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A brief look at biomarkers

Intestinal health is widely recognised as central to overall animal health and productivity. There remains uncertainty regarding a precise definition for intestinal health and, importantly, how we measure 'it'. However, the identification of biomarkers that provide a reliable indication of the state of the intestine are important steps towards understanding, defining and assessing intestinal health. There are a variety of potential biomarkers, a selection of which are outlined below.

Microbiota and metabolites

The intestinal microbiota and its metabolic activity are clearly central to gut health status and thus should provide useful biomarkers to reflect this. The gut microbiota is complex and the evidence suggests that each individual has a unique microbiome, making it somewhat challenging to determine whether specific components are more or less important. Certain bacterial phyla (e.g. Firmicutes), families (e.g. *Lachnospiraceae*), genera (e.g. *Faecalibacterium, Ruminococcus*, etc.) or species (e.g. *F. prausnitzii, B. fragilis*, etc.) may be particularly relevant, as well as the products of their metabolic pathways, for example short-chain fatty acids and/or amino acid metabolites, but more research is required to fully understand and apply their significance.

Acute phase proteins

Acute phase proteins (APP) are/have often been employed as indicators of 'health' status. APP are produced by the liver in response to inflammatory mediators. APP can be classified as major, moderate or minor based on their fold changes (e.g. 10 - 1000 fold, 4 - 10 fold and 2-3 fold, respectively), or positive or negative depending on whether they are increased or decreased, respectively, during an acute phase response. Different APP may have differing significance in different species. APP would generally be considered relevant for systemic acute phase status but, recently, faecal APP (i.e. ovotransferrin) have been correlated with intestinal disease/damage and thus proposed as potential gut health biomarker(s) (Goossens et al., 2018). Intestinal damage is suggested to allow leakage of circulating APP into the gut lumen. Further work is likely to be required to appreciate the sensitivity of faecal APP and whether leakage into the gut lumen occurs in the earliest stages of compromised intestinal health or only once significant gut damage has occurred.

Indicators of barrier failure

Various factors may be detected in serum that indicate gut barrier failure, including endotoxin and carotenoids (organic pigments). Endotoxin, otherwise known as lipopolysaccharide(s), is derived from the outer membrane of Gram-negative bacteria. As the blood is largely deemed sterile, the presence of endotoxin is considered to originate from the largest source of (Gram-negative) bacteria in the body, the intestine, and correlate with increased gut permeability. When gut health is compromised the ability of the intestine to absorb nutrients, including fat-soluble carotenoids, is impaired and thus serum carotenoid concentrations should correlate with this gut function.

Immune factors

There are numerous immune-related factors that could provide important biomarkers. Immune signalling molecules, specific cell subsets, as well as host-derived antimicrobial compounds (e.g. defensins, cathelicidins) maybe relevant but more work is needed for their application as reliable biomarkers. In addition, calprotectin is a major cytosolic protein of neutrophils. Neutrophil migration to the intestinal tract can release calprotectin into the gut lumen and its concentration in faeces has been shown to correlate significantly with neutrophil infiltration of the gut mucosa and thus provide an indication of intestinal inflammation (Lehmann et al., 2015).

Others

Intestinal histomorphometry to quantitively assess the structure of the mucosa, including villus height, crypt depth, their ratio, surface area, etc., can be considered very informative but also very invasive, which limits their practical use for general gut health monitoring.

Citrulline is an amino acid primarily produced by enterocytes (with the liver's contribution deemed negligible). Mucosal damage has been associated with significantly reduced plasma citrulline concentrations and thus may provide a reliable biomarker for enterocyte number/mass.

Fatty acid binding proteins (FABP) are cytoplasmic proteins and Intestinal FABP is excreted in urine and blood specifically from damaged intestinal epithelial cells.

Discussion

There are various factors to consider when attempting to utilise biomarkers to assess intestinal health status, which include their relative merit, species relevance, accessibility (i.e. invasiveness), stability, etc. The most ideal biomarker(s) will be those that correlate strongly with the desired interpretation of intestinal health in the species of interest, are known to remain stable until analysis and can be readily accessed with minimal invasiveness or welfare considerations. Faecal or intestine-related biomarkers are, generally, likely to be more indicative of gut state than those obtained systemically, as systemic markers are probably influenced by a conglomeration of inputs from tissue/region states that are unrelated to the intestine. An ideal, future, scenario will be the ability to employ detection methods in the production environment that are able to monitor relevant biomarkers in real-time and thus allow rapid identification of compromised intestinal health and enable timely interventions.

References

Chen, J., G. Tellez, J. D. Richards, J. Escobar. 2015. Identification of potential biomarkers for gut barrier failure in broiler chickens. Front. Vet. Sci. 2:14.

Ducatelle, R., E. Goossens, F. De Meyer, V. Eeckhaut et al. 2018. Biomarkers for monitoring intestinal health in poultry: present status and future perspectives. Vet. Res. 49:43.

Goossens, E., G. Debyser, C. Callens, M. De Gussem et al. 2018. Elevated faecal ovotransferrin concentrations are indicative for intestinal barrier failure in broiler chickens. Vet. Res. 49:51.

Lehmann, F. S., E. Burri, and C. Beglinger. 2015. The role and utility of faecal markers in inflammatory bowel disease. Ther. Adv. Gastroenterol. 8: 23-36.

Pang, T., S. T. Leach, T. Katz, A. S. Day, C. Y. Ooi. Faecal biomarkers of intestinal health and disease in children. Front. Pediatr. 2:6.