



**STUDY ADAPTOGEN**

# EVALUATION OF THE EFFECT OF AN ADAPTOGENIC SUPPLEMENT ON THE PERFORMANCE AND ON THE NON-SPECIFIC IMMUNITY OF THOROUGHBREDS

**THIS STUDY WAS SUPPORTED  
BY PAVESCO TWYDIL®**

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**P**hysical effort creates a much higher bodily stress than normal and natural conditions. For a race horse, this means increases in the heart rate (up to 240bpm), the respiratory rate (up to 120bpm), the body temperature (up to 41°C in the muscles), the ratio of the red blood cells and the circulating leucocytes (sometimes x 2), the lactic acid in the muscles and in the blood (the pH may be lower than 7.000), and the hormonal secretions etc. Recent studies have shown that, in horses as well as in humans, this effort-related stress leads to a decrease in the quality of immune responses – for example, the body's defences against infections days after the race ("open window period"), leading to illnesses, principally respiratory infections, during that sensitive period.

Training, a regular repetition of effort, is meant to lower the intensity of stress caused by physical work by adapting physiological responses to this kind of extreme investment of the body. Apart from training, feeding and supplements can have so-called "adaptogenic" effects as protective effects against stress. In the present study, a supplement contain-

ing specifically vitamin C, probiotics, and ginseng, amongst other ingredients, has been tested for its possible effects on race performance, stress by physical effort and resistance to respiratory infections.

## PRESENTATION OF THE STUDY

The study is a close examination of horses at rest and under test of strenuous physical effort on a treadmill before oral supplementation and 12 days later.

8 untrained, clinically healthy Irish thoroughbreds (3-4 years old) were used for the study. These horses went through a four week familiarisation routine where they were trained and progressively accustomed to a treadmill.

They stayed in the same stable and received the same feeding (daily 2 litres of concentrated feed and unlimited fodder) through the whole experiment. During the test, every horse's VLA4 was evaluated during a treadmill performance – VLA4 being the race speed at which the blood rate level of 4 mmol/l lactate is reached.



## TESTS

Different evaluations were carried out to measure (1) performance, (2) stress, and (3) immunity to infections. Systematically both horse groups (placebo and supplement) were evaluated. No treatment information was revealed to the team members involved in the handling of the horses and in the collection of the data.

Evaluation at rest: transcriptomic study of 23,000 leucocyte genes by the chessboard technique (see box page 9).

Evaluation under effort: direct parameters (lactates, heart rate, muscular enzymes) and indirect parameters (VLA4, V200) of sports medicine based on heart rate and lactatemia were measured (for more details, see HPH 08-09, p. 45-48). These parameters provide objective measures of the intensity of the effort and the corresponding stress in an individual.

### Tests before and after effort

- Hormones: plasma stress hormone variations, for example beta-endorphins and cortisol, reflect the intensity of the stress. A rise in the cortisol ratio is one of the factors responsible for the decrease in the immune response.
- Variations of the immune response of the pulmonary leucocytes (see box page 12). To study this, white blood cells were sampled from the pulmonary liquid and placed in a growth medium. The white blood cells' response to simulate viral or bacterial attack was then evaluated.

## TIMING OF MEASURINGS AND SAMPLINGS

2 days before and after the test, the horses were given a tranquiliser and a broncho-alveolar lavage (BAL) carried out. White blood cells were sampled from this BAL for an *in vitro* test of their immune response to specific stimulations.

The tests took place before and after every test of physical effort, i.e. on the days D1 and D27 (evaluation of effort's effects), D10 and D36 (evaluation of supplement's effects) and D13 and D39 (evaluation of supplement's effect on effort's effect).

On day D0 blood was sampled to provide white blood cells for the transcriptomic study.

In the next step, the horses were equipped with a device for continuous ECG-measurement and put on a treadmill (test 1) for a warm-up, a gallop (3,500m) at the speed of VLA4 (preset).

After 2 minutes of recovery, the horses performed again a 1-minute gallop at the speed VLA4, then 3 incrementally higher levels of 1 m/sec. each, with 1 minute for the first 2 levels and then up to full fatigue for the third level, i.e. the horses' inability to keep the necessary speed no matter how intensively they were encouraged to do so by the supervisor.

The data of "time to fatigue" (TTF), the duration (expressed in seconds) of the last level were recorded for each horse.

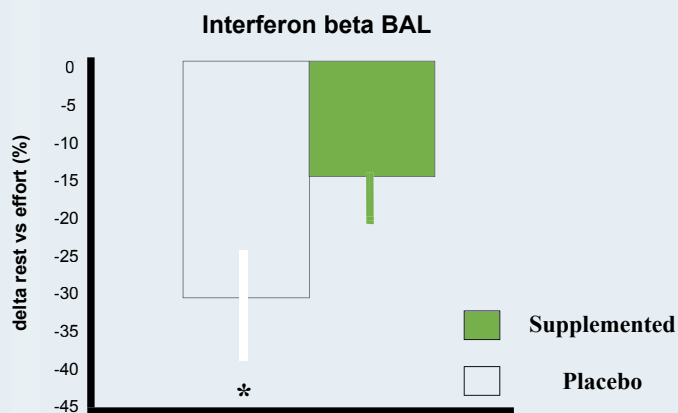


TATIANA ART (DVM, PHD, DIPL ECEIM)

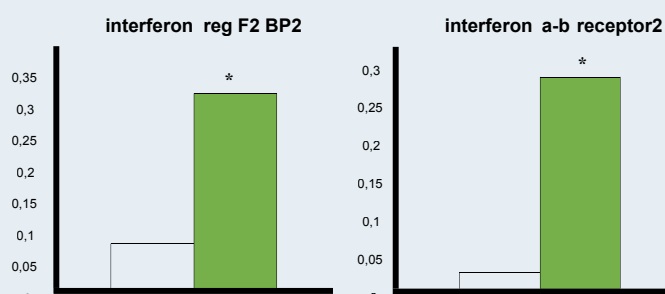
Professor of Physiology (muscular exercise) at the Veterinary department of ULg.

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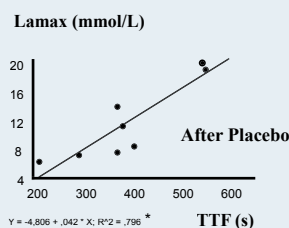
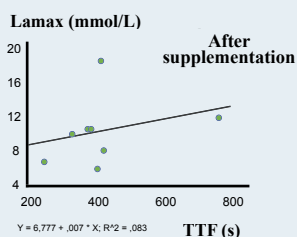
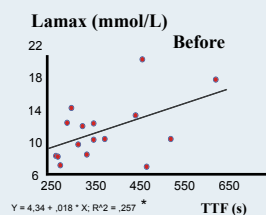
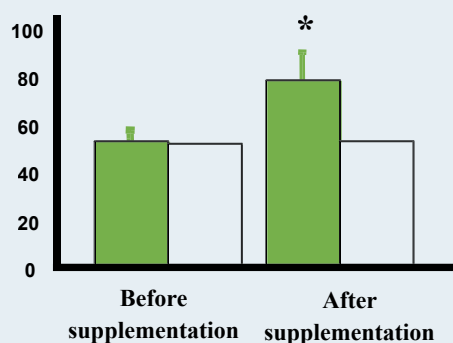




### Blood lymphocyte genes expression(Log2)



### TTF/lactate (s.(mmol/L)<sup>-1</sup>)



2 random groups each of 4 horses were chosen for the next step: a treatment group and a reference group. On day D1 and the following 12 days, the treatment group received a vitamin supplement in the feed (1 dose daily) and the reference group received a placebo. On day D12, both groups went once again through the same effort test as on day D0 (test 2).

The horses were then kept for 15 days in a paddock for a 15-day wash-out.

On day D26, the effort test was carried out again (test 3).

The administered content (supplement or placebo) was then reversed for the groups. 12 days later, i.e. on day D38, the last effort test was carried out (test 4).

## MATERIAL AND DESCRIPTION OF ANALYSIS

### *Physiological parameters under effort:*

Heart rate was continuously recorded with a Holter system, vein blood lactatemia with a portable analyser.

Blood samples were taken before and 1 hour after effort to measure the red blood cell ratio, the CK (muscle enzymes) and the stress hormones (beta-endorphins and cortisol).

### *Bronchoalveolar lavage (BAL), cytology and extraction of white blood cells.*

The horses were given a light tranquiliser for bronchoalveolar lavages (BAL) with a fiberscope while. 360 ml physiological serum was introduced into the lungs of the horses and re-collected.

The collected solution was subsequently used to count and isolate the cells by successive centrifugations. After isolation, the cells were exposed to virus or bacteria-like stimulation. Their response was then “read” in their mRNA (see box 9 and 11).

### *Transcriptomic study of the white blood cells*

10 ml blood was sampled on 4 horses on D0 and D26 as well as on D12 and D38, just before effort, for RNA extraction.

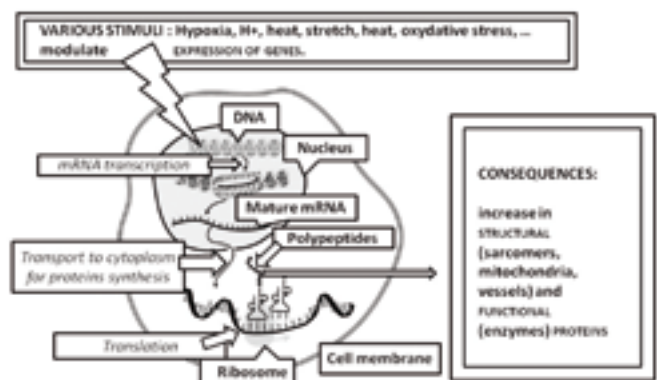
## RESULTS

### *Supplement's effect on aptitude to effort*

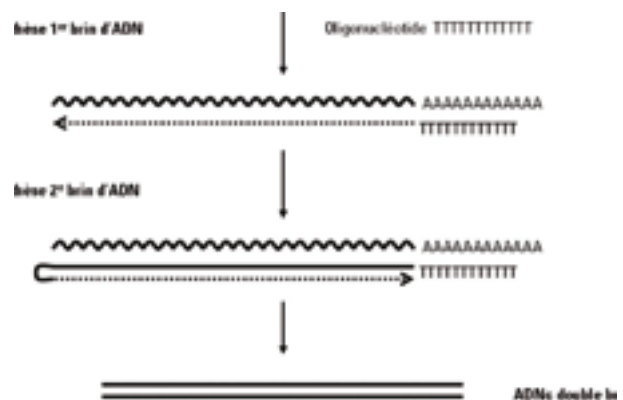
The whole outcome of the effort test confirms that high effort performance (up to fatigue) causes a significant physiological stress as can be seen e.g. in the heart rate, lactatemia, the rise of the stress hormones ratio etc.

## → WHAT IS TRANSCRIPTOMICS

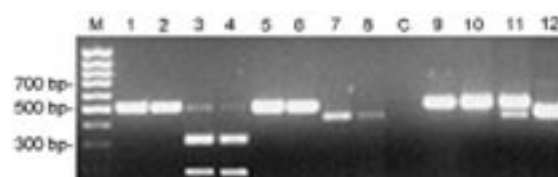
A transcriptomic study uses sophisticated techniques of molecular biology to find out if specific parts of a genome are expressed and translated. Basically, the genome in a cell is a kind of instruction of use for the construction and the operation of any organism. Construction and operation orders are transmitted by the mRNA which can be seen as carriers sent by the genome into the cell to trigger the synthesis of proteins necessary for the production of new tissue, enzymes, cytokines or hormones e.g.



A transcriptomic study "intercepts" the message (extraction of the mRNA from the cells) and reads it (transcription and PCR, or chessboard reading) to detect at a very early and precise stage physiological changes caused by a modification of conditions (e.g. by effort, training, feeding, inflammation or infection), and sometimes even before functional and clinical signs of such adaptations can be observed.

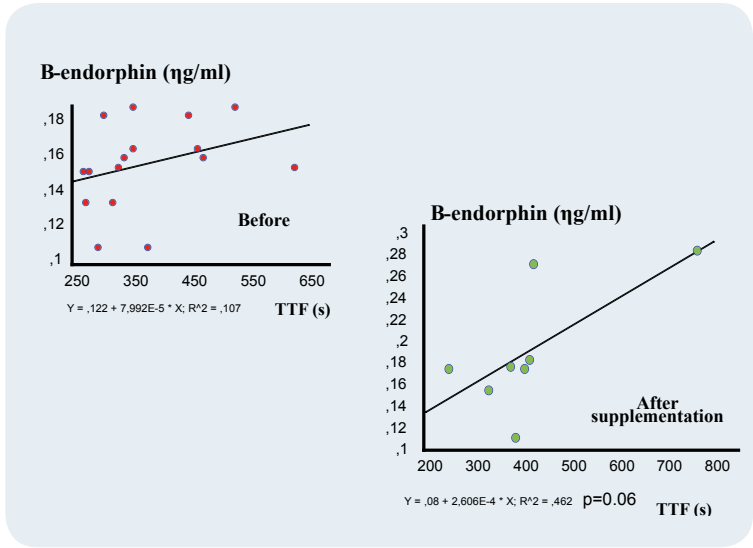


That is why transcriptomics were chosen to control the effect of the supplement in the test. Variations of gene expression were studied in the circulating leucocytes by a transcriptomic analysis on equine homologous chessboard (see HPH 04-05, 46-49).



The adaptogenic supplement lowers the maximal production of lactates and keeps the TTF very high. Statistically, the result is a significant change of the TTF/lactate ratio: the initial value of this ratio (the longer the TTF, the higher the lactate production) remains unchanged for the placebo group but is modified for the supplement group.

Hormonally, effort raises the level of the plasma cortisol but the ratio of beta-endorphins remains stable. The TTF increase in the 2nd test causes a rise in the secretion of beta-endorphins virtually associated with this parameter in the treatment group ( $p < 0.60$ ).



*Supplement's effect on the post-effort decrease of innate immune response.*

Effort causes a decrease in the response of pulmonary blood cells to a simulation of virus attack. This means that the cells' response to a polyIC stimulation (polyIC having the same double-string as a virus RNA), as measured in the gene expression of some pro-inflammatory cytokines, is weaker than 24h after effort.

A 12-day supplementation of the horses tends to break down this weakening of the immune response as can be seen for example in the beta-interferon gene overexpression in the samples from supplement horses 24h after effort.

*Supplement's effect on the gene expression of white blood cells*

Based on the results, the supplement horses show, compared to the placebo horses, an overexpression of some immune genes (higher immunity) and of some genes favouring metabolic stress resistance.

**CONCLUSIONS**

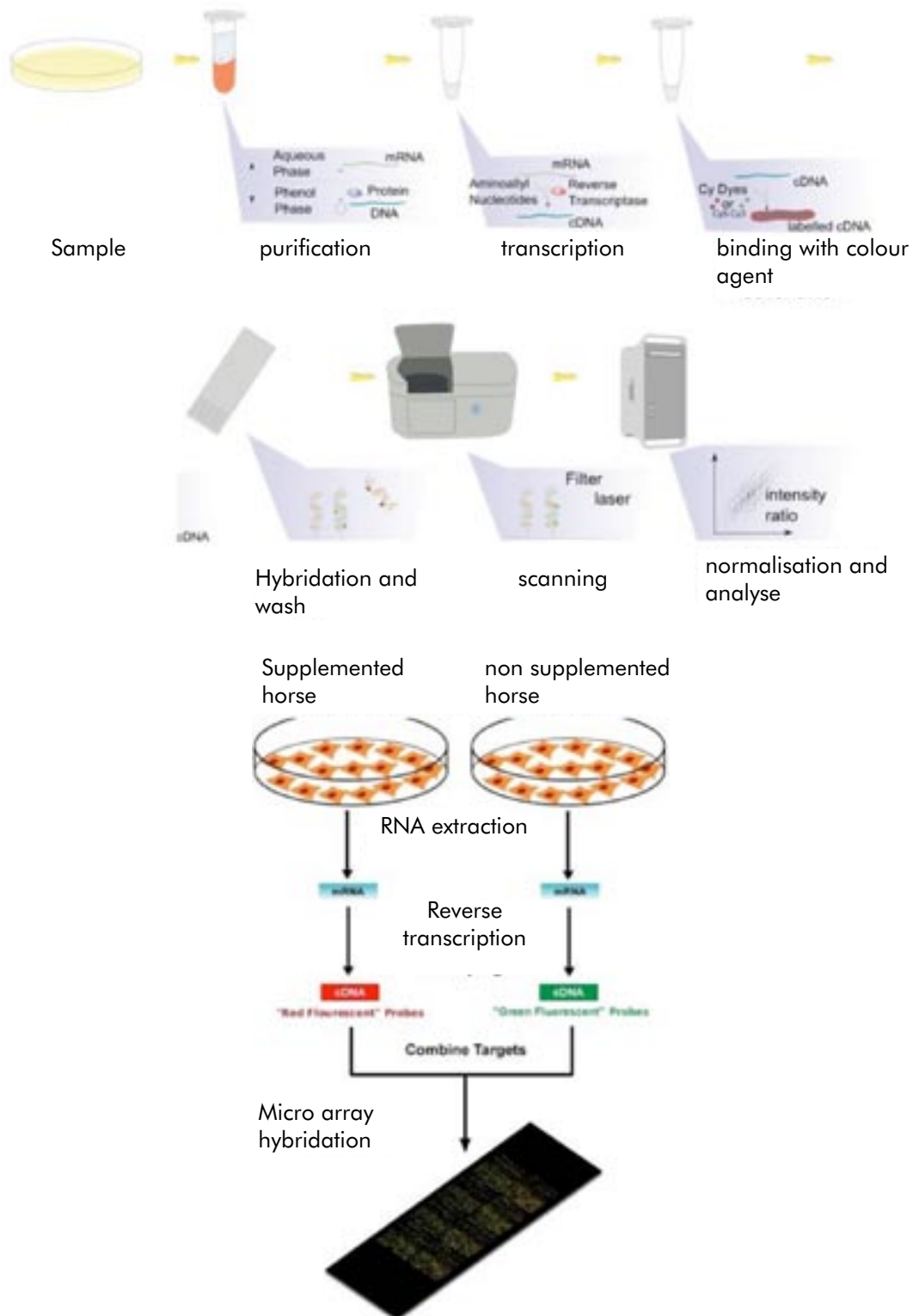
The adaptogenic supplement used for this study seems to have protective effects against the process of decreasing immunity caused by effort both in the blood and in the lungs. Furthermore, it seems to limit the production of lactates during intensive effort, which makes it comparable to training, in that it enables the development and the use of aerobic metabolic resources.





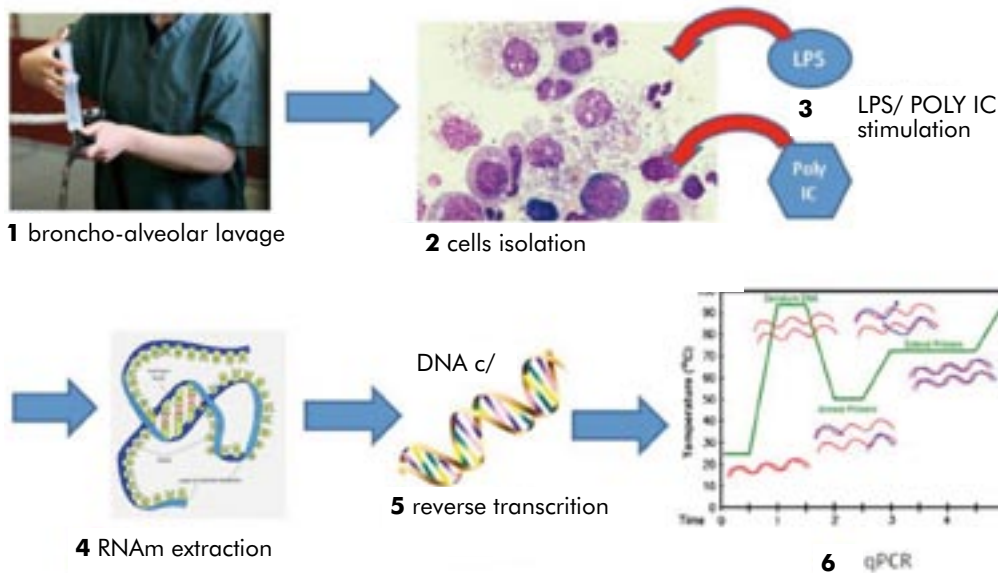
## → CHESSBOARD TECHNIQUE

As described in the diagram on page 9, the expression of some genes must be quantified. With RT-PCR, the presence of mRNA is detected by targeting genes that may be overexpressed or underexpressed. Rather, the chessboard procedure is an analysis of the whole genome's response. The disadvantage of this procedure is the huge response examination of 23,000 genes implying the use of sophisticated statistics. On the other hand, with the chessboard method, every single modification of gene expression can be detected.



## → STEPS OF THE STUDY OF THE INNATE IMMUNITY IN WHITE BLOOD CELLS OF THE DEEPER RESPIRATORY

### Study of the pulmonary immunity



## PROFESSOR PIERRE LEKEUX ANSWERS

Professor of Physiology at the Faculty of Veterinary Medicine of Liège

### 1/ How would you explain to a non-scientific horse professional what is new in this study?

The adaptogenic supplement tested in this study seems to have a double beneficial effect on race horses.

Firstly, it seems to improve their ability to ward off numerous pathogenic agents in their respiratory system when they are under competition stress.

Secondly, it seems to improve their efficiency of muscular energy production and, consequently, performance.

### 2 / Why is immunity so important for horses and why did the study choose to focus selectively on the lungs?

A race horse is exposed to a double risk of diseases (viral and bacterial ones)

which affect predominantly the main entrance into the body, i.e. the airways. On the one hand, a race horse travels a lot and meets many other horses, which increases the spread and, thus, the contamination risk of infectious diseases. On the other hand, training and competition stress has a negative effect on horses' immune system, i.e. their ability to ward off a viral or bacterial aggression.

That is why, though race horses are inoculated, they still present so frequent sub-clinical respiratory infections altogether with the corresponding harmful effects on their performance.

### 3/ Practically speaking, can you say that the supplementation somehow

### simulates the effects of training?

The adaptogenic supplement does not replace training, but it seems to enable positive effects, i.e. a better use of oxygen and, thus, a lesser production of lactates for a particular effort.

Performance and post-effort recovery should theoretically be improved.

### 4/ Concretely speaking, in which disciplines and when would you recommend to use the feed supplement?

This kind of adaptogenic supplement should be indicated for horses who deliver intense and repeated efforts – without consideration of discipline.

The most adequate time for administration should be intense training phases and competition periods.