FRA® BUTYRIN HYBRID DRY TRIAL

Stimulate intestinal health and effective pathogen control

TRIAL REPORT

- Intestinal development
- Egg quality & Shell strength
- Feed efficiency

- Brazil, 2019
- Trial code: CL_Fra_160012a
- Additional info: R&D@framelco.com

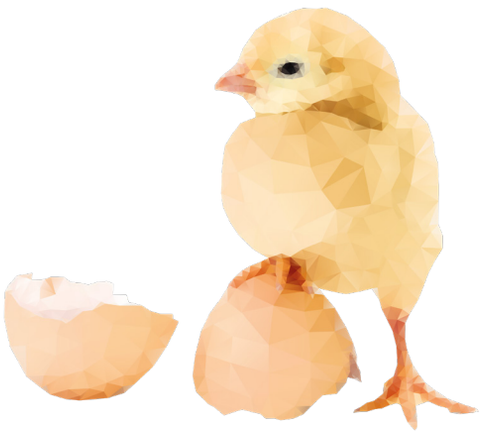
FRA® MELCO

1 INTRODUCTION

The main concern in egg production is a decrease in eggshell quality as laying hens age. With 96%, the avian eggshell's main component is calcium carbonate. Eggshells are built up from nanoparticles of calcium carbonate arranged into ordered crystals by proteins, forming a calcite shell. During aging, egg size and weight increases, without increasing the calcium carbonate deposition in the eggshell. Consequently, shells become thinner and the risk of broken eggs is increased.

Egg quality
Shell strength
Ileum development
FCR

Dietary calcium is essential for eggshell synthesis. Calcium can be absorbed from the diet via the intestinal cells of the bird. However, during aging gut inflammation is more common and intestinal epithelial cells of layers become weaker and the villi shorten, which results in impaired nutrient absorption, eggshell abnormalities and reduced shell strength. Therefore, a well-developed healthy gut without inflammation is essential. A reduction in eggshell quality is not only a concern in older laying hens, but it is a problem during the entire production cycle. Feed-related problems, diseases and bad management practices can result in a decreased laying rate and in eggs which are abnormal in shape and colour. This results in lower profit for the farmer. A feed additive could be a tool to improve laying hen performance and egg quality.



FRA®melco B.V. is specialized in the production of glycerides from glycerol and fatty acids like butyric acid. Butyrate glycerides come in multiple forms of which tributyrin and alpha-monobutyrin are the most potent. Tributyrin contains 3 molecules of butyric acid and is therefore concentrated in butyric acid. Tributyrin is known for its anti-inflammatory, anti-oxidative, gut developing and microbiota improving properties. Alpha-monobutyrin is obtained by esterifying butyric acid to the first position of a glycerol backbone and is known for its anti-microbial effects against different pathogenic bacteria. FRA® Butyrin Hybrid Dry combines the positive effects of tributyrin and alpha-monobutyrin in one product. It is expected that the combination of tributyrin and alpha-monobutyrin improves gut development and the health status of the animal. The aim of this trial was to study the effect of FRA® Butyrin Hybrid Dry on general performance and egg quality of laying hens aging from 51 to 70 weeks.

2 MATERIALS & METHODS 1/2

This trial was conducted at the University of São Paulo (USP) located in Brazil. Hundred-and-eighty Hisex White laying hens of 48 weeks of age were purchased and randomly assigned to the control and treatment group. Ten layers of each group were culled upon arrival at the trial facility to determine the histological state of the ileum, indicated by villi height (VH), crypt depth (CD) and villi height to crypt depth (VH:CD) ratio. The remaining 160 laying hens were housed in cages in a masonry shed with open sides with screens and were equipped with fans for temperature control. The hens were evenly distributed over the control and treatment group, with 10 repetitions of 8 layers each.

The first 3 weeks of the trial (48-50 weeks of age) served as adaptation period and all layers received the same basal diet based on corn and soybean meal (Appendix I). After the adaptation period, the treatment group received the experimental diet which consisted of the basal diet where 2 kg of sand per ton feed was replaced by 2 kg FRA® Butyrin Hybrid Dry per ton of feed. Hens were offered water and feed *ad libitum*. A lighting program of 16 hours of light was applied. The trial lasted for 20 weeks (51-70 weeks of age), divided over 5 cycles of 28 days. Performance parameters were evaluated per replicate at the end of each cycle. The following parameters were determined: mortality, laying rate, average daily feed intake, egg mass, feed conversion ratio per dozen eggs and feed conversion ratio per kg egg mass.

Laying rate (%)	=	(Daily egg production / number of hens) * 100
Average daily feed intake (g/hen/day)	=	Feed intake / number of hens / 28 days
Average egg mass (g/hen/day)	=	Average egg weight * laying rate (%)
FCR/dozen eggs	=	(Av. daily feed intake (kg) / laying rate (%)) x 12
FCR/g of egg mass	=	Av. daily feed intake (g/hen/day) / egg mass (g/hen/day)

2 MATERIALS & METHODS 2/2

In order to evaluate internal and external egg quality parameters two eggs per repetition were collected on day 27 and day 28 of each cycle, making a total of 40 eggs for each group per cycle. Eggs were individually analysed in a Digital Egg Tester (DET6000, Nabel, Japan) for egg weight, eggshell strength, albumen (egg white) height, yolk colour and Haugh units. The Haugh unit is a parameter to measure the internal quality of an egg and is an indication of freshness of the egg, but does not provide other information such as micronutrient or vitamins concentrations in the egg. The higher the number, the better the quality of the egg. At the end of the trial one hen of each repetition was killed to evaluate gut morphology of the ileum.

All statistical analyses were performed using the Statistical Analysis System (SAS Institute, 2012), and results were considered statistically significant at 95% probability level ($P < 0.05$).

3 RESULTS 1/2

The intestinal epithelium is organized into villi-crypt units to maximize the surface area for nutrient absorption. Stem cells in the crypt of the intestinal epithelium renew itself every few days. Cells originating from the crypt move upwards and form the villi. A decrease in crypt depth indicates a faster renewal of the epithelium. A higher VH:CD ratio indicates a larger surface for nutrient absorption which generally induces a positive effect on laying hen performance and egg quality.

The effect of FRA® Butyrin Hybrid Dry on ileum morphology is presented in Table 1. At the start of the adaptation period no differences were observed in villi height, crypt depth and VH:CD ratio, proving all layers had the same initial histological state of the ileum. After 20 weeks of supplementing FRA® Butyrin Hybrid Dry, villi height was numerically increased by 11.3% in the ileum. Moreover, crypt depth was significantly decreased (P=0.010) and in combination with longer villi, a significantly improved VH:CD ratio was obtained (P=0.024). In literature also higher villi are observed in the upper part of the GIT when tributyrin is administrated to broilers. Although in this trial only the effect on the ileum was studied, it is assumed that FRA® Butyrin Hybrid Dry also has a positive effect on gut morphology of the duodenum and jejunum, resulting in overall improved gut development and consequently better animal performance.

T1 **The effect of FRA® Butyrin Hybrid Dry on intestinal morphology of the ileum**
Table 1

		Control	FRA® Butyrin Hybrid Dry	Difference
Week 48	Villi height (µm)	510	512	+0.4 %
	Crypt depth (µm)	83.54	84.15	+0.7 %
	VH:CD ratio	6.10	6.08	-0.3 %
Week 70	Villi height (µm)	515	573	+11.3 %
	Crypt depth (µm)	85.57[b]	80.13[a]	-6.4 %
	VH:CD ratio	6.03[b]	7.17[a]	+18.9 %

[a,b] Different superscripts in the same row indicate significant differences (P≤0.05).

The average effect of FRA® Butyrin Hybrid Dry measured during 5 cycles of 28 days on performance and egg quality is summarized in Table 2. During each production cycle (data not shown) and over the entire trial period no effect was seen on laying rate. An average laying rate of 94.97% was observed in the control group and 94.38% in the treatment group. On average feed intake was significantly decreased with 6.8% when FRA® Butyrin Hybrid Dry was fed (P<0.001). This significant reduction in feed intake was observed in each production cycle (data not shown). A reduction in feed intake without compromising laying performances could be the result of the improved gut development as indicated by the improved gut morphology of the ileum. Consequently more nutrients could be absorbed from the feed. Thus, thereupon layers may have reduced their feed intake. As a result, FCR per dozen eggs was improved by 10 points from 1.51 to 1.41 (P=0.001).

3 RESULTS 2/2

T2 The effect of FRA® Butyrin Hybrid Dry on laying hen performance and egg quality from 51-70 weeks of age
Table 2

	Control	FRA® Butyrin Hybrid Dry	Difference
Mortality (%)	0.00	0.00	
Laying rate (%)	94.97	94.38	- 0.6%
Feed intake (g/hen/day)	**118[a]**	**110[b]**	**- 6.8 %**
FCR/dozen eggs	**1.51[b]**	**1.41[a]**	**- 10 points**
Egg mass (g/hen/day)	62.69	62.73	+ 0.06%
FCR/g of egg mass	**1.89[b]**	**1.76[a]**	**- 13 points**
Egg weight (g)	66.01	66.47	+ 0.7%
Albumen (mm)	7.62	7.66	+ 0.5%
Haugh unit	85.22	85.46	+ 0.24
Yolk colour	5.33	5.26	- 0.07
Shell strength (kgf)	**4.33[b]**	**4.48[a]**	**+ 3.5%**
Shell thickness (mm)	0.42	0.41	- 2.4%

[a,b] Different superscripts in the same row indicate significant differences (P≤0.05).

Compared to the control group no significant effect was seen on egg weight when FRA® Butyrin Hybrid Dry was supplemented to the diet. However, egg weight was numerically higher at the end of each production cycle (data not shown), which resulted in a slight numerically higher egg weight over the entire trial. Egg mass was calculated based on laying rate and egg weight, and was found similar in both groups. However, as a result of the lower feed intake in the treatment group, a significant improvement of 13 points in FCR/g of egg mass was obtained (P=0.001).

No differences were found in albumen height, Haugh unit and yolk color between the control and treatment group. Yolk color does not affect nutritional quality, taste, nor it is a marker for freshness, but it is an important measure for consumers acceptance. Egg yolk color is produced by carotenoids (pigments) which are naturally present in feed ingredients such as corn but are also added seperately as a supplement to laying hen diets. Different nutritional and non-nutritional factors influence yolk color. For example, pigments are fat-soluble, and high fat absorption including pigments can darken yolk color. Furthermore, yolk color can directly be affected by an increased or decreased feed intake, whereby an increased feed intake will increase the amount of pigments absorbed. In this trial feed intake was significantly reduced when FRA® Butyrin Hybrid Dry was fed, but no negative effect was seen on yolk color. A healthier and better developed intestine and therefore an improved absorption, which is normally impaired during aging, may be a possible explanation why yolk color was not affected by the lower feed intake of laying hens receiving FRA® Butyrin Hybrid Dry.

Shell thickness was similar for both the control and treatment group with on average 0.41-0.42 mm. Thickness is the main factor contributing to the mechanical strength of the eggshell. Despite a similar shell thickness, eggshell strength was significantly stronger in the treatment group (P=0.047). As mentioned before, dietary calcium is essential for eggshell synthesis. As laying hens age, intestinal epithelial cells become weaker and the villi shorter which results in impaired calcium absorption and consequently reduced eggshell strength. The significantly higher eggshell strength found in the treatment group, could be the result of a better developed intestine and improved calcium absorption.

4 CONCLUSIONS

The aim of this trial was to study the effect of FRA® Butyrin Hybrid Dry on laying hen performance and egg quality. From this trial it could be concluded that inclusion of a combination of tributyrin and alpha-monobutyrin in the diet improved ileum development. Feed intake was lower in the treatment group but laying rate and egg mass were comparable. This resulted in an improved FCR per dozen eggs and FCR per g egg mass by 10 and 13 points respectively. Egg quality was also improved as indicated by increased eggshell strength.

- Egg quality
- Shell strength
- Ileum development
- FCR

Additional info: R&D@framelco.com
FRA®melco B.V. / R&D department / Ruisvoorn 5, NL-4941 SB, Raamsdonksveer, The Netherlands

5 TRIAL SUMMARY FRA® BUTYRIN HYBRID DRY

Stimulate intestinal health and effective pathogen control

TRIAL

Animals » **Laying hens**
Product » **FRA® Butyrin Hybrid Dry**
Dosage » **2 kg / ton**

BRAZIL

Farm » **University of São Paulo**
Period » **January – June, 2019**

EXPERIMENTAL SET-UP

Animals » **160 laying hens (White Hisex)**
System » **Cages**
Treatments » **Control**
FRA® Butyrin Hybrid Dry @ 2 kg/ton
Set-up » **2 treatments x 10 repetitions x 8 laying hens**
Feed & water » ***ad libitum***

INTESTINAL INTEGRITY

SHELL STRENGHT +3.5%

Stronger eggshell

VH:CD RATIO +18.9%

Villi Height:
Crypt depth
ratio

FCR -10pnts

Reduced
FCR / dozen eggs

FCR -13pnts

Reduced
FCR / g of egg mass

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6 APPENDIX

A1 Basal diet
Appendix 1

	Control
Corn	57.640
Soybean meal	20.920
Limestone	10.340
Wheat bran	4.030
Soybean oil	3.870
Dicalcium phosphate	1.490
Salt	0.450
Premix	0.400
DL-methionine	0.290
L-Lysine	0.150
L-Threonine	0.110
L-Valine	0.110
Sand	**0.200**
Total	100.000
ME, kcal/kg	2900
CP, %	15.610
Arg. dig.	0.909
Fen. dig.	0.671
Fen. + Tir. dig.	1.142
His. dig.	0.379
Iso. dig.	0.568
Leu. dig.	1.262
Lys. dig. %	0.780
SAA dig. %	0.720
Met. dig. %	0.503
Thr. dig. %	0.601
Trp. dig. %	0.160
Val. dig. %	0.725
Ca. %	4.430
AvP %	0.374
Na. %	0.211
CF %	2.729
Linoleic acid %	3.410