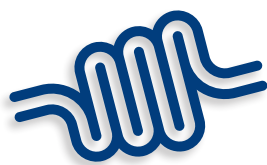


Better performance
Less inflammation & lower medication

Lumance[®]

Intestinal Health & Medication Control



What is the Lumance® Platform ?

1 A product Technology with 4 proven Modes of Action:

1. Reinforces the intestinal integrity
2. Balances the intestinal microbiota
3. Protects against Reactive Oxygen Species (ROS)
4. Reduces gut inflammation



2 A series of well-established *in-vitro* models for Proof of Concept and Mechanistic approaches and *in vivo* biomarkers service assessing:

1. Anti-Inflammatory model:
Monocytic murine macrophage cell line RAW 264.7
2. Bacterial growth inhibition model:
ISO 10932/IDF 223 standard protocol
3. 'Tight-junctions' model:
Porcine gut epithelium cell line IPEC-J2
4. *In vivo* biomarkers:
Acute Phase protein in plasma, IL-6, NF-kB etc.

3 A liquid intervention for practical drinking water application

Powerful at critical periods



4 A Science based offering:

1. Rigorous field & research trials' data across several geographies & species
2. Published technical articles
3. Research-proven true synergistic effect
4. Research-proven heat stability – adequate protection against pelleting & extrusion processes
5. Natural rather than synthetic essential oils inside the Technology with superior anti-inflammatory effect

5 Tailor-made application programs that address specific challenges and needs



Intestinal health is the most determining factor for animal health in general, herd performance and eventually farm profitability.

Pathogenic bacteria can colonise the gastrointestinal tract and result in sub-clinical and clinical disease. Reduced feed intake and daily gain, inactivity and decreased performance are all observed in animals with bacterial infections.

Low quality feed

Livestock producers are often forced to use feed materials (carbohydrates, proteins and oils) of lower quality. This means that animals are constantly challenged not only with changing diet compositions but also with feed quality. Both pose great risks for their health.

Stress

Stress (both biotic and abiotic) often leads to health challenges and intestinal disorders and is linked with the 'leaking gut' syndrome. The end-product is **subclinical inflammation** which can cost up to **30%** of the animal's energy.

Restrictions of antibiotic and ZnO usage

Regulation, food safety and animal welfare are setting up as well new trends towards limitations or restrictions of antibiotic usage for disease treatment, medicated feed, zinc oxide etc.

Lumance® offers a comprehensive **INTESTINAL HEALTH MANAGEMENT** program that achieves growth promotion with concomitant medication reduction by lowering gut inflammation, strengthening the intestinal integrity of the epithelium and regulating beneficially the microbiota of the lumen by protecting against some key pathogens.

Lumance® is a proprietary complex technology which incorporates:

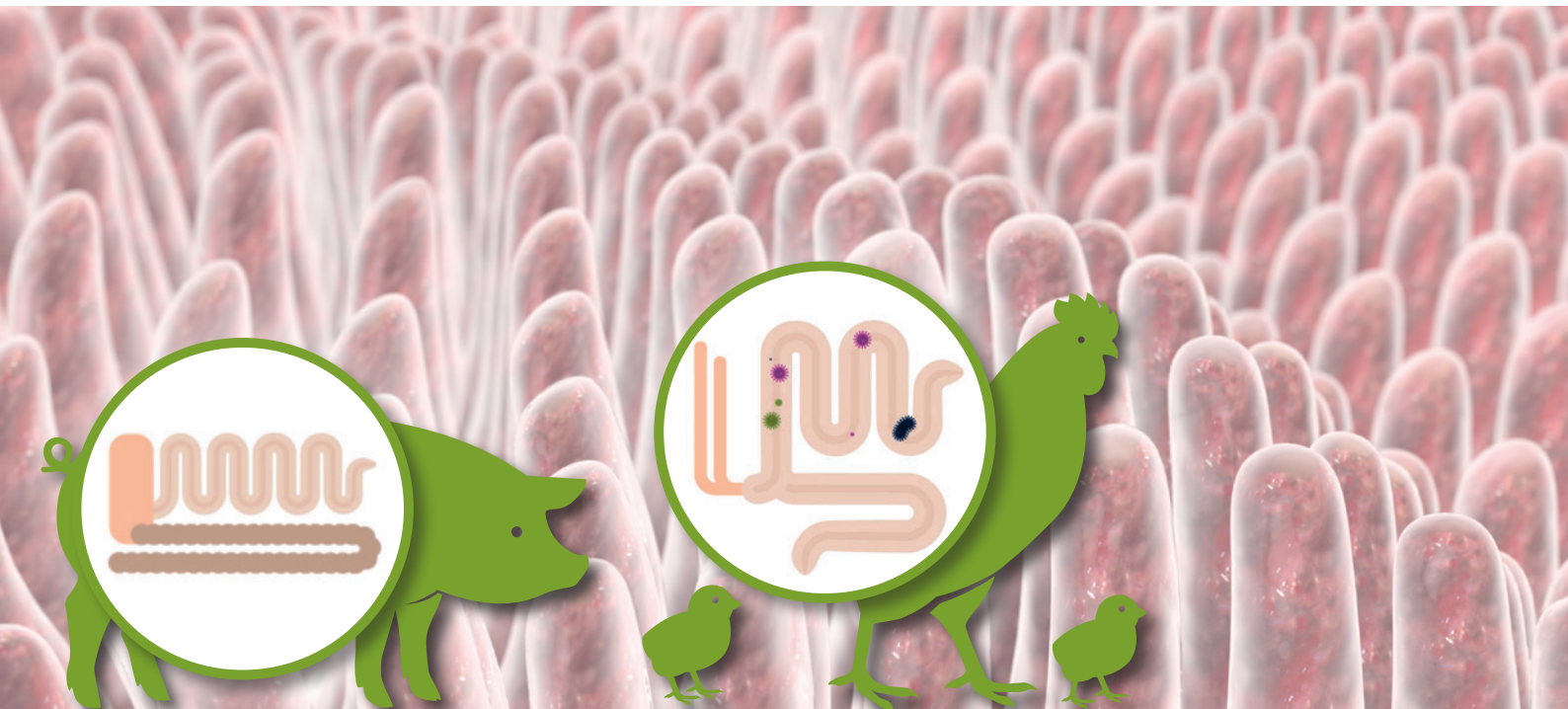
- **The newest generation of butyrate for slow and targeted release throughout the gut**
- **Protection technologies to ensure that:**
 - Short Chain Fatty Acids,
 - Medium Chain Fatty Acids,
 - Essential Oils,
 - Plant Extracts rich in polyphenols

**Are actively delivered in the Gut and,
Can withstand pelleting and extrusion processes**

Lumance®

An effective and powerful Technology to reduce inflammation, promote villi growth, tighten intestinal junctions and regulate beneficially the gut microbiota.

Lumance® - Product features and modes of action:



1. Reinforcing the gut integrity

Slow and targeted release of butyrate throughout the intestinal tract.

Butyrate:

- Provides energy to colonocytes enhancing the gut development. Thus, it stimulates the growth of villi and micro-villi as it is their preferred energy source.
- Enhances the intestinal barrier by facilitating tight junction assembly.
- Reinforces the intestinal defense by:
 - stimulating release of Host Defense Peptides and,
 - inducing antimicrobial activity in intestinal macrophages *in vivo* and increasing resistance to enteropathogens, without triggering tissue damaging inflammation
- Acts as an intestinal cell multi-mediator regulating multiple functions of gut cells and beyond, including gene expression, cell differentiation, gut tissue development, immune modulation, oxidative stress reduction, and diarrhoea control.
- Reduces intestinal inflammation

In brief, butyrate demonstrates positive effects on animal production including: enhancement of gut development, immunomodulation, control of enteric pathogens, reduction of inflammation, improvement of growth performance and improvement of carcass composition and meat quality and, positive modulation of the gut microbiota.

However, Lumance® is not just a source of butyric acid.

Lumance® is a complex proprietary and comprehensive Technology and its additional constituents contribute to an enhanced gut integrity. Research at University of Ghent, Belgium, demonstrated that Lumance® enhanced significantly the gut epithelial tight junctions even under bacterial enterotoxin (LPS) challenge (Fig1).

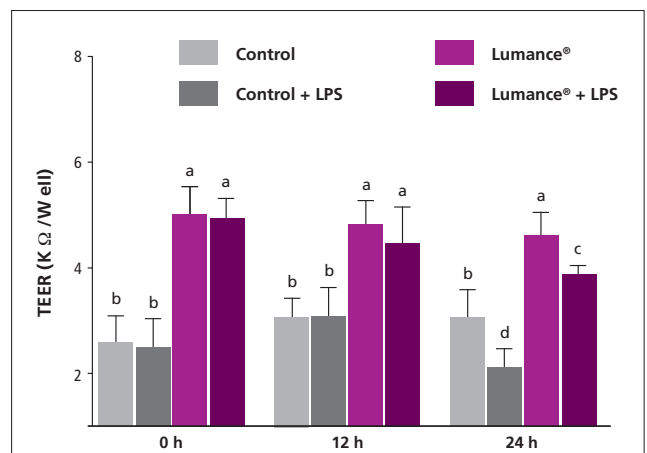


Fig 1. Effects of Lumance® and LPS on intestinal barrier integrity measured by TEER in IPEC-J2 cells. The intestinal cells were incubated for 5 days in case of Lumance prior to challenging with LPS on day 5 post-differentiation; TEER was measured at 0, 12, and 24 h after LPS challenge, respectively. Values are means \pm SD, n = 3. Different superscript letters (a, b, c, d) indicate statistically significant differences of mean values, p < 0.05.

This is of particular importance, taking into account that several stressors including poor diet (high in anti-nutritional factors such as NSP, oil rancidity etc.), pathogens and toxins can increase the gut permeability and lead to chronic inflammation and often disease such as Necrotic Enteritis in poultry. The experimental results suggest that **Lumance®** can potentially improve cases of 'leaky gut syndrome', diarrhoea and wet litter in the field.

2. Proven slow and targeted release of butyric acid in the gut

For any externally supplied form of butyric acid to act, it is imperative that it reaches the hindgut. **Lumance®** has been tested in an advanced *in-vitro* model which mimics the digestion in the gastro-intestinal tract (stomach, duodenum and hind gut). The sophisticated three-step enzymatic incubation did prove that the esterified n-butyric acid within **Lumance® a)** by-passes the stomach with practically no losses, **b)** gets activated only at the duodenum level and, **c)** reaches the hind gut (**Fig. 2**).

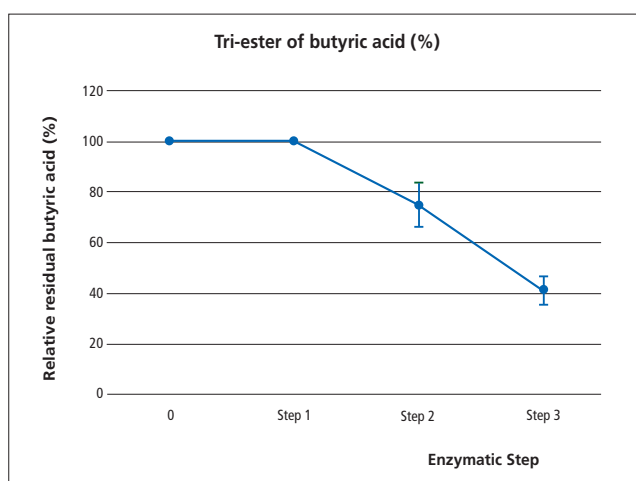


Fig 2. *In vitro* model mimicking digestion in a three-step enzymatic incubation simulating the gastro-intestinal tract (stomach: step 1, duodenum: step 2 and hind gut: step 3). (Method by Boisen & Fernandez, 1997).

3. Reducing gut inflammatory responses

The anti-inflammatory properties of butyric acid are complemented by selected plant extracts rich in alkaloids and essential oils found in the unique **Lumance®** Technology. Their efficacious anti-inflammatory and synergistic effects have been demonstrated both *in vitro* and *in vivo*. Research at University of Ghent, Belgium showed that **Lumance®** exerted *in vitro* significant anti-inflammatory effect in a dose response manner against macrophage cells challenged with bacterial enterotoxin (LPS) (**Fig.3a**). Research at Oklahoma State University, USA demonstrated also that **Lumance®** increased significantly the immune response *in vivo* in chickens by triggering the production of anti-inflammatory Host Defense Peptides (**Fig 3b**).

This is of great importance as recent findings demonstrate that the control of pathogens at subclinical level is only a minor mechanism of Antibiotic Growth Promoters (AGPs) and that reduction of inflammation is indeed the main mode of action behind the growth promoting activity of AGPs.

4. Exerting a synergistic effect in the gut

The data in **Fig. 3c** from the Research conducted at the University of Ghent, Belgium shows that the anti-inflammatory effect of **Lumance®** is superior to any of its single groups of components (fatty acids, plant extract and essential oils). In fact, the anti-inflammatory activity of **Lumance®** was increased by 25% ($p < 0.05$) compared to the theoretical sum of its groups of components (**Fig. 3c**).

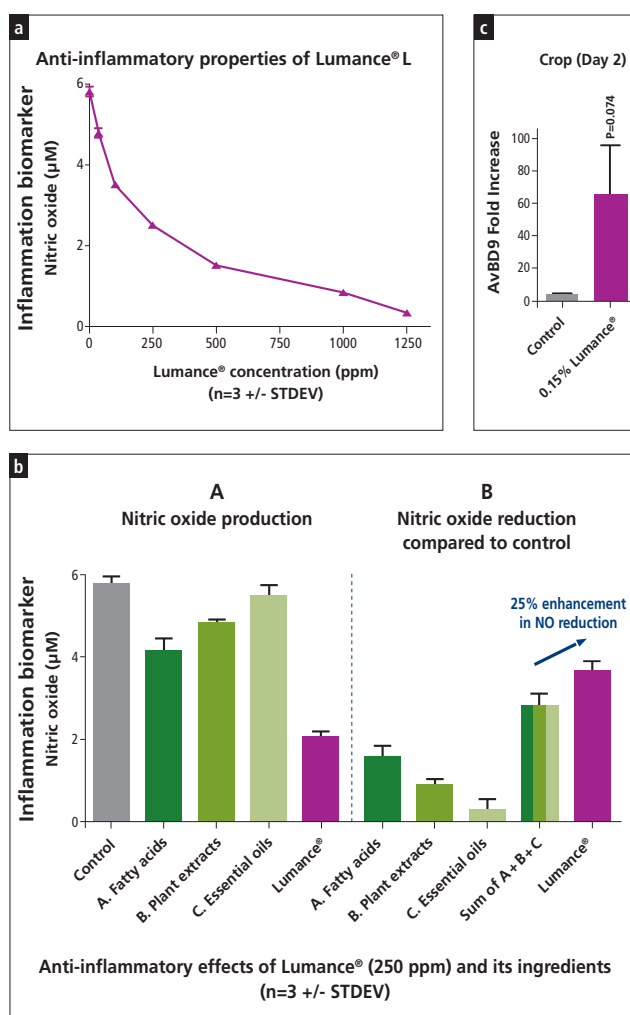


Fig 3. a) *In vitro* inhibition of LPS induced inflammation in macrophage producing cell lines by **Lumance®** expressed as reduction of Nitric Oxide, **b)** *In vivo* demonstration of the anti-inflammatory effect of **Lumance®** (gene expression of the chicken Host Defense Peptide, β -defensin 9 - AvBD9) **c)** Demonstration of the synergistic effect of **Lumance®** in macrophage producing cell lines.

Lumance® can potentially improve cases of 'leaky gut syndrome', diarrhoea and wet litter



Additionally, Lumance® succeeded to counteract, in a dose response manner, the negative effects on Growth Performance in Broiler Chickens (Ross 308) in a recent Feed Challenge Trial in Belgium. More specifically, Lumance®, both at 1 kg/ton and 2 kg/ton, increased significantly the body weight (BW) (3.5% and 6.0%, respectively – $p < 0.05$) and reduced significantly the FCR ($p < 0.05$). The broilers were challenged with a diet in which corn was completely replaced with mash wheat or with mash wheat and rye (starter: 60% wheat; grower and finisher: 60% wheat + 5% rye) without the use of coccidiostats and NSP enzymes (Fig. 4).

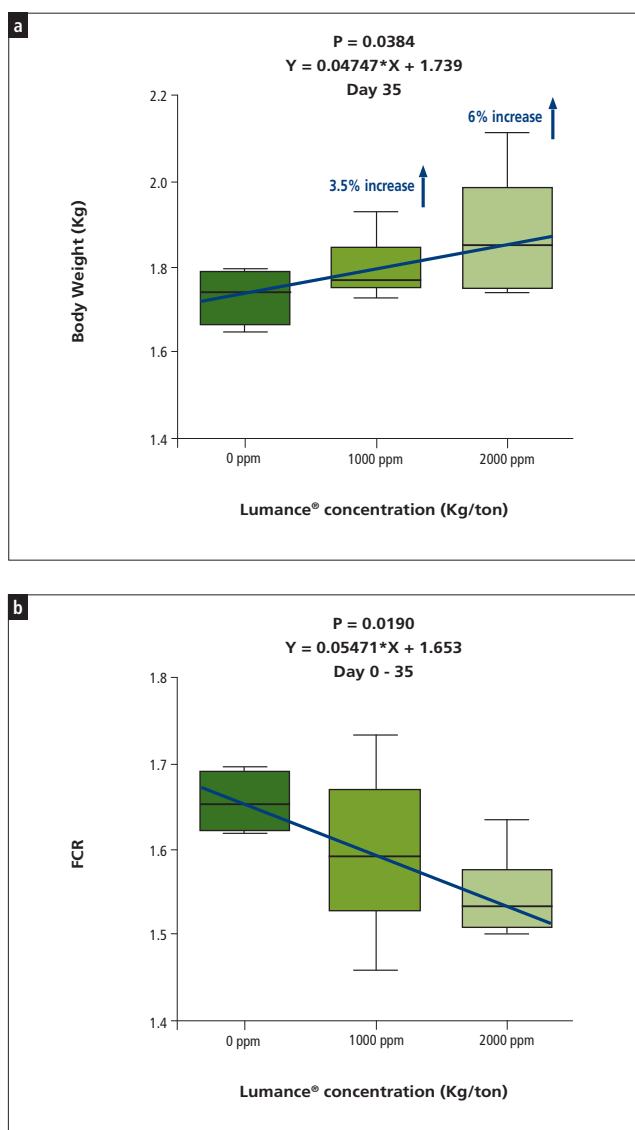


Fig 4. Regression analysis of **a**) body weight (BW) and **b**) feed conversion ratio (FCR) of broiler chickens (Ross 308) fed with a Feed Challenge Diet* containing different doses of Lumance® (mean values ± SD are reported; $n = 6$, 30 birds per treatment).

* Commercial diets: **starter**; 1 to 14d, **grower**; 15 to 28d; and **finisher**; 29 to 35d. All diets were: mash wheat without coccidiostats and without NSP enzymes. **Starter**: 60% wheat; **Grower and Finisher**: 60% wheat + 5% rye.

5. Balancing the lumen and its gastrointestinal microbiota

Lumance® has been carefully designed to reduce the impact of key pathogens and support the growth of beneficial bacteria in the lumen. *In vitro* tests performed at the University of Ghent, Belgium revealed significant antipathogenic activity against key pathogens such as *Salmonella Typhimurium* (SL1344) and *Clostridium perfringens* (CP56) in a dose response manner (Fig. 5).

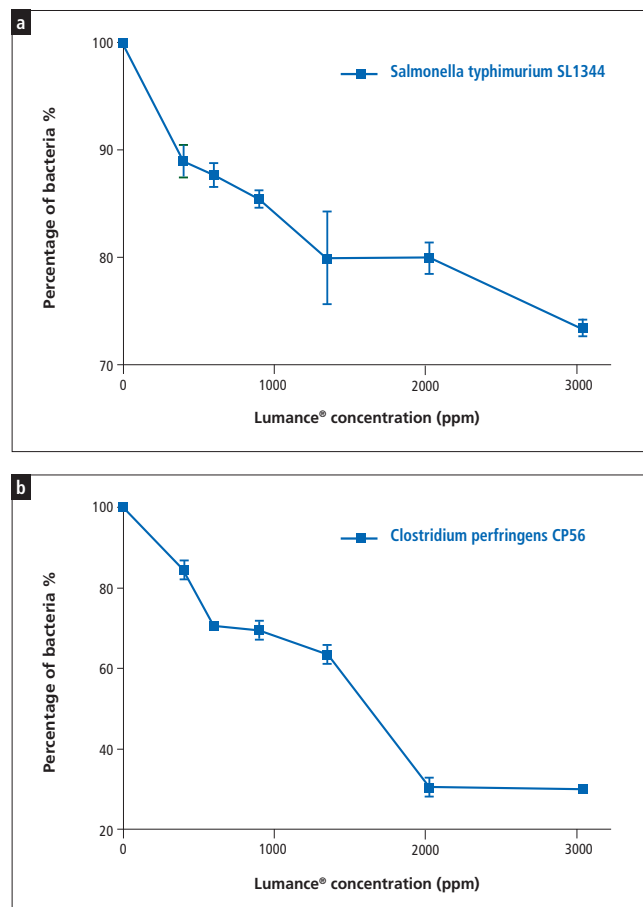



Fig 5. *In vitro* direct antibacterial effect of Lumance® against **a**) *Salmonella Typhimurium* (SL1344) and **b**) *Clostridium perfringens* (CP56) expressed as percentage (%) recovery when compared to the growth in the control treatment. The broth microdilution assay based on the ISO 10932/IDF 223 standard protocol was conducted ($n=3$, +/- STDEV).

6. Protecting against Reactive Oxygen Species (ROS)

Butyrate stimulates endogenous glutathione release which is the most potent antioxidant within the animal's metabolism. Importantly, Lumance® as explained previously, meets a key requirement and the esterified butyrate in its composition, reaches the hindgut.

In addition, the plant extracts and essential oils added in Lumance® have been selected for their antioxidant activity and oxidative stress inhibitory effect, which complement those exerted by butyrate (data not shown).



Lumance® has proved until now the only effective program for simultaneous replacement of both ZnO and antibiotics (neomycin), used traditionally to prevent post-weaning diarrhoea in pigs

Need for Rigorous Field Data

Lumance® has been used successfully in different geographies, farming conditions, animal species (including aqua species), genetics, climates and legislation frameworks in terms of use of AGPs.

To our knowledge, Lumance® has proved until now the **only effective program** for **simultaneous replacement** of both **ZnO and antibiotics (neomycin)**, used traditionally to prevent **post-weaning diarrhoea in pigs**. It is estimated that more than 14 million pigs have been reared successfully (as of December 2019) in real large-scale farming conditions in Spain, whilst improving their performance.



Go Natural or Synthetic?

In the recent years, the feed additives market has been flooded by **myriads of blends** comprising of plant-based (inspired) ingredients; both plant extracts or essential oils. However, their origin, quality and consistency of such materials remain, most of the times, unclear and several marketing terms such as 'natural', 'natural-identical', 'natural-like' are coined.

Innovad® has contracted out its exclusive production of 100% natural Greek oregano (*Origanum vulgare spp. hirtum*) in Greece (Fig 6). This is of great importance as naturally occurring **herbs** found in the wild **unless cultivated** in the original climatic conditions where they have evolved, they **cannot produce** essential oils with the same composition. Moreover, the **natural Greek oregano** exhibited **superior anti-inflammatory activity** when compared against synthetic carvacrol (Fig 7).

The **natural Greek oregano** reduced significantly the inflammation in a gut epithelium cell line challenged with bacterial enterotoxin (41% versus Control), whereas, the **synthetic carvacrol exerted no anti-inflammatory activity** (Fig. 7).



Fig 6. Innovad®'s exclusive production of 100% natural Greek oregano (*Origanum vulgare spp. hirtum*) in Greece.

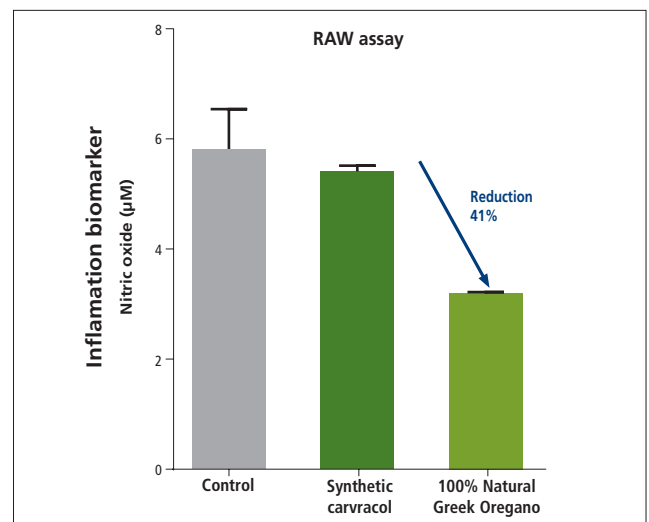


Fig 7. Nitric Oxide (NO) levels in RAW 264.7 cells (10^6 cells/ μ l in 96-well plates) treated with synthetic carvacrol and 100% Natural Greek Oregano cells. Levels of NO were measured by RAW assay after incubation with LPS.

**Chronic Subclinical inflammation due to biotic and abiotic stress
can consume up to 30% of the animal's energy**



Advanced 'Formulation' Properties



Lumance® has undergone rigorous testing after heat treatment at different temperatures and times, with practically no losses during pelleting and extrusion processes for both its essential oils and fatty acids (Table 1).

Table 1. Mean values and standard deviation (n=3) of the percentage (%) recovery of a) the essential oils (EOs) and b) total-esters of butyric acid from three different batches of the Lumance® Technology, after heat treatment at different temperatures and times.

	ESSENTIAL OILS								BUTYRIC ACID	
	EO No1		EO No2		EO No3 (Oregano)		EO No4		Total-esters	
	Mean %	St. Dev.	Mean %	St. Dev.	Mean	St. Dev.	Mean %	St. Dev.	Mean %	St. Dev.
1 min at 90°C	101.0	7.7	93.9	7.3	94.9	6.6	101.1	8.6	99.7	2.26
1 min at 120°C	96.7	2.1	96.7	3.3	92.3	3.1	98.3	2.8	103.3	0.99
5 min at 90°C	97.7	2.0	98.9	2.2	94.9	1.0	99.5	1.5	104.3	2.13
1 min at 120°C	96.7	2.1	96.7	3.3	92.3	3.1	98.3	2.8	103.3	0.99
5 min at 120°C	101.5	6.7	101.6	3.9	99.0	4.9	105.4	7.3	105.8	2.75

What about a 'Liquid Application'?



Liquid intervention of novel feed additives is another important feature, whereby exerting similar modes of action and added to the drinking water for short periods of time without any restrictions, unlike AGPs. Lumance® L is such an intervention, offering practically the same positive effects when most needed i.e. at critical periods during the production.



Dosage and application

- In feed: 0.5 - 2.0 kg/t depending on species, challenge and objective
- Calf milk replacer: min 1 ml/liter of milk
- Via drinking water: min 0.5 ml/liter of water

Lumance® research:

The synergistic effect of Lumance® compared to its single components

SCAN ME

Technical Bulletin

The synergistic effect of Lumance® is superior to any of its single components

Abstract: ...

Introduction

... the synergistic effect of Lumance® compared to its single components ...

Materials and methods

... of ...

Field trials

PIGS



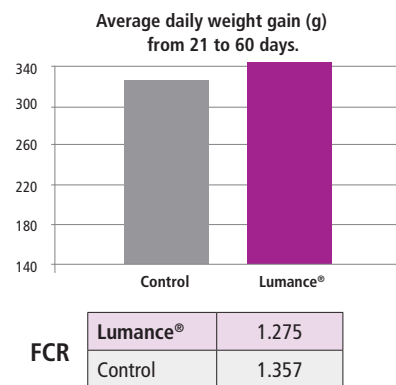
Replacement of antibiotics and ZnO

Commercial farm: Spain

- 40/40 piglets at weaning (21 days),
- Stop of regular neomycin treatment, stop ZnO & Performance improvement

GROUP	TREATMENT (IN FEED)
Control	Prestarter: Neomycin 150 ppm, ZnO 2500 ppm Starter: ZnO 1500 ppm
Lumance®	Lumance® 4 kg/t

Results



No clinical symptoms of post weaning diarrhoea in Lumance® group. Digestive disorder in one box of control group.

Conclusions

Lumance®:

- Replaced completely and successfully antibiotics and ZnO
- Outperformed the Control group when comparing ADWG and FCR



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AVIAN - BROILERS



Replacement of antibiotics

Research trial:

- Oklahoma State University, Department of Animal Science, USA
- 360 male D0 chicks (Cobb 500)
- Trial period: D0 - D21

GROUP	TREATMENT	ADG (g/d)	BW (g)	FCR
Control group	Standard feed (antibiotic free)	31.23	698	1.97
Tylosin group	Standard feed + Tylosin (20 mg/kg)	39.72	876	1.61
Lumance® group	Standard feed + Lumance® (1 kg/t)	41.10	905	1.50

Conclusions

Lumance®:

- Improved significantly the overall performance of broilers in the critical period from D0 to D21
- Proved a successful alternative to antibiotic growth promoters



Replacement of 3 alternatives for non-antibiotic ever program

Field (large scale) trial:

- 7.3 million broilers
- Control of coccidiosis - Amprol in the feed in both programs (control & trial)
- Trial period: D0 - D36 (the age when the birds were sold)

GROUP	TREATMENT	ADG (g/d)	BW (kg)	FCR
Control program	– Ca butyrate – A Probiotic – Natural anticoccidial product	54.5	1.96	1.68
Lumance® program	Starter: 1.5 kg/t Grower : 1 kg/t Finisher : 0.5 kg/t	56.7	2.06	1.69

Conclusions

Lumance®:

- Eliminated the risk of Necrotic Enteritis (NE): No cases of NE reported during the Lumance® program. Cases of NE were reported during the Control program
- Successfully replaced 3 feed additives used as alternatives to antibiotic growth promoters and for the control of NE
- Improved performance and production costs of broilers

