

# Faecal biomarkers of gastrointestinal functionality in animals

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**ABSTRACT.** Effective functioning of the gastrointestinal (GI) tract is essential for determining and sustaining animal health, welfare and performance. A balanced diet, efficient digestion and absorption, normal and stable microbiota, a robust immune system, and a healthy gut mucosa are the primary key components determining optimal GI functionality. These pivotal factors offer an opportunity to explore potential biomarkers for evaluating the performance of the GI system in animals. Among various biological samples, faecal samples emerge as advantageous due to their easy collection, non-invasiveness, applicability in wild animals, minimum stress, and their contribution to animal welfare. While literature on faecal biomarkers in animals is scarce, some studies have investigated such indicators in monogastric, wild, and laboratory animals; these include lactate and succinate (indicators of fermentative diarrhoea), sialic acid (indicative of intestinal damage), glucocorticoid (stress marker), intestinal alkaline phosphatase (associated with pathogenic intestinal damage), and lipocalin-2 and calprotectin (indicating intestinal inflammation). Faecal glucocorticoid metabolites are particularly useful for evaluating environmental or captive stress in wild animals. The aforementioned faecal biomarkers offer insights into events influencing GI functionality and may pave the way for developing nutritional interventions aimed at modulating the GI system to enhance animal welfare and performance.

## Introduction

For several years, the concept of intestinal health has attracted significant attention amongst nutritionist, veterinarians, and scientists (Kogut and Arsenault, 2016; Celi et al., 2019). This interest stems from the need to develop nutritional interventions that would modulate the gastrointestinal (GI) functionality towards optimal animal health, resulting in increased animal production performance (growth, milk yield, meat and eggs etc.) (Celi et al., 2019). GI functionality, at its core, represents a harmonious symbiotic equilibrium between microbiome and the

intestinal tract (Celi et al., 2017). The major components of the proper GI function include balanced diet, effective digestion and absorption, a stable intestinal microbiome, strong immune status, healthy gut mucosa, as well as neuro-endocrine and motor function (Celi et al., 2017). These components collectively play pivotal roles in GI physiology, animal health, welfare and performance. There is a pressing need to identify biomarkers for gut functionality, yet the development and validation of such biomarkers pose significant challenges. These difficulties may arise from the absence of certain markers in individual species, as well as the absence of necessary

reagents for assay development and validation (Niewold, 2015). In order to better understand the factors affecting the intestinal barrier, its functioning, and the ecology of the GI microbiota, it is essential to develop biomarkers of GI functionality. The purpose of this article is to introduce several indicators of GI functionality to animal nutritionists and veterinary scientists, with a focus on non-invasive markers and highlighting their specific potentials.

## **Factor influencing gastrointestinal functionality**

### **Diet**

Dietary ingredients, nutrients, and additives can influence the growth and function of the GI tract, as well as its immune system and microbiota (Conway, 1994). Dietary anti-nutrient factors (alkaloids, certain types of dietary fibre, trypsin inhibitors, phytate, lectins, undigested protein in the distal GI tract, glycosides, mycotoxins, and others) can affect both pro-inflammatory and anti-inflammatory effects, causing disruption of the structural and functional integrity of the gut (Celi et al., 2017; Broom and Kogut, 2018). Conversely, dietary nutrients, both macronutrients (carbohydrate, protein and fat) and micronutrients (minerals and vitamin), have the capacity to modulate and regulate the inflammatory response during environmental stress, nutritional challenges and diseases. Therefore, they play an important role in immune-modulation (Klasing, 2007) and improve animal health, welfare, and productivity (Pluske, 2013; Starkey, 2014).

### **Effective digestion and absorption**

Digestion is a process that breaks down larger and complex feed molecules, including macronutrients and micronutrients, into smaller and simpler compounds for absorption through various physical and biological processes. Optimal digestion and absorption are closely linked with effective GI tract functions. Inflammation in the GI tract can reduce the efficiency of digestion and absorption of nutrients (Celi et al., 2017). Maldigestion involves inadequate breakdown of nutrients, often due to insufficient enzyme secretion. Malabsorption, on the other hand, pertains to issues with the absorption of the end products of digestion. The outcomes of ineffective digestion and absorption are insufficient nutrient absorption and the transfer of surplus nutrients, especially protein and fat, to the distal regions of the GI system, where the microbiota may ferment them improperly. The decomposition of nutrients

and their absorption into the bloodstream can be determined using biomarkers of digestion and absorption. These biomarkers serve as indicators of the GI system's effectiveness in carrying out fundamental digestion and absorption functions. Despite the lack of current technologies or equipment capable of providing real-time assessments of digestion and absorption on a farm, analysing biomarkers in faeces proves to be a valuable method for gauging the efficiency of these processes.

### **Normal and stable microbiota**

The GI microbiome is emerging as an exciting and powerful field not only for the management of GI health but also for the well-being of entire organism. Often referred to as the 'fifth organ', current literature underscores the pivotal role of the microbiome in processing and distribution of environmental signals throughout the organism (Dietert and Silbergeld, 2015). A noteworthy aspect of the GI microbiome is its role in orchestrating a mutual relationship with the host's immune system. This symbiotic relationship is integral to the microbiome's function of 'teaching' the immune system, facilitating a harmonious coexistence (Dietert and Silbergeld, 2015). The intricate dynamics of this association form the primary pathways through which the GI microbiome regulates the functions of various organs, including brain, and the immune system.

The gut microbiota regulates a variety of physiological processes, including digestion and absorption, metabolism, immune system development, and infection prevention (Willing and Van Kessel, 2010; Lee and Hase, 2014; Marchesi et al., 2016). These numerous and complex interactions between the microbiome and the host have a major impact on the characteristics and functions of the gut microflora. The development and availability of high-throughput techniques are rapidly advancing our understanding of changes in phylogenetic composition (16S sequencing), functional capability (metagenomics), gene expression in specific condition (meta transcriptomics), and metabolic impact (metabolomics) of the intestinal microbiota (Ji and Nielsen, 2015). The GI microbiome is characterised by a significant degree of functional redundancy (Moussavi et al., 2007), implying that various bacteria can perform similar functions, such as metabolising the same substrates and producing similar metabolites. Therefore, it may be more relevant to examine the efficacy of eubiotic therapies by scrutinising the activity of the gut microbiome

rather than merely its composition and structure. The integration of 'omics' technologies plays a pivotal role in unravelling the complex ecology of the GI microbiome. These technologies contribute significantly to our comprehension of the factors driving dynamic shifts in microbiota composition and activity.

### Effective immune status

More than 70% of immune system cells are located in the GI tract, making it the largest immune organ in the body (Vighi et al., 2008). According to Yegani and Korver (2008), and Celi et al. (2017), the increasing understanding of the relationship between the immune system and the GI tract, in addition to the gut functionality, reveals its crucial importance for health, well-being, and disease prevention. The digestive tract serves as a crucial barrier against diseases and antigens, making it the largest interface between the host and the external environment. The GI barrier is a dynamic and functional structure that not only separates the digesta from the host, but also serves as a site for extensive sampling and communication between the host and the gut content, emphasising the relevance of its role. Consequently, the evaluation of the GI barrier necessitates consideration of a diverse array of assays and indicators to comprehensively assess its structural integrity and functional efficacy.

Biomarkers of GI inflammation and immune function can provide important information regarding the interactions of the GI tract with the environment and the functionality of the GI barrier (Celi et al., 2019). The GI tract possesses two types of barriers: a structural barrier composed of the vascular endothelium, epithelial cell lining, and mucus layer, and a functional immunological barrier comprising digestive secretions, immune molecules, cell products like cytokines, inflammatory mediators, and antimicrobial peptides. These are primarily produced by Paneth cells in the crypts of the small intestine (Bischoff et al., 2014). The determination

of intestinal inflammatory activity is essential for the assessment of the GI barrier because intestinal inflammation can impair the gut function by inducing changes in the structure and function of the intestinal mucosa.

### Gut mucosa

Maintaining optimal intestinal barrier function plays an essential role in optimal GI functionality, ensuring animal health and welfare (Celi et al., 2017). The first layer of the intestinal barrier is composed of a mucus layer, which consists of an outer layer associated with the microbiota and an inner layer rich in sIgA and mucin (Bischoff, 2011). The innate immune system of the GI tract is composed of multiple elements, each contributing to the fine balance between tolerance to commensal bacteria and response to pathogens (Celi et al., 2017). Despite continuous exposure to the GI microbiota, the GI mucosa is able to maintain the integrity of the intestinal barrier, protecting against damage caused by toxins, bacteria, their cell debris, and anti-nutritional factors, while allowing the selective entry of essential nutrients (Bischoff, 2011). Various methods and approaches are available for assessing intestinal permeability and integrity (Bischoff et al., 2014). These techniques can be implemented *in vitro* or *in vivo*, they have been validated across diverse animal models, and are capable of determining the levels of a wide range of molecules and cells (ions, carbohydrates of different sizes, macromolecules and antigens, bacterial products and bacteria themselves) in several biological matrices (peripheral blood, portal vein blood, urine).

### Advantages of faecal biomarkers

- a) Non-invasiveness: In contemporary veterinary research, invasive methods, often involving slaughter techniques, are commonly employed to test specific parameters. Consequently, there is a pressing need to develop non-invasive methods for sample collection and analysis;

**Table 1.** List of selected faecal biomarkers and their role

Biomarker	Comments	References
Lactate and succinate	Fermentative diarrhoea	Ushida (2001); Ewaschuk et al. (2004); Shimomura and Sato (2006); Sato and Shiogama (2009)
Sialic acid	Intestinal damage	Fernando et al. (2011); Huang et al. (2015)
Glucocorticoid metabolites	Stress	Messmann et al. (1999); Morrow et al. (2002); Allwin et al. (2016); Jurkovich et al. (2017)
Intestinal alkaline phosphatase	Pathogenic diarrhoea	Thomas et al. (1985); Lalles (2010)
Lipocalin-2	Intestinal inflammation (mild)	Chassaing et al. (2012); Abella et al. (2015); Hsieh et al. (2016)

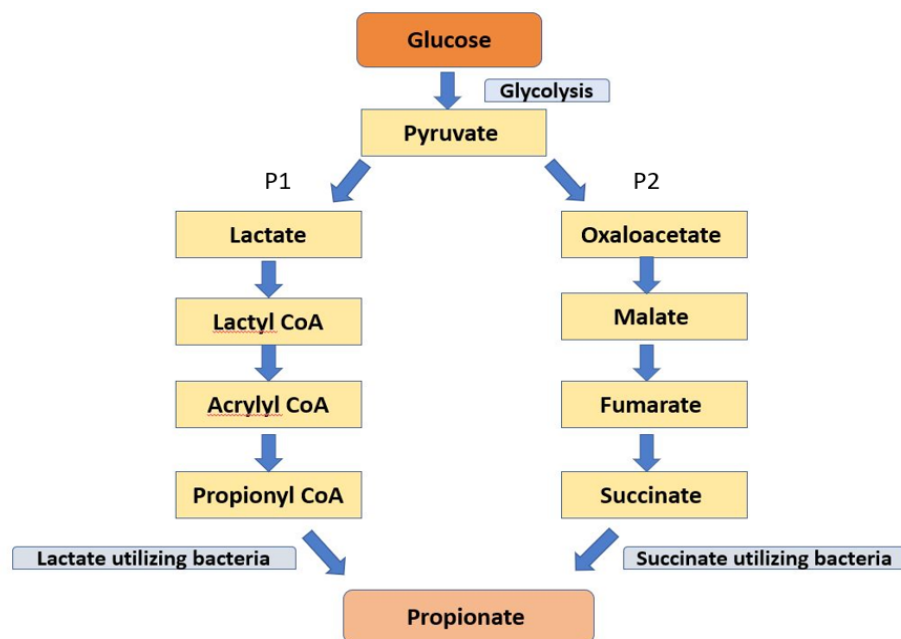
- b) Easy sample collection: Compared to other biological sample, faecal sampling is very easy;
- c) Role in wild animals: Collecting samples, especially blood or urine, from wild animals poses considerable challenges. However, faecal sampling presents a practical solution as it allows for observation from a distance, making it easier to detect defecation and collect samples;
- d) Animal welfare: Traditional sample collection methods may require restraining animals, thereby inducing stress that can adversely impact their performance. On the other hand, collecting faeces is a much more straightforward procedure without inflicting any stress on animals.

### Lactate and succinate

One of the richest and most productive habitats on Earth is the rumen of dairy animals. In this specific environment, microbes convert plant organic compounds, such as carbohydrates, into volatile fatty acids (acetate, propionate, and butyrate). These fatty acids serve as the main source of energy and fat for milk production. Additionally, the microbial community in the rumen converts non-protein nitrogen compounds into high-quality microbial protein, as well as metabolise certain plant toxins. The transition period is a critical time for dairy animals, during which animals experience abnormal dietary and hormonal changes, leading to metabolic diseases at and after parturition (Wrzecińska et al., 2021). During the dry period, dairy animals are typically fed with low-quality feed, while on the onset of parturition, they are transitioned to a high concentrate diet to meet their increased energy requirements associated with elevated milk production. This shift in dietary composition leads to a change in the microbial population i.e., an increase in the population of lactate-producing bacteria (*Streptococcus bovis*, *Lactobacillus*) and a decrease in the population of lactate- and succinateutilising bacteria (*Megasphaera elsdenii* and *Selenomonas ruminantium*) (Hernández et al., 2014). When ruminant animals are fed easily digestible carbohydrates, lactate-producing bacteria ferment these carbohydrates and generate more lactate, resulting in a lower ruminal pH. Lactate has a low Pka value and damages the rumen papilla, which reduces volatile fatty acids absorption. As a consequence of the high rate of acid production, the pH of the rumen contents and blood decreases, triggering several significant physiological changes (Counotte et al., 1979). This phenomenon is referred to as ‘lactic acidosis’, and clinically, this condition is characterised by intoxication, haemoconcentration, dehydration, rumenitis, and laminitis.

In young ruminants, due to an underdeveloped rumen and liquid diet, most organic compounds are fermented in the lower GI tract. The immature flora present in the lower GI tract during early life produces large quantities of intermediates such as lactate, succinate, and alcohols, resulting in diarrhoea in calves (Shimomura and Sato, 2006; Sato and Shiogama, 2009). Under normal physiological conditions, intermediate products, i.e., lactate and succinate, are metabolised to volatile fatty acids (acetate, butyrate, and propionate), leading to their particularly low concentrations in the GI tract (Macfarlane and Macfarlane, 2007). However, if abnormal metabolism occurs or a high grain diet is administered, there can be an increase in the concentrations of lactate and succinate, rather than their conversion to acetate, butyrate, and propionate (Figure 1). Fermentative diarrhoea is caused by high levels of lactic and succinic acids in the large intestine, as their luminal accumulation creates an osmotic load that increases the mucosa’s capacity to secrete water. Strong organic acids like lactic and succinic acids are thought to reduce luminal pH. Additionally, the mucosa does not secrete bicarbonate in response to lactic or succinic acids, exacerbating the acidity of the lumen and causing damage to the large intestine, leading to reduced water absorption. As a result, excess lactate and succinate are excreted in the faeces, and their concentrations can be measured spectrophotometrically. Lactate is converted into acetaldehyde by heating with sulphuric acid in the presence of copper and then reacts with p-hydroxybiphenyl to form a coloured complex, which can be measured at 565 nm (Barker and Summerson, 1941).

In healthy calves, faecal lactate concentration was shown to range from 0.1 to 2.8 mmol/l (Sato, 2003), while during diarrhoea episodes, faecal lactate levels were found to be elevated, ranging from 8.4 to 22.8 mmol/l (Sato and Koiwa, 2008). Comparable findings by Ewaschuk et al. (2004) indicated faecal lactate concentrations of 4.5–23 mmol/l in healthy calves versus 3.7–51.3 mmol/l in diarrhoeic calves. Faecal lactate and succinate levels can be used to distinguish pathogenic diarrhoea from non-pathogenic or fermentative diarrhoea. Tsukahara and Ushida (2001) conducted a study on piglets with pathogenic and non-pathogenic diarrhoea, revealing that faecal lactate and succinate concentrations played a crucial role in differentiation the two conditions. Faeces of piglets raised under normal conditions contained less than 2–4 mmol/kg of lactate and succinate. However, piglets fed a high-starch diet exhibited increased



**Figure 1.** Two pathways of propionate synthesis  
P1 – lactate pathway, P2 – succinate pathway

concentrations of faecal lactate (15 mmol/kg) and succinate (25 mmol/kg) without displaying signs of colibacillosis, in contrast to piglets with colibacillosis-positive diarrhoea.

### Sialic acid

The GI mucosal barrier consists of epithelial and immune cells that work in concert with the resident microbiota to establish a barrier against harmful substances. Goblet cells produce a thick layer of mucus that protects epithelial cells and serves as the first line of innate host defence (Cornick et al., 2015). This mucus layer acts as a physical barrier, preventing microorganisms and harmful substances from accessing the epithelial surface. Mucins, which are high molecular weight glycoproteins, are the key components of the mucus gel (Hollingsworth and Swanson, 2004). Mucins contain five distinct monosaccharides: N-acetyl-galactosamine, N-acetylglucosamine, galactose, fucose, and sialic acids. Microbes may come into contact with sialic acid-coated structures on cell surfaces or released mucin glycoproteins on mucosal surfaces, such as in the respiratory or GI tracts. Mucins, the primary structural components of the mucus layer covering the epithelial surface, are a significant source of sialic acids in the gut. The outermost layer of mucus hosts a variety of commensal bacteria that have adapted to feed on mucin proteins, which contain 80% carbohydrates. When the intestinal mucosa is damaged or a foreign pathogen utilising sialic acid for growth penetrates the barrier, the concentra-

tion of sialic acid, a component of mucin, increases in the GI lumen and is excreted with faeces (Huang et al., 2015).

The concentration of sialic acid in the faeces was determined by Jourdian et al. (1971), who used a colorimetric method, where N-acetyl neuraminic acid at concentrations ranging from 0.05 to 0.3  $\mu\text{mol}$  was employed as the standard. A mixture of test reagents and distilled water without N-acetyl neuraminic acid served as the blank. Colour absorbance was measured at 610 nm. There was an increased faecal concentration of sialic acid ( $>7 \mu\text{mol/g}$  vs. 4–7  $\mu\text{mol/g}$ ) in the potato protein diet compared to the soybean protein feed administered to broilers. Potato protein showed lower digestibility and higher toxigenic effect related to increased production of *Clostridium perfringens* alpha toxin, leading to necrotic enteritis (Fernando et al., 2011).

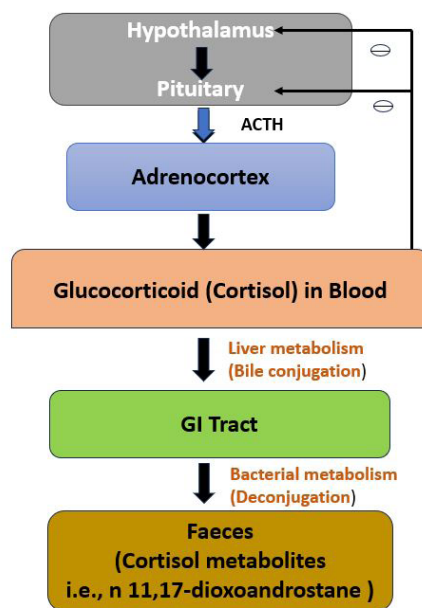
### Glucocorticoid metabolites

According to Phillips et al. (2010), cattle are the most common farm animals utilised in milk production for human consumption. Recent advancements in cattle production have led to increased scrutiny from the public and animal rights organisations, emphasising the need for improved management practices and humane interactions between humans and cattle (Lynch, 2010). The public and animal rights organizations argue, that intensive animal agriculture and experiments may have compromised animal welfare, urging a reassessment of practices (Dohms and Metz, 1991). Animal welfare is defined by the

condition of an animal in relation to its immediate surrounding environment, with the animal's ability to respond to external stimuli determining its overall health and well-being. Maintaining high reproductive efficiency in the animal production system is important from an economic point of view, and to sustain it, there must be a balance between the pursuit of increased production and the elimination of the adverse effects of environmental stressors.

Stress is a reflex response that arises from an animal's inability to adapt to its environment and can manifest in a variety of negative effects, from discomfort to death. It involves the behavioural and biological reactions to a variety of abiotic stressors, including rough handling, social interactions, common farming practices (castration, dehorning, teeth clipping, crowding, weaning etc.), inadequate feeding, exposure to unfavourable climatic conditions, exercise, work, and transportation (Endris and Feki, 2021). Both internal and external stressors can originate from individuals or their environment. When a stress stimulator triggers a signal to the hypothalamus, which in turn transmits the signal to the anterior pituitary gland to secrete corticotrophic releasing hormone. This hormone acts on the adrenal gland, prompting the secretion of corticosteroids. This glucocorticoid i.e., cortisol, exerts adverse effects on immune response, glycogen metabolism, behaviour, and reproduction (Sapolsky et al., 2000). The sympathetic-adrenomedullary axis and the hypothalamic-pituitary-adrenal (HPA) axis assist in the process of linking the initial perception of the stress to an adequate response (Lynch, 2010). Cortisol, a primary glucocorticoid in cattle, is released from the adrenal cortex and distributed via the circulatory system to various target tissues, organs or body systems (Burdick et al., 2011). Glucocorticoids secreted by the HPA axis and circulating in the plasma are primarily metabolised in the liver (Taylor, 1971). Further metabolism of steroids excreted in the bile by bacterial enzymes may occur in the intestine, and metabolites with a n 11,17-dioxoandrosterane (11,17 DOA) structure may be excreted with the faeces (Figure 2) (Messmann et al., 1999). Faecal glucocorticoid metabolites i.e., 11,17 DOA, can be extracted and their concentration determined using group-specific enzyme immunoassays (Palme and Möstl, 1997; Palme et al., 1999; Morrow et al., 2002).

The basal level of 11,17-DOA was determined by Messmann et al. (1999) to range 34–445, 93–1031, 2.3–35.3 and 6.9–19.1 nmol/kg faeces of cattle, sheep, horse and pig, respectively. The latter authors observed an increased concentration of



**Figure 2.** Endocrinal secretion of glucocorticoid (cortisol) in blood and its metabolism in faeces

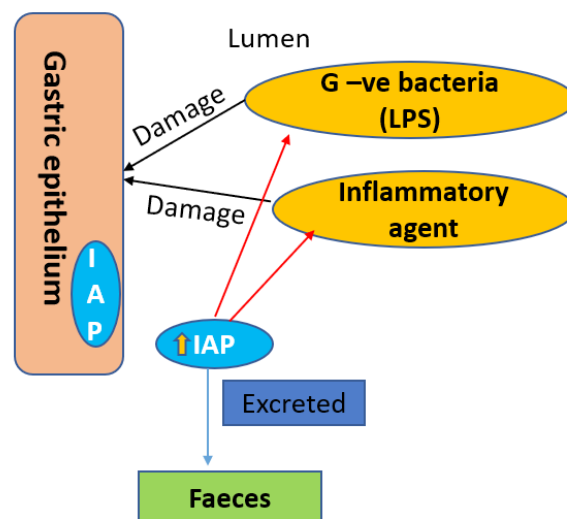
ACTH – adrenocorticotrophic hormone, GI – gastrointestinal tract

faecal glucocorticoid metabolites in dairy cows after the administration of adrenocorticotrophic hormone and concluded that determining the levels of glucocorticoid metabolites in dairy cattle could be a reliable method to detect acute adrenal activity. Moreover, when combined with other physiological and behavioural markers, it can also be used to monitor the welfare and general health of cattle (Messmann et al., 1999; Morrow et al., 2002). Jurkovich et al. (2017) conducted a study to assess stress in relation to the type of milking system: parlour milking, automatic milking system, and the frequency of human interaction in 27 Holstein Friesian dairy cows. To this end, these authors determined faecal glucocorticoid metabolites using the method described by Csernus (1982). The mean faecal glucocorticoid metabolite concentrations were higher during parlour milking (58 ng/g faeces) compared to automatic milking (19 ng/g faeces). The results suggest that automatic milking may be less stressful for cows than parlour milking, possibly due to the shorter duration of restraint after milking and less human interaction. Additionally, glucocorticoid concentrations in faecal samples of pigs from the Eastern and Western Ghats of Tamil Nadu, India, were determined using the DSI-EIA kit. An increased mean faecal glucocorticoid metabolite concentration was found in Western Ghat pigs (343 ng/g faeces) compared to Eastern Ghat pigs (224 ng/g faeces), attributed to lack of food, human interference, and environmental changes (Allwin et al., 2016).

## Intestinal alkaline phosphatase

Alkaline phosphatase (AP) is a family of metalloenzymes that catalyse the hydrolytic elimination of phosphate from a wide range of compounds (Millán, 2006). The mammalian AP family includes multiple isozymes that can be divided into tissue nonspecific APs (TNAP), found in bone, liver, and kidney, and tissue-specific APs present in the intestine, placenta, and germ cells (Millán, 2006; Yang et al., 2012). Intestinal alkaline phosphatase (IAP) is located in the intestines, with the highest levels of expression in the duodenum and significantly lower levels in the jejunum, ileum, and colon, but it has also been found in the stool. IAP, which is found in the apical microvilli of the brush borders of enterocytes, and is secreted into both the intestinal lumen and the bloodstream, has long been considered a crucial component of intestine mucosal defence, playing a significant role in maintaining gut homeostasis (Lallès, 2010). According to Fawley and Gourlay (2016), who reviewed data from a number animal and human studies, exogenous IAP has been found to protect against intestinal and systemic inflammation in many diseases. The most significant functions of IAP in the GI tract include the control of bicarbonate secretion and pH on the duodenal surface, modulation of the absorption of intestinal long-chain fatty acids, and detoxification of endotoxin lipopolysaccharide (LPS), which results in local intestinal and systemic anti-inflammatory effects. The ability of IAP to inactivate LPS appears to be particularly important in the aforementioned contexts. LPS is a key component of the outer membrane of Gram-negative bacteria, which constitute a large part of the mammalian gut microbiota (Yang et al., 2012). LPS possesses two phosphate groups in its toxic lipid A moiety that are required for its biological effect (Reitschel et al., 1994). IAP is involved in the LPS dephosphorylation, which increases mucosal tolerance to gut microbes. Thus, the concentration of IAP was shown to increase in order to protect the gastric epithelium from damage caused by pathogens and other inflammatory agents. Subsequently, it was excreted in faeces along with the damaged epithelium (Figure 3).

Faecal IAP was evaluated as a marker of intestinal damage in rats treated with bleomycin (40 mg/kg body weight intra-peritoneally). IAP levels in faecal samples were determined spectrophotometrically using *p* nitrophenyl phosphate as a substrate. Bleomycin was demonstrated to exert toxic effects on the intestine, resulting in increased faecal IAP levels (200–300 units/24 h) compared to the control group (<100 units/24 h) (Thomas and Henton, 1985).



**Figure 3.** Excretion pathway of intestinal alkaline phosphatase in faeces

IAP – intestinal alkaline phosphatase, LPS – lipopolysaccharide

## Lipocalin-2

White adipose tissue is now recognised as a *bona fide* endocrine organ capable of secreting a diverse array of adipose-derived factors collectively termed ‘adipokines’. Lipocalin (LCN)-2, one of the adipokines, is known as a pleiotropic molecule involved in a wide range of physiological and pathological processes, including inflammation, infection, immunological response, and metabolic homeostasis (Abella et al., 2015). LCN is a small secreted protein derived from adipose tissue, hence also referred to as an adipose derived cytokine, and it helps in the transport of small lipophilic molecules. LCN, also known as neutrophil gelatinase-associated LCN, plays a crucial role during mild inflammation. It contributes significantly to reducing infections by preventing bacterial growth and enhancing immunity through inflammatory modulation (Rathore et al., 2011; Chakraborty et al., 2012). A major challenge for pathogenic bacteria during infection and post-infection inside the host is acquiring sufficient amounts of iron. When deprived of a readily available iron source, pathogens upregulate siderophore biosynthesis. However, the host cells respond by increasing the concentration of neutrophils as the first line of defence. These neutrophils contain LCN-2, which captures siderophores, leading to growth inhibition of pathogenic bacteria (Wang et al., 2019). Therefore, increased secretion of neutrophils during intestinal inflammation results in the release of LCN-2 into the intestinal lumen, subsequently excreted with faeces, and its faecal concentration can be measured using ELISA. A linear increase in

LCN-2 concentration (10 to 10000 ng/g faeces) was observed in mice treated with gradually incrementing doses of DSS (0.225–4%) (Chassaing et al., 2012). Similarly, Radhakrishnan et al. (2015) reported higher faecal levels of LCN-2 and calprotectin in pigs fed a high calorie diet (25% fat) for 13 weeks. These animals showed oxidative stress, inflammation and a colonocyte proliferation zone, resulting in higher LCN-2 levels in the faeces. In mice with colitis induced by 2,4,6-trinitrobenzenesulfonic acid, faecal LCN-2 level was found to be 10 000-fold higher than serum LCN-2, rendering it a significantly more sensitive biomarker capable of distinguishing between mild, moderate, and severe colonic inflammation. This heightened sensitivity may be attributed to a localised inflammatory reaction in the colon, whereby granulocytes release LCN-2 at the site of inflammation (Hsieh et al., 2016).

### Calprotectin

Calprotectin (S100A8/A9 heterodimer) is a calcium and zinc binding protein, found primarily in neutrophils, and to a lesser extent in monocytes and epithelial cells. It constitutes approximately 60% of the total cytosolic protein content in neutrophils and 5% of their total protein content (Foell et al., 2004; Paduchova and Durackova, 2009; Judd et al., 2011). Calprotectin is released during cell disruption or death, and a portion is also actively secreted (Rammes et al., 1997; Voganatsi et al., 2001). As a result, its presence in faeces implies neutrophil migration to the intestinal tract and its infiltration (Vermeire et al., 2004; 2005). Calprotectin is a positive acute phase protein that plays an important role during inflammation (Vaos et al., 2013). It stimulates neutrophils to express receptors involved in motility, adhesion, and phagocytosis. Quantification of calprotectin in stools can be easily achieved using a commercially available ELISA immunoassay. It is increasingly employed in diagnostic procedures for stomach inflammation due to its high sensitivity and specificity, relative simplicity, rapid turnaround time, and long stability at room temperature (up to seven days) (Alibrahim et al., 2015). Increased faecal calprotectin levels were observed in piglets soon after birth (33 mg/g faeces), which was dependent on the sanitary status of the animals (Lallès and Fagarhol, 2005). Similarly, in weaned pigs challenged by enterotoxigenic *Escherichia coli*, a higher incidence of diarrhoea and increased jejunal calprotectin expression (390 unit) was observed compared to the control (327 unit) (Xiao et al., 2014). In dogs with chronic inflammatory enteropathies, faecal calpro-

tecin appears to be a potentially effective variable for assessing the degree of GI inflammation. Faecal calprotectin concentrations effectively discriminate dogs with SRE/IRE (steroid or immunosuppressant responsive enteropathies) and FRE or ARE (food or antibiotic responsive enteropathies) (Heilmann et al., 2018).

### Conclusions

Faecal biomarkers play a crucial role in identifying events that affect gastrointestinal functionality. They act as non-invasive indicators, eliminating the need to slaughter animals for sample collection. This is particularly valuable in the case of wild animals, where restraining and sample collection (blood, urine) is difficult. The ease of collecting faecal samples without causing stress to the animals contributes significantly to their welfare. Despite the high potential of faecal biomarkers in assessing gastrointestinal functionality in animals, there is a scarcity of literature on this subject, especially regarding the validation of methods for processing and analysing such biomarkers. There is a pressing need for the development of such indicators in the coming future to ensure effectiveness, reliability, and animal welfare.

### Conflict of interest

The Authors declare that there is no conflict of interest.

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