

# Proline and Heat-tolerance

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Dietary proline supplementation is useful for enhancing heat-tolerance and semen quality of rabbit bucks affected by heat-stress. These effects are dose-dependent and were achieved at doses ranging between 50 and 100 mg proline/kg DM. Higher proline concentrations likely have undesirable effects on redox status and sperm quality.

Keywords: proline ; antioxidants ; heat stress ; metabolites ; semen ; rabbits

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## 1. Introduction

Proline is an amino acid with a unique chemical structure and multiple biological functions; it acts as a proteinogenic amino acid, an osmolyte, and an antioxidant <sup>[1][2]</sup>. In addition, proline regulates several pathways that are active under various stress conditions <sup>[3]</sup>. Among these pathways, proline dehydrogenase/proline oxidase (PRODH/POX) signaling is upregulated under oxidative stress and other stress conditions <sup>[4]</sup>. Proline also regulates the mammalian target of rapamycin activation pathway <sup>[5]</sup>, which integrates signals regarding nutrients (glucose and amino acids), cellular energy status, and growth factors, thereby controlling cell proliferation and function <sup>[6][7]</sup>. Kang and coworkers <sup>[8]</sup> found that dietary supplementation with 1% proline increased the expression of heat shock protein 70 (HSP70) in weaned piglets that were challenged with lipopolysaccharide. In adults, Ma et al. <sup>[9]</sup> observed a relationship between decreased blood plasma proline concentrations and increased prevalence of semen abnormalities and male infertility. Accordingly, proline can be considered a functional amino acid for mammalian species <sup>[10]</sup>. In most mammals, proline is classified as a nonessential amino acid, although under conditions such as heat stress and cold stress, several amino acids that are synthesized by animals can be considered as conditionally essential because their rates of catabolism are not compensated by rates of anabolism <sup>[11][12]</sup>.

## 2. Effects of Proline in Rabbits

There is increasing evidence of the role of proline as a biologically versatile functional amino acid, particularly of its functions under environmental stress conditions. Thus, in the present study, we assessed the potential of proline to enhance heat stress tolerance in rabbit bucks exposed to an average THI of around 31.99 (i.e., extremely severe heat stress conditions; <sup>[13][14]</sup>). We acknowledge the lack of a control group without exposition to heat stress but the negative effects of heat stress on physiology and metabolism of rabbits are already well-known and described and, in light of animal welfare, the inclusion of such a control group would have increased the number of rabbits in the experimental procedure. Dietary supplemental proline at a level range between 50 and 100 mg/kg DM reduced heat stress-related indicators like rectal temperatures, respiratory rates, and blood plasma cortisol concentrations <sup>[15]</sup>, which suggests improvement in the heat tolerance of animals. Rabbits are physiologically adapted to lose body overheat by increasing panting rate (respiratory rate) as they have a low number of functional sweat glands <sup>[16]</sup>. In our study, the reduction in rectal temperature, however, was not accompanied by an increase in respiratory rate, indicating implications of other adaptive mechanisms.

The results also showed that increasing LDP up to 70 mg/kg DM improved blood plasma total protein and albumin concentrations. This could be mediated by the proteinogenic and chaperone properties of proline <sup>[1]</sup>. In addition, albumin and proline have demonstrated osmoprotective properties in plasma and cells <sup>[17][18]</sup>. This property is crucial under environmental heat stress conditions since it regulates water balance and facilitates the maintenance of protein and enzyme stability. It is worth noting that the relationship between proline levels and either total blood plasma protein levels or blood plasma albumin levels was cubic. In fact, these relationships are difficult to interpret, however they at least highlight the biological complexity between these variables and the importance of adjusting proline level supplementation to achieve desired biological effects. On the other hand, increasing LDP up to 110 mg/kg DM decreased blood plasma ALT activity. High extracellular ALT activity is considered a negative indicator of liver function <sup>[17]</sup>, thus proline might have a hepatic-protective effect. Also, increasing LDP up to 100 mg/kg DM improved red blood cell counts and haemoglobin

concentrations. These enhancements are also required adaptive mechanisms during heat stress to improve oxygen circulation during panting process, improving the panting process efficiency and thus heat loss [15].

Heat stress impairs energy status and energy utilization in rabbits [19]. It was reported that availability of blood plasma glucose during heat stress allows energy utilization with lower metabolic heat production compared to other energy-yielding nutrients [17][15]. Glucose is the main source of electrons for ATP and NADPH production in most mammalian cells, and it is a glycerol precursor and intermediate for nonessential amino acid synthesis [19]. Interestingly, increasing LDP up to 100 mg/kg DM increased blood plasma glucose concentrations, which may be ascribed to the contribution of proline to gluconeogenesis [20], the regulation of proline-dependent cellular energy signaling pathways, such as the rapamycin activation pathway [5], which integrates signals from nutrients such as glucose and amino acids [6], and/or improved cellular glucose uptake due to proline serving as a structural component [21][22] or inducer of genes encoding glucose transporters (GLUT; [23]).

Proline supplementation improved the redox status of naturally heat-stressed rabbit bucks by increasing TAC and SOD activity in blood plasma. However, these effects on redox status were only observed with low proline concentrations (30 mg/kg DM), and the relationship was cubic. Proline also has oxygen radical scavenging activities that may contribute to these observations [3][10]. However, conversion of proline into pyrroline-5-carboxylate (p5C) by the PRODH/POX catalyzing system in the inner mitochondrial membrane generates superoxide anions, which participate in ROS-dependent apoptotic pathways [2]. These diverse effects of proline suggest that a complex relationship between proline supplementation and redox status and further specific studies are therefore necessary. According to our results, low proline concentrations could improve the redox status by improving TAC and SOD activity, whereas high proline concentrations may act as a source of free radicals.

Finally, assessment of libido and spermatogenesis after dietary proline supplementation showed linear improvements in libido, semen ejaculate volume, and sperm concentration. These results highlight the positive effects of proline on reproductive traits. In bucks, the major facilitative neurotransmitters that control sexual motivation and copulatory performance are dopamine, glutamate, and norepinephrine [24]. In addition, a high-affinity L-proline transporter is expressed in mammalian brain tissues, where it mediates high-affinity uptake of neurotransmitters, including norepinephrine, dopamine, serotonin, glutamate, and glycine [25]. Proline is also a dietary precursor for the amino acid arginine [26], which contributes to nitric oxide (NO) synthesis. NO has been previously shown to improve libido and erectile function by increasing blood flow throughout the genital tract [27]. Thus, the present effects of dietary proline supplementation may be related to sexual neurotransmitter signaling pathways and blood flow throughout the genital tract, leading to improvements in the overall sexual performance of rabbit bucks. Besides, proline contributes to multiple signaling pathways that are involved in the molecular regulation of spermatogenesis process. For example, in Sertoli cells, the activation of proline rich regions of androgen receptors (amino acids 352–359) associating with the SH3 domain of Src has been found to be a critical pathway for maintaining spermatogenesis [28]. Also, proline has been found to be a specific component of seminal plasma and germ cell proteins. For example, proline is a major structural amino acid component of nucleoproteins and polyamines [10], which are required extensively during mitosis and meiosis in male germ cells [27]. Among such proteins, FNDC3A is a proline-rich amino-terminal protein that, as an integral membrane protein in male germ cells, may contribute to spermatogenesis by mediating the mandatory adhesion between Sertoli cells and round spermatids [29].

The enhancements in libido and spermatogenesis found in proline-supplemented rabbit bucks may be also attributed to improved blood plasma testosterone concentrations (steroidogenesis). Testosterone is required for several critical processes during spermatogenesis including maintenance of the blood-testis barrier, meiosis, Sertoli-spermatid adhesion and sperm release as well as sexual activity [28][30]. Again, proline is a structural component of a specific family of proline-directed serine-threonine kinases known as mitogen-activated protein kinases (MAPKs) including three major subfamilies: p38, ERK, and JNK. These kinases are important cellular signaling components that are involved in the regulation of steroidogenesis in germ cells of mammals [31].

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