Exercise influence on gene-expression profile in endurance horse PBMC

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ABSTRACT

It is known that moderate physical activity may have beneficial effects on health, while strenuous physical effort induces an inflammation-like state. The molecular mechanisms that underly the cellular response to exercise are still unclear. Instead it is plain that the immune system plays a key role in this phenomenon. On the basis of these issues the physio-pathological condition, which develops in athletes subjected to heavy training (i.e. overtraining syndrome), could be derangement the result of the cellular immune regulation.

The purpose of the study was to have a snapshot of endurance horse transcription in strenuous conditions, in order to identify candidate genes for this immune system derangement. This is an essential prerequisite for planning an appropriate training schedule to obtain better performance and preserve animal welfare. We choose peripheral blood mononuclear cells (PBMCs), Considering them the best candidate cell type to investigate the physiological changes connected to exhaustive exercise. We perform a wide gene expression scan, using microarray technology on 10 horses chosen among high-level participants in national and international endurance races. Time course strategy with three different time points allowed us to reveal changes of gene expression comparing the post-effort samplings (“immediately after the race” and “24 h after the race”) with the basal one (“at rest”). Gene expression analysis was performed using an equine oligonucleotide microarray which included 384 equine transcripts of the mitochondrial and nuclear genome. A biological interpretation of the whole data set was performed using two types of analysis to identify the main biological functions and the main metabolic pathways revealed by the expression profile. The categorization of the gene functions was generated through the use of Ingenuity® Pathway Analysis: the significant genes associated with biological functions were considered for the analysis. Protein synthesis, apoptosis, cellular movement, growth and proliferation were the main cellular functions associated with the modulated genes. A GO (Gene Ontology) annotation was performed using Blast2GO software. qRT-PCR to validate ongoing changes in gene expression.

After filtering, 110 genes were significant (p<0.05) for the comparison “race vs basal” and 108 for the “24h vs basal”. IL1R2, MMP1 and MMP13 achieved respectively the maximal expression values after race (fold 7.92, 6.43 and 4.58). These three genes remain the most expressed in the second comparison, 24h after race, but with a lower level of transcription (fold 1.49, 1.30 and 1.23). On the contrary, the maximum down-regulation after race was found for COX7A1, COX7A2L and CCL6 with -34.86, -20.80 and -12.97 fold changes respectively. Regarding the comparison basal-24h after race, the three most down-regulated genes were the following: TRAK1, HIP1 and SLC25A10 with -2.7, -21.1 to -20.1 fold changes respectively.

Funded by SELMOL-MIPAF.
Preliminary survey on gross composition and nitrogen fraction distribution of Ragusano donkey milk with different protein patterns

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ABSTRACT

In the last few years in Sicily the consumption of donkey milk arised in importance: it is often well tolerated by infants and adults affected by cow’s milk protein allergy, probably thanks to its protein fraction and its low casein-to-whey protein ratio, as confirmed by few clinical trials. A recent survey on the protein fraction of donkey milk highlighted a remarkable variability of individual milk samples of Ragusano breed: the occurrence of four different isoelectrophoretic patterns was reported. This heterogeneity, associated with both caseins (αs1-casein) and whey proteins (β-lactoglobulin II), might affect milk protein composition and potentially its nutritional and allergenic properties. In this preliminary research the gross composition and nitrogen fraction distribution of donkey milk samples with different isoelectrophoretic patterns were investigated. In a farm, included in the functional controls conducted by the Italian Breeders Association (AIA), 7 donkeys (3 producing “reference” milk with a normal protein profile and grouped in pattern A, 1 producing milk without αs1-casein and identified as pattern B and 3 lacking β-lactoglobulin II grouped in pattern C) were selected. Monthly, individual milk samples were collected from the donkeys mechanically milked twice a day, following the procedure provided by the AIA experimental protocol. Milk yield was recorded and the two samples taken from the same donkey each month were pooled before analysis. Crude protein, fat, lactose and urea content as well as somatic cells count were measured by a Foss Milkoscan in the milk laboratory of AIA. Total nitrogen (TN), non casein nitrogen (NCN) and non protein nitrogen (NPN) contents were obtained according to the procedure AOAC International (Official Methods of Analysis) and determined by Kjeldahl’s method. Casein content (CN) was calculated as (TN - NCN) x 6.38 and total whey protein (WP) as (NCN - NPN) x 6.38. Average milk yield was 1.90 kg/day. Ragusano milk, which resulted poor in protein (1.51 g/100 g) and fat (0.13 g/100 g) and rich in lactose (6.52 g/100 g), appears more similar to mare and human milk than to other milks. In the whole sample, somatic cells count was low (about 13.500), urea content was 26.41 mg/ml. The fat content of donkey milk confirmed to be very low, even lower than those previously reported by other authors. During the lactation, proteins and caseins gradually decreased, whereas whey proteins increased in the last months. In the first seven months, the average milk yield was 1.754, 1.919 and 2.033 kg/day in pattern C, A and B respectively. Fat content resulted lower in pattern C and B (0.09 and 0.11 g/100 g) than in reference pattern A (0.18 g/100 g); the same trend (C<B<A) was observed for protein content (1.48, 1.49 and 1.56 g/100 g). Lactose was lower in reference pattern A (6.47 g/100 g) than in pattern C and B (6.52 and 6.58 g/100 g). Nitrogen distribution, compared between defective (B and C) and the reference pattern, showed that the milk sample lacking the αs1-casein had a lower casein content (0.62 vs 0.64 g/100 g), while the few tested milks without β-lactoglobulin II accounted for a lower whey proteins amount (0.61 vs 0.66 g/100 g).
Influence of extruded linseed supplementation on ass milk quality

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ABSTRACT

Donkey breeding is attracting increasing interest due to the spread of donkey therapy and, recently, an increase in ass milk production. Ass milk has interesting nutritional properties, including low allergenicity, that are useful in children's diet. PUFA levels are also good, although they vary according to environmental, diet and physiological conditions. In this research, conducted in the framework of a project aimed at a greater diffusion of Romagnola ass, we tested the effect of an extruded linseed supplement, rich in C18:3 n3, on some blood parameters and on ass milk fat composition. The pasture-based diet administered at the beginning of the trial had to be gradually replaced with hay, due to a sudden whether change 2 weeks into the trial. Exceptionally early snowfall then required an all-hay ration to be provided. After a 2-week control period, pregnant asses (6-7 month) received 50 g/d linseed supplementation for 3 weeks and 100 g/d in the following 3 weeks. The research, involving 5 subjects bred on the high Apennines around Forlì, began on the last week of September. Early snowfall reduced the third phase of the trial to a single week. Milk was sampled 6 times (day 0, 6, 15, 30, 37, 44) and blood twice (day 0, 44). Milk PUFA, particularly C18:3 n3 and C18:2 n6, were high (21-23% and 8-10%, respectively) in the first two samples—collected during the control period—due to the pasture-based diet. Their levels, especially C18:3 n3, fell (11.67%) when the pastures were exhausted and were substituted with hay; when the lower linseed supplement was started (day 15), they rose again (17.66%) in the sample collected midway through the administration of the 50 g/d supplement (day 37). Levels fell again, to ca 10%, in the samples taken in the first week of the third phase. The fluctuation may be explained either by changes in the diet (exhaustion of pastures) and/or by the fact that in the last 3 months of gestation there is a physiological shift in the use of n3 fatty acids from milk production to the development of the foetal nervous system. Similar fluctuations in choline levels lend support to the hypothesis. The blood parameters considered in this study (total WBC, platelets and total triglycerides) had an opposite trend to n3 fatty acids, which have anti-inflammatory, platelet-disaggregating and triglyceride-lowering effects. Diet supplementation with n3 rich feeds may thus play a useful role, especially in animals fed dry forage.
Effects of artificial photoperiod and β-carotene administration on the resumption of the mare’s oestrous activity

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ABSTRACT

The resumption of the mare’s oestrous cycle is regulated by the interaction of the photoperiod (equids are long-day breeders) with some nutritional factors, mainly β-carotene levels. The study tested the single and combined effects of of β-carotene and the induction of an artificial photoperiod on anoestrous mares in winter, considering previous investigations in this direction. The mares were exposed to fairly stressful artificial light (300W from 0200 to 0400 am), envisaging the prolongation of the natural photoperiod, differently from other studies. Since the last 10 days of December blood progesterone (P4) level of 40 Italian trotters was monitored. Eight trotters showed changes in P4 and were excluded after 4 weeks. The remaining mares were randomly assigned to one of 4 groups [Contr (control: n=6); Beta (β-carotene: n=6; 500 mg/d β-carotene); ContrLux (artificial photoperiod: n=10); and BetaLux (β-carotene and artificial photoperiod: n=10)] and monitored for plasma progesterone and oestrogen, twice in a week, until the end of the research (25th April). In all groups progesterone levels showed an early resumption of the oestrous cycle, which however was earliest in “β-carotene and artificial photoperiod mares” (BetaLux: 27.0±20.3; ContrLux: 42.1±22.8; Beta: 37.1±6.9; Contr: 56.4±37.4 days from the beginning of the study). Cycling activity in BetaLux mares resumed on average one month (16th February) before than in the Contr group (17th March). Oestrogen profiles were consistent with P4 levels, with a peak in all groups 2 days before the rise of P4. In conclusion, combined β-carotene supplementation and exposure to an artificial photoperiod successfully induced resumption of cyclic activity in February, the latest possible time allowing parturition in the early days of the following year.
Preliminary results on the donkey salami made in Sicily

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ABSTRACT

In a dairy jennet farm the meat, which can be produced using the foals in excess, could be another profitable income. Equine meat is very thin and with high protein content; above all, the fatty acids are unsaturated, being a monogastric animal product. The donkey meat processed products are sold in niche market and are very appreciated by the European consumers. A study on the characteristics of the donkey salami made in Sicily was carried out using a 12 months old foal derived from Ragusano breed. Two different mixes were compared: only donkey meat (A thesis) and donkey meat plus the 10% of Nero Siciliano pig fat (AS thesis). The meat was husked of fat and nerves and mixed with 3.5 kg/q of Aromil D.S+Nisal, 1 l/q of Nero D’Avola wine, 150 g/q of not crushed white pepper, and the following crushed spices: 150 g/q of black pepper, 150 g/q of white pepper and 60 g/q of garlic. The mixes were gloomed with a holed plate (Ø 8 mm). After that, the chopped fat pig in the AS mix was added. A and AS mixes were bagged in mutton gut (Ø 24/26). The fresh salami weighed on average 205 g, and for 4 days were dried, modifying the temperature (T) from 22 to 16°C and the relative humidity (R.H.) from 60 to 75%. After that, for 30 days the products were cured at T=14°C and R.H.=78%. At the slaughtering the lived animal and the carcass weights were registered. At the salami production the weights of the bones, the meat and the other tissues of each carcass quarter, while at the beginning and at the end of the drying period and at 15 and 30 days of the curing period the salami weights were registered. In the same time samples of salami were collected for pH determination and microbiological analyses. The salami chemical composition was determined at the bagging and at the end of the curing. A sensorial analysis test was carried out with a panel of 91 potential consumers, to value A, AS and C salami (only Nero Siciliano pig meat) at 16 days of cured, with a 3 point hedonic scale (1=low; 2=medium; 3=high) on colour, odour, taste and chewing parameters. The lived animal weight was 245 kg. The slaughtering, the meat, the bones and the fat yields were respectively 58, 53, 24 and 23%. During the curing the weight decrease was on average higher in A than in AS salami (final value: A=55% vs AS=49%). This result could be affected by the higher water content in the muscle tissues than in the fat ones. The pH trend was for both theses on line with the values of a normal curing process, but the A thesis achieved more rapidly a pH value >6, in relation with a higher weight decrease. This result shows the possibility to reduce the curing period at <30 days for the salami produced by only donkey meat. The protein and the fat contents of the cured products were respectively equal to 59.0 and 26.0% in A, and 42.7 and 45.3% in AS, in accordance to the fat pig addition in the AS mix. The microbiological analyses showed the absence of Listeria monocytogenes and Salmonella spp. and the presence of coliforms, Escherichia coli and enterococcus. Their concentrations were higher in the mixes and decreased during the curing. This result requires an improvements of hygienic environmental conditions during the making process. The 39.6%, the 26.4% and the 31.9% of the consumers appreciated respectively A, AS and C salami, while the 2.1% did not give any preference. The hedonic parameters on average showed better values in the thesis A (2.6) than in AS (2.4) and in C (2.3).
Histological characteristics of the *longissimus dorsi* muscle in the italian and polish crossbreed horse meat

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**ABSTRACT**

The meat produced by local horse does not meet internal market demand for the high costs. For that, slaughter horses mainly imported from Eastern Europe Countries. Horse meat consumption is very high in some Italian regions such as Apulia and in the last few years in considerably increasing. The fibre type composition deeply influences the post-mortem changes in the conversion of muscle to meat, as well as the product quality. Many recent studies found correlation between fibre type or size, and eating quality. The aim of this study was to investigate histological characteristics (surface and diameter) of muscle fibres and adypocytes associated with two different genetic types sold in Italy. Ten horse for each genetic type (TPR x Abruzzese and TPR x Polish) were slaughtered at the age between 24 and 26 months. After slaughtering the carcasses were chilled at 3°C for 24-36h. Samples of *longissimus dorsi* (LD) muscle were collected from all animals for the histochemical characterization of the muscular fibres. Cross sections were cut and mounted on spindles before sectioning 15 mm thick using a Reichert-Jung freezing microtome. Serial sections mounted on glass microscope slides were stained with NADH-Tr, myofibrillar ATPase reacted at alkaline pH to differentiate muscle fibre type, according to their oxidative and glycolitic capability, and hematoxylin in order to stain intracellular fat content. Fibers were classified on the basis of stain reactions. Sections of each fiber type were analysed using an Image Analyzer Vidas by Zeiss, to determine fiber diameter and fiber percentage type. The two horse genetic types showed little variation in the fiber type of the meat, considering the least square means of all 3 fiber types (red, intermediate and white) found in the samples muscle. The alpha-white fibers were larger than beta-red and alpha-red (P<0.05). Significant differences in distribution were found for most fibre types within breeds. A higher proportion of slow fibers was found in the longissimus muscle of the TPR x Abruzzese (P<0.05). The diameter of the adypocytes was larger in TPR x Abruzzese (P<0.05). The histological results showed that 2 genetic types had small effect on the size of the LD fibers, while TPR x Polish had the amount and the size of adypocytes respectively proved to be lower and smaller than the other genetic type.
Infrared video-thermography of sport horses on a high speed treadmill: a pilot study

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ABSTRACT

The reduction of performance in sport horses often leads to a severe economic loss. Despite this, mechanisms of performance reduction and chronic maladaptation in sport horses are still poorly understood.

It is possible that horses, suffering from sub-clinical diseases or not properly trained, respond with a higher muscular, cardiac and respiratory effort, than well-trained subjects, when tested for physical work. During these tests, the animals need to be not disturbed by invasive instruments, to avoid changes in their physiological responses.

Infrared thermography meets these requirements, being a non-invasive technique. The infrared thermography may be used to remotely assess skin temperature at distance, without interfere with animal’s behaviour.

Single thermographic images have already been used in veterinary medicine as a diagnostic method for lameness and inflammations in sport horses. Considering the authors’ knowledge, thermographic videos have never been taken in horses running on a high speed treadmill.

The aim of the study was to verify the possibility of recording properly thermographic videos of sport horses tested on a treadmill and investigate their significance.

A digital video thermography of two horses on treadmill, using a thermocamera AVIO TVS 500, which is a very sensitive and portable instrument, was successfully created videos were taken for the entire duration of tests, from a lateral and a dorsal position, showing the heating of different skin regions of the horse, moving at different gaits.

The next step will be to optimize and standardize the video-thermography technique, and determining the heating to be considered normal, in subjects without disease and well-trained, working on a greater number of horses. The contemporary use of other techniques (such as heart rate variability measure, blood tests, ultrasound) will allow to recognize which diseases cause the thermal anomalies detected.

Information obtained about skin temperature evolution during physical efforts may be very useful to optimize training and performance of sport horses; moreover, they will objectively allow to recognise when a horse is in the range of a good condition. They also promote a better study of those diseases that modify muscle blood flow and thermoregulation, as well as allow the early identification of inflammations related to musculoskeletal problems.
Epidermal Growth Factor (EGF) in mare and ass milk: a preliminary investigation

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ABSTRACT

Mare and ass milk are nowadays increasingly used in human nutrition, especially for infants suffering intolerance or allergies to other foods such as cow milk or derived from soy (Businco et al., 2000). The milk of these two equine species seems to have also generic and non-defined dietary/healing properties, especially with regards to the gastrointestinal tract. Human milk contains Epidermal Growth Factor (EGF), a mitogenetic protein that has been shown to promote growth and maturation of infant intestinal mucosa (Odle et al., 1996). Since EGF is not destroyed by proteolytic enzymes, it may be absorbed in the infant’s gut and thus affects other tissues of the suckling infant with several beneficial effects (Playford et al., 2000; Warner and Warner, 2005). Furthermore, EGF is considered to be a “surveillance peptide” able to promote the repair following injury to the gastrointestinal mucosa (Petrides et al., 1985).

The aim of this preliminary study was to assess the presence of an epidermal growth factor consisting of 53 amino acids in mare and ass milk. The study was undertaken on milk collected from 4 Murgese breed mares and from 4 Martina Franca asses reared according to the traditional system. The milk samples were collected by manual milking every 10 days, with a total of 13 samples collected for each animal. Milk samples were centrifuged at 4000 x g for 15 minutes in order to remove the fat and the remainder was frozen at -80°C until analysis was performed (ELISA sandwich). Milk collected from 2 cows according to the same mare and ass sampling scheme and Phosphate Buffered Solution (PBS) served as negative controls while the synthetic peptide used for the production of the hyper immune serum, a sequence made of 14 of the 53 amino acids constituting the horse EGF, served as the positive control. There were no aspecific reactions between the polyclonal antibodies of the hyper immune serum and the negative controls. The polyclonal antibodies used for the ELISA sandwich were obtained following inoculation of the synthetic peptide conjugated to a carrier into rabbits. The substrate used to reveal the occurrence of the reaction antigen/antibody was the Sigma Phosphatase Substrate.

The antigen-antibody reaction between EGF present in milk and the hyper immune serum containing polyclonal antibodies anti-horse EGF enabled to detect the presence of the growth factor both in mare and ass milk during the first four months of lactation. The positive reaction detected by the ELISA technique not only puts in evidence the presence of EGF in ass milk but it also suggests a possible similarity between the amino acid sequence of the EGF in these two species, at least for the 14 amino acids sequence used as antigen.

The presence of such a surveillance peptide in ass milk could be a key to understanding the properties of some dietary/therapeutic milk of this species.
Morphological traits of the “Indigeno Siciliano” horse

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ABSTRACT

From a historical perspective, the “Indigeno Siciliano” horse constitutes an invaluable heritage for the island of Sicily where, in former times, it was reared both by farmers and by big landowners who appreciated its morphology and temperament. In ancient times, the Greeks (known all over the world for their excellent horses) praised the Sicilian horse and, over the centuries, the rearing of the “Sicilian horse” on the Mediterranean island continued under the various dominations and cultures that traditionally admired horses as noble animals. The present autochthonous population of Indigeno, acknowledged by the Mediterranean Genetic Centre, exhibits homogeneous characteristics that reflect not only its oriental origins (Asian, African), but also hints of Anglo-oriental and English blood and its derivates. The spreading of this population within a confined insular territory such as Sicily – which at the same time represents an extremely heterogeneous environment – constitutes a precious reservoir of genetic variability capable of responding to an increasing variety of requirements due to an extended equestrian tourism. The remarkable morphological and genetic variability that characterizes the “Indigeno Siciliano” horse, and the renewed interest in this breed have triggered the effort to systematically identify these subjects in order to be able to develop a breeding programme which aims to the recovery and protection of an equine population which, actually, has not a National Pedigree Register. Due to its characteristics and nature, the “Sicilian horse” is particularly versatile and (considering its acknowledged rusticity) eco-friendly. The purpose of our study was to determine the morphological type of this equine population in order to provide useful data for its ethnic identification. The most significant morphometric traits of the 74 “Indigeno Siciliano” horses examined (67 brood-mares, 7 stallions) were measured in erect position, using Lydtin’s stick, calliper and flexible meter, and the most significant morphometric indices were calculated. The mean values (±SD) obtained (withers height: 152.60±4.03 cm; rump height: 151.52±4.50 cm; chest height: 68.42±4.21 cm; chest width: 39.48±3.59 cm; body length: 162.27±8.54 cm; lateral body conformation index: 94.25±4.74 cm; body index: 86.70±4.88; traversal body index: 25.53±3.75; dactyl-thoracic index: 10.68±1.34; cephalic index: 32.49±5.06; pelvic index: 44.37±5.76) indicate that the “Indigeno Siciliano” horse has been used in various ways and can be categorized as meso-dolichomorphic type. Further research is needed in order to bestow on the Indigeno Siciliano” horse the attention and appreciation it deserves.

Research financed by the Regional Ministry of Agriculture and Forestry for the Region of Sicily.