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## Thymosin $\beta_4$ Bulletin

The Racing Medication and Testing Consortium (RMTC) has received a number of inquiries regarding thymosin  $\beta_4$  (thymosin beta) and products purported to contain the active ingredient or substances related to it. Thymosin  $\beta_4$  is a naturally occurring protein which is an endogenous substance in all mammals including the horse. Thymosin  $\beta_4$  consists of a number of smaller peptide chains that are covalently linked to each other. One of these chains is referred to as LKKTETQ based on its amino acid sequence and represents the active site within the protein thymosin  $\beta_4$ .

A peptide with this amino acid sequence is being synthesized as its N-acetylated derivative and is being marketed for use in the horse. This peptide is marketed on a number of websites and is sometimes referred to generically as TB-500. Some marketing materials include claims that the substance can build muscle, speed muscle recovery, stop bleeding, increase red blood cells, and decrease inflammation. The cost of the substance ranges from \$150-300 per 10 milligrams. The recommended dosing regimen is subcutaneous injections of the contents of one vial containing 10 mg of thymosin  $\beta_4$  once a week for six weeks then monthly indefinitely. The protocol also includes a recommendation to administer TB-500 one day after an intense workout.

The University of California-Davis, Kenneth L. Maddy Laboratory has analyzed a number of "TB-500" labeled substances and has reported to RMTC that many of these substances do not contain any thymosin  $\beta_4$  or any peptide derived from thymosin  $\beta_4$ . The laboratory reported that most did not contain any proteins, peptides, or amino acids.

However, the Kenneth L. Maddy Laboratory reported that one of the samples tested actually did contain N-acetylated LKKTETQ, the chemical derivative of a peptide that is found in thymosin  $\beta_4$ , and which is purported to be the active component of TB-500. While there is limited peer-reviewed scientific literature to support various marketing claims made about the substance, studies have shown that thymosin  $\beta_4$  and peptides derived from it *may* have some effects in the horse.

Several research studies have investigated the effects of introducing thymosin  $\beta_4$  and peptides derived from it into animals via injection.<sup>1</sup> Specifically, they have found that injecting the peptide corresponding to the amino acid sequence LKKTETQ in thymosin  $\beta_4$  may have a role in wound healing. Additionally, they have determined that this peptide may also be responsible for angiogenesis – the development of blood vessels (not to be confused with erythropoiesis – the development of new red blood cells). Furthermore, it may also be responsible for cross-linking with fibrin at sites of blood

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<sup>1</sup> Sonse, G., *Biological activities of thymosin  $\beta_4$  defined by active sites in short peptide sequences*, The FASEB Journal, 24: 2144-2151 (July 2010); Philp, D., *Animal studies with thymosin  $\beta_4$ , a multifunctional tissue repair and regeneration peptide*.

coagulation in wound healing.<sup>2</sup> It appears, however, that the anti-inflammatory properties are attributed to another peptide that has not been found in TB-500 or similar products.

The Hong Kong Jockey Club Racing Laboratory has reported that it has developed a test to detect a race-day (or same-day) administration of N-acetylated LKKTETQ from TB-500.<sup>3</sup> In this study, two horses were administered a single dose of N-acetylated LKKTETQ. The investigators were able to detect a metabolite of N-acetylated LKKTETQ, unique to the synthetic peptide, at 11.3 hours in plasma but only for 9.7 hours in urine samples. However, based upon the recommended treatment regimen for TB-500 described above, a race-day administration would be highly unlikely. Therefore, in order to control the use of N-acetylated LKKTETQ the RMTC recommends regulatory agencies perform out-of-competition testing. This testing approach will discourage would be abusers from using these products between race dates.

The FDA has not assessed this peptide for safety and efficacy in the horse. The only products available under this generic label are compounded which requires no proof of consistency of content, purity, or safety. Accordingly, under the ARCI guideline regarding unclassified medications and foreign substances the RMTC recommends that any administration of the N-acetylated LKKTETQ be treated as a prohibited act and that a finding for N-acetylated LKKTETQ or its metabolites be treated as a finding for a prohibited substance. As such, it should be treated as an ARCI Class 1 substance with Class A penalty guidelines attached.

If you have any additional information regarding these substances please do not hesitate to contact Dionne Benson with RMTC at [dbenson@rmtcnet.com](mailto:dbenson@rmtcnet.com) or 859-224-2844.

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<sup>2</sup> Mannherz, H.G., *The  $\beta$ -Thymosins: intracellular and extracellular activities of a versatile actin binding protein family*, Cell Motility and the Cytoskeleton 66: 839-51 (2009).

<sup>3</sup> Ho, E.N., *Doping Control analysis of TB-500, a synthetic version of an active region of thymosin  $\beta_4$ , in equine urine and plasma by liquid chromatography-mass spectrometry*, Journal of Chromatography A, 1265: 57-69 (2012).