic Millions conducts sales each year corresponding with some of these large carnivals. You can visit their website at www.magicmillions.com.au and find out dates of the next sales and organize catalogues to be sent or view details on the website. What a great holiday it would make to come to Australia to a sale where some of the best bloodstock in the world is on offer and participate in all the fun and excitement of one of our many carnivals. You could go home a winner and an owner of an Australian yearling with a huge future.

Australia is also a land of opportunity for international people wishing to come to Australia and be involved in the industry.

There is a shortage of many occupations within the industry such as jockeys, jumps jockeys, track riders, stable hands and suitable people for stud management work.

Those who have worked or holidayed in Australia know what a great lifestyle we have, if you want

a part of it, the welcome mat is always out!



Clive Harris of CYBERBOX PTY Ltd, exclusive distributor of TWYDIL® in Australia, together with Valère Henry.

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WINNER OF TWYDIL® QUIZ AT FRENCH NATIONAL EQUINE CONGRESS 2004

Céline Bourzac, winner of TWYDIL® quiz AVEF 2004.



Céline BOURZAC, winner of TWYDIL® quiz during the AVEF-Congress 2004, has sent us this picture of her stay of 4 days in the beautiful ski resort of Arosa where she stayed in the 5 stars hotel Kulm.

Céline BOURZAC is a student in the last year at the veterinary school of Lyon (F).

Vincent Druet, another student in

the last year at veterinary school, also answered all the questions correctly.

The skiing holiday sponsored by TWYDIL® was allocated through a lucky draw of all who answered the quiz correctly, undertaken by Dr Richard Corde, President of the French Association of Equine Veterinarians.



Hotel Kulm, Arosa



NEW RESEARCH & DEVELOPMENT DIRECTOR

Dr Briec de Moffarts will have authority over TWYDIL®'s department of science, research and development as from 1 October 2005. Dr de Moffarts will be in charge of:

- control of the range of products and their development programme of research and the appropriate assignment of funds
- relation with different research centres
- contact with our partners PRO-BIOX SA
- global scientific contacts
- transmission of scientific information

Academic history

After graduating in veterinary medicine at the University of Liège (B) in 2001, Dr de Moffarts went on to obtain his DEA in 2002 and his PhD in 2005.

Dr de Moffarts has rapidly become accepted as a specialist in the study of oxidant stress in performance horses in the department of physiology of Professor Lekeux at the University of Liège (B).

In this position, he has attended numerous international congresses (United States, Great Britain, France, Germany, Canada etc.) and has written or co-authored several articles published in prestigious international journals such as the Veterinary Journal and the Equine Veterinary Journal.

Competent Rider

A passionate horse lover, Dr de Moffarts is also a competent rider.


He has been "Maître d'équitation" and instructor at the sports centre of the Belgian army for several years. He has also trained with Filipe de Figueiredo (Dr Graciosa), [Director of the Portuguese School].

He was a student of Frederic



Dr de Moffarts receiving in presence of Prince Philippe of Belgium the prize of the Thomas Lermusiaux Foundation in June 2003 awarded for his diploma thesis in the third stage of DEA, entitled: "Effect of exercise on markers of oxidant stress in trotting horses" (*Effet de l'exercice sur des marqueurs du stress oxydant chez le cheval trotteur*).



Demoulin, a former rider of the Belgian Horsemanship Academy and has ridden with several famous riders in Belgium and Portugal such as Nuno Palm, Luis Valencia, Patrick Leroland, Alex Wims, Catherine Henriquet and Sue Oliveira. 

Age	28 years
Civil Status	married
Nationality	Belgian
Residence	Chaufontaine (Belgium)
Diplomas	Veterinary surgeon, DEA (2002) and PhD (2005)
Languages	French, English, Dutch, Portuguese, Spanish
Passions	Horses, wine, good food
Early education	Primary school at the French Lycée in Baghdad (Iraq)

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TWYDIL® Organisation chart

President
Finance
Production
Science, research and development
Marketing
Legislation
Administration

Valère Henry
Barbara Borer
Peter Bommer
Briec de Moffarts
Philippe Henry
Michael Putnam
Sandra Schubiger



OBITUARIES

The TWYDIL® management was deeply saddened by the sudden death on 28 January 2005 of Hans Bont who, for many years, acted as outside controller of our book-keeping.

This remarkable, widely respected professional was also our friend. He was a man of great fairness and compassion for others.

We are all very sad.

NEW PRESENTATION

The TWYDIL® range has now extended its assortment of pack sizes:



TWYDIL® HIPPACAN+C

(10 sachets)



TWYDIL® HEMATINIC

(10 mouth syringes)



TWYDIL® TWYBLID

(10 sachets)



TWYDIL® CALMIN

(21 sachets)



TWYDIL® PROTECT PLUS

(10 sachets)



TWYDIL® ELECTROLYTES

(10 mouth syringes)

These various products are now available in packs of 10 or 21 units corresponding to an application for one horse.

Of course, the larger packs of 50, respectively, 100 units, remain available.

NEW RANGE OF HIGH QUALITY DIAGNOSTIC TOOLS



BLOOD TESTS ALLOWING TO OPTIMISE COMPETITION HORSES

After years of scientific studies showing the efficacy of their methods, the companies **TWYDIL®** and **PROBIOX®** have joined forces to introduce a new range of high quality diagnostic tools in clinical biology to veterinarians to help breeders and trainers keep their champions optimally fit.

Who is PROBIOX SA?

PROBIOX® is a spin-off of the University of Liège, founded in 2001 for the purpose of utilising years of productive research on oxidative stress. Established as a share holding company, **PROBIOX®** is now the European leader in blood test equipment for high precision measurement of oxidative stress (i.e. the ratio of pro- to anti-oxidants).

For several years, **PROBIOX®** has been monitoring some of the best football and tennis players in the world.



WHAT IS OXIDATIVE STRESS?

Oxidative stress occurs when the body's antioxidant defence system is overwhelmed and unable to counterbalance pro-oxidant aggression. It has been demonstrated that intense exercise and competition increase the amount of pro-oxidants in the body, which may destabilize the oxidative balance. If this imbalance is not compensated or corrected by the supply of additional antioxidants, it could harm performance or even induce a pathological reaction.

Pro-ox → Oxid → Pathologies

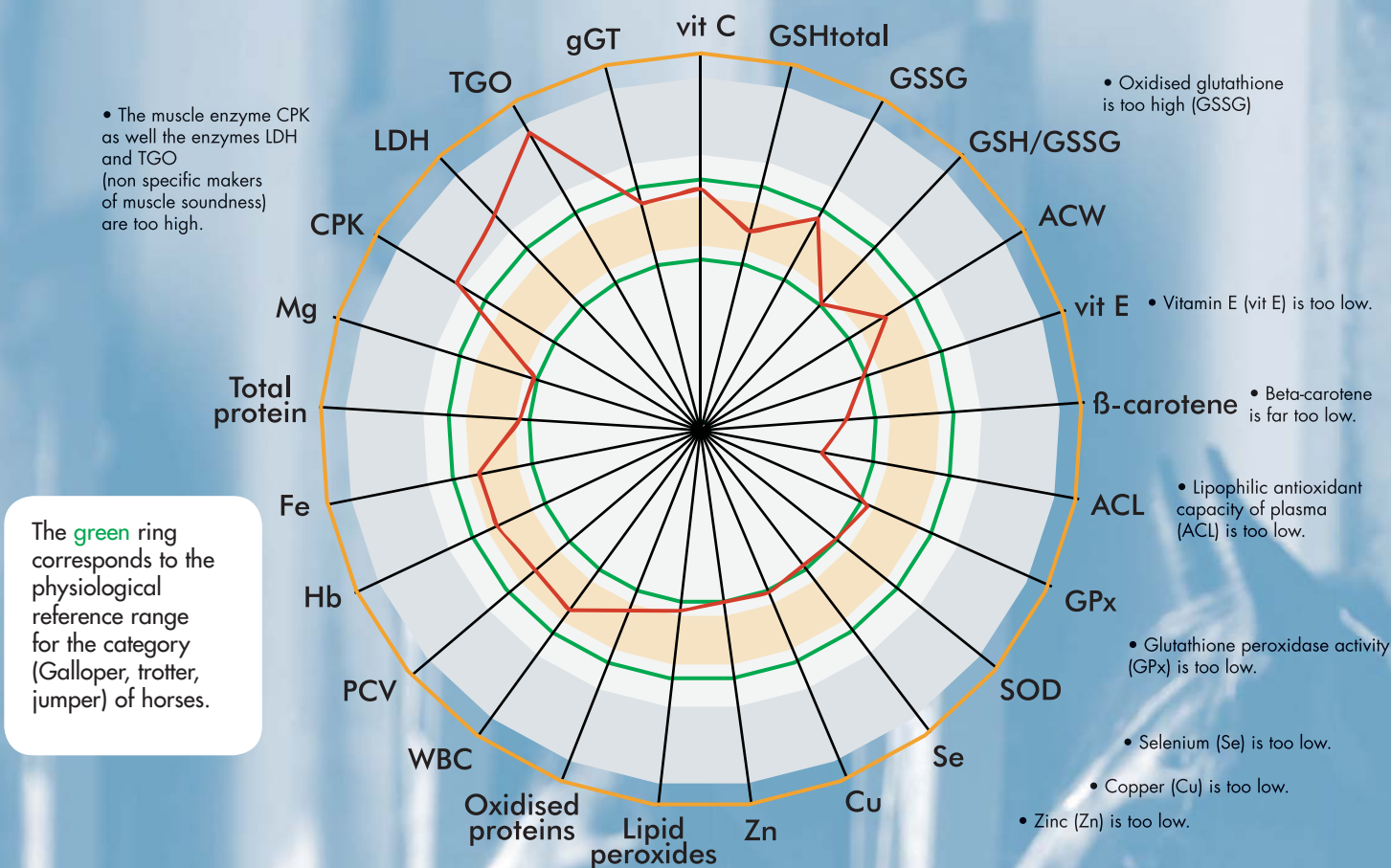


It has been shown that oxidative stress can result in the development of problems associated with:

- the locomotor system
(myopathies, arthrosis, ...)
- the respiratory system
(tracheobronchitis, small airway inflammation)
- the vascular system
(exercise-induced pulmonary haemorrhage)
- the nervous system
(motor neuron disease, grass sickness)

PRACTICAL EXAMPLE

STEP ONE: THE HORSE UNDERGOES OXIDANT BLOOD PROFILE.



STEP TWO: A VETERINARIAN, SPECIALISED IN OXIDANT STRESS, DELIVERS HIS SCIENTIFIC COMMENTS TO THE PRACTITIONER.

In this specific case:

This horse shows a certain number of values outside normal. Its oxidant balance is not optimal at the moment. In particular, magnesium, trace elements and lipophilic vitamins are required. Supplementation with certain of these nutrients could help to improve its pro-oxidant/antioxidant balance.

The muscle enzymes should be carefully monitored.

STEP THREE: TWYDIL® OFFERS A NUTRITIONAL RECOMMENDATION

On the basis of the record of deficiencies, and on the conclusions of the University, TWYDIL® proposes, by separate mail, a suitable supplement "à la carte" with the purpose of trying to restore each parameter to the optimum. In this particular case, the horse should receive a supplement consisting of vitamin E, β -carotene and trace elements in the appropriate concentrations.

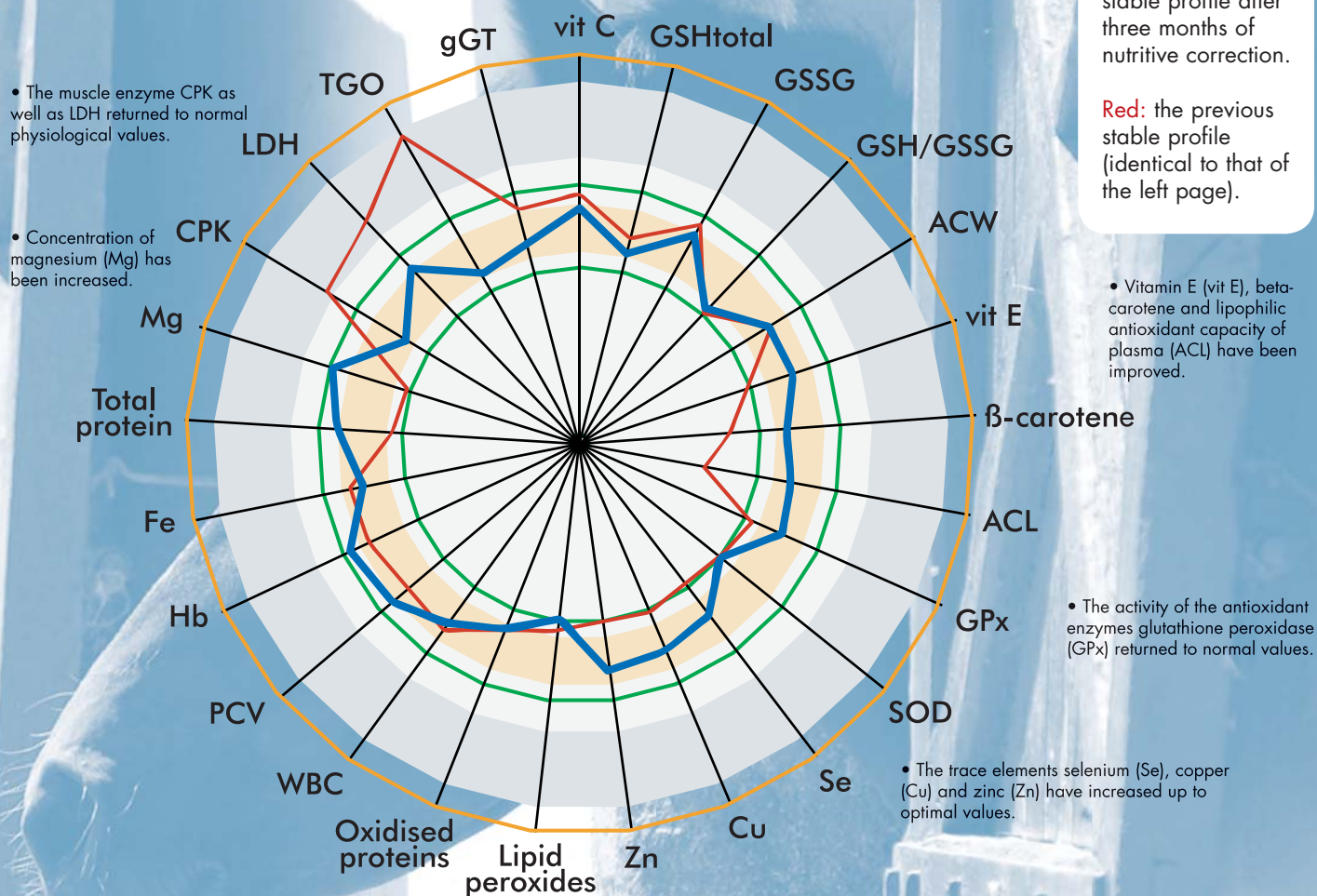


In addition to the existing large range of products, TWYDIL® has developed 5 specific nutritional correction supplements specially for horses whose oxidant profiles TWYDIL® PROBIOX® show small specific deficiencies.

STEP FOUR: THE HORSE RECEIVES THE APPROPRIATE NUTRITIONAL CORRECTION MEASURES



STEP FIVE: THE NEXT BLOOD SAMPLE MEASURES THE EVOLUTION OF THE OXIDANT STATE OF THE HORSE



This horse has now an optimum profile and should be maintained under the same nutritional conditions.

CONCLUSION:

This new approach to stable management, uses accurate scientific data to prevent several problems of the competition horse and enhance its maximum potential during the whole season.

PRACTICALLY:

HOW TO PROCEED FOR A **TWYDIL®** - **PROBIOX®** OXIDATIVE STRESS PROFILE ?

OPTION 1 : DESPATCH OF A TECHNICIAN

Where there is a sufficient number of horses requiring blood test analyses, **TWYDIL®** - **PROBIOX®** sends a technician anywhere in the world. The technician undertakes the centrifuging and stabilisation of the blood samples collected by the veterinarian and subsequently forwards them to the laboratory in Belgium.



OPTION 2 : READY TO USE PACKS

The veterinarian may obtain a carton **TWYDIL®** - **PROBIOX®** containing all the necessary material for 20 champion profiles. Following exactly the instructions in the carton, the veterinarian takes the blood samples, stabilizes them and arranges their shipment at controlled temperature to Belgium.



OPTION 3 : VETERINARY FACULTY OF THE UNIVERSITY OF LIEGE (B)

The horse may be sent (by prior arrangement) to the "Centre de Médecine Sportive" at the University of Liège (CEMESPO) where not only **TWYDIL®** - **PROBIOX®** profile tests will be undertaken but also other tests if necessary (test of exertion on treadmill, endoscopy, ultrasonic testing Doppler etc).



OPTION 4 : RACETRACK MONS-GHLIN (B)

Also by prior arrangement, a horse can be sent to the veterinary satellite of the University of Liège at the racetrack of Mons-Ghlin where it could undergo a test of exertion on the track, endoscopy and a **TWYDIL®** - **PROBIOX®** profile.



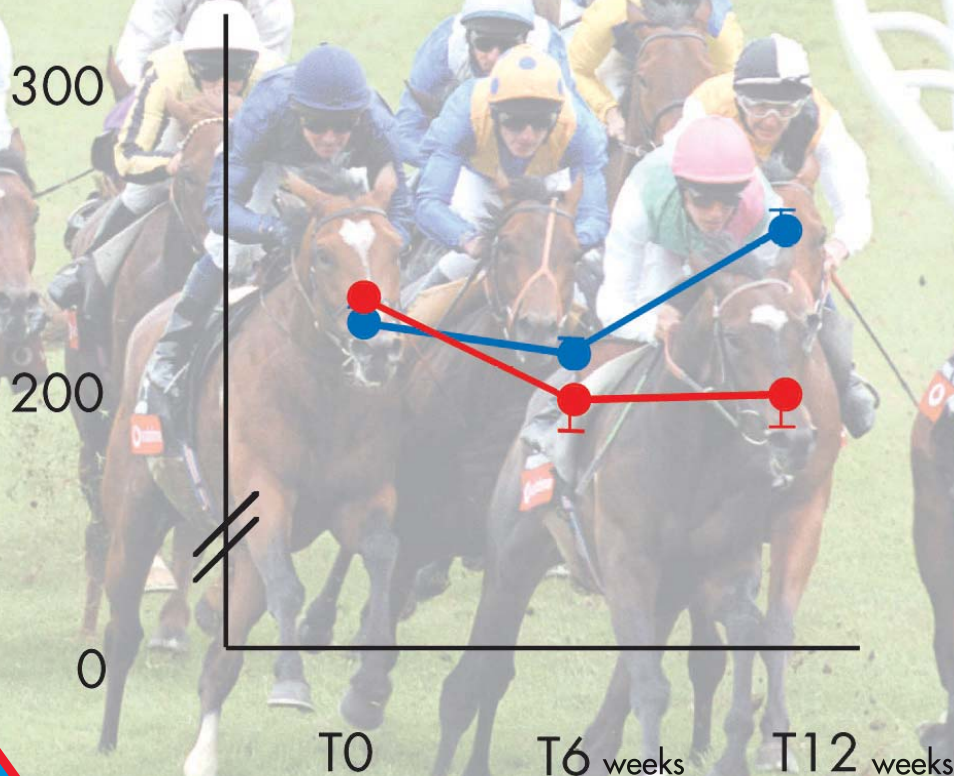
FOR ALL INFORMATION ON **TWYDIL® - **PROBIOX®** PROFILES, CONTACT**

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BETTER THAN WORDS!

Glutathione Peroxidase (IU/gHb)



**WITH GLUTATHIONE
PRECURSORS**

Red: Control
Blue: TWYDIL®

<http://www.twydil.com>

TWYDIL®



TWYDIL® PROTECT PLUS NEW

Thanks to its balanced combination of vitamins C, E and β -carotene, L-carnitine, glutathione precursors, and other essential vitamins and minerals, TWYDIL® PROTECT PLUS NEW helps to fortify natural antioxidant defences of the athlete horse. Helps to improve recuperation, to provide extra muscular protection (less CPK), and to prevent disorders coming from an oxidative unbalance due to intensive training.

- Officially certified by LCH (on final product, urine and blood) :
can be used without risk up to the day of the competition.

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Fax (41)(61)272 23 88

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Needham, Harleston, Norfolk IP20 9LG
Tel. (01379) 85 28 85
Fax (01379) 85 41 78

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321 N, 22nd Street
St. Louis, MO 63166, USA
Tel. (314) 421 0300
Fax (314) 421 3332



e-mail: info@twydil.com

TWYDIL® ANTI-DOPING PRECAUTIONS FURTHER REINFORCED



Dr Yves Bonnaire, Director LCH

In addition to the controls undertaken over many years on the final product and on the urine of a horse having received an overage of the TWYDIL® product, we have now asked LCH also to analyse the horse's blood.

Each production batch of all TWYDIL® feed supplements is checked in three different ways by LCH before being released.

This triple certificate established by LCH is available at www.twydil.com - just type the name of the product and its batch number.

HPH : Why is this supplementary analysis of the blood of a horse receiving an overage of the feed supplement advantageous?

Yves Bonnaire : The analysis of the blood is indeed a complement to the analysis of the urine. Without going into complex technical details, a

brief review of drug metabolism reveals that urine is, above all, a way of detoxifying molecules received by the body. Consequently many molecules are eliminated as metabolites in the urine while unchanged molecules may still be present in the blood for a further period (depending on the half-life of these molecules). Further, some molecules such as peptides (for example EPO or growth hormones) can more easily be found in the blood as their large size only allows slow renal excretion. These considerations lead us to conclude that blood testing is essential for complete anti-doping control and it has to be done on both biological fluids at the same time.

urine and the blood of a horse receiving an overage of the product)?

Yves Bonnaire : In summary, the analysis of natural contaminants undertaken on the final product gives a statistical control of the production processes and therefore allows an overall control of the risks of contamination of the raw materials and of the final product during production, conditioning and transportation with regard to known contaminants. The control of urine and blood provides a much more complete answer regarding the total content of prohibited substances including the active principles normally present in the given products.



HPH : Does this mean that analysis only of the final product would not detect all prohibited substances?

Yves Bonnaire : The analysis of the final product is not inherently a sufficient guarantee inasmuch as the product can contain biological precursors, i.e. molecules susceptible to transformation into prohibited substances through different metabolic pathways which take place after ingestion of the product by the animal. Under these conditions, analysis of the biological fluids after an overage presents an additional security.

HPH : Can a feed supplement supplier give the maximum guarantee of security to the user only by means of these three different controls (on the final product, on the

LCH is the official French laboratory for the animal anti-doping controls.

LCH is the only authorised laboratory in France to control the races of thoroughbreds, trotters and the jumping competitions.

LCH is also the FEI Central Laboratory supervising the four major control laboratories in Hong Kong, Sydney, Newmarket and New York.

LCH also controls horse competitions in many other countries.

FEI CENTRAL LABORATORY		
ANALYSIS REPORT: RAPPORT D'ANALYSE		
The following product: Le produit suivant:		
PRODUCT / PRODUIT	BATCH NUMBER / N° DE LOT	
TWYDIL® HPPACAN®	050663	
has successfully passed the three following controls, under the operating process in duty at the laboratory, a passé avec succès les trois contrôles suivants, selon les modes opératoires référencés au laboratoire :		
WHAT HAS BEEN CONTROLLED OBJET DU CONTRÔLE	SUBSTANCES SEARCHED SUBSTANCES RECHERCHÉES	DATE
FINAL PRODUCT Produit fini	Contaminants naturels**	13.07.2005
URINE of a horse having absorbed an overage of the product.	Toutes molécules prohibées**	03.08.2005
URINE of a horse kept absorbed on a supplement of the product.	All prohibited substances**	
BLOOD of a horse having absorbed an overage of the product.	Toutes molécules prohibées**	03.08.2005
SAME of a horse kept absorbed on a supplement of the product.	All prohibited substances**	
<small>** Caffeine, theobromine, theophylline, atropine, scopalamine, morphine, methylsulfonylmethane, amphetamines, lidocaine, Carbuterol, theobromine, theophylline, atropine, scopalamine, morphine, methylsulfonylmethane, amphetamines, lidocaine. ** under the operating process in duty at the laboratory, at the mentioned date, selon le mode opératoire référencé au laboratoire en date des analyses.</small>		
Dr Yves Bonnaire - Director of LCH		

TWYDIL® PRODUCTS* CAN BE USED WITHOUT RISK UP TO THE DAY OF COMPETITION

WHAT IS DONE FOR EACH BATCH OF PRODUCTION



REPRESENTATIVE SAMPLE

During the manufacturing process samples are taken during the different stages of production and mixed, following a precise method, to obtain a final sample as representative as possible of the whole batch.



LCH SEARCHES FOR CONTAMINANTS IN THE FINAL PRODUCT

LCH checks that, even at the limits of detection, the final product is free of all the 9 natural contaminants (morphine, atropine, butofenine, caffeine, theophylline, theobromine, scopolamine, methylbufotenine, dimethyltryptamine) for which vigilance must be maximum because of their possible presence in feed ingredients.



A HORSE FED WITH AN OVERAGE OF THE FINAL PRODUCT

The Laboratory of Hormonology in Marloie, Belgium, directed by Dr Philippe Delahaut, follows a strict and precise protocol quality control experiment (specific for each product).

Under this protocol, the Laboratory gives a horse 3 times the normal dosage of the product for a minimum of 3 days. Samples of urine and blood are taken and sent to LCH.



LCH ANALYSES THE URINE OF THE HORSE

LCH submits the urine to a complete screening for all the prohibited substances (stimulants, analgesics, narcotics, anabolic steroids, beta-blockers, diuretics and not just the likely contaminants).



LCH ANALYSES THE BLOOD OF THE HORSE

LCH submits the blood to a complete screening for all the prohibited substances (stimulants, analgesics, narcotics, anabolic steroids, beta-blockers, diuretics and not just the likely contaminants).



LCH ESTABLISHES A TRIPLE ANTI-DOPING CONTROL CERTIFICATE

Certificate available on: www.twydil.com



SAMPLES SEALED

A sealed urine sample is kept refrigerated by the Laboratory of Marloie for one year beyond the expiry date of the product and TWYDIL® keeps in Switzerland a sealed sample of the final product for the same period.

* There is one exception to the above TWYDIL® LIQUID BLISTER for which we recommend 5 whole days between the last treatment and the day of competition!

2004 AVEF RESEARCH AWARD

OXIDATIVE STRESS AND ERYTHROCYTE MEMBRANE FLUIDITY IN EVENTING HORSES

Dr Karine Portier, DMV, DEA



Dr Karine Portier, 2004 AVEF research award winner and Valère Henry, President of TWYDIL®.

This article is based on Dr. Karine Portier's masters degree thesis at the Lyon National Veterinary School: "Oxidative stress and erythrocyte membrane fluidity in sport horses: effects of exercise and of antioxidant supplementation", for which she received the AVEF Research Award at the 2004 Congress of the French Equine Veterinary Practitioners Association. This work was partially sponsored by TWYDIL®, and was carried out with the collaboration of Professor Pierre Lekeux's team, from the University of Liège.

The aim of the study, was to evaluate the effects of exercise and antioxidant supplementation enriched with omega-3 fatty acids in 12 eventing horses.



The assessment of these high level athletes showed that:

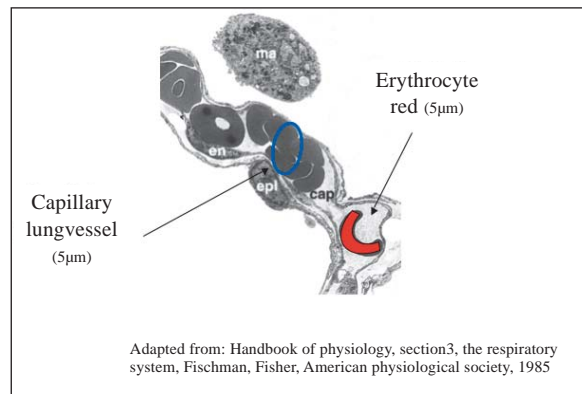
- 1) Horses suffer oxidative stress and a decrease of erythrocyte membrane fluidity due to exercise.
- 2) These effects can be reduced by supplementing the diet with antioxidants and omega-3 fatty acids.

During effort, the ability of the horse to carry oxygen rapidly from its lungs to its muscles is essential. The cells involved in this transport are the erythrocytes. To manage their mission, these specialized cells constantly distort, in order to pass easily through the finest blood vessels. (photo 1).

Recent studies have shown that the erythrocyte's ability to distort can be affected by oxidative stress (Baskurt, 1999). This link is now established in man as well as in horses.

Oxidative stress (cf. HPH 02/03) is defined as the loss of balance between the attacks coming from pro-oxidant products (like free radicals) and the antioxidant defences of the

Photo 1





organism, for instance during respiration. The potential consequence of this oxidative stress is an increase in cellular and tissue lesions. During exercise, oxygen consumption increases, leading to the production of free radicals, which can cause a loss of balance between anti- and pro-oxidative molecules in sport horses (de Moffarts *et al.*, 2004 ; Mills *et al.*, 1997 ; Kirschvink *et al.*, 2002a).

In man and laboratory animals, exercise or illness seem to induce a decrease of erythrocyte membrane fluidity, as well as a drop in their ability to distort (Senturk *et al.* 2005 ; Cazzola *et al.* 2003 ; Yalcin *et al.* 2000 ; Keddad *et al.*, 1996)

In the same way as it has been shown that antioxidant supplementation can help the body defend itself during exercise and limit the deleterious effects of oxidative stress in sport horses (de Moffarts *et al.* 2005 ; Deaton *et al.*, 2002, Kirschvink *et al.*, 2002b), different studies have shown that antioxidant or omega-3 fatty acids supplementation influence erythrocytes' ability to distort (Jeukendrup and Aldred, 2004).

The following study aimed at evaluating the oxidative status of a group

of national and international eventing horses, at rest and working, with and without antioxidant supplemen-

tation enriched with omega-3 fatty acids, by measuring their erythrocyte membrane fluidity as well as by analysing indirect markers.

PRESENTATION OF THE STUDY:

All horses were kept in a racing stable in Belgium. The TWYDIL® supplementation programme had never been used in this stable. The trainer selected 12 trained and healthy horses from amongst the stable population. They were randomly divided into two groups. The first group of 6 horses received the antioxidant and omega-3 fatty acids treatment; whereas the second group, also of 6 horses, was given a placebo. The supplementation period lasted for three weeks. At the end of these three weeks, the horses were given an effort test, equivalent to the work put in during competition. Four "oxidative stress" checkups were made, in order to study the effect of exercise and of supplementation. The first check-up, called (T0), was undertaken before the start of the supplementation period, the second check-up (T1) was made after this period; the third (T2) and fourth (T3)



Figure 1

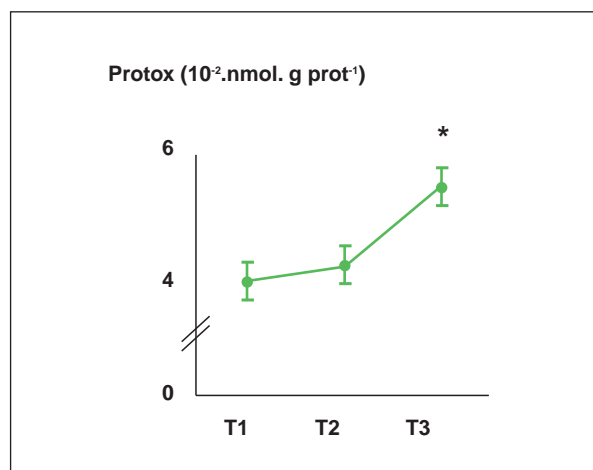
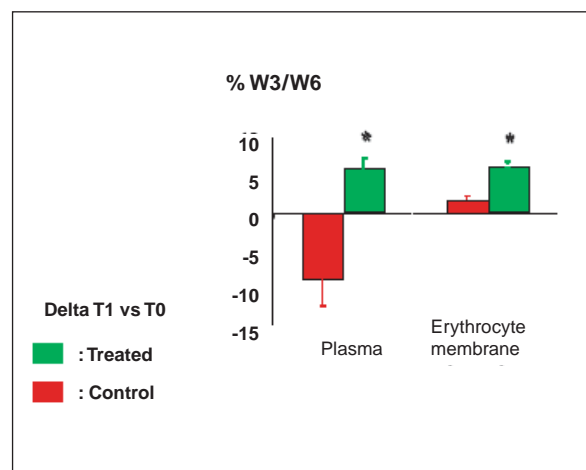


Figure 3



respectively fifteen minutes and 24 hours after exercise.

RESULTS OF THE STUDY

1. EXISTENCE OF AN EXERCISE-INDUCED OXIDATIVE STRESS

The study confirmed that horses suffer not only changes in their pro- vs. anti-oxidative balance, but also modifications to their erythrocyte mem-

brane fluidity during exercise. These changes were determined by measuring the levels of the antioxidant defences and of derivate products of free radical attack, as well as by observing the behaviour of the erythrocyte cell membranes by membrane fluidity studies.

Firstly, *the antioxidant supplies in the horse decreased during the rest period*. Reductions of glutathione (GSH) and vitamin E (vit. E) levels were also observed.

Next an *oxidative process appeared* during the study. This was mainly indicated by an increase of the level of oxidized proteins (protox) (figure 1).

Eventually, the erythrocyte membrane fluidity decreased (figure 2).

During exercise, the combination of increased oxidative activity and decrease of antioxidative capacity acted on the erythrocyte membrane fluidity. This phenomenon, if not compensated, could cause even more serious oxidative stress and micro-circulation disorders during the next exercise.

2. EFFECTS OF SUPPLEMENTATION

At rest, analysis results showed that the treated horses significantly maintained their GPx (the regenerating enzyme of the antioxidative glutathione) activity, as well as their copper/zinc balance, which confirmed the antioxidative effect of the supplementation.

Also, the increase of plasma and erythrocyte omega-3 fatty acid level and the improvement of the omega-3/omega-6 fatty acids balance (figure 3) in both matrices were significant in the treated group.

The erythrocyte membrane fluidity (figure 2) significantly reduced at T2 and T3 in the control group, whereas it was not significant at T2 in the treated horses. This indicates that the antioxidative omega-3 fatty acids supplementation replaced the exercise-induced loss of erythrocyte membrane fluidity.



AVAILABLE SOON !

TWYDIL® OMEGADIL



Colored oil.



Extremely sophisticated formula, based mainly on carefully selected **omega-3** fatty acids, plant extracts and various micronutrients, including 3 000 mg vitamin E, L-carnitine, and orotic acid, **to assist the body's natural defences.**

Ideal concentration of **omega-3** fatty acids (EPA and DHA) to ensure their absorption in cell membranes.

Synergic action with other **TWYDIL®** supplements.

Efficiency scientifically proven compared to placebo.

Superior quality of **omega-3** acids extracted from fish oil.

Production, filling and sealing under nitrogen atmosphere (to avoid oxidation of active substances). Better palatability thanks to deodorized procedure and vanilla addition.

Triple doping control by LCH on final product and on urine and blood of a horse having received an overage of **TWYDIL® OMEGADIL.**



TWYDIL® OMEGADIL is protected in its pack by a complex 'multiwalls' film assuring a permanent barrier against oxygen.

Recommended supply : add 60 ml per day to the daily ration at least for one month.



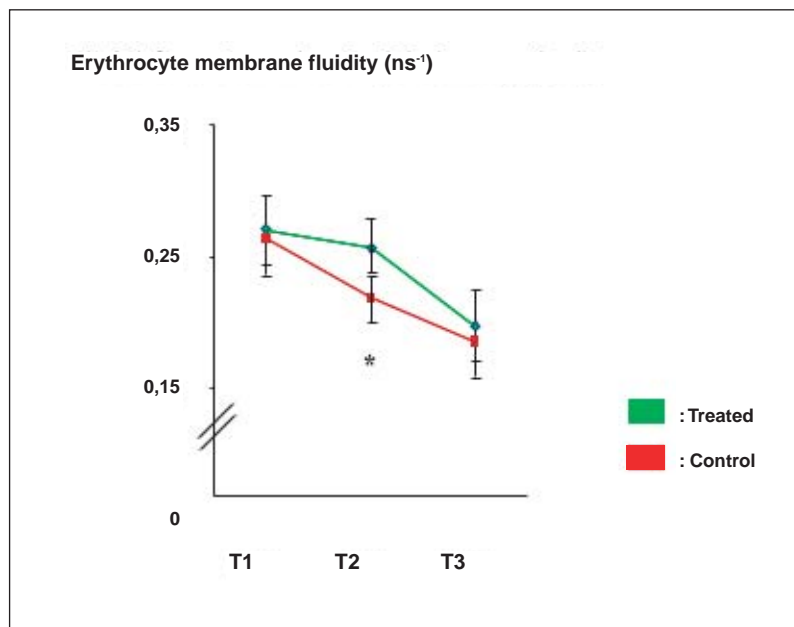
+ TWYDIL® **+**

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e-mail: info@twydil.com

Figure 2



Before drawing conclusions, it must be stressed that an oral fatty acid supplement can only be beneficial if the products used are of perfect quality and contain an adequate concentration of antioxidants. It has been clearly shown that this kind of oral supplementation can have the opposite effect when using products of lower quality (Song J H *et al.* 2000, Palozza *et al.*, 1996).

GENERAL CONCLUSION

Exercise in horses induces oxidative stress, associated with erythrocyte membrane fluidity modifications.

These phenomena can be partially controlled by using a sophisticated mix of omega-3 fatty acids and antioxidants (editor's note: TWYDIL® OMEGADIL).

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MEMBERS OF THE TWYDIL®-TEAM



BENEDICTE MAEMOTO

Bénédicte Maemoto

Bénédicte Maemoto, who is a professional interpreter, is now the permanent local anchor point of TWYDIL® in Japan and is the connecting link between Switzerland and the five Japanese TWYDIL® distributors. Bénédicte Maemoto regularly visits our numerous clients in Japan. She is married to a Japanese and has been living since 1984 in a suburb of Osaka; they have three children: Takefumi, Kensakou and Noémi.



ADRIAN SIMMONDS-MEAD

Adrian Simmonds-Mead

Adrian Simmonds-Mead has joined the PAVESCO UK team. Like his colleagues, Adrian receives telephone enquiries and orders and helps with the distribution of TWYDIL® products in the UK. Formerly an engineer with a prestige sports car manufacturer, Adrian is now married and father of two children, Samuel (age 10) and Frances (age 7).

Adrian is also currently restoring the 15th century family home and any spare time that is left, is spent either with a guitar in his hands or a surfboard at his feet.

DO THE LABELS COMPLY WITH WHAT IS REALLY IN A PRODUCT ?



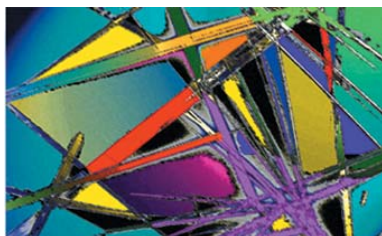
Declaration of content of antioxidants per daily dose :	COMPETITIVE PRODUCT	TWYDIL® RACING + TWYDIL® PROTECT PLUS
Vitamin E	250 mg	7,000 mg
Vitamin C	1,150 mg	11,500 mg
Selenium	2 mg	7 mg

INDEPENDENT TRIALS UNDERTAKEN DURING 2003 ON 45 HORSES

OBJECTIVE : to compare blood concentrations of antioxidants of horses having received a placebo with horses having received either TWYDIL® products or competitive products daily for 3 weeks.

ANALYSIS OF PRODUCTS : an independent laboratory analysed the actual contents of all ingredients (both TWYDIL® and competitive products). The results have shown significant differences between declarations on the label and the actual contents of active substances.

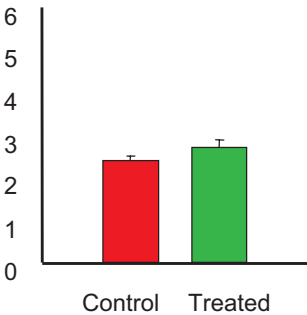
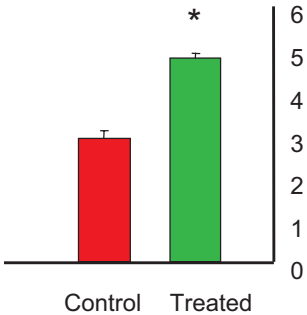
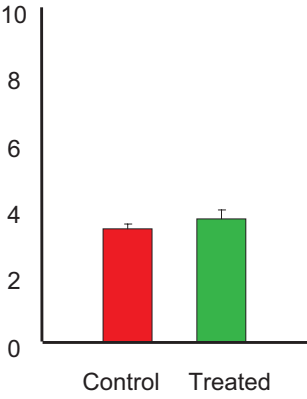
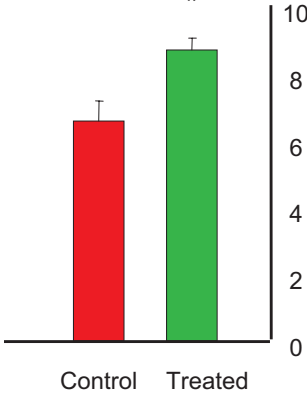
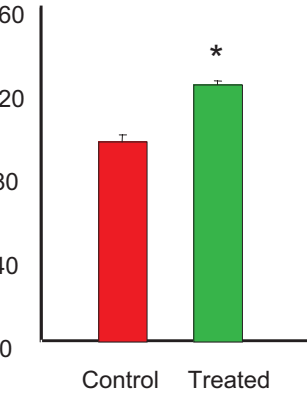
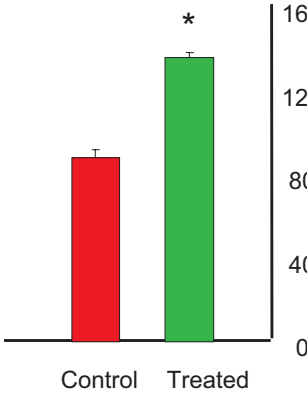
CONFORMITY OF LABEL TO WHAT THE PRODUCT REALLY CONTAINS :	
QUANTITY FOUND BY AN INDEPENDENT LABORATORY IN	
COMPETITIVE PRODUCT	TWYDIL® RACING + TWYDIL® PROTECT PLUS
Between 15 and 74 % of declaration.	Between 91 and 129 % of declaration.



Vitamin E



COMPARISON OF BLOOD ANALYSIS OF HORSES HAVING RECEIVED EITHER A PLACEBO OR A COMPETITIVE PRODUCT OR TWYDIL® PRODUCTS DAILY FOR THREE WEEKS.

Antioxidants	What are the differences in blood between a group of horses having received a placebo or a supplementation (TWYDIL® or competitive products) (blood taken the 1st day and after 3 weeks) :	
	COMPETITIVE PRODUCTS	TWYDIL® RACING + TWYDIL® PROTECT PLUS
Vitamin E Vit E (mg.g chol⁻¹)	No difference between the control group and the treated group 	
Vitamin C Vit C (µg.mL⁻¹)	No difference between the group control and the treated group 	
Selenium Se (µg.L⁻¹)		

* Means that the difference is significant.

CONCLUSIONS :

WITHOUT A VITAMIN GUARANTEE, WHAT IS WRITTEN ON THE LABEL IS NOT NECESSARILY CONTAINED IN THE FINAL PRODUCT.

Certain vitamins are so unstable that without taking due precaution, they can almost disappear during manufacture and storage.

Thanks to know-how and experience over more than 40 years, to the highest production standards and to the purchase of raw materials of best quality as well as to a carefully

calculated overage, TWYDIL® is able to guarantee 100% content of vitamins in its products up to the date of expiration of shelf life.

TWYDIL® can also scientifically demonstrate the absorption of most of the active substances by the horses.





Valère HENRY, President of TWYDIL® presenting the race trophy to Prince Khaled bin Abdallah in Riyadh.



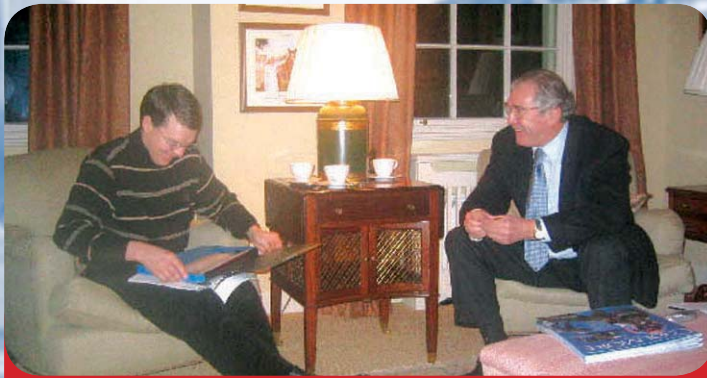
Car of the local sales staff of 'Champions Choice' (TWYDIL® exclusive distributor in the United Arab Emirates).



TWYDIL®



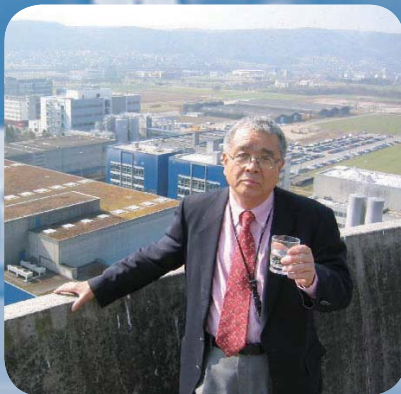
TWYDIL® around the world



Aidan O'Brien in discussion with Valère Henry, President of TWYDIL®.



Mr and Mrs Oluk, exclusive TWYDIL® distributors in Turkey, together with Philippe HENRY, Marketing Director of TWYDIL®, in front of their shop in Istanbul.



Visit of DSM (former Roche) together with one of the five TWYDIL® distributors in Japan.



TWYDIL® PROBIOX® team taking blood samples in Chantilly.



e-mail: info@twydil.com

OPTIMISATION OF HAEMATOLOGICAL PARAMETERS IN HEALTHY SPORT HORSES

Dr Brieuc de Moffarts, DMV, DEA, PHD



The current study aimed to evaluate the effect of a dietary supply of the vitamins and trace minerals involved in the functioning and regeneration of red blood cells and was carried out under the auspices of Professor Pierre Lekeux.

In healthy sport horses, the trial showed that:

- 1) the supply of the trace elements under test increased the blood levels indicating good bioavailability ;
- 2) the observed modification of the levels of these elements and vitamins appeared to favour red blood cell regeneration, as haematological parameters also increased.

During effort, the horse's ability to transport oxygen quickly from its lungs to its muscles is decisive. The red blood cells are the main carriers of oxygen. Physical exercise induces stress in sport horse's red blood cells (Hanzawa, *et al.*, 1999). Some of the red blood cell components are recycled. As red blood cell turn-over is especially important when the body is subject to exercise stress, components can be under-supplied and optimal red blood cell production cannot be maintained. Consequently, the horse can suffer from anaemia, which will generally be indicated by low haematocrit and blood haemoglobin levels (picture). Rose and Hodgson

(1997) suggest that a drop in haematocrit level below the norm negatively influences sport horses' performances.

The current study evaluated the influence of a dietary supply of the vitamins and trace minerals involved in red blood cell functioning and regeneration.

INTRODUCTION

All horses were confined in a Belgian eventing horses' stable. The coach selected 12 healthy, trained horses from among the total group. These 12 animals were divided randomly into two groups. The first six animals received a dietary supply of TWYDIL® HEMATINIC containing vitamins E, B₁, B₆, B₁₂, folic acid, biotin and trace elements including iron, copper, cobalt, manganese and zinc. The second group was a control group that was only given a placebo. The supply period lasted for 10 days. Two blood tests were undertaken in order to evaluate the bioavailability of the trace elements as well as the change in the haematologic parameters of each horse. The first sample (T0) was taken before the start of the supply period, and the second (T1) was taken at the tenth day of dietary supply. Deltas (T1-T0) of treated and control groups were compared.

RESULTS

1. ABSORPTION AND BIOAVAILABILITY OF THE DIETARY SUPPLY

The current study confirmed that the administration of a dietary supply of a vitamin and trace mineral supplement increased the levels of the given trace elements in all biological fluids. These increases were significant for vitamin E, folic acid, vitamin B₆ and cobalt (figure 1 and figure 2).

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Oral paste, with key vitamins (E, B₁, B₆, B₁₂, folic acid and biotin) and trace elements (iron, copper, cobalt, manganese, and zinc) - an ideal supplementation to support haematological parameters.

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- Vitamin stability 100 % guaranteed for shelf life

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Figure 1

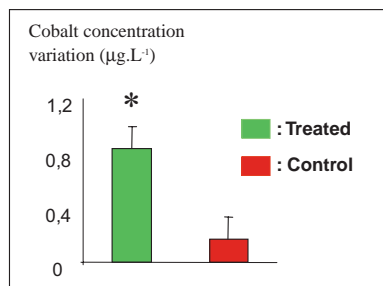


Figure 2

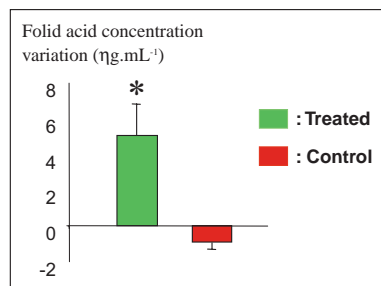


Figure 3

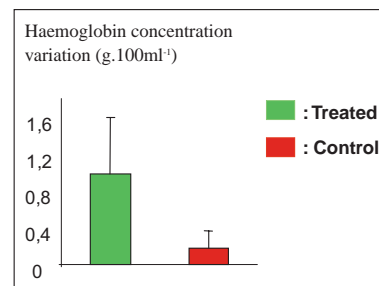


Figure 4

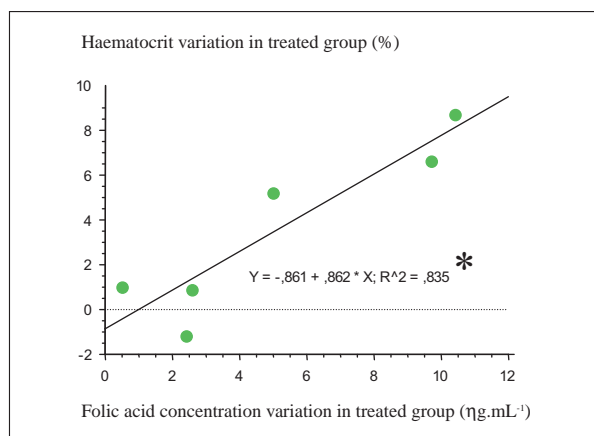
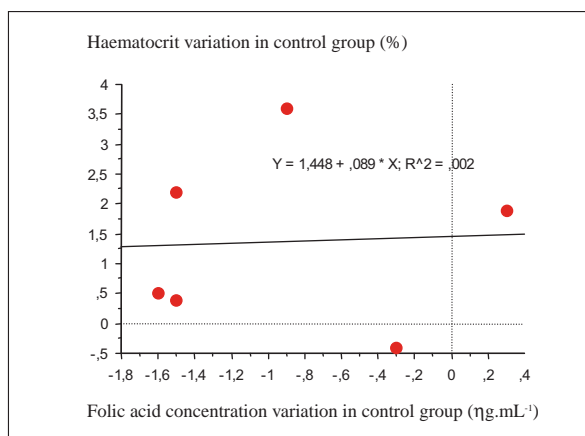


Figure 5



The results indicate that the chemical conformation and concentrations in the supplement are suitable for intestinal absorption and result in blood level increases of those particular components.

2. EFFECTS OF THE DIETARY SUPPLY ON THE HAEMATOLOGICAL PARAMETERS

The association of vitamins and trace minerals appears to induce an increase of haematological parameters such as haematocrit, blood haemoglobin and corpuscle numbers (see graph). Rises in haematocrit and haemoglobin levels were positively correlated to the folic acid concentration (figure 3). However only 80% of the supplemented horses reacted completely to the treatment, whereas, to be significant, 95% of them should have responded. We can thus only indicate a statistical tendency for the general effect on haematological parameters (figure 4 and figure 5).

The results of the study on healthy sport horses suggest that the tested

dietary supply positively influences red cell production.

GENERAL CONCLUSION

The tested dietary supply (editors note: TWYDIL® HEMATINIC) provides certain red cell components and, in so doing, favours red cell regeneration.



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QUESTIONS TO PROFESSOR PIERRE LEKEUX

*Professor of Physiology and Dean of the Veterinary Faculty of Liège
where TWYDIL®'s scientific trials are undertaken.*



HPH: *Do you have new scientific proof that an oxidative imbalance (oxidative stress) can affect horse's performance?*

PL: Formal proof does not yet exist for the horse. However studies undertaken on the treadmill with rats have shown that they have less resistance to tiredness when a deficiency of anti-oxidant enzymes is present. Considered from the other direction, a recent study with horses in training has shown a positive correlation between the increase of an anti-oxidant enzyme and the increase of the maximum consumption of oxygen - an important parameter of performance.

HPH: *Has it been scientifically proved that very precise nutritional adjustments may correct oxidative imbalance?*

PL: Different studies have shown that an oxidative imbalance, appearing during intensive work or inflammatory problems, may be reduced, if not corrected, by administering specific nutritional supplements which would be beneficial to the well being,

health and performance of horses.

HPH: *Does the study on prevention of ulcers show an alternative to treatment with efficient but expensive omeprazol?*

PL: Omeprazol is the usual treatment for gastric ulcers. However, its high cost limits its use to the short term treatment of most severe cases and does not prevent relapses in competition horses. The product tested in the study presented in the HPH is therefore not a competitor but a complementary product to the administration of proton pump inhibitors. Compared to the omeprazol treatment, its potential utilisation is either upstream (to prevent appearance of ulcers) or downstream (to prevent relapses).

HPH: *In your opinion, what are the major conclusions of the study (HPH page 24 to 28) made on a combined supplement of anti-oxidants and omega-3 fatty acids?*

PL: This study shows that the oral administration of a cocktail containing anti-oxidants and high quality omega-3 fatty acids significantly increases the ratio of omega-3/omega-6 acids in the red blood cell membranes and improves their fluidity.

HPH: *In your opinion, knowing the effects of a supplement enriched with omega-3 fatty acids, what is the potential for such a supplement?*

PL: The potential interest in such a supplementation is twofold. Firstly, a better fluidity of the red blood cells in the pulmonary capillaries could result in a

reduction of the incidence or the severity of exercise-induced pulmonary haemorrhage. Secondly, the increase of the omega-3/omega-6 ratio in the cellular membranes could result in a reduction of the incidence or the damage caused by chronic inflammatory conditions. These encouraging perspectives have, however, to be confirmed by further studies.

HPH: *Do you believe in the importance of genomic studies in the horse and if yes, what in practice exactly will they comprise?*

PL: In general genomic studies and, in particular, micro-array techniques, open very promising perspectives for the veterinary follow-up of competition horses. In preventative medicine, the routine analysis of the expression profile of horses' genes would reveal their weak points and any predisposition to pathological conditions. This could be used to propose individually-adapted preventive strategies. On the curative side, the identification of horses' genes that are over- or under-expressed could result in early, targeted treatment, which, potentially, should be more efficient than blanket treatment.



SAUDIA ARABIA: REMARKABLE ACHIEVEMENTS IN A SHORT PERIOD OF TIME

Dr. Mubarak Al-Hurasen



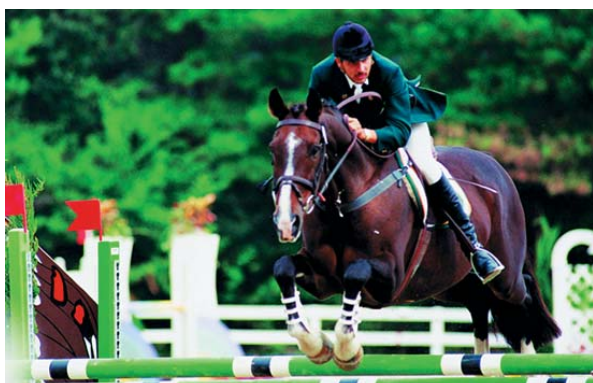
Saudi horsemanship has recently begun to emerge on the map of the advanced world in this field after showing effective and tangible presence in local and international horse racing events. During the last 40 years Saudi horsemanship passed through important stages which further accelerated the pace of its achievements. The establishment of the Equestrian Club in Riyadh under the chairmanship of King Abdullah bin Abdulaziz is considered as the launching step in Saudi equitation trip towards better achievements. The past 40 years of the Equestrian Club history have witnessed a lot of success stories in thoroughbred horses' races beginning with organizing the random spontaneous horse races which were common in the past, and recording and documenting these races. With the passage of time the prizes set for these races improved, and so did the number of competitors. In the past, very low prizes were offered. The government support given to this type of sport proved to be the main incentive behind the present success stories in horse racing in the Kingdom. Now the lowest prize

offered to participants in the horse race by this outstanding club is the equivalent of \$10,000. Other prizes are higher in line with the level of the race and the type of participating horses. The prize may be as high as \$150,000 for one race only. Many people believe that the prizes will continue to go up still further due to the fact that more and more people are buying horses, and many businessmen also began showing a great deal of interest in this type of sport, especially that the number of horse race events is rising continuously as shown by the fact that every week there are two racing events of 7 races each. The achievements of the Saudi horse racing are many and it is not easy to sum them up in this brief account. It is true that Saudi horse racing achievements were not focused on the first position, yet they were really positive - taking into account the short span of attaining these achievements and the relatively short life of the local production industry in the Kingdom. The production of the breeding farms began to improve only in the last 15 years. Among locally produced Saudi race

horses which have shown good results in horse racing events held here and abroad were Mirkhan, owned by Prince. Sultan bin Mohamed and Naim, owned by the sons of King Abdullah bin Abdulaziz. It is worth mentioning that there have been a lot



King Abdullah bin Abdulaziz



Khaled Al Eid



Valère Henry with Mr Saud bin-Saad Al-Qahtani

of great achievements made by Saudi horses owners abroad, in America and Europe, like Prince Khaled Alabdullah and the two late Princes, Fahd and Ahmed bin Salman.

The second official agency which promotes horse racing sport is the Saudi Equestrian and Archery Federation which was established in 1985. This federation, which is chaired by H.R.H. Prince Nawaf bin Faisal bin Fahd, pays special attention to horse racing events like show-jumping and endurance horse races. The Federation achieved a number of Arab and world titles and successfully graduated many leading and well-known horsemen like Khaled Al Eid, the silver medalist of Sydney Olympiad, Ramzi Aldahami, the world amateurs' champion, and Kamal Bahamdan in addition to Abdullah Sharbatly the promising shining star who will represent the Kingdom in the regular world show-jumping. Moreover, this Federation has graduated many champions of endurance horse races – most prominent among them was Tarik Taher, the world champion of 1998 Olympiad in France, and many other future champions.

The third agency which is paying a great deal of attention to horsemanship in the Kingdom is King Abdulaziz Center for Original Arabian Horses. The center pays special attention to Arabian horses and organizes horse races from time to time. The Saudi produced horses, have shown great successes especially in races organized abroad despite the fact that they were trained only for short periods. Earlier, most of good

results were scored by horses from the Buwaibia Stable owned by Prince Sultan bin Abdulaziz and Al Khalidiya Stables owned by Prince Khaled bin Sultan. The participation of horses from the two stables was mostly confined to world events and as a result many of the titles they won were international titles. They won four championships in France along with camel races. Similarly, they won

Arabian horses' races organized in Morocco, Europe and America.

Being able to join the world champions of the International Equestrian Federation in such short time is a great achievement of Saudi equitation. This achievement has been the result of the well planned and continued support of the patron of the horse and the horseman, King Abdullah bin Abdulaziz.



**Dr. Mubarak Al-Hurasen of AL FADER TRADING EST,
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- Officially certified: can be used without risk up to the day of competition.

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TWYDIL® 4LEGS

OBJECTIVES:

Soothing and relaxing cream to be applied daily to the legs.

INDICATIONS:

Apply systematically after work.

Especially recommended where there is articular swelling.

The presence of a cutaneous erosion is not a counter-indication to the use of TWYDIL® 4LEGS.

COMPOSITION:

Contains notably plant extracts, arnica, calendula, kaolin, thymol, eucalyptus and *Aesculus hippocastanum*.

HOW TO USE IT:

After work, wash and dry the legs, then apply without rubbing about 5 mm of the cream to each leg.

Do not cover with cotton or bandage. If possible, leave until next day then brush before work.

For heavy cases, put 1 cm of cream, leave for 2 hours, wash, dry and then renew the application before leaving overnight.



PRESENTATION:

Pail of 2 kg.



• OFFICIALLY CERTIFIED: CAN BE USED WITHOUT RISK UP TO THE DAY OF THE COMPETITION.

VITAMINS AND THEIR BIOAVAILABILITY ORALLY AND BY INJECTION

Michael E. Putnam



There are both advantages and disadvantages to giving vitamins by injection.

Advantages of injection

This method is normally reserved for animals suffering veterinary-diagnosed severe hypovitaminosis or where there has been a toxic interaction that can be reversed by specific vitamins. Most vitamin injections are intended for intra-muscular use; rarely are they given intravenously. In Europe, only licensed products may be used and care should be taken to use only sterile material.

The main advantages are that injected vitamins bypass the digestion/absorption route and are 100% effective. They produce a sudden, large surge of that specific micronutrient in the blood that may have pharmacodynamic effects. Bypassing the alimentary canal is not particularly important for water soluble vitamins, which tend to be 100% absorbed anyway. It is much more rele-

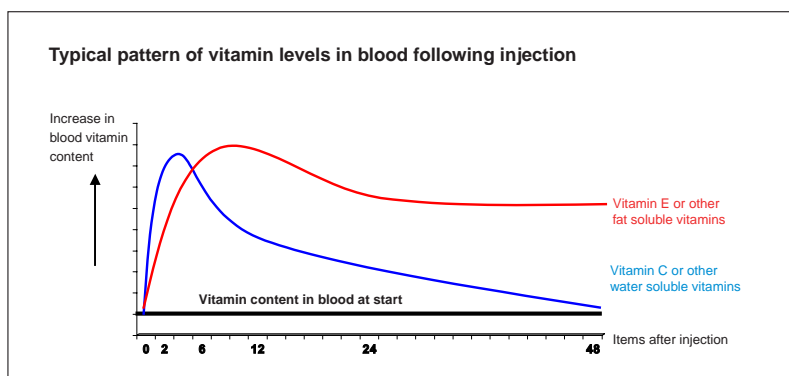
vant for fat soluble vitamins where the efficiency of intestinal absorption is probably only 25%. There are also disadvantages that may far outweigh the advantages.

Disadvantages of injection

The greatest problem of all is sensitization – either to the vitamin substance or to an adjuvant. For example, several companies used to have combined vitamins A, D and E injection. There were several cases of death in horses and sheep from anaphylactic shock brought on by sensitization to one of the ingredients, usually the emulsifier used to ensure that the fat soluble vitamins could be carried in suspension in the blood plasma. Another difficulty – particularly with single vitamin injections – is that they are given in large enough quantities to unbalance the vitamin/hormone/enzyme system, leading to inefficiencies in the utilisation of the other vitamins. A further disadvantage is that adjuvants used in the preparation of injectable products may produce positive reactions to dope tests; there are no guarantees or stringent quality control tests used by manufacturing companies to ensure that injectable products can be given to performance horses without risk of positive test results.

The immediate surge in blood contents falls away fairly rapidly after the injection; the rate of decay varying between vitamins. The water soluble vitamins are lost far more rapidly than the fat soluble vitamins. This is generally because the homeostatic mechanisms in liver and kidneys remove excess levels and excrete them as rapidly as possible. Within 24 to 48 hours the water soluble vitamin contents in the blood will be almost back to where they were before the injection unless steps are taken to renew the injection on a daily basis. No one likes the idea of





sticking needles in horses every day, particularly if there is an on-going risk of sensitization and anaphylactic shock. Equally, there is always a risk of septic reactions since few stables practice good asepsis.

Looking at the individual vitamins, the possible concentrations in injections vary enormously depending on solubility and the need for adjuvants.

We do not recommend the use of injections on a regular basis to provide supplies of nutrients and strongly advise against the administration of vitamins by injection except where absolutely necessary. It is much more economic and effective to give vitamins and other micronutrients orally. For example, the effect of 15 injections is lower than a daily dosage of 75 g of TWYDIL® RACING.

However, there are some circumstances where injections cannot be avoided. When horses are so seriously debilitated that they are not eating, repeated multivitamin injections may aid rapid recovery, whereas oral applications are ineffective. The other situation is where the vitamin is needed as an antidote to a toxic reaction. Two examples are given below:

Two concrete cases where injections cannot be avoided:

1) Poisoning with coumarin rodenticides

Oral coumarin anticoagulants, used in many rodenticides, act by interfering with vitamin K metabolism in liver cells producing haemolysis and death. Should non-target species

ingest rodenticides the effects can be neutralised by repeated vitamin K injections. Vitamin K₁ (phyloquinone or phytomenadione) is effective as an antidote to coumarin anticoagulants whereas vitamin K₃ (menadione) is ineffective and should not be used. The normal feed supplement is vitamin K₃.

Horses that have been poisoned with coumarin rodenticides should be given 0.5 – 1.0mg vitamin K₁ per kg liveweight daily for seven days in divided doses intramuscularly.

2) Thiaminase ingestion

Horses are unlikely to suffer from a deficiency of thiamine because there are normally adequate supplies in plant products. However, horses that have access to bracken may ingest thiaminase, an enzyme that destroys vitamin B₁ and results in acute symptoms of hypovitaminosis. Treatment is to supply excessive amounts of thiamine to overcome the effects of the enzyme.

The usual injectable form is thiamine hydrochloride, which is extremely soluble. It is possible to dissolve 100 g in 100 ml of water. This produces a very acidic solution (pH

about 2). Concentrations of between 1 and 10% have been used in injectables with buffering agents to reduce the acidity to make the solution compatible with blood plasma; 10% is fairly common in injectable solutions suitable for horses. This means that each 1 ml contains 100 mg thiamine hydrochloride which is equivalent to about 80 mg vitamin B₁ measured as thiamine. The normal treatment for horses that have consumed bracken and have started to show deficiency symptoms such as excessive excitability or unexplained paralytic spasms is 0.25 – 1.25 mg thiamine per kg liveweight by intramuscular or slow intravenous injection twice daily for up to 7 days.

Conclusions

There are some cases where it is absolutely essential to give vitamins by injection. Horses that are under veterinary care for severe hypovitaminosis or debilitation, or where there has been a toxic interaction, need the rapid response from injectable material.

Users should note that injectable products do not come with any guarantees that they are without risk of positive doping test results if given to performance horses; in fact, some adjuvants are known to give positive results.

Horses require daily supplies of all the vitamins and oral applications are convenient, fully bioavailable and inexpensive. However, there are differences between that quality of various vitamin products and manufacturing processes, so it is essential to select the best and most suitable product with a quality control guarantee.

There is good scientific proof of the excellent bioavailability of the products in the TWYDIL® range. Scientific trials have shown that, after only three weeks, horses receiving a TWYDIL® product had significantly more active vitamins and antioxidants than those receiving a placebo. In addition to this excellent bioavailability, each batch of every TWYDIL® product is supplied with a triple guarantee of a nil response to doping tests (analysis of the product, and tests on urine and blood of a horse receiving a triple daily supply) and a shelf life guarantee.



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TWYDIL® MINERAL COMPLEX

A horse receiving a traditional diet on an oats and hay base presents a calcium/phosphorus ratio of about 1:1 instead of the ideal ratio of 2:1.

It is important to realise that the total ration and not just the supplementation should have a 2:1 calcium/phosphorus ratio.



A calcium-phosphorus supplementation has to take into consideration two parameters:

1. The nutritional deficiency: the supplementation has to correct this deficiency which, in most diets, is calcium.
2. Calculation shows that a feed supplement to be given at a maximum of 80 g per day can only improve the phospho-calcic ratio of the total feed if it is extremely concentrated in calcium. Effectively, giving a mineral supplement with a ratio of only 2,5:1 does not really change the ratio in the total diet.



THE 10 BENEFITS OF TWYDIL® MINERAL COMPLEX:

1. A very high calcium/phosphorus ratio, supplementing traditional feeding or a complete mixed feed, provides the horse with the best possible Ca/P ratio.
2. Provides 17 g calcium daily.
3. Excellent bioavailability thanks to three different sources of calcium including calcium gluconate.
4. An appropriate supply of phosphorus.
5. Supply of sea salt.
6. Appetence tested before being launched.
7. Each batch of production triple tested against doping reactions by the leading French laboratory LCH (on product, urine and blood).
8. The TWYDIL® quality guarantee.
9. An attractive price/quality ratio.
10. A final optimal balance calcium/phosphorus.

IN CONCLUSION:

TWYDIL® MINERAL COMPLEX is the perfect phospho-calcic supplementation to traditional feeding, to complete feeds or to vitamin supplements like **TWYDIL® RACING** or **TWYDIL® STUD**, for horses of all ages.

MICROARRAY (BIO-CHIP) : A FUTURE TOOL IN COMPETITION HORSES MONITORING ?

Aurélie Thomas, Tatiana Art & Pierre Lekeux

University of Liège, Faculty of Veterinary Medicine, Equine Sports Medical Center
20, Bvd de Colonster, B-4000 Liège



Picture 1 : Aspect of a classical microarray (modified from Le Crom & Marc, 2004).

INTRODUCTION: WHAT IS THE WEAK LINK IN COMPETITION HORSES MEDICAL MONITORING?

High level competition horses' performances are based on three key-stones: the genetic potential of the animal, its excellent health and its optimal training. Nowadays, two ways to evaluate performances exist: performance control itself and veterinary monitoring. The latter classically consist of a clinical check-up and complementary analysis, as for instance blood analysis, radiography,



or ultrasounds. In spite of the regularity of the veterinary check-ups, in lots of situations, health problems cannot be prevented or controlled in time, which has a negative influence on the horse's performances. So for

instance pathologies are detected when they have already induced irreversible lesions, as it is the case when joint inflammation has already started to damage the joint cartilage. It also occurs when the pathology is

Table 1. Examples of abnormalities in gene expression potentially related to pathologies in racing horses.

PROTEIN	EXPRESSION ABNORMALITY	CONSEQUENCES	EXAMPLE OF ASSOCIATED PATHOLOGY	NON SPECIFIC TREATMENT	SPECIFIC TREATMENT * Thanks to the use of microarray
GPx antioxidant Enzyme	Under expression	Oxidative stress	Myopathy	Anti-oxidant supply in alimentation	Specific stimulation of the GPx gene expression.
LOX Pro-inflamma- tory mediator	Over expression	Excessive inflammation	Chronic pharyngitis	Anti-inflammatory treatment	Specific inhibition of the LOX gene expression.

* The specific treatment has the advantage of being either more efficient, or to generate less undesirable effects

not determined with enough precision: the proposed treatment can then be little specific and often not very efficient. The perfect example here is the inflammation caused either by mechanical stress, allergy or infection: the symptoms are often similar, but the three aetiologies require specific treatment.

In order to propose solutions to the limiting factors of the medical monitoring, a tool, born from modern technologies, is being elaborated. It's the microarray or bio-chip, also called « DNA chip » in the Anglo-Saxon literature. This new technology should permit:

- to detect certain pathologies earlier, i.e. before they provoke lesions and disrupt performances ;
- to determine more specifically the origin of the pathologies, in order to use a better targeted, and thus, more efficient therapy ;
- to detect horses predisposition to certain pathologies by genetic profiles, in order to set up appropriate preventive care.

WHAT IS MICROARRAY TECHNOLOGY ?

Micro-draughtboard technology permits to quickly determine the level of gene expression by analysing a sample of the horse's cells (cf. box). Genes can be normally-, but also under- or over expressed. In the ideal situation, each gene of the organism should be expressed in function of the horse's needs in each moment. If the gene is under expressed, too low levels of the corresponding proteins will be synthesised. On the contrary, if the gene is over expressed, too high levels of the corresponding proteins will be synthesised. Now proteins just happen to be fully efficient when synthesised in the exact needed amount, at the exact needed moment and at the exact needed place. Too high levels of proteins are therefore just as bad as too low levels (Table 1).

WHY SUCH A FANCY FOR MICROARRAYS ?

The middle term development of microarrays applied to eventing horses veterinary medicine is moti-



vated by the many advantages offered by this new technology.

1. The possibility to analyse hundreds, even thousands of genes

Nowadays, the genetic molecular methods can only analyse one gene at once. The actual microarrays, thanks to miniaturization techniques, are able to analyse several thousands of genes on only one glass strip of a few centimetres wide (Picture 1). Here for, it is now possible to understand interactions between different genes, of which many had been unknown until today. This makes the global understanding of the horse's pathology much easier for the veterinary practitioner

2. Obtaining new information: gene expression

Microarrays give an instant image of the exact way each cell gene is expressed. This makes possible under- or over expressions of genes easily detectable. The gene expression level can thus be measured in different situations, like for instance before and after effort.

3. Precision of the obtained information

By studying the expression level of each gene, the micro-draught allows the diagnostic and the characterisation of a pathology at its immediate source. The obtained information brings a very precise diagnostic, as it

is considering every gene. This is nevertheless not possible in a simple blood sample, as only the actually present molecules in the blood are usable.

4. Individualisation of the proposed treatment

As the diagnostic is extremely precise, the treatment also is. The approach indeed consists of pharmacologically stimulating the expression of the under expressed genes, and conversely, inducing the inhibition of the over expressed ones. This way, only the proteins implicated in the pathology are concerned by the treatment. The microarrays thus permit a fast and specific treatment of the concerned horse. Actually in order to reduce the risk of complication, the microarray could be used as an acute tool in the early stage of the disease.

5. Precocity of pathology detection

Microarrays diagnostic is not based on a detection of symptoms, but on a modification of the gene expression. This makes pathology detection possible even before the onset of its clinical manifestation. The physical capital of the horse is protected and its performances are not affected.

6. The prevention of certain pathologies

It is indeed possible to imagine a microarray analysis on a regular base before and after training, in order to verify the high level eventing horse's

health and to determine its potential predispositions to develop pathologies. The preventive measures can be adapted in function of the characteristics and specific needs of every horse.

WHICH ARE THE CURRENT LIMITING FACTORS?

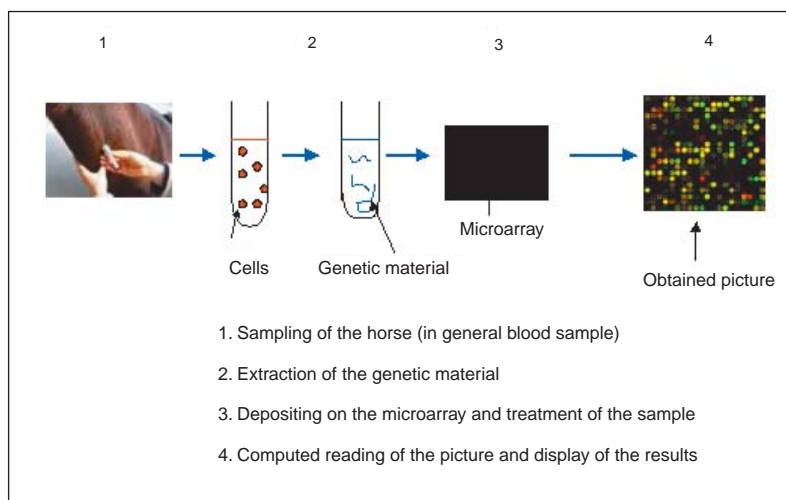
Up to now, two limiting factors exist in the development of the microarray technology.

1. Horse's genome is not yet completely decoded. This means not all equine genes are known. Nevertheless, research teams discover new genes every day. Equine genome will thus be decoded in the near future
2. The cost of a microarray is still substantial and its use is still limited to scientific research. On the other hand, it's obvious that the price will drop dramatically and the use of microarrays will certainly be affordable for racing horses monitoring after its commercialisation.

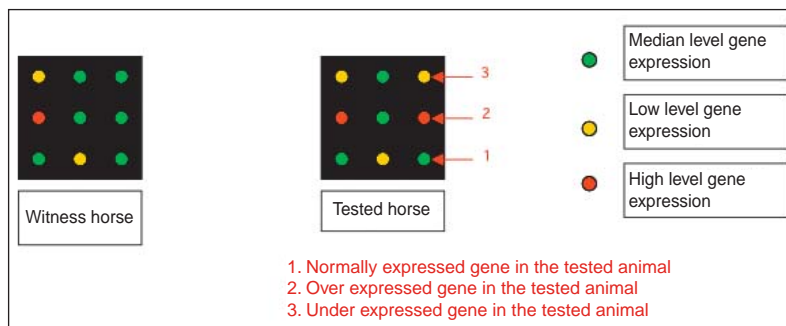
WHICH ARE THE STEPS IN A MICROARRAY ANALYSIS?

The analysis is carried out on horse cells. These can come from blood, joint liquid puncture, tracheo-bronchial wash or uterine wash. The genetic material is extracted and put on the micro-draughtboards in the laboratory, in order to be treated (Picture 2). Thanks to a very sophisticated technique (for more details, see Thomas *et al.*, 2005 ; Venkatasubbarao, 2004), a characteristic picture of the studied genes' expression is obtained. This picture will then be compared to one obtained from a witness horse on the same microarray (Picture 3). Every colour spot represents a specific gene. In our example, the microarray indicates two abnormally expressed genes. Marked gene #2 is over expressed, and gene #3 is under expressed in the tested animal in comparison to the witness horse. A therapeutic strategy should thus be established to decrease the expression of gene #2 and to stimulate the expression of gene #3.

Picture 2: The four steps in a microarray analyse.



Picture 3: Simplified example of a microarray reading.



WHICH IS THE CONCRETE USE OF MICROARRAYS IN EVENTING HORSES?

The majority of applications of microarrays in competition horses is based on the analysis of the gene expression level, in order to detect under- and over expression (Table 1). It then becomes possible to evaluate the adaptation of the horse to its training. For instance, genes corresponding to anti-oxidative enzymes as Glutathione Peroxidase (GPx) can be analysed. The synthesis of this protein should be proportional to the horses needs before and after effort. Under expression of these genes induces oxidative stress (de Moffarts *et al.*, 2005) and can lead to effort-linked muscular dystrophy. If such an under expression were detected, it could be compensated pharmacologically by specifically stimulating the deficient genes expression. On the

other hand, it remains possible to complement the animal with anti-oxidants on a daily basis in its food for instance. The horse will reach its best performances again, and recover optimally.

According to the same principle, microarrays can also very precisely diagnose inflammation, by analysing joint liquid for example. The evidence of the over expression of genes corresponding to pro-inflammatory mediators can indeed permit the characterisation of the exact type of inflammation (acute or chronic; mechanical, allergic or infectious ;...). The immediate interest is that the active principle to use in order to stop the inflammation process can be quickly identified. This way, time is won, and transformation of an acute into a chronic inflammation, which would have diminished short term performances and induced median term irreversible lesions, can be

avoided. Targeting the treatment increases its efficiency and decreases its secondary effects.

DO OTHER APPLICATIONS EXIST FOR MICROARRAYS?

Yes, other applications for microarrays exist. It can for instance be interesting to follow genetic profiles in a champion's lineage. These profiles can be associated to positive characteristics or excellent performances. On the other hand, champion's profile's selection could favour the outbreak of certain pathologies, if preventive measures are not taken precociously enough. Eventually, microarray technology can also be used to identify exactly pathogens like bacteria or viruses, which has positive repercussions on the veterinary public health.

CONCLUSION

Microarrays are molecular tools which should quickly find their place in veterinary medicine, in complement to the current diagnostic techniques.

Thanks to progress in the equine genome decoding research and to molecular technology, reliable and cheap microarrays should be commercialized in the near future. This would contribute to the increase of the well-being, health and performances of our horses.



1. What's a gene ?

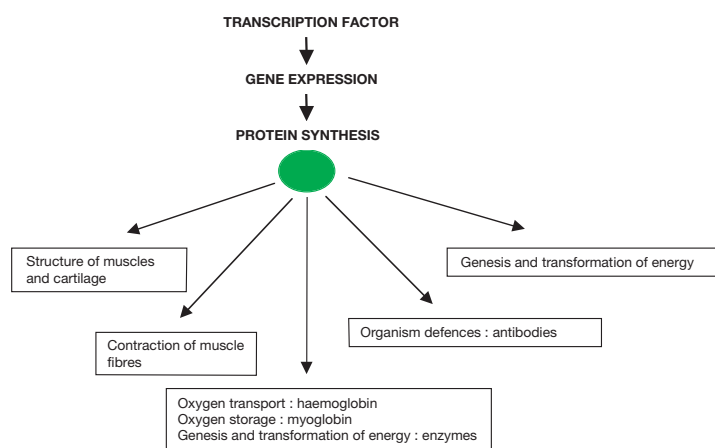
A gene is a part of the genetic cell material containing all the necessary information for protein production. The complete set of genes represents the genetic program of a horse, and is identical in each and every cell of its organism. This genetic program is the essential support to the correct growth and functioning of every animal.

2. What are genes for ?

The expression of the information contained in a specific gene permits the production of a particular protein. By analysing simultaneously the expression of thousands of genes, a simple microarray checks the correct synthesis of thousands of proteins.

3. What are proteins for ?

Proteins are omnipresent in the organism where they carry out many functions. They are indispensable to the horse's health, and to its ability to achieve performances.



To correctly achieve its function, a protein has to be synthesized in quantities proportional to the horse's needs at that precise moment. Excessive protein production can be as harmful as insufficient protein production. Thus, gene over- and under expression are both as disastrous. This gives a special interest to microarrays, as they detect these abnormalities, which could cause many pathologies and dysfunctions in horses.

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Aurélie Thomas



Tatiana Art

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Head Office
CH-4010 Basel, Switzerland
Tel. (41) (61) 272 23 72
Fax (41) (61) 272 23 88

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Needham, Harleston, Norfolk IP20 9LG
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The screenshot shows the TWYDIL website with a blue header. At the top left, it says 'New' next to an image of a TWYDIL syringe. The main banner features the TWYDIL logo with Swiss crosses and a photo of a horse race. Below the banner, there's a text block stating: 'TWYDIL® is a world renowned range of feed supplements, cosmetic and medications for top performance horses, produced in Switzerland.' To the left of this is a 'ProbiOX' logo. Below the text block is a 'SUMMARY IN OTHER LANGUAGES' section with flags for German, Spanish, Italian, Japanese, Turkish, Serbo-Croatian, and Polish. At the bottom of the page, it says 'The use of this website is subject to our Terms and Conditions'. On the right side, there's a search bar with the text 'Search :', a 'Search' button, and a navigation menu with links: 'Who are we?', 'TWYDIL® Label', 'Horses and vitamins', 'Range of products', 'Application', 'Programmes', 'FAQ, documentation', 'Orders', 'How to reach us?', and 'Blood tests'. Below the menu is an 'Anti-doping certificate' section with a 'Lot number:' field and a 'Submit' button. At the bottom right, contact information for PAVESCO AG is listed: 'CH - 4010 BASEL', 'TEL: +41612722372', 'FAX: +41612722388', and 'EMAIL: info@twydil.com'. Copyright notices are also present: '© Copyrights belong to TWYDIL®' and '© Copyrights of pictures belong to photographers.'

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