

## ***Lactobacillus acidophilus* D2/CSL (CECT 4529): history, safety, efficacy (1985-2016)**

The history of the strain *L. acidophilus* D2/CSL is similar to that of many strains produced by the CSL. In fact, the CSL's activity regards the search, isolation and selection of strains of lactic acid bacteria (LAB) from their natural habitats (feeds, foods, intestine, etc.). Once the selection phase (*in vitro* and *in vivo*) is completed, the most promising LAB strains for the specific use "in the field" are tested by the pilot laboratory, so they are selected again according to their ability to be successfully replicated in industrial scale. Normally, only one strain of 4 pre-selected ones is also suitable for the industrial production. All strains are still deposited at the CSL's strains collection, so since 1948 the Company boasts a collection of 4000 LAB strains.

The history of *L. acidophilus* D2/CSL starts in 1984-85 (Bianchi Salvadori B. et al., 1985) with the isolation of 19 strains of lactobacilli (of which 6 of *L. acidophilus*) from the gastrointestinal tract of a healthy adult chicken. In the preliminary *in vitro* selection, the strains of *L. acidophilus* seem the most promising ones for the use as probiotic for the poultry husbandry, particularly in light of their quick rate of growth/acidification on "chicken feed" medium and the ability to adhere to the crop epithelium in the presence of bile. Subsequent *in vivo* tests on broilers confirmed the outstanding ability of the species *L. acidophilus* and *L. salivarius* (administered as fresh washed cells) to colonize the gastrointestinal tract of the chicken and to positively modify the balance of the intestinal microbiota (Bianchi Salvadori B. et al., 1985). Finally, the CSL's pilot laboratory tested the different intestinal lactobacilli and selected the strain *L. acidophilus* D2/CSL for its best performance in terms of mass of viable cells (CFU) collected after growth, centrifugation and lyophilization. Since then, the freeze-dried cells of *L. acidophilus* D2/CSL produced on a pilot scale have been subjected to a series of efficacy trials on hens, broilers and turkeys, then marketed.

In fact, *L. acidophilus* D2/CSL has undergone a number of *in vivo* scientific studies, some published, others made public through conferences, or not yet published. Overall, the strain has a substantial wealth of evidence regarding its efficacy, especially on broilers and laying hens, with administration by water or feed (Giardini A. et al., 1993; Giardini A. et al., 1994; Giardini A. et al., 1995; Gatto V., 2007; Gallazzi D. et al., 2008; Marelli S.P. et al., 2008; Cesari V. et al., 2012; Cesari V. et al., 2014; Forte C. et al, 2015; Forte C. et al, 2016; Manfreda G. et al., 2016).

Laying hens fed *L. acidophilus* D2/CSL had an improvement of their "gut health", measured in term of:

- reduction of faecal moisture and faecal stickiness, as well as of toxic putrefaction compounds (NH<sub>3</sub>, H<sub>2</sub>S, etc.);
- improved egg production rate;
- better feed conversion ratio (kg feed/kg eggs);
- better shell quality (specific gravity) and albumen quality (Haugh units);
- reducing waste eggs (thin shelled, cracked, or with faecal stains).

After the EFSA was founded (2002), the CSL began to pursue the road of the European authorization of *L. acidophilus* D2/CSL (CECT 4529) as a feed additive for laying hens in the functional group 4/b, "gut flora stabilizers" pursuant to Regulation (EC) 1831/2003. By definition, these additives "favourably affect animal production, performance or welfare, particularly by affecting the gastro-intestinal flora" (art. 5,f of Reg. 1831/2003).

*L. acidophilus* D2/CSL (CECT 4529) received the first European approval as an additive for laying hens in 2003, the second in 2007, and the third in 2015 (EU Implementing Regulation 2015/38). The product is licensed for the use via feed and water for drinking. The minimum dose is 1.0 \*10<sup>9</sup> CFU/kg feed and 5.0\*10<sup>8</sup> CFU/L water. However, there is not a maximum dose. In fact EFSA, states that *L. acidophilus* have the QPS status and therefore it is absolutely safe for the consumers, the animals, and the environment. This approach requires the identity of the strain to be conclusively established, and evidence that it does not show resistance to antibiotics of human and veterinary importance (EFSA

Journal, 2014, 12, 7: 3789). EFSA stated that “*Lactobacillus acidophilus* D2/CSL has the potential to increase laying intensity and significantly improve the feed-to-egg mass ratio at the proposed dose of  $1 \times 10^9$  CFU/kg feed. The



FEEDAP Panel considers that the results are independent of the mode of administration, provided that exposure is the same and that the equivalent dose in water for drinking would be  $3-5 \times 10^8$  CFU/L”.

Now, after 30 years of *in vitro* and *in vivo* trials, we can declare that the strain *Lactobacillus acidophilus* D2/CSL is:

- \* a natural friend of the chicken: it colonizes the *habitats* of the chicken gut and assists the establishment of the autochthonous microbiota, positively stimulating the GALT;
- \* a natural competitor of many pro-inflammatory, pathogenic and putrefactive bacteria: in these ways it prevents the dysbiosis;
- \* a leader of the useful intestinal fermentations: it improves the digestive functions by fermenting carbohydrates – not the amino acids – and producing lactic acid, which is an additional source of energy for the host.

It is well known that many birds into a reared flock are continually subject to gastro-intestinal dysbiosis. The final outcome will be a true disease (acute inflammation), or more commonly, a sub-clinical disease, with no recognizable clinical findings, but highlighted by reduced animal performance (growth, egg production, feed conversion) and poor quality of the products (dirty eggs, thin shells, etc.). The dysbiosis adversely affects the digestive functions, as well as the vitality of the animals; also, it degrades the quality of the environment within the shelter (more humidity, ammonia, and pathogens of fecal origin, etc.). Finally, it reduces the hygienic quality of meat and eggs (increase of pathogenic bacteria loads). The intestinal microbial balance, or eubiosis, can be restored in a natural way through the administration of selected indigenous (autochthonous) lactobacilli, such as *L. acidophilus* D2/CSL. This is a natural (not GM) and safe strain, so it leaves no toxic residues in the poultry products, and it can be used in the organic farms. The use of *L. acidophilus* D2 / CSL meets the needs of the modern consumer, which is focused on more “natural” foods.

Concluding remarks:

1. *L. acidophilus* D2/CSL is an indigenous strain able to early colonize the chick intestine, creating a favourable *habitat* for the establishment of a more balanced gut microflora;
2. taking into consideration a reared flock, *L. acidophilus* D2/CSL primarily protects the “at risk” subjects (prophylaxis) and the “sub-clinically sick” ones (metaphylaxis): that is why we can see an increase in the overall performance of the flock as well as in the quality of products;
3. from the sanitary point of view, please remember this rule of thumb: when using a safe and protective strain such as *L. acidophilus* D2/CSL, you have to do the opposite of how the “sense of hygiene and health” is suggesting to you, namely: (1) do not fear “to contaminate” the feeds, the drinking water, the animals and the environment, and (2) prefer a higher bacterial load (a more powerful dose) to a lower one!

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