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Harnessing heat shock protein responses: It's about more than heat stress

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> *Heat shock proteins (Hsps) are essential for cell homeostasis and therefore, animal health. Despite their name, Hsps are for an animals' response to a wide range of stressors other than heat including pathogens, nutritional stress and even exercise. Using cactus extract, we can modulate the Hsp response, bolster disease resistance and enhance bird productivity.*


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HEAT SHOCK PROTEINS AND THEIR DISCOVERY

The concept of biological molecules related to genetic responses to thermal stresses were first shown in the early 1960's by Ritossa in *Drosophila busckii*. In 1962, he observed that temperature shocks induced changes in both the metabolic activities and puffing patterns of the salivary gland chromosomes of *Drosophila* larvae (Ritossa, 1962). The puffs indicated that genes were being activated in that region of the genome and the encoded proteins synthesised. Ritossa further identified the puffing pattern in response to other stressors such as endotoxins and hypoxia. Later work by other researchers identified the main group of proteins corresponding to the individual puffs, calling them "heat shock proteins" (Hsps). It was later demonstrated that Hsps, in particular members of the Hps60 and Hsp70 families, were exceptionally well conserved across organisms. For example, Hsp70 in humans is 99% identical to the mouse (*Mus muscularis*), 74% identical to *D. melanogaster* and 44% identical to *Escherichia coli* (Desai et al., 2010).

HSP CLASSIFICATION

Currently, Hsps are classified based on their molecular masses into the following major groups: small heat shock proteins (sHsps) family (molecular masses 10-30 kDa); Hsp40 family (40 kDa); Hsp60 (or chaperonins) (molecular mass close to 60 kDa); Hsp70 family (70 kDa); Hsp90 family (83-90 kDa); and Hsp100/110 family (≥ 100 kDa). Each family can also include homologues with similar or slightly different functions which may significantly differ by molecular masses (up to 10 kDa). Furthermore, Hsps can also be classified based on expression patterns, constitutive cognate (Hsc) or inducible (Hsp). Hscs share 50-80% homology with their Hsp counterparts but are either silent or play a housekeeping function. Hsps on the other hand, are synthesized at very low levels in the cell under normal conditions but on exposure to a stressor, induction of Hsp synthesis increases hundreds fold.

HSP FUNCTION

What are these highly conserved group of proteins and what do they do? Typically, when Hsps are mentioned in the same moment as livestock, it is often assumed that heat stress is the topic of discussion. This is unfortunate, as Hsps are involved with so much more than just heat stress; they are integral to cellular homeostasis.

Hsps act as molecular chaperones with a role in protein secretion and maintenance of cellular protein structures, for example, refolding proteins that denature due to cellular stress, helping to prevent cell apoptosis and contributing to cell survival. Hsps are upregulated in response to numerous stressors including nutritional deficiency, toxins, UV radiation, pathogens, microbial damage or, indeed, any cellular stress. When the cell is stressed, there is up-regulation of the Hscs to produce newly formed HSPs, which can be detected in the cell at concentrations significantly greater than the constitutive chaperones. Hsps act as an interconnected network of cell signals, a cascade of Hscs and Hsps driving cellular responses. For example, numerous Hsps are involved in the innate and adaptive immune response (e.g., Hsp60, Hsp70 and Hsp90; a response which is also dependent upon Hsp regulation (e.g., Hsp70) of oxidative stress via the Nrf2-Keap1 pathway. Crucially, Hsps have also been observed to have a protective immune function e.g., restoring tolerance in autoimmune diseases, in part due to their upregulation during inflammatory stress (Van Eden et al., 2017). Malfunction of HSPs is related with many diseases, including cancers, neurodegeneration, and other diseases.

HARNESSING THE POWER OF HSP USING PLANT EXTRACTS

As discussed earlier, Hsps are not confined to higher organisms. In fact, Hsps are also found in plants, and a good example is nopal or the prickly cactus pear (*Opuntia ficus indica*) and its fruits (Figure 1). *O. ficus* tends to be found in arid and semi-arid regions and is rich in phytosterols, flavonoids and phytols and in many cultures was a source of food, drink and medicine for centuries. Importantly, the environment where *O. ficus* is found can be exposed to extremes of temperature. It was observed that *O. ficus* rapidly produces high levels of Hsps (including 71-75kDa and 62 kDa) on exposure to extreme temperature (Somers et al., 1991). Recently, the potential of *O. ficus* cactus extract (CE) to stimulate Hsps in cell lines and subsequently aquatic and terrestrial species was explored with interesting results. Using sustainable practices, the fruits of *O. ficus* are harvested, and the cactus juice extracted, concentrated and combined with a novel carrier to increase bioavailability for use in animal production (as the product Opuntech, Nutri Biotech Services, Malta).

When (CE) was applied to *Artemia*, Hsp70 was expressed within 1 hour of application and provided protection against abiotic stressors as well as increasing resistance to disease infection (Baruah et al., 2012; 2014). In horses, exercise was shown to increase the production of Hsp72 after 120 minutes. However, when horses were fed CE prior to exercise, Hsp72 was significantly increased within 30 minutes of the start of exercise (Martinod et al., 2007). In exercising horses, Hsp72 mRNA expression in skeletal muscles has been positively correlated with the peak concentration of blood lactate, indicating the role of Hsp 72 in exercise endurance and the potential for CE to improve resistance to physical stress. These two extremes of aquatic and terrestrial species, suggest the conserved nature of Hsps could extend to the stimulation of a protective response in many other species. Furthermore, a protective cellular response could also lead to improved growth, particularly in livestock species, since less energy might be diverted into mounting immune responses and the impact of disease could perhaps be mitigated.

Figure 1. *Fruits of the prickly cactus pear (Opuntia ficus indica)*

APPLICATION OF CE IN POULTRY

Like *Artemia*, the application of CE to day-old chicks via spray leads to a large increase in Hsps (Hsp72) after 1 hour (Figure 2). Furthermore, Hsp72 remains elevated for at least 24 hours (Parker et al., 2014).

Figure 2. *Hsp72 serum levels (via ELISA) in day-old chicks at hatch, 1 hour and 24-hours after CE application*

To explore the potential performance benefits of CE in broilers, trials were undertaken in Brazil over consecutive months on several different farms. Poultry houses were naturally ventilated, bedding comprised wood shavings and birds fed a corn-soy ration with a nicarbazin/salinomycin anticoccidial shuttle. Over 3 trial periods, birds given CE at day-old via spray were compared to the production average (Control). CE was administered in addition to the standard programme and no other changes made (neither feed nor management were changed to implement cost savings, for example). Different farms were selected each time for CE application. Across all trials, birds administered CE at day-old via spray were on average 6.3% heavier, had a 1% lower mortality and FCR with an associated 9% improvement in EPEF (Table 1).

Further trials where only the early stage of production was followed (112,000 birds), showed that CE administration led to 21%

and 24% lower mortality at 7 and 14 days, respectively, and 5% and 8% higher liveweight for the same periods. A key factor in the adoption of any additive (feed or water) in livestock production, is return on investment; can the producer return a profit when applying the additive? The use of CE had an average ROI 2 when applied to over 120,000 birds across 3 production cycles, including CE crops that broke with infectious bronchitis. This financial benefit of using CE is attributed mainly to the improved liveability and additional body weight, particularly noteworthy when 10,000 birds were reared in temperatures > 33°C and still achieved 6 points lower FCR, 100g higher liveweight and 1.5% higher liveability.

SUMMARY

Hsps are recognised for their importance in cell homeostasis and protecting the cell against stressors. Due to the conserved nature of Hsps across taxonomic classes, stimulation of Hsps via a plant extract such as cactus extract (CE) can stimulate similar protective responses in vastly different animals. We can potentially enhance endurance in exercising horses, survivability of aquatic species and improve the performance of livestock species.

For poultry producers, the use of cactus extract presented as Opuntech could be game changer. A single application at day-old can improve early chick viability and weight and lead to more efficient, healthier birds with the potential to reduce medicine usage, even when there is no heat stress. Hsps serve several essential and protective functions in the bird; we are simply giving nature a helping hand!

About Dr David Harrington

Dr David Harrington is the Managing Director at teamTWO Solutions (Denmark). He obtained his BSc (Hons) and MSc from Edinburgh University, UK, and his Ph.D from Newcastle University, UK researching avian immune responses to ectoparasite infections. His early career was focused on the development and commercial support of swine and poultry vaccines mainly in the EU before moving into feed additives in both commercial/strategic and technical roles globally.

About Dr Reginaldo Teixeira Filho

Reginaldo Teixeira Filho, graduated in Animal Science in 1990 from UNESP- Botucatu, MBA in commercial management, specialist in monogastric animals, with experience in national and multinational companies, currently CEO of Natturo Agronegócios focused on the southern region of Brazil, co-author of publications presented at world congresses mainly in the line of non-antibiotics.

About Dr Charles Saliba

Dr Charles Saliba is an entrepreneur with a strong technical background in the area of biotechnology and more than thirty years experience. After graduating in Applied Biological Sciences and undertaking a Ph.D thesis in vitro modelling he worked in the Agribusiness and Life Sciences sector setting up a number of research institutes and production facilities in various countries He also lectures at the University of Malta and has supervised over forty theses focusing on stress related issues in animal welfare. He has also served as an advisor on the board of various companies, education establishments and governmental agencies.

TAGS animal health | cactus extract | feed additive | heat shock proteins | heat stress | Hsps | livestock species | Natturo Agronegócios | natural feed additives | Nutri Biotech Services | phytogenics additives | plant-based products | teamTWO Solutions

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