Technical Bulletin

Better performance through a synergistic combined approach in broilers raised without antibiotics.

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Abstract

Societal concern and government regulations related to potential development of antibiotic resistance have increased the need for effective non-antibiotic growth promoters. A combination of several active ingredients acting synergistically with multi-functional activity on pathogenic bacteria, intestinal epithelial layer integrity and inflammation may hold the best promise. The objective of this study was to assess the use of a commercial blend of esterified fatty acid and plant extract as an effective antibiotic replacement in broiler chickens. Using a two-way ANOVA model, 32 pens with 62 Cobb 500 male chicks were split into four groups: control **(T1)**, coccidiostat **(T2)**, antibiotic + coccidiostat **(T3)** and Lumance® **(T4)**. Body weight (BW), feed intake (FI) and FCR ratio were determined on days 7, 14, 21 and 28. On day 22, gut morphology and morphometry parameters were determined. Overall, **T4** and **T3** fed chicks showed a significantly higher BW and lower FCR compared to the control group. No differences were found between **T3** and **T4** groups concerning BW, FI and FCR. Regarding the gut morphology and morphometry parameters, only **T3** and **T4**, but not **T2**, decreased intestinal inflammation. No differences were found between **T2** and **T1** groups for all measured parameters. In conclusion, Lumance®, a unique synergistic blend of feed additives, proved a promising replacement strategy to antibiotics by alleviating at the same time some mild inflammation seen in the Control (T1) group.

Introduction

The growth-promoting effects of antibiotics administered at sub-therapeutic concentrations in animal feed (termed antimicrobial growth promoters, AGPs) were first discovered by Moore et al (1946), who reported the beneficial effects of streptomycin on chicken growth and feed efficiency. Since then, many researchers have studied and documented the growth promoting effects of AGPs in production animals (Castanon, 2007; Dibner & Richards, 2005). Since the early in the discovery of antibiotics the development of resistance was always a concern, particularly in humans consuming animal products that were fed antibiotics (Aarestrup , 2013; Stanton 2013; Carnevale 2001). The decision by the EU to ban the use of AGPs and the knock-on effect in other countries around the world has triggered the need for affective alternatives. Since then a broad range of natural replacements for AGPs have been proposed and tested both in vitro and in vivo, but most of these replacements have generally proved less efficient than AGPs with variable success and lack of consistency under field conditions (Gaggia et al, 2010; Allen et al, 2013). However, withdrawal of AGPs within EU has resulted in animal performance issues, increase in feed conversion, as well as a rise in the incidence of certain animal diseases, such as necrotic enteritis in poultry (Huyghebaert et al., 2011; Papatsiros et al., 2013). Definitions of poultry 'raised without antibiotics' vary around the globe as for example in the USA the use of ionophores to control coccidiosis is permitted.

The emergence of multi-drug resistance and concerns regarding residues in poultry products have led to the search of more effective and safer alternatives to control coccidiosis. Thus, there is high need for alternative solutions to synthetic drugs both for coccidiosis and pathogens control related to intestinal health, with potential immunomodulatory activity. The majority of research evaluating potential non-antibiotic feed additives has mainly focused until now on the response of individual ingredients/ additives and not in combinations of different types of additives.

However, combinations of different ingredients exerting synergistic action may hold the most promising strategy for replacement of antibiotics in animal feed. It has been well established that butyrate (a short chain fatty acid - SCFA), plays a crucial role in the maintenance of gastrointestinal homeostasis and overall health status in production animals (Van Der Wielen et al., 2000; Timbermont, 2009). In addition, several plant extracts and essential oils (EOs) have shown to exert antimicrobial, antioxidant, anti-inflammatory and antiviral properties (Liu et al., 2012; Rathee et al., 2009). For example, polyphenols are considered a major fraction of plant extracts with bioactive properties and their composition and concentration is highly dependent on the plant, environmental conditions, geography, and the method used for harvesting and processing (Burt, 2004).

Medium chain fatty acids (MCFA) have also been reported to exert antibacterial and antiviral properties (Cochrane et al., 2016; Cochrane et al., 2017). Lumance® (Innovad® SA/NV Belgium) is a blend of the newest generation of butyrate, MCFAs, essential oils and polyphenols incorporated into a matrix that protects and ensures slow release of the active ingredients. Khadem et al (2017, 2018 a) demonstrated the anti-inflammatory and synergistic effects of Lumance® both *in-vitro* and *in-vivo* study. The objectives of this study were to investigate the efficacy of the commercial product Lumance® a) on the performance of broilers as a growth promoter and b) on controlling intestinal lesions as a natural anticoccidial agent.

Materials and methods

A total of 1984 male broiler chickens (Cobb 500, Commercial strain) of similar mean body weight, vaccinated for Newcastle disease, were obtained from a commercial hatchery (Biomaster, Campeche, Mexico) at day one of hatch. The chicks were housed in pens with dimensions of 2 × 3 m2 each, supplied with a water belt and a feeder (adapted to the age of the birds), and raised on wood shavings in the experimental poultry facility at the Centre of the Applied Animal Research, Chablekal, Mexico. The monthly mean environmental temperature during the 4-week period of the study was 26°C, whilst the monthly relative humidity and rainfall were 85% and 208 mm, respectively. Temperature was controlled with heaters at 35°C upon arrival of the newborn chicks, followed by a reduction of 0.5°C each day until 26°C was reached at the 4th week of age. The litter from the previous flock was used as natural challenge to the birds. The performance trial was carried out in 32 pens with 62 birds per pen and 8 pens were assigned per treatment.

From D1 onwards, the chicks were fed with the following four treatments:

T1 - Control group: Diet without coccidiostat and antibiotic growth promoters from D1 to D28

T2 - Coccidiostat group: Diet with 125 ppm of Nicarbazine from D1 to D21 and with Salinomycin (66 ppm from D22 to D28) without antibiotic growth promoters.

T3 - (Antibiotic + Coccidiostat) group: Diet with 125 ppm of Nicarbazine and 55 ppm of Bacitracin Methylene disalicylate (BMD) from D1 to D21 and with Salinomycin (66 ppm from D22 to D28).

T4 - Lumance group: 1.5 Kg per ton of Lumance® from D1 to D21 + with 1.0 Kg per ton of Lumance® from day 21 to 28, without and AGPs or coccidiostats.

Body weights and feed intake were recorded on a pen basis at weekly intervals (D7, 14, 21 and 28). Mortality was recorded daily. Feed gain values per unit were corrected for the number of birds that died during the course of the experiment.
At D22, one bird per replicate cage was randomly selected, killed by cervical dislocation, and lesions were scored by an experienced avian veterinarian who was blind to treatment allocations. Each euthanized bird was given a score between 0 and 4 for the intensity of coccidiosis lesions in different segments of the gut based on textbook definitions of coccidiosis related to Eimeria tenella (E. tenella), E. acervulina and E. maxima, according to the following scale: 0 = no lesions; 1 = maximum 5 lesions per cm² (mild lesions); 2 = More than 5 lesions per cm² (moderate lesions); 3 = Coalescence of lesions (severe lesions); 4 = Complete coalescing of lesions (extremely severe lesions). Furthermore, five (5) additional gut morphology/morphometry parameters as indicators of bird health were assessed and scored either as '0' when absent and '1' when present: 1) intestinal thickness/ fragility 2) intestinal inflammation/hyperemia 3) hemorrhage 4) intestinal abnormal contents 5) excessive mucus production.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). The pen was considered the experimental unit for the measurements of BW, FI and FCR. For lesion scores, individual chicks were considered as the experimental unit. Body weight, feed intake and feed conversion ratio were subjected to a two-way ANOVA with repeated measures, applying the following variables as fixed effects: treatment, time (repeated option) and the treatment x time interaction. When treatment or time effects were significant ($P < 0.05$), the means were separated using Tukey's adjustment test.

The model applied was as based on the following formula:

$$Yij=\mu + A\,i + Bj + ABij + eij$$

where μ is the overall mean; Ai is the effect of treatment; Bj is the fixed effect of time; ABij is the interaction of time per treatment; and eij is the random residual error. Chi-squared analysis was used to determine significant differences between treatment groups for intestinal lesion scoring.

Results

The effects of the four dietary treatments on BW, FI and FCR ratio across the duration of the study are summarized in Table 1. Overall, the treatments exhibited a significant impact on body weight and feed conversion ratio (BW, P = 0.011; FCR, P = 0.011) (Table 1), whereas they had no influence on feed intake (P = 0.926). No interaction was seen between time and treatments for BW, FI and FCR (P = 0.052, P = 0.102 and P= 0.961 respectively, Two-way ANOVA).

However, T3 (antibiotic + coccidiostats) and T4 (Lumance®) enhanced significantly the BW of broilers compared to the Control (T1) (P = 0.048, P = 0.046, respectively), whereas no differences were found between T2 (coccidiostat group) and the Control (P = 0.978) (Table1). Overall, T4 (Lumance®) and T3 (antibiotic + coccidiostat) fed chicks exhibited a significantly lower FCR than the control group (P = 0.014 and P = 0.015, respectively), whereas there was no difference between treatment T2 (coccidiostat) and the Control group (P = 0.374, Table1). No significant differences were found between all four treatments regarding the feed intake over the whole period (P > 0.05). Furthermore, no differences were seen between the Lumance® (T4) and T3 (antibiotic + coccidiostat) group regarding the BW, FCR and FI over the whole period (P > 0.05). For the determination of lesion severity 32 chicks were used in total i.e. 8 chickens per treatment. No lesions due to coccidiosis cases were reported in the intestinal contents of all four treatments (data not shown). Additionally, the thickness and fragility of intestine were not affected by the different dietary treatments tested including the Control group (P > 0.05). Moreover, intestinal haemorrhage, abnormal contents and excessive production of mucus were not observed in any treatment (P > 0.05). However, there was a significant difference between the treatments with regards to intestinal inflammation and hyperaemia (P = 0.015) at day 22. More specifically, no intestinal inflammation and hyperaemia were observed for the Lumance® group (T4) and treatment T3 (antibiotic + coccidiostat) followed by whereas, the Control and T2 (coccidiostat) groups demonstrated moderate levels of abundance of intestinal inflammation and hyperemia (3 out of 8 cases, Table 2). The lack of instestinal inflammation and hyperemia in Lumance® (T4) and T3 (antibiotic + coccidiostat) groups was in good agreement with their improved performance when compared to the control at D21 in terms of BW (1.072 ± 0.009 and 1.074 ± 0.009 vs. 1.012 ± 0.016, respectively) and FCR (1.244 ± 0.008 and 1.234 ± 0.011 vs. 1.299 ± 0.016 respectively) ***(Figure 1)***.

Figure 1. Effect of dietary treatments on **A)** BW, **B)** FCR and **C)** FI of broilers at day 21. Mean values with SEM are shown (n = 8 replicates per treatment. Each replicate (pen) consisted of 62 birds).

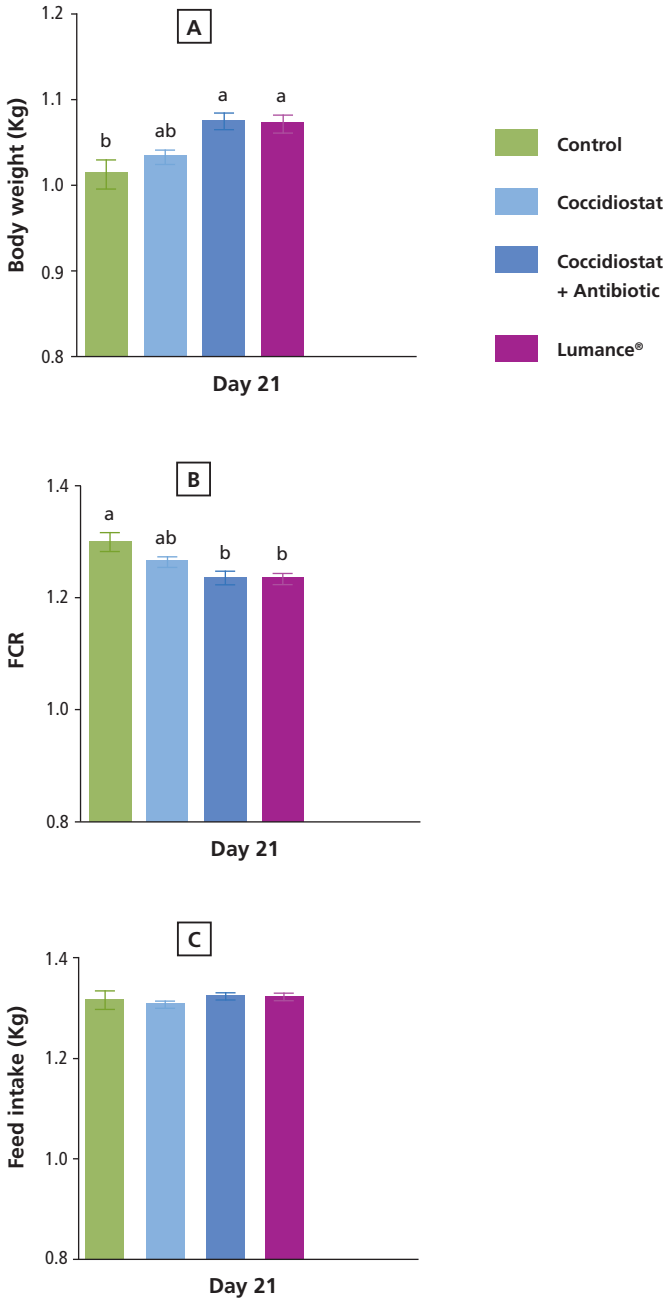

Table 1. Effect of dietary treatments on BW, FCR and FI of broilers (with Two-way ANOVA Test).
Mean values with SEM are shown (n = 8 replicates per treatment. Each replicate (pen) consisted of 62 birds).

Treatment	Time	BW	FCR	FI
Control	d 7	0.169 ± 0.002	0.946 ± 0.009	0.161 ± 0.002
	d 14	0.493± 0.006	1.210 ± 0.020	0.576 ± 0.002
	d 21	1.012 ± 0.016	1.299 ± 0.016	1.316 ± 0.017
	d 28	1.668 ± 0.014	1.399 ± 0.017	2.342 ± 0.026
Coccidiostat	d 7	0.168 ± 0.001	0.947 ± 0.012	0.159 ± 0.002
	d 14	0.493 ± 0.006	1.171 ± 0.015	0.574 ± 0.002
	d 21	1.033 ± 0.007	1.263 ± 0.008	1.307 ± 0.006
	d 28	1.663 ± 0.013	1.398 ± 0.009	2.333 ± 0.009
Antibiotic + Coccidiostat	d 7	0.171 ± 0.002	0.936 ± 0.011	0.161 ± 0.002
	d 14	0.504 ± 0.008	1.162 ± 0.013	0.575 ± 0.002
	d 21	1.074 ± 0.009	1.234 ± 0.011	1.323 ± 0.006
	d 28	1.687 ± 0.012	1.384 ± 0.009	2.336 ± 0.006
Lumance®	d 7	0.172 ± 0.002	0.940 ± 0.005	0.162 ± 0.001
	d 14	0.510 ±0.006	1.150 ± 0.012	0.577 ± 0.001
	d 21	1.072 ± 0.009	1.234 ± 0.008	1.322 ± 0.006
	d 28	1.683 ± 0.019	1.382 ± 0.012	2.332 ± 0.011

Source of Variation	BW (P-value)	FCR (P-value)	FI (P-value)
Treatments	0.011*	0.011*	0.926
Time	< 0.001*	< 0.001*	< 0.001*
Interaction (Time x Treatment)	0.0519	0.102	0.961
Tukey's multiple comparisons			
Control **vs.** Coccidiostat	0.978	0.374	0.946
Control **vs.** Antibiotic + Coccidiostat	0.048*	0.025*	> 0.999
Control **vs.** Lumance®	0.046*	0.014*	> 0.999
Coccidiostat **vs.** Antibiotic + Coccidiostat	0.111	0.521	0.935
Coccidiostat **vs.** Lumance®	0.108	0.385	0.954
Antibiotic + Coccidiostat **vs.** Lumance®	>0.999	0.995	0.999

* Significant differences (P<0.05)

Table 2. The effect of dietary treatments at day 22 on intestinal five gut morphology/morphometry parameters as indicators of bird health. One bird per replicate cage (n =8 per treatment) was randomly selected, euthanized and scored by an experienced avian veterinarian who was blind to the treatment allocations. The differences were compared with the use of the Chi square test.

	Control		Coccidiostat		Antibiotic + Coccidiostat		Lumance®		
	absence	presence	absence	presence	absence	presence	absence	presence	P-value
Gut morphology/morphometry parameter									
Thickness/ fragility	7	1	8	0	8	0	8	0	0.172
Inflammation/hyperemia	5	3	5	3	8	0	8	0	0.015*
Hemorrhage	8	0	8	0	8	0	8	0	1.000
Abnormal contents	8	0	8	0	8	0	8	0	1.000
Excessive mucus production	8	0	8	0	8	0	8	0	1.000

* Significant differences (P<0.05)

Discussion and conclusions

Despite the addition of bird litter from the previous flock the experimental set up in this study failed to produce significant challenges to the birds as evidenced by the absence of lesions in all treatments including the Control group.

This could be attributed to

a) the 'cleaner' environmental conditions present in the experimental facility,

b) the more easily controlled and thorough cleaning and disinfection procedures,

c) the lower density stocks and

d) the high quality of the standard diet across all treatments when compared to real farming conditions. As such, no signs of coccidiosis were manifested in this trial and the investigation of the effect of the interventions was not feasible.

Interestingly though, the present investigation revealed that the dietary supplementation of Lumance®, a synergistic approach of the latest generation of feed additives, ensured top performance, achieved optimum feed conversion ratio whilst exhibited no signs of intestinal inflammation and hyperaemia when compared to the one of the medication treatments (T2, a single coccidiostat -Nicarbazine- throughout the study) and the Control group.

In fact, Lumance® succeeded in replacing fully a combined coccidiostats and antibiotic growth promoter intervention (Nicarbazine + BMD from D1 to D21 followed by Salinomycin from D22 to D28) without compromising anything in terms of broiler perfomance.

The findings here are in agreement with a large scale *in-vivo* field trial where Lumance® successfully replaced three different commercial non-antibiotic feed additives that were combined to maintain performance and control necrotic enteritis (NE) in the absence of antibiotic growth promoters, offering thus significant economic benefits (Khadem et al., 2018b). However, further studies are required to determine the direct anti-coccidial activities of Lumance® in broilers.

In conclusion, (Lumance®) – a unique synergistic blend of feed additives demonstrated an ability for enhanced performance and use in starter, grower and finisher diets in broilers by eliminating any signs of inflammation and hyperemia. At the same time Lumance® demonstrated a fully effective replacement application to routine medication alternatives, proving thus a long term environmentally sustainable approach with no risks to antibiotic resistance and its detrimental consequences.

References:

Aarestrup, F.M. (2000) Characterization of glycopeptide-resistant Enterococcus faecium (GRE) from broilers and pigs in Denmark: genetic evidence that persistence of GRE in pig herds is associated with coselection by resistance to macrolides. J. Clin. Microbiol. 38, 2774–2777.

Allen, H. K., Levine, U. Y., Looft, T., Bandrick, M., & Casey, T. A. (2013). Treatment, promotion, commotion: Antibiotic alternatives in food-producing animals. Trends in Microbiology, 21(3), 114–119. https://doi.org/10.1016/j.tim.2012.11.001.

Burt, S. Essential oils: their antibacterial properties and potential applications in foods: a review. 2004. Int. J. Food. Microbiol. 94:223-253.

Carnevale, R. 2001. Antibiotic resistance and food producing animals—Views of the industry. http://www.ahi.org/ Accessed: July 2002.

Castanon, J. I. R. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. Poultry Science, 86, 2466–2471. https://doi.org/10.3382/ps.2007-00249

Cochrane, R.A. M. Saensukjaroenphon, S.S. Dritz, J.C. Woodworth, A.R. Huss, C.R. Stark, J.M. DeRouchey, M.D. Tokach, R.D. Goodband, J.F. Bai, Q. Chen, J. Zhang, P.C. Gauger, R. Main, and C.K. Jones. 2016. Evaluating the inclusion level of medium chain fatty acids to reduce the risk of PEDV in feed and spray-dried animal plasma. J. Anim. Sci. 94(Suppl. 2): 50.doi:10.2526/msasas2016-107.

Cochrane, R.A., S.S. Dritz, J.C. Woodworth, A.R. Huss, C.R. Stark, M. Saensukjaroephon, J.M. DeRouchey, M.D. Tokach, R.D. Goodbankd, J.F. Bai, Q. Chen, J. Zhang, P.C. Gauger, R.J. Derscheid, R.G. Main, and C.K. Jones. 2017. Assessing the effects of medium chain fatty acids and fat sources on PEDV RNA stability and infectivity. J. Anim. Sci. 95(Suppl. 2):196 (Abstr.).

Dibner, J. J., & Richards, J. D. (2005). Antibiotic growth promoters in agr culture: history and mode of action. Poultry Science, 84, 634–643. https://doi.org/10.1093/ps/84.4.634.

Gaggìa, F., Mattarelli, P., & Biavati, B. (2010). Probiotics and prebiotics in animal feeding for safe food production. International Journal of Food Microbiology, 141(SUPPL.), S15–S28. https://doi.org/10.1016/j.ijfoodmicro.2010.02.031.

Huyghebaert, G., Ducatelle, R., & Immerseel, F. Van. (2011). An update on alternatives to antimicrobial growth promoters for broilers. Veterinary Journal, 187, 182–188. https://doi.org/10.1016/j.tvjl.2010.03.003.

Khadem A. Al-Saif J., Letor B, Bauwens S., Sevastiyanova M, Combes F, Zhang G, Sanders N. 2017. A commercial mixture of plant extracts and fatty acids modulates immune responses, through inhibition of nitric oxide production, and induction of host defense peptide synthesis in an in vitro and in vivo model, respectively. PSA Annual meeting USA.

Khadem A. Al-Saifi J., Letor B, Bauwens S., Sevastiyanova M, Al-Saifi M, Sanders N. 2018 b. The synergistic effect of Lumance® is superior to any of its single components. Poultry World Magazine.

Khadem A. Al-Saifi J., Letor B, Bauwens S., Sevastiyanova M, Al-Saifi M, Van Belle J, Gougoulias C. 2018 b. Prevention of necrotic enteritis by a synergistic non-antibiotic feed additive in broiler chickens. Gut health Proagrica Magazine December.

Liu, Y., M. Song, T.M. Che, D. Bravo, and J.E. Pettigrew. 2012. Anti-inflammatory effects of several plant extracts on porcine alveolar macrophages in vitro. J. Anim. Sci. 90:2774-2783.

Papatsiros, VG, Katsoulos P.D, Koutoulis, KC, Karatzia, M, Dedousi, A & Christodoulopoulos, G. (2013). Alternatives to antibiotics for farm animals. CAB Reviews Perspectives in Agriculture Veterinary Science Nutrition and Natural Resources. 8. https://doi.org/10.1079/PAVSNNR20138032.

Rathee, P., Chaudhary, H., Rathee, S., Rathee, D., Kumar, V., & Kohli, K. (2009). Mechanism of action of flavonoids as anti-inflammatory agents: a review. Inflammation & Allergy Drug Targets, 8, 229–235. https://doi.org/10.2174/187152809788681029.

Stanton, T. (2013) A call for antibiotic alternatives research. Trends Microbiol. 21, 111–113.

Timbermont, L., 2009. A contribution to the pathogenesis and treatment of Clostridium perfringens associated necrotic enteritis in broilers. PhD thesis, Faculty of Veterinary Medicine, Ghent University.

Van Der Wielen, P.W., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B.A., Van Knapen, F., 2000. Role of volatile fatty acids in development of the cecal microflora in broiler chickens during growth. Applied and Environmental Microbiology 71, 2206–2207.

Van Immerseel, F., De Buck, J., Pasmans, F., Huyghebaert, G., Haesebrouck, F., & Ducatelle, R. (2004). Clostridium perfringens in poultry: an emerging threat for animal and public health. Avian Pathology: Journal of the W.V.P.A, 33 (September 2014), 537–549. https://doi.org/10.1080/03079450400013162.