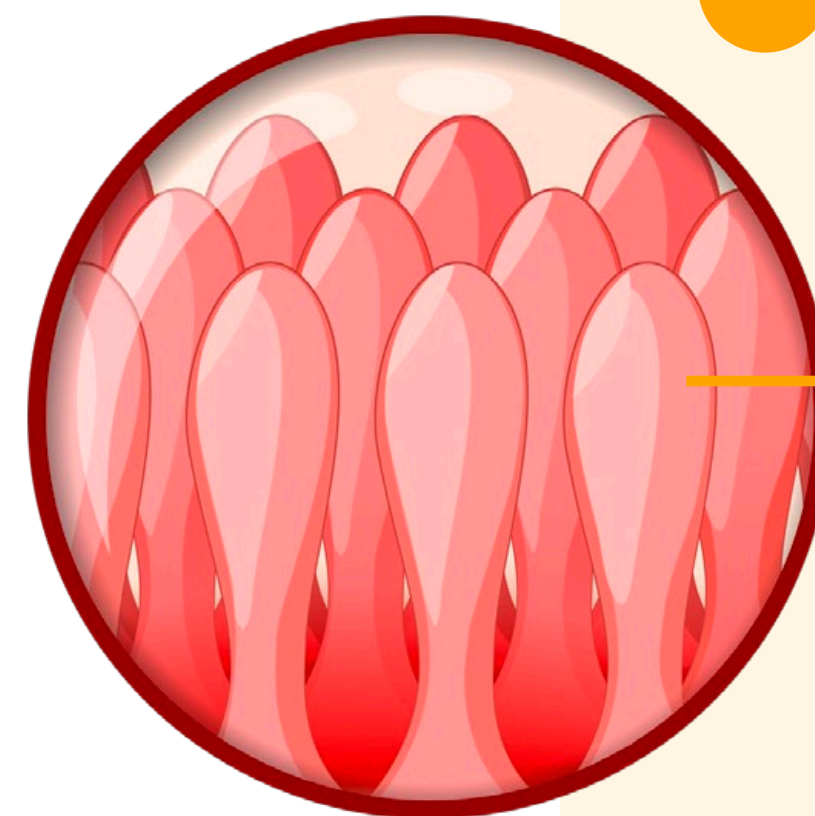


GUT HEALTH IN PULLETS AND LAYING HENS



ENG

In 2030, **expected global population growth is estimated at 8.6 billion people** (UN, 2017). In turn, global demand for food will increase with **an estimated growth in egg production of 50% between 2015-2035**. (FAO; OECD; Rabobank; FAPRI © Statista 2018).

In this sense, genetic selection during the last years has provided hens with a higher production capacity.



Gut health is a **key factor in achieving maximum productive potential**, not only because it's a key factor for digestion and the absorption of nutrients but also because it's an essential component of the bird's immune system.



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Concerns are also growing among consumers on the **use of antibiotics in relation to microbial resistance**.

This, along with pressure from a demand in increased welfare and food standards in particular from the increase of cage free production would lead us to a strong emphasis and focus on the challenges of maintaining a healthy gut.

Several factors must be considered about Gut health (**Figure 1**):

▼ **Figure 1.** Factors affecting gut health





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Feed and water

Both vectors create a connection between the external and internal environment of the hen, increasing the possibility of a negative effect on the intestinal balance. Some common influences:

- **Anti-nutritional factors** (non starch polysaccharides and anti-trypsin factors)
- **Water, raw material and feed contaminants** (*E. Coli*, Salmonella, mycotoxins, etc.)
- **Sudden changes in formulation**
- **High density diets – excess of nutrients**



Bird physiology

How **different organs and the endocrine system** respond against challenges.

Gut microbiota

Represented by the balance between pathogenic and commensal flora. Latter being the one involved in the development of intestinal morphology and structure, immune modulation and supporting digestion and absorption processes.

► **Figure 2.** Effect of mycotoxins on gut integrity. Cheng et al., 2012. Gut integrity is affected by increasing deoxynivalenol (DON) concentration in feed.

Control diet



2mg DON/kg



5mg DON/kg





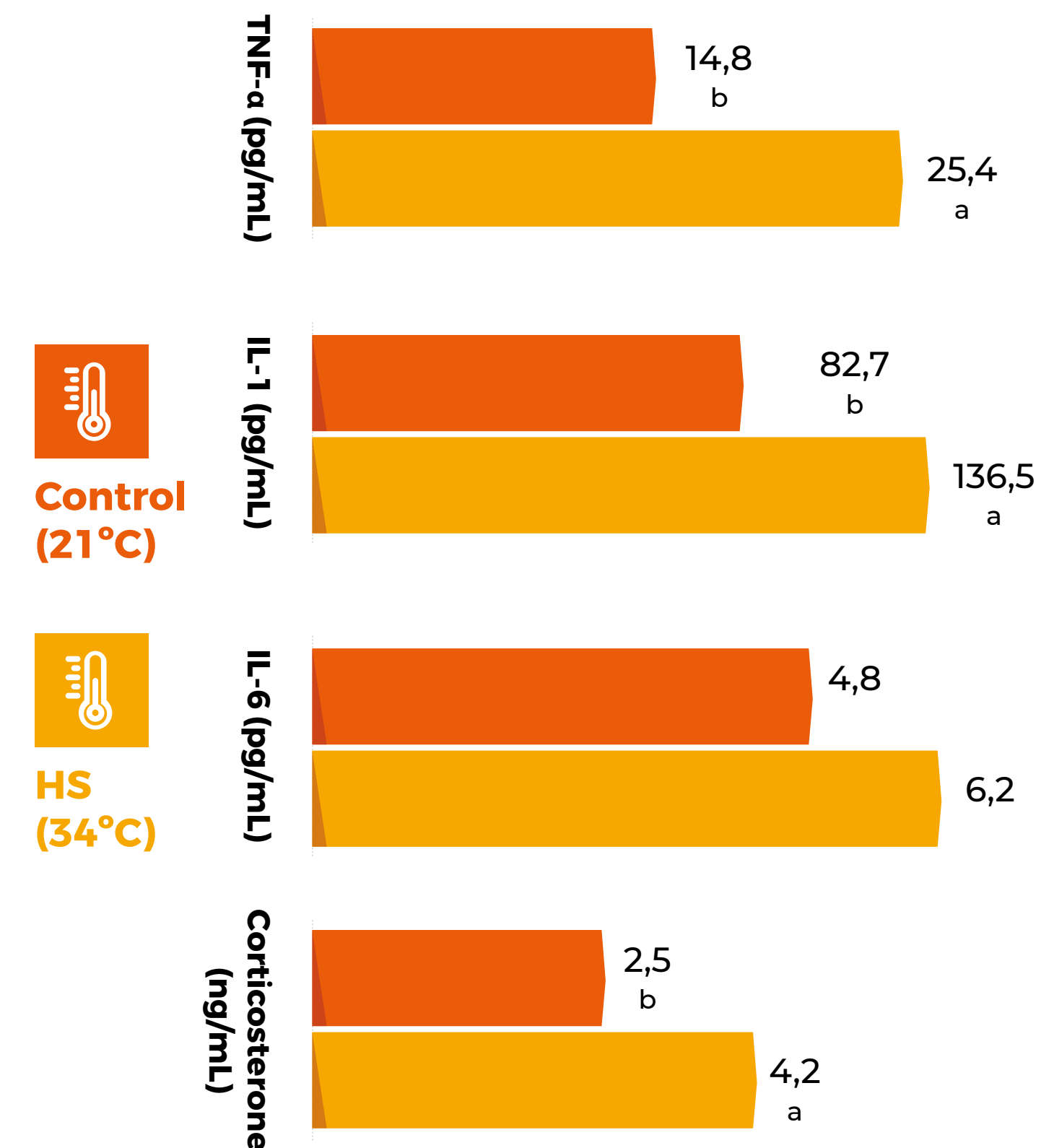
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Also, factors like temperature (**Figure 3**), light intensity, poor ventilation, transport, vaccination, high stocking density, gut pathogens and the presence of virus may trigger a gut imbalance leading to inflammation processes becoming prevalent.

All these factors represent the basis for the establishment of optimal gut health.

► **Figure 3.** Effect of temperature on TNF- α production (related with acute inflammatory phase stimulation), IL-1 (cytokine produced in response to stress situations), IL-6 (cytokine with both, pro-inflammatory and anti-inflammatory activity) and corticosterone (hormone that mediates under stress situations) in hens of 60 weeks. Continued exposition to high temperatures causes changes of gut microbiota and morphology. Deng et al., 2012.





Strategies for gut health maintenance

The best practice to maintain an optimal gut health and therefore the hen's productivity is **PREVENTION**. Some aspects to be considered are:

- Proper **management, hygiene and biosecurity**
- **Optimal feed quality, format and presentation**
- **Water quality. Monitoring water to feed ratio would act as an indicator.**
- **Establishing adequate vaccination programs**
- **Reduction in crude protein levels** may help to reduce undigestible protein fermentations. **Inclusion of synthetic amino acids and proteases will help to control these undesirable fermentations.**
- **Feed additives**

The recent focus on reducing or restricting the use of antibiotics has been accompanied by a rise in intestinal problems often leading to a **loss of productivity** that has led to the development of several feed additives with the potential to exert beneficial effects on intestinal microbiota, **impede pathogens adhesion to epithelial cells and to improve the immune response.**

The market is now awash with **probiotics, prebiotics, organic acids** and blends of them (either protected or not), **phytobiotics** and existing feed enzymes.

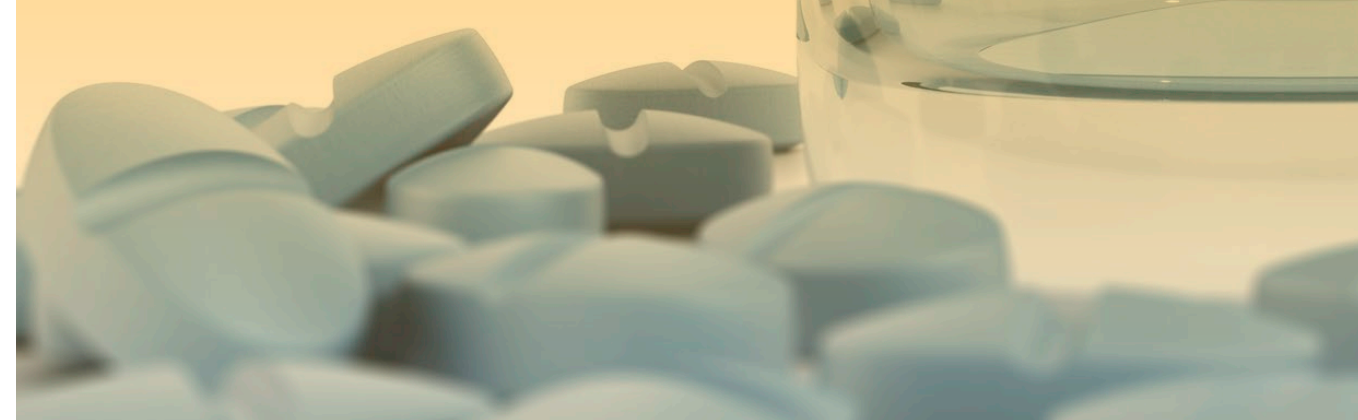
Always remember that the efficacy of feed additives relies on additional factors such as age of the hen, management, production system, genetics, etc.



Format and feed presentation

Feed particle size and format are extremely important for gut development. Providing mash feed with optimal feed distribution particles, improves:

- **Feed consumption**
- **Nutrient digestibility**
- **Promotes digestive organ development**
- **Improved gut health for optimizing performance**



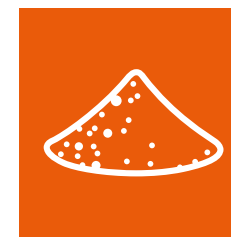
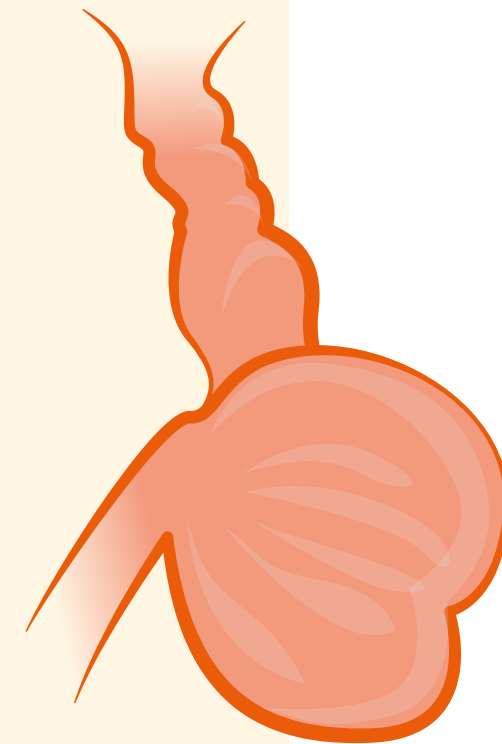


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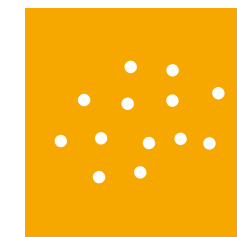
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When feed contains too many fine particles, it negatively affects gizzard and proventriculus development, these organs are important due to their essential role in nutrient utilization and gut health maintenance. (Zaefarian et al., 2016)

Gizzard development is key. A well-developed gizzard, besides reducing feed particle size entering the duodenum, acts as a barrier for microbiota due to pH reduction.



Mash



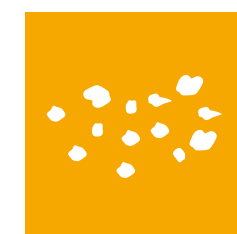
Crumble

▼ **Figure 4.** Influence of feed format and presentation on digestive organs relative weight (g/100g of body weight) in layers. Ege et al., 2019

Gizzard



Fine



Coarse

Gizzard





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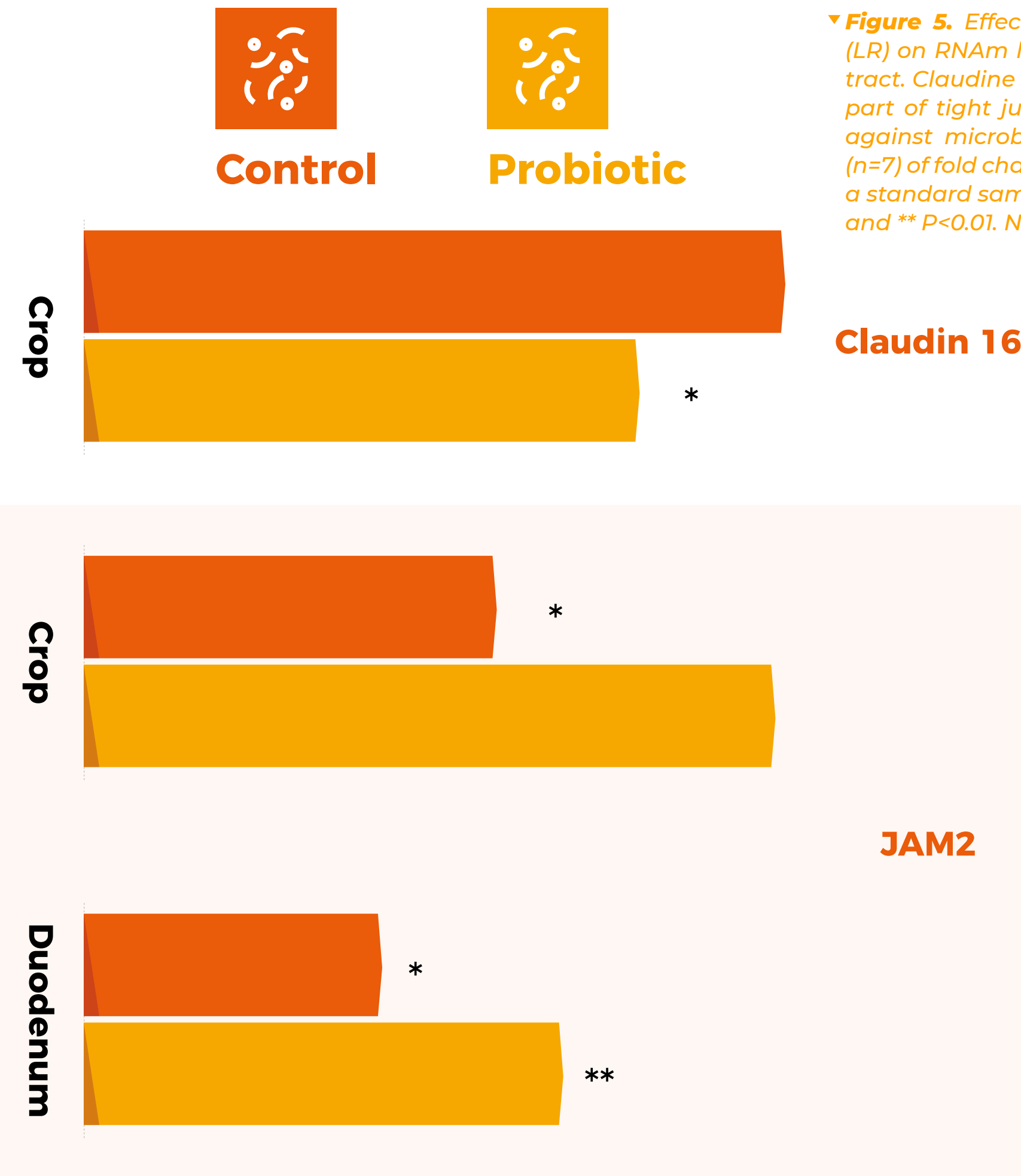
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Probiotics

Probiotics are viable microorganisms able to promote a beneficial effect on birds' gut health.

Mechanisms of action include:

- **Improvement on intestinal microbiota balance**
- **Source of energy** through short fatty acids (SCFA's)
- **Reducing the capacity of pathogens to adhere** to the intestinal epithelium by competitive exclusion
- **Improving enterocyte's tight junctions**
- **Modulating the immune response**
- **Inhibiting tumour necrosis factor** at epithelial cells



▼ **Figure 5.** Effect of supplementation of *Lactobacillus reuteri* (LR) on RNA levels related with tight junctions on intestinal tract. Claudine increases intestinal permeability while JAM2 is part of tight junctions controlling permeability and protects against microbiota invasion. Values represent mean SEM $\pm$ (n=7) of fold change in the target gene expression compared to a standard sample of the cecum in the control group. * $P < 0.05$ and ** $P < 0.01$. Nii et al., 2020.





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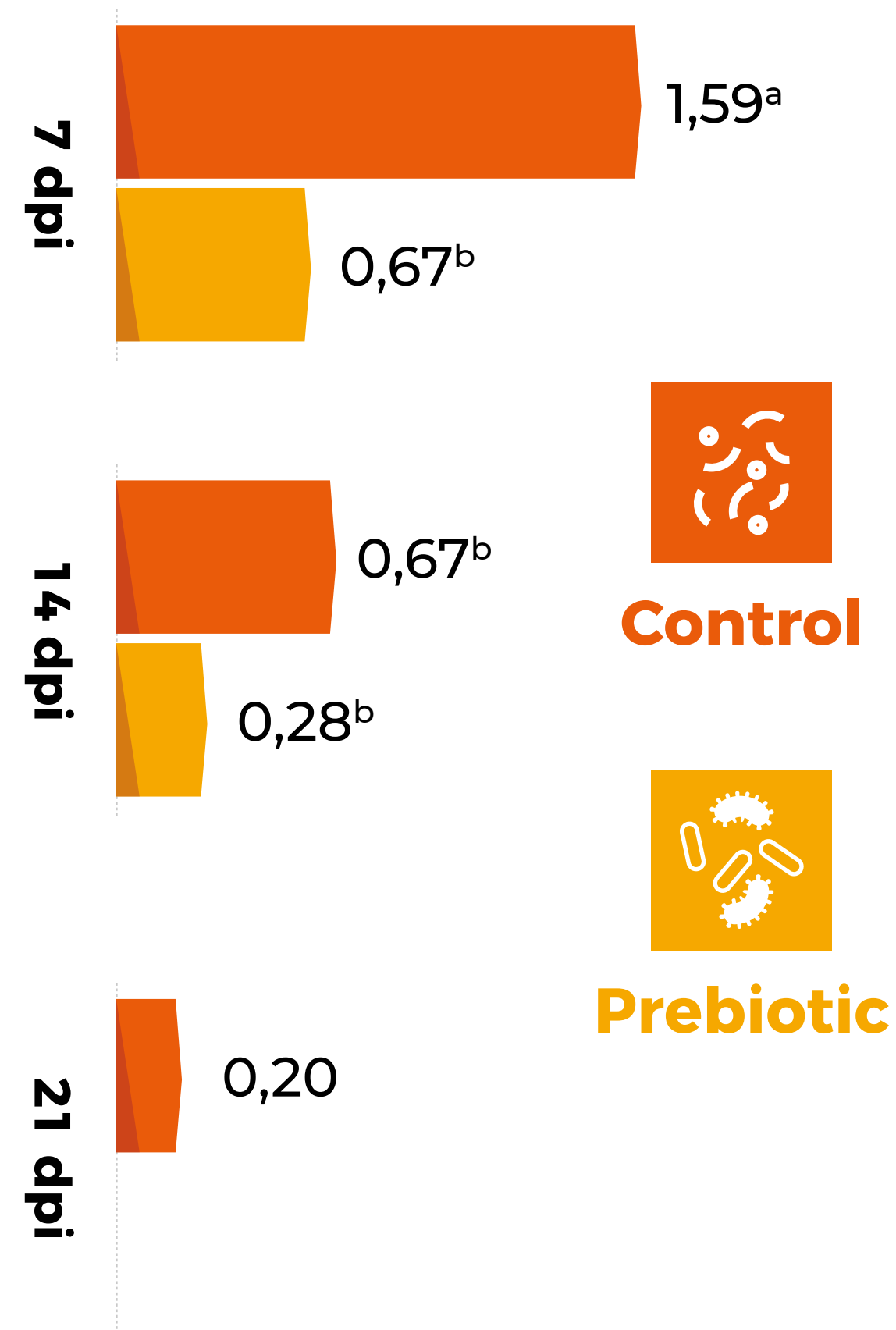
Prebiotics

Prebiotics are characterized by their **capacity to modify (in a beneficial way) the composition of intestinal microbiota** exerting a positive effect on gut health. They're different oligosaccharides whose main feature is that they are undigestible by endogenous enzymes. For that reason, they reach the distal part of intestinal tract where they can be used as a substrate by beneficial bacteria like bifidobacterial or acidolactic bacteria (*Ricke, 2018*).

Normally, oligosaccharides include carbohydrates like fructooligosaccharides (FOS), galactooligosaccharides (GOS) and mannoooligosaccharides (MOS).

▼ **Figure 6.** Effect of prebiotic on hens challenged with *Salmonella Enteritidis*. Murate et al., 2015.

log10 cecal of *S. Enteritidis*



Here it's worth mentioning dietary fibre, the sum of NSP's (non starch polysaccharides) and lignin. **Some of these polysaccharides represent a substrate for intestinal microbiota besides a source of energy.** Increasing the inclusion rate of crude fibre would improve intestinal microbiota diversity and tract physiology.

During the rearing period, the first 5 weeks of age are key due to the development of the digestive and immune system as well as being the period when intestinal microbiota establishment takes place. The inclusion of moderate levels of insoluble fibre in pullet and layer diets stimulates development and physiology of the intestinal tract therefore improving bird's productivity.



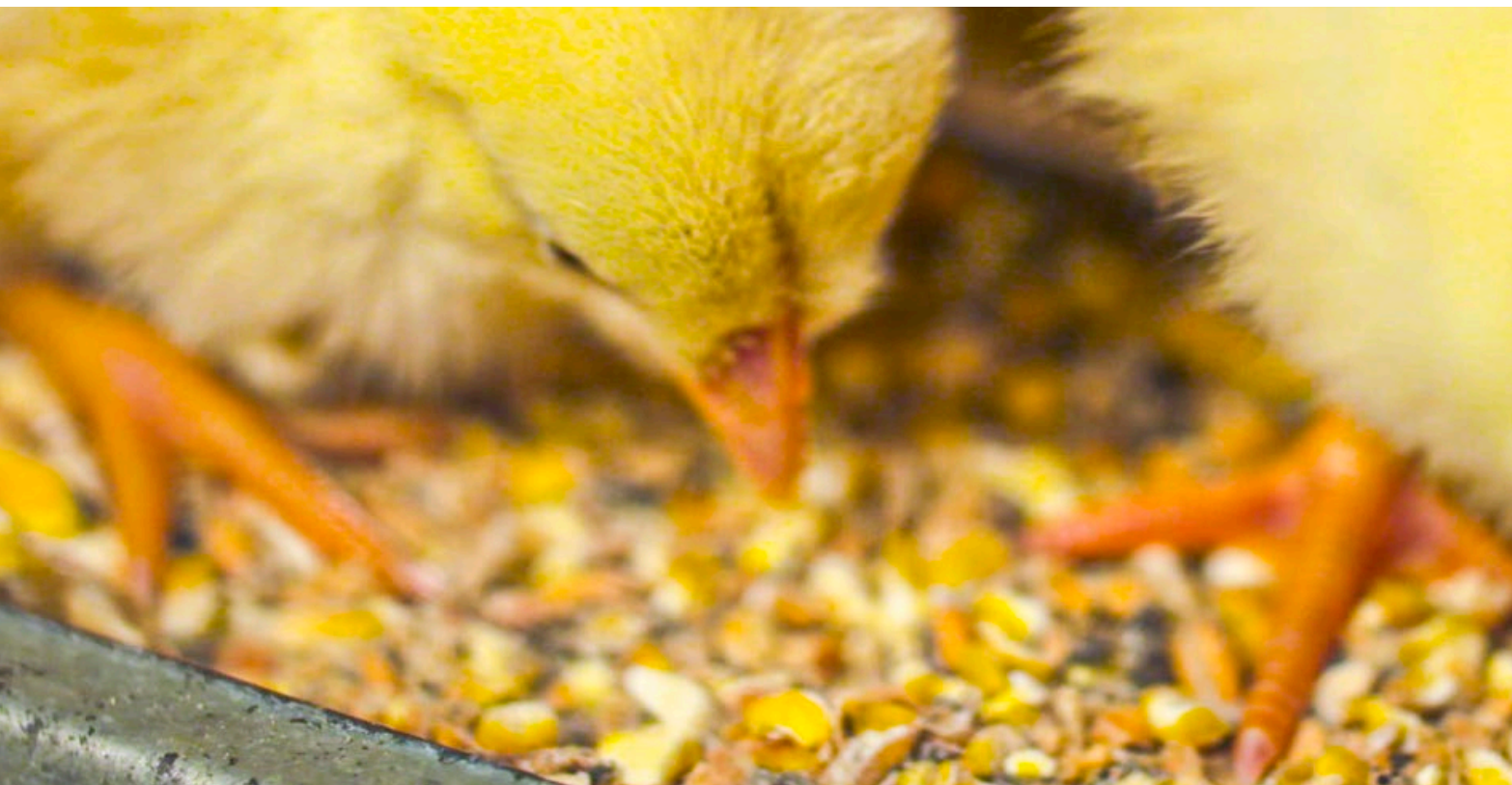


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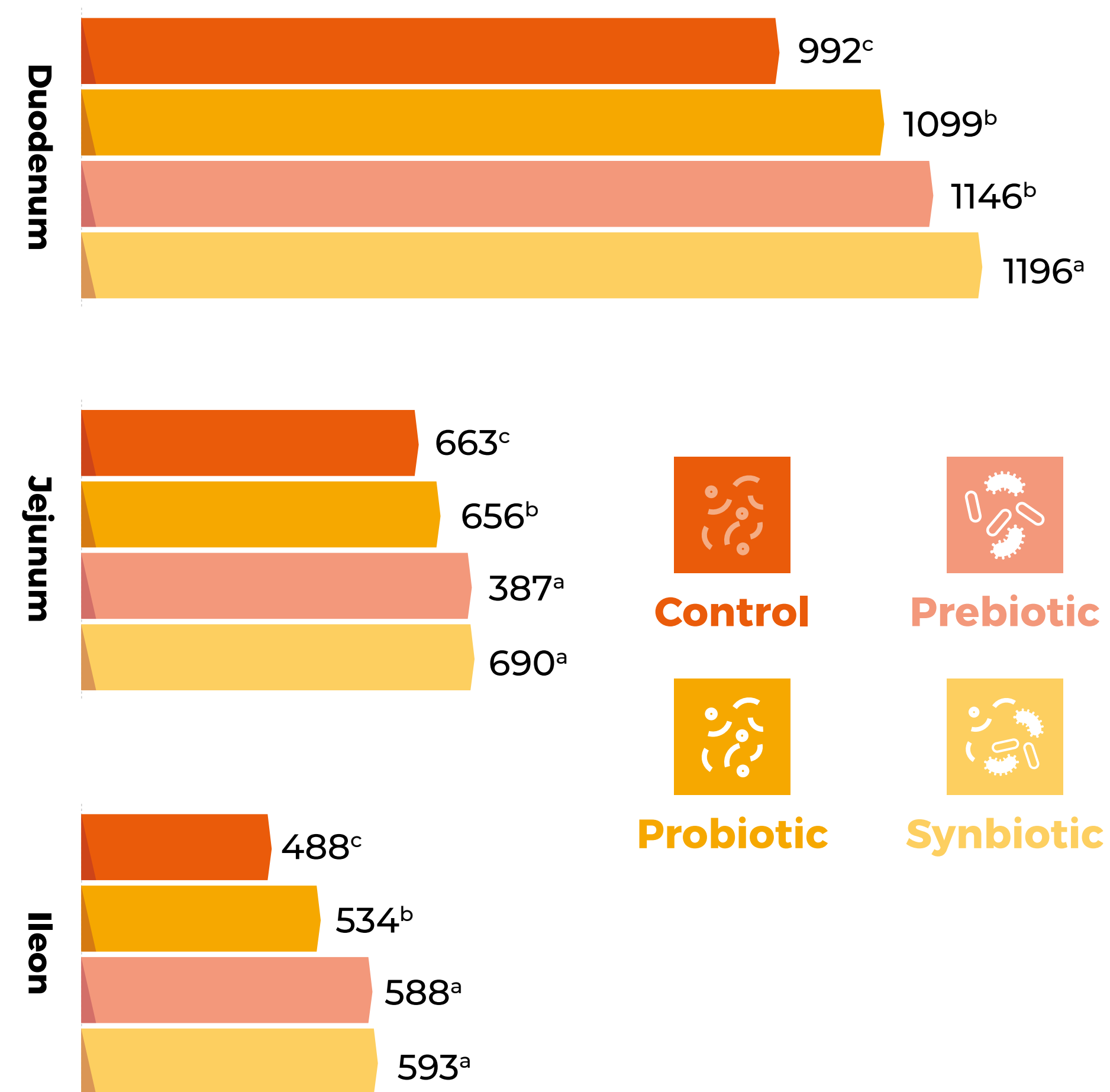
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Synbiotics

Is defined as the **combination of prebiotics and probiotics** where the latter selectively enhances probiotic growth thereby showing a synergic effect.



Villi height, μm



◀ **Figure 7.** Effect of synbiotic on intestinal morphology of laying hens. Abdelqader et al., 2012. Higher villi height is associated with a higher absorption capacity and intestinal integrity.

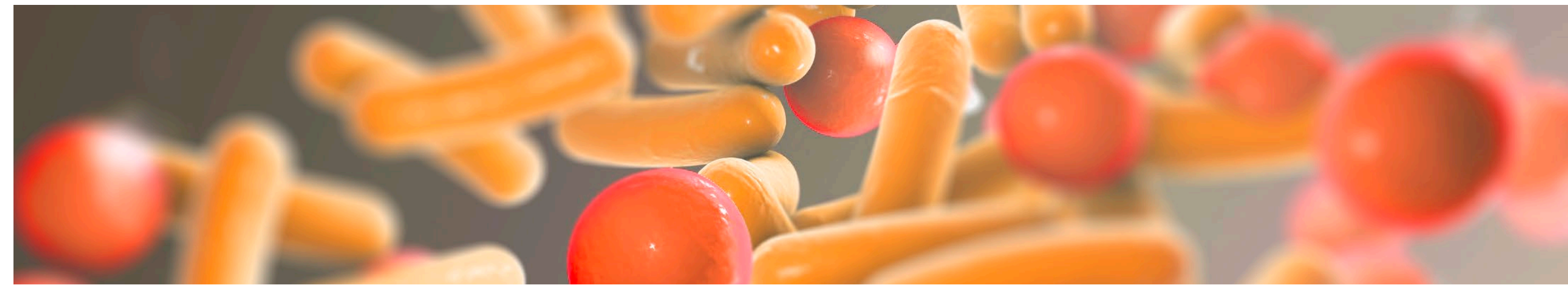
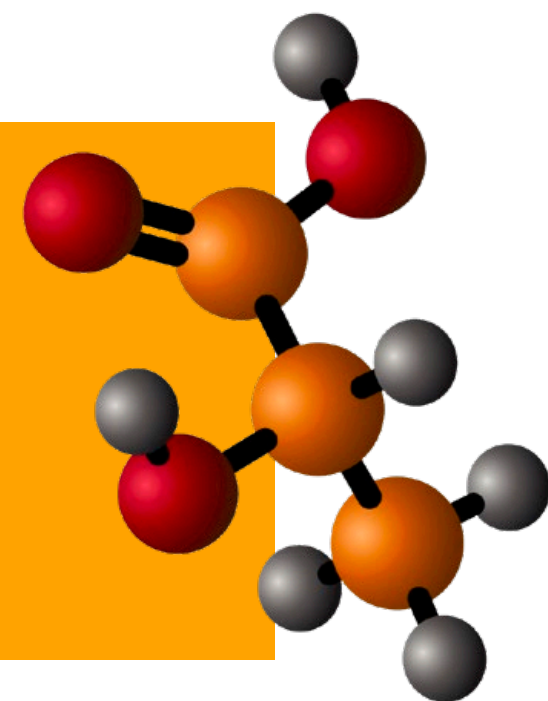


Organic acids

Organic acids are additives that, besides being effective reducing or controlling microbiological contamination of the feed (Theron y Rykers Lues, 2011), **have been proven to exert beneficial effects on gut health and performance.**

Their inclusion in hens and pullets' diets **stimulate** endogenous enzyme activity, **improves** mineral solubility and can have an antibacterial effect therefore **supporting** gut intestinal integrity.

There are a variety of organic acids available. Some examples are acetic acid, formic acid, lactic acid, fumaric acid, etc. or combination of them. They differ on physic-chemical characteristics and can be included in feed or drinking water.



▼ **Table 1.** Minimum inhibitory concentration of some organic acids. Strauss and Hayler, 2001.

	Formic acid (%)	Propionic acid (%)	Lactic adic (%)
<i>Salmonella typhimurium</i>	0,10	0,15	0,30
<i>Escherichia coli</i>	0,15	0,20	0,40
<i>Campylobacter jejuni</i>	0,10	0,20	0,25
<i>Staphylococcus aureus</i>	0,15	0,25	0,40
<i>Clostridium botulinum</i>	0,15	0,25	0,30
<i>Clostridium perfringens</i>	0,10	0,25	0,30



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Their effectiveness depends on the kind of acid (pka and molecular weight), concentration, dosage and pH of the environment.

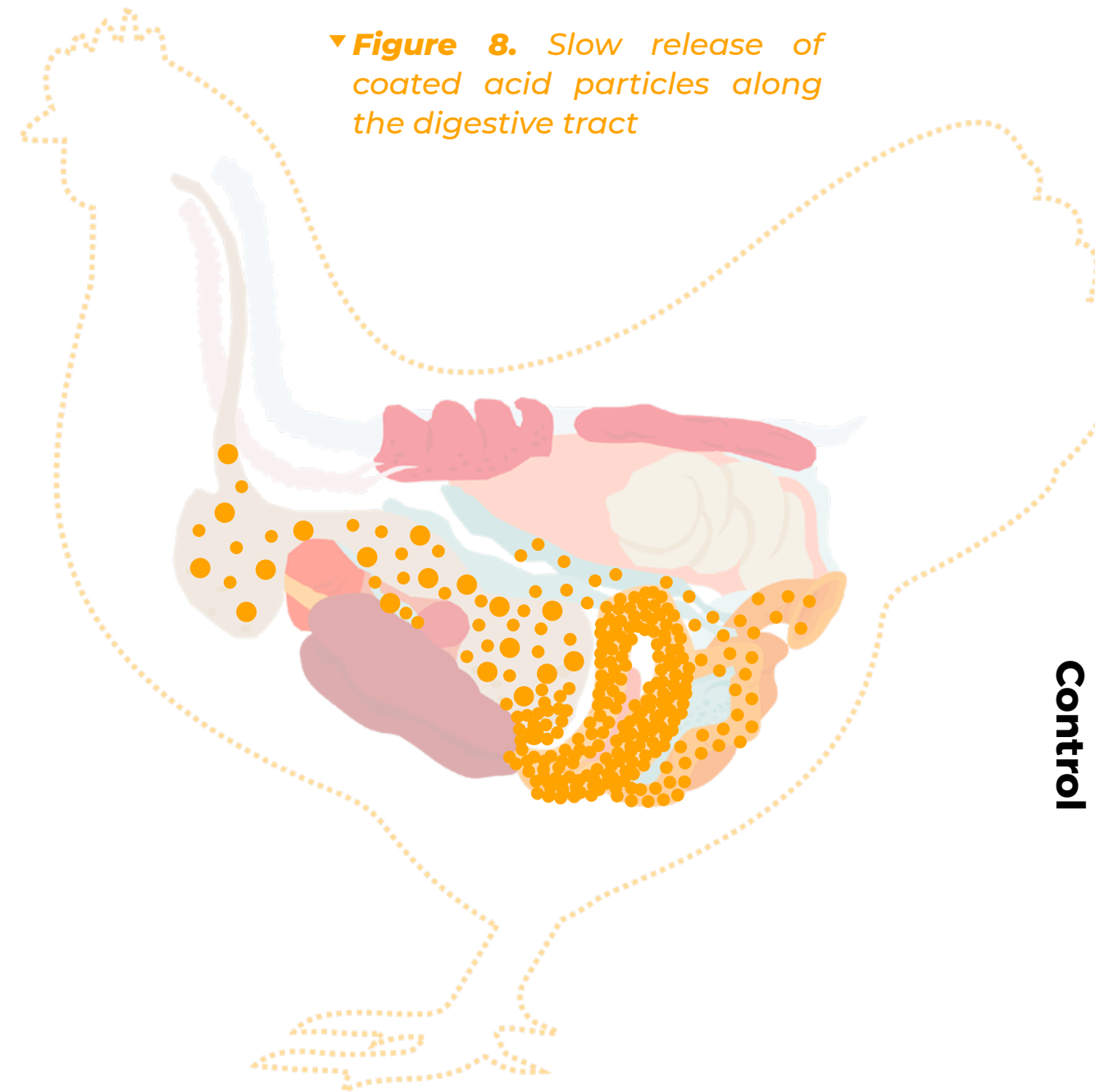
At lower levels of pH, a higher concentration of non-dissociated form can be expected. This allows them to penetrate inside the bacteria reducing their proliferation and sometimes even killing them directly.

Therefore, their effectiveness will be higher at the foregut (crop, proventriculus and gizzard) where pH values are lower.

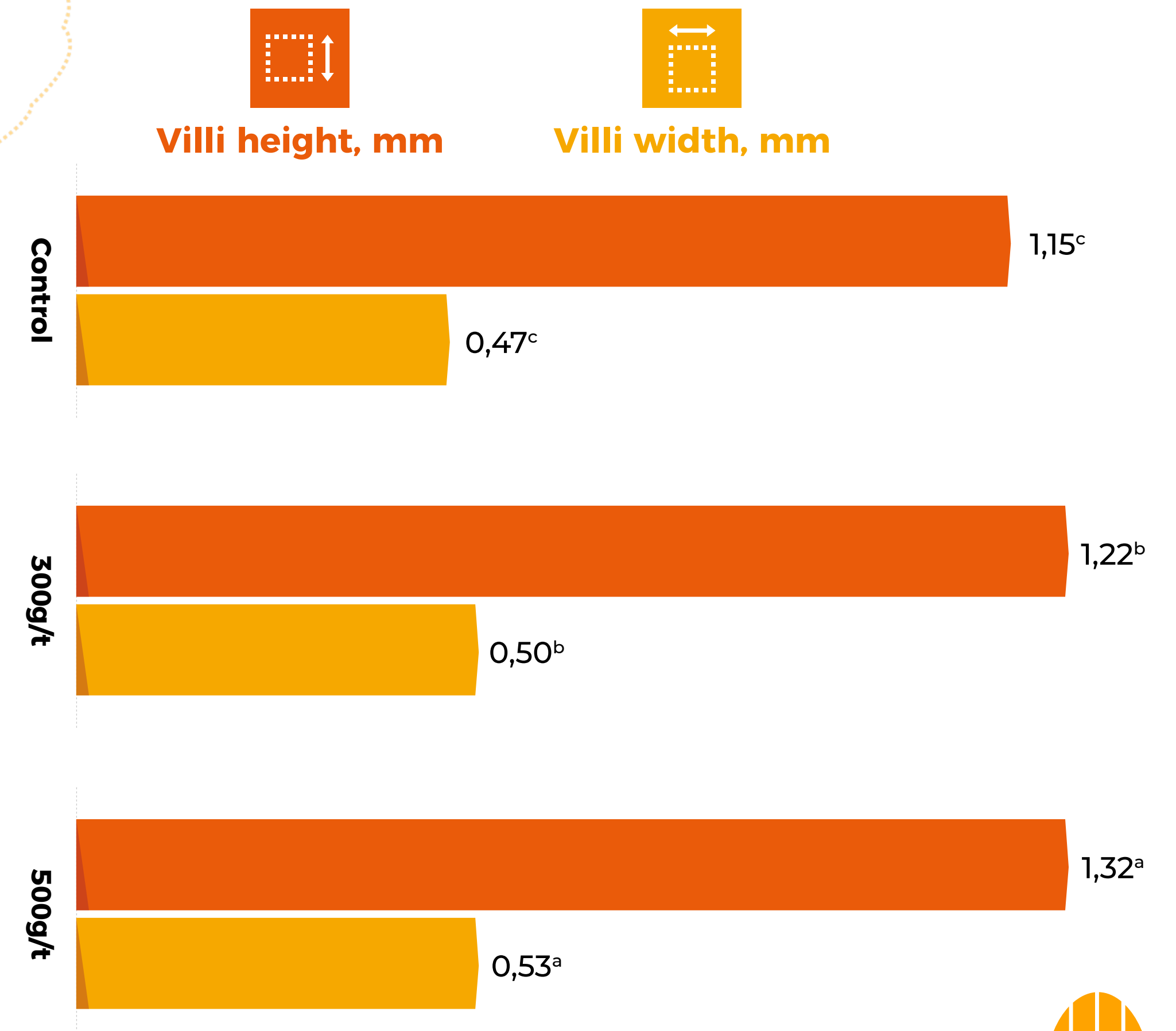
When the desired effect is to reach the distal part of the intestinal tract, we find that organic acids or blends that have been coated work more effectively. **Coating them prevents the realisation of the acid before duodenum (Figure 8) avoiding the proliferation of pathogenic microorganisms.**

Butyric acid/butyrate is one example. Besides being an energy source for colonocytes, it is also a cellular mediator that helps to regulate multiple functions like intestinal tissue development, oxidative stress reduction and immune system modulation.

▼ **Figure 8.** Slow release of coated acid particles along the digestive tract



▼ **Figure 9.** Effect of butyrate on intestinal morphology. Van Vugt Pna et al., 2007.



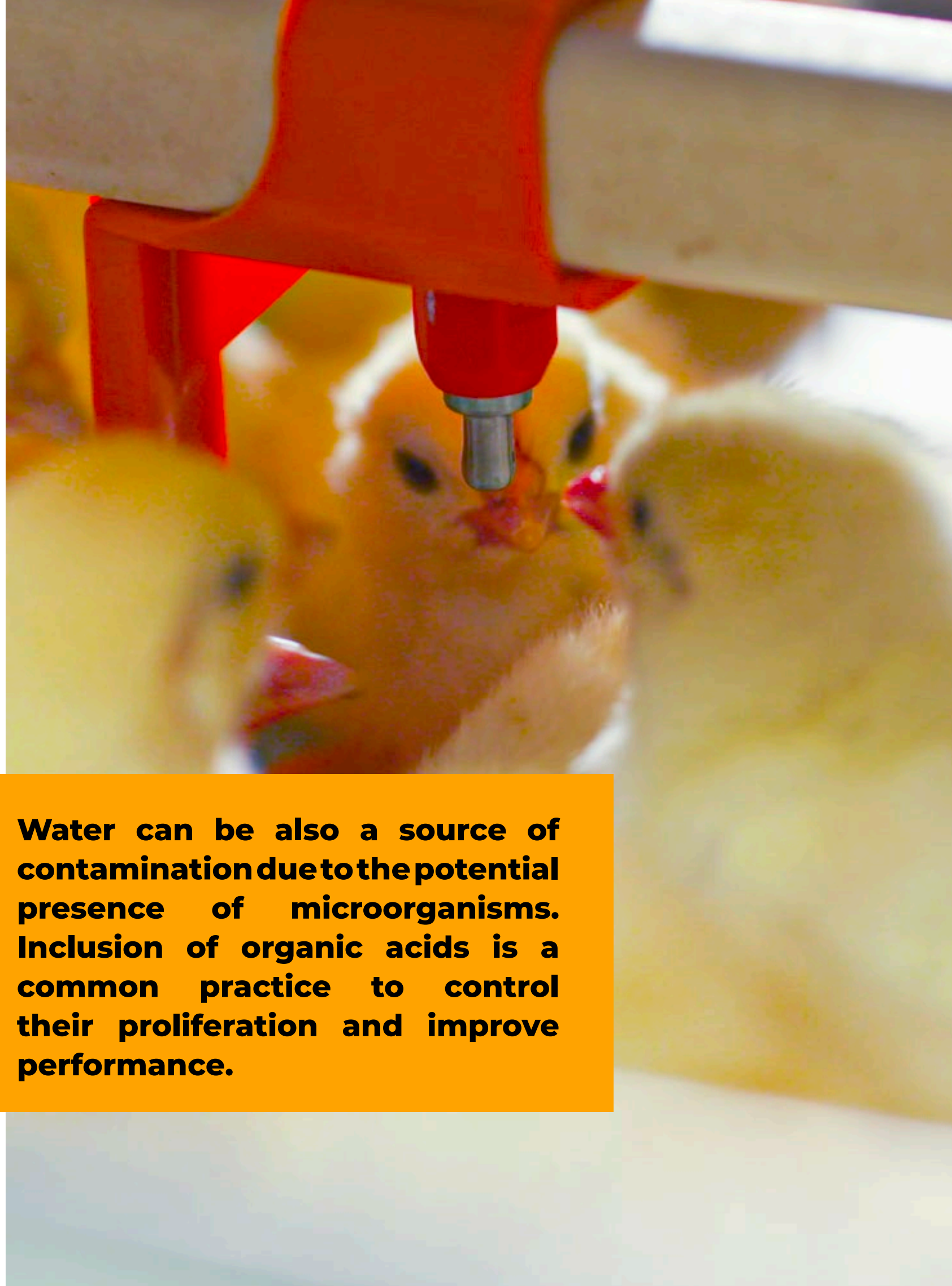


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Water can be also a source of contamination due to the potential presence of microorganisms. Inclusion of organic acids is a common practice to control their proliferation and improve performance.



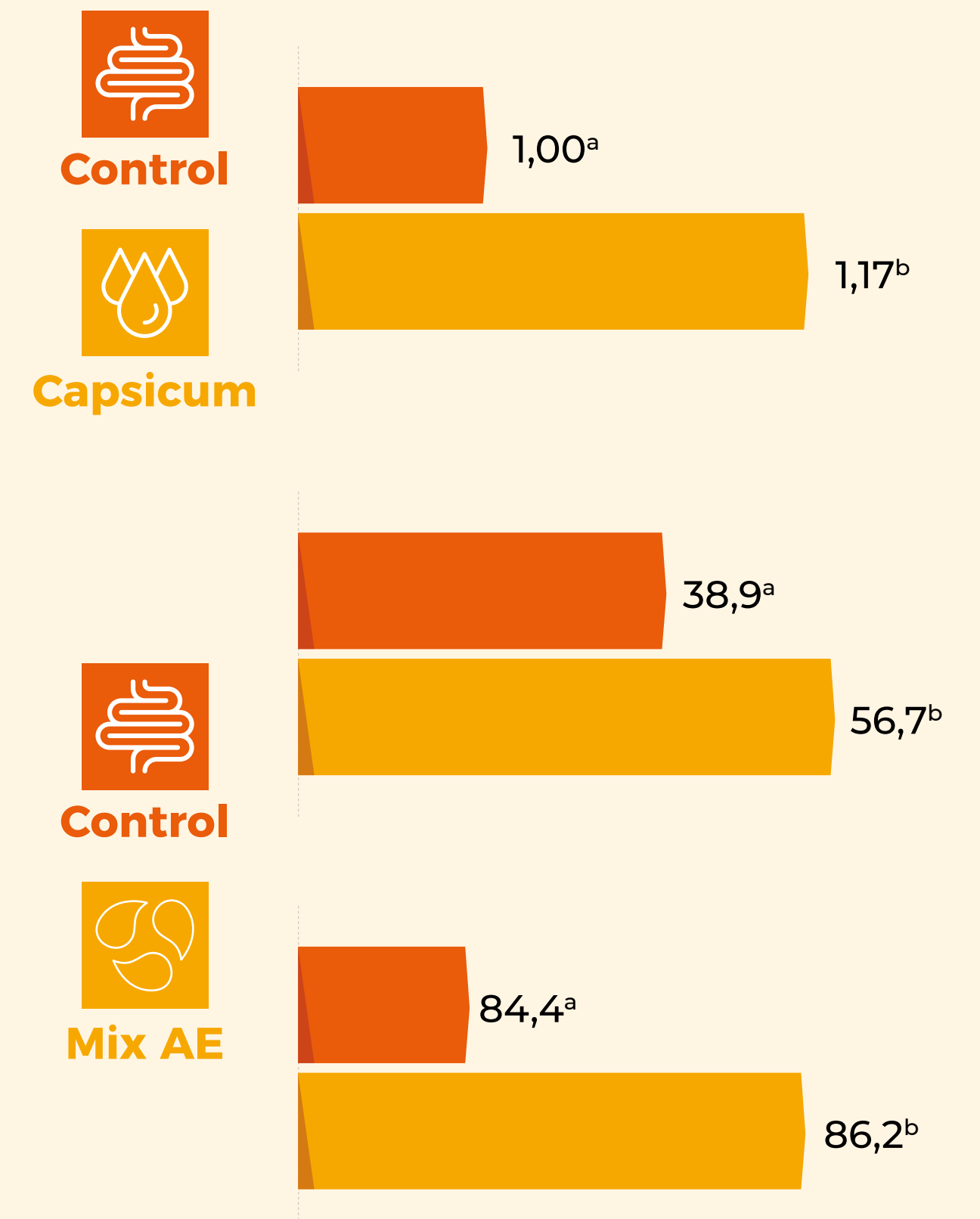
Phytobiotics

Phytobiotics represent natural plant derived compounds **associated with antimicrobial, antifungal, antiparasitic, anti-inflammatory, antioxidant and immunomodulatory properties.**

Essential oils are within this group.

Antioxidant properties of essential oils will influence the immune response however there can be a high variability of results from different essential oils.

▼ **Figure 10.** Effect of different essential oils on nutrient digestibility. Ganesh & Bhat y col., 1984; Jamroz y col., 2005; Bravo, Pirgozliev et al., 2014



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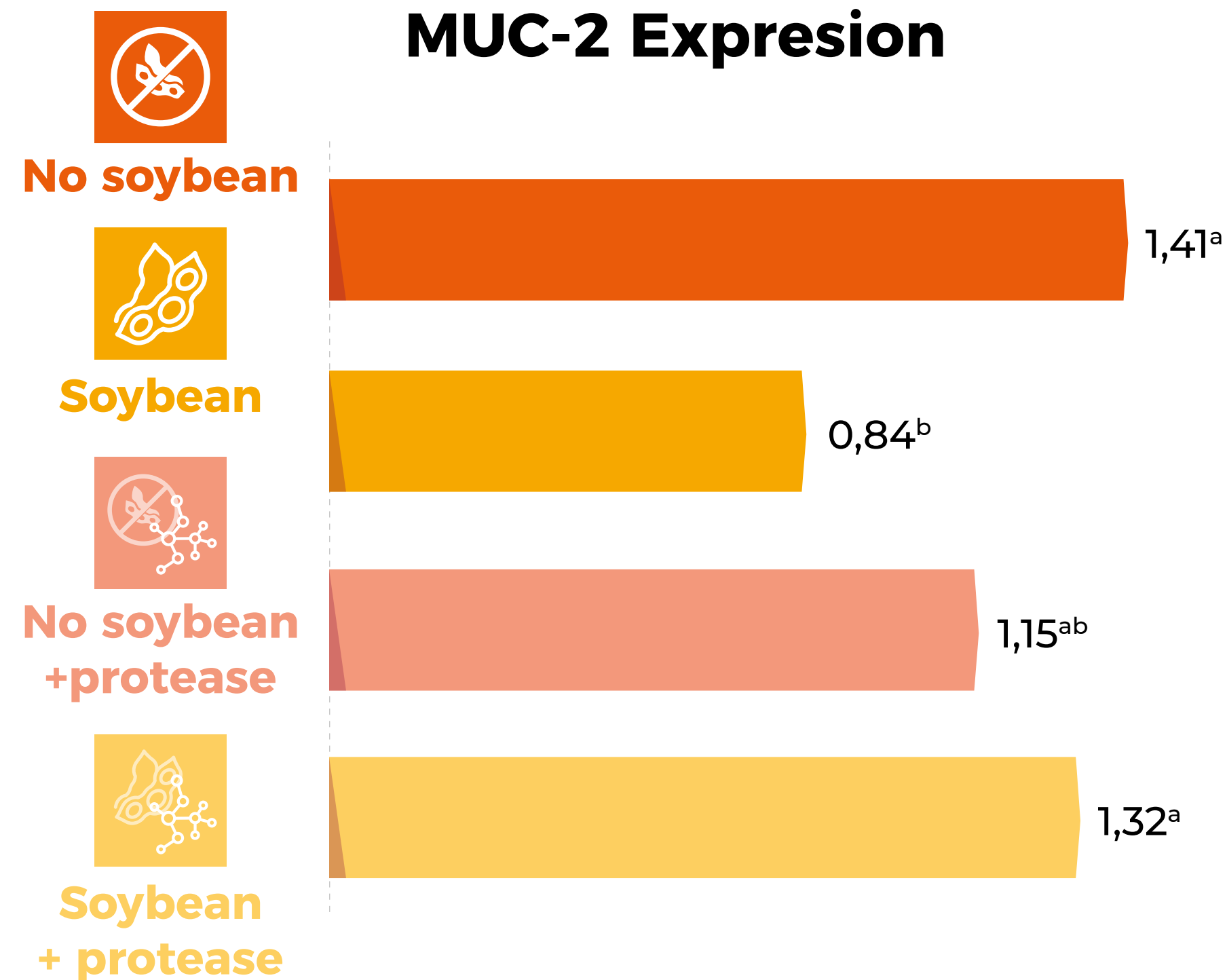
Exogenous enzymes

For many years the inclusion of exogenous enzymes in hen's diets has been standard practice. **Xylanases, β -glucanases, mannanases, lipases, proteases, phytases to name a few, or indeed, combinations of exogenous enzymes are often supplemented dependent on raw material availability and quality in order to reduce the impact of antinutritional factors, such as NSP's, phytic acid or proteases inhibitors.** As they may affect the process of digestion and the absorption of nutrients with the potential of compromising gut health.

The inclusion of Carbohydrase's can have a positive impact on intestinal microflora due to its different mechanism, ultimately reducing the antinutritional effect of NSP's by producing different oligosaccharides with a potential prebiotic effect.

Similarly, the inclusion of proteases reduces the undigestible protein concentration in the intestine that would otherwise trigger fermentations that may affect the microbiota composition.

▼ **Figure 11.** Expression of MUC-2 gen (associated with mucin production that protects the intestinal epithelium against pathogenic bacteria and serves as substrate for commensal ones) in diets in presence or absence of soybean meal with & without addition of protease. Cowieson et al., 2016



In addition, it has also been suggested that they have the capacity to degrade antigenic proteins present in soybean meal and/or degrade antinutritional factors like trypsin inhibitors and lectins (Cowieson et al., 2016).





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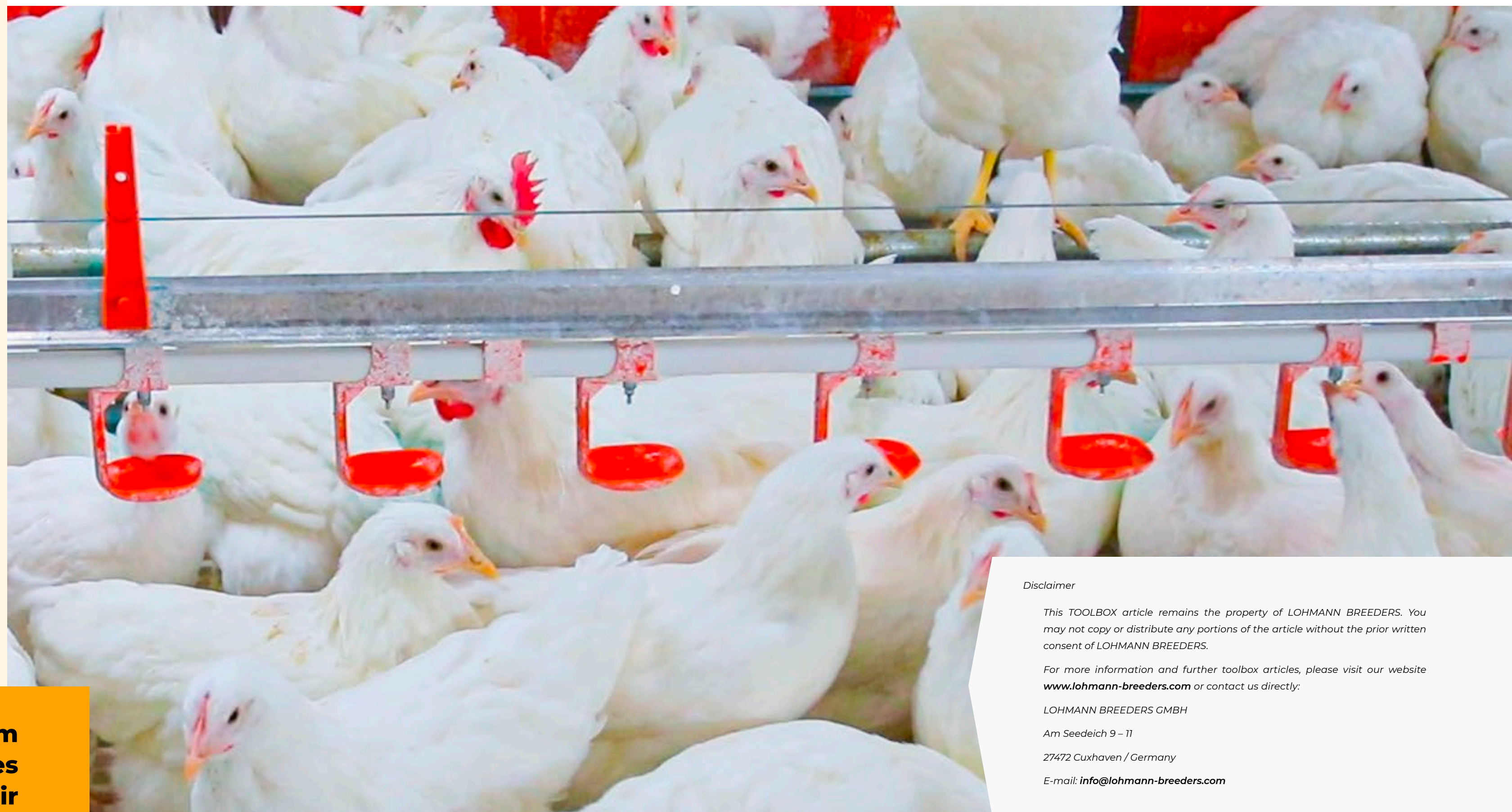
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Summary

The avian intestinal tract is a series of organs that performs two important functions: digestion and supporting the immune system. **The microbiota present at the digestive tract is key to maintaining gut health and, therefore, productivity.**

Factors like management, diet, etc. **influence the presence and proliferation of pathogens** that pose a risk factor that may trigger infections. Any action should be addressed that will help maintain the balance between commensal and pathogenic flora.

Within strategies, prevention will be our best ally. From a nutritional standpoint there are a variety of additives that have proven, through different mechanisms, their potential to support intestinal ecosystem maintenance and immune function.



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