Summary of mode of action research for improved adaptive capacity with Anco® Fit Poultry

Two studies presented during the Poultry Science Association meeting in San Antonio, TX in July 2022 focused on response of a feed additive containing plant extracts and functional carriers (Anco® Fit Poultry (AFP), Pancosma, ADM), on inflammatory, and antioxidant genes expression along with an overall layer performance evaluation. This research added new understanding to a previously conducted work in broilers with AFP (Mountzouris et al., 2019 and 2020).

In their newest work, the University of Athens researchers (Brouklogiannis *et al.*, 2022) have shown that increasing *AFP* inclusion in layer diets between 500 to 1500 ppm enhanced laying rate with 1000 ppm providing significantly (P<0.01) better response than control (0 ppm). Albumen height and Haugh unit increased significantly with 750 and 1000 pm of *AFP*. Both parameters are typically associated with egg quality (e.g. freshness, higher quality eggs tend to have thicker whites) while bird age (Silverside *et al.*, 1993), mycotoxin levels (Lee et al., 2012), temperature and storage time (Davis and Stephenson, 1991) negatively influence egg quality and significantly reduce the Haugh units. Shell mass increased quadratically with *AFP* dose, peaking at 1000 ppm. Shell quality issues typically appear in older laying hens or during environmental challenge.

In ovaries, the expression of most studied genes of the Nuclear factor-kappa B (*NF-κB*) pathway were significantly downregulated particularly at 1000 and 1500 ppm. *NF-κB* is a ubiquitous transcription factor that is involved in pro-inflammatory and immune responses (Giuliani *et al.*, 2018).

From aryl hydrocarbon receptor (*AhR*) pathway genes, the expression of Cytochrome P450-B1 (*CYP1B1*) was significantly reduced with increasing *AFP* levels. *AhR* was found to be responsible in mediating the onset of a plethora of deleterious health effects associated with exposure to environmental pollutants (Nebert *et al*, 1975). *CYP1B1* metabolizes a number of procarcinogens, including polycyclic aromatic hydrocarbons, N-heterocyclic amines, arylamines, amino azo dyes and several other compounds (Shimada *et al*, 1996).

AFP cytoprotective potential was demonstrated via beneficial changes seen for the majority (9 out of 11) of the Nuclear factor (erythroid-derived 2)-like 2 (*Nrf2*) pathway genes with 1000 ppm treatment displaying most significant differences from control. *Nrf2* is a protein, a transcription factor, that serves as a key regulator of cellular homeostasis. In stress conditions, *Nrf2* activation responds by biding to the antioxidant response element (*ARE*), which initiates the transcription of enzymes involved in counteracting oxidative stress, endobiotic and xenobiotic metabolism, regulators of inflammation. Certain bioactive components derived from plants have proven to modulate *Nrf2* pathway in the poultry gut and increase the bird's adaptive capacity to cope with stressors (Mountzouris *et al*, 2020; *AFP* research).

The underlying mechanism of inflammatory and antioxidant responses in duodenum and ceca of laying hens were studied further in a follow up study (Anagnostopoulos *et al*., 2022). In duodenum, increasing *AFP* contributed to the expression downregulation (P<0.05) of most inflammatory genes involved in *NF-κB* signaling pathway. On the other hand, most of the antioxidant genes implicated in *Nrf2* pathway were increased (P<0.05) with increasing *AFP* levels. Similar results were found at the cecal level. From a performance perspective, egg mass was significantly increased (P<0.01) with differences up to 4% with 1000 ppm *AFP* compared to control.

The authors concluded that their research demonstrated that *AFP* contributed to downregulation of layer inflammatory genes expression responses, whilst increasing expression of antioxidant response genes along with an overall layer performance enhancement, with 1000 ppm displaying optimal benefits. This supports contemporary knowledge that phytogenic compounds derived from various vegetables, fruits, spices and herbs activate a signaling pathway known as the Kelch-like ECH-associated protein-1 (*Keap1*)/*Nrf2*/*ARE*) and induce the expression of antioxidant enzymes (Stefanson and Bakovic, 2014; Ahmed *et al.*, 2017; Lee *et al.*, 2017).

This new layer research complements earlier work conducted by the same group with broilers (Mountzouris *et al.*, 2019, 2020). In the 2020 research, total antioxidant capacity (*TAC*), *Nrf2*, antioxidant enzymes genes expression: catalase (*CAT*), superoxide dismutase 1 (*SOD1*), heme oxygenase 1 (*HMOX1*), glutathione peroxidase (*GPX2*) and NAD(P)H quinone dehydrogenase 1 (*NQO1*) were determined in various sections of gastrointestinal tract of 42-day old broilers. Inclusion of *AFP* increased ($P \leq 0.05$) the expression of cytoprotective genes against oxidation, except *CAT*. In particular, the cytoprotective against oxidation genes were up-regulated primarily in the duodenum and the ceca and secondarily in the jejunum. Most of the genes were upregulated in a quadratic manner with increasing *APF* inclusion level with the highest expression levels noted in treatments 750 and 1000 ppm compared to control. From the assessed genes relevant for inflammation and stress, *NF-κB1*, toll-like receptor 4 (*TLR4*) and heat shock 70kDa protein 2 (*HSP70*) expression was reduced in treatments with increasing *AFP* level. Intestinal *TAC* was significantly higher at 1000 ppm in the duodenum and in the ceca. Additionally, Mountzouris *et al*. (2019) have also shown improved *TAC* with *AFP* inclusion in liver, breast and thigh meat.

The researchers concluded that their work has shown that *AFP* inclusion primed the expression of critical genes for host protection against oxidation and down-regulated genes relevant for inflammation and stress. Gut profiling revealed that the duodenum and the ceca as the most responsive intestinal segments to modulation by *AFP*, followed by the jejunum. When the Keap1/Nrf2/ARE pathway genes were upregulated, *NF-κB1* expression was downregulated (Mountzouris *et al.*, 2020). This finding is in agreement with the general notion that activation of the *Keap1/Nrf2/ARE* pathways inhibits *NF-κB1* activation resulting in increased protection against oxidative stress and inflammation (Wardyn *et al.*, 2015).

Considering all the available research, a consistent up-regulation of cytoprotective genes and down-regulation of stress and inflammation related genes in birds without any specific challenge has been seen with *AFP* inclusion. Collectively, the studies highlighted the potential of phytogenics combined with functional carriers to beneficially stimulate baseline bird cytoprotective response with further investigation under stress-challenged conditions warranted. However, commercial and research experiences with *AFP* have already shown performance improvements under various challenge situations including heat stress and increased laying persistence in layers at the later stage of the laying cycle.

Explanation of terms:

AFP	Anco® Fit Poultry	Plant extracts, functional carriers , yeast (Anco® Fit Poultry (*AFP*), Pancosma, ADM) – ginger, lemon balm, oregano, thyme, 1m558 bentonite and clinoptilolite.
NF-κB	Nuclear Factor-kappa B	Protein transcription factor regulating the expression of various genes involved in inflammation induced by various stressors.
AhR	Aryl hydrocarbon receptor	Protein transcription factor. Mediating the onset of a plethora of deleterious health effects associated with exposure to environmental pollutants.
CYP1B1	Cytochrome P450-B1	Protein, enzyme. Metabolizes numerous endobiotics and xenobiotics – majority of these processes result in largely non-toxic quantities of metabolites, some reactions with *CYP 450* family of proteins result in toxic compounds that can directly elicit liver damage and activation of inflammation responses.
Keap1	Kelch-like ECH-associated protein-1	Protein coding gene. Inhibitor of *Nrf2*, regulating the *Nrf2* signaling pathway.
Nrf2	Nuclear factor (erythroid- derived 2)-like 2	Protein transcription factor that serves as a key regulator of cellular homeostasis. Key transcription factor considered as the master regulator of cellular antioxidant response and xenobiotic metabolism.
ARE	Antioxidant response element	*Nrf2* target gene. Initiates the transcription of enzymes involved in counteracting oxidative stress, endobiotic and xenobiotic metabolism, regulators of inflammation
ROS	Reactive oxygen species	Key signaling molecules and also mediators that play an important role in the progression of inflammatory disorders. Example of *ROS* is superoxide.
TAC	total antioxidant capacity	Total antioxidant capacity (*TAC*) is the measure of the amount of free radicals scavenged by a test solution used to evaluate the antioxidant capacity of biological samples.
SOD	superoxide dismutase	Antioxidant enzyme. Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. E.g. *SOD* catalyzes the conversion of extremely reactive superoxide into stabilized dioxygen and hydrogen peroxide. Results of the *Keap1/Nrf2/ARE* pathway.
HMOX1	heme oxygenase 1	Antioxidant enzyme. Known for its detoxification function. A highly protective enzyme that directly inhibits pro-inflamatory cytokines and activates anti-inflammatory ones.
NQO1	NAD(P)H quinone dehydrogenase 1	Antioxidant enzyme. Known for its detoxification function.
CAT	Catalase	Antioxidant enzyme
GPX2	Glutathione peroxidase	Antioxidant enzyme. Known for its antioxidant function. *CPX2* can reduce hydrogen peroxide to water and lipid peroxides to their analogous alcohols.
TLR4	toll-like receptor 4	Involved in inflammation and stress processes. A number of phytogenic components have been identified to be involved in reducing *TLR* signaling.
HSP70	heat shock 70kDa protein 2	Involved in stress – serves as a classical marker of stress in animals. Expressed as a response to physical, chemical, or biological stresses and play an important role in the protection and repair of cells and tissues. E.g. HSP70 has been shown to be capable of protecting the intestinal mucosa from heat-stress injury by improving antioxidant capacity and inhibiting the lipid peroxidation production.

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