ZOOTESTS

VOTRE PARTENAIRE R&D
POUR LES PRODUCTIONS ANIMALES !

"ZT1832" EXPERIMENT

Effect of GREENFEED CHORELLA on layer performance

July 20th, 2018

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TABLES

SPONSORS LIST

Sponsor:

René Jean Guillard
CDL ALGAE
LA CROIX DES LANDES
22600 TREVE, France

Director for experiment:

ZOOTESTS SAS
ALLENO Christophe
Parc Technologique du Zoopôle
5, rue Gabriel Calloet Kerbrat
22440 Ploufragan, France

Supervisor for experiment:

ZOOTESTS SAS
COMBEAU Céline
Parc Technologique du Zoopôle
5, rue Gabriel Calloet Kerbrat
22440 Ploufragan, France

1. PROTOCOL

PROTOCOL ABSTRACT

The aim of the study is to evaluate the impact of GREENFEED CHLORELLA water supplementations on commercial layers performance at the end of lay. Supplementation starts at 77 weeks of age. The effects are studied between 77 and 81 weeks of age.

Experiment is managed on 2 batches of 1300 hens that receive a different treatment:

- T1 = No water supplementation,
- T2 = GREENFEED CHLORELLA water supplementation (0.2 %).

Experiment is divided into 2 periods:

- 1st period (from 76 weeks-old) is placed before supplementation start. The aim of the comparison before starting of supplementation is to test comparability of the initial groups.
- 2nd period (from 77 to 81 weeks-old) is the supplementation phase.

Zootechnical criteria (mortality and egg production) are recorded between 77 to 81 weeks of age. Egg quality traits (individual egg weight, shell strength and Haugh Unit) are measured at 2 different ages: 77 and 81 weeks of age.

1.1. CALENDAR FOR THE STUDY

Approval for protocol : May, 2018
Data collection : between week 2018/22 and week 2018/28
Data delivery : August, 2018

1.2. LOCATION FOR EXPERIMENT

Laying house: P1 House
Le Lostec Pascale
Kerlay
22480 Saint-Gilles-Pligeaux
Contact: Mrs Pascale Le Lostec (06 82 14 59 74)

1.3. EXPERIMENTAL DESIGN

1.3.1. Global design

The aim of the study is to evaluate the impact of GREENFEED CHLORELLA water supplementations on commercial layers performance at the end of lay. Supplementation starts at 77 weeks of age. The effects are studied between 77 and 81 weeks of age.

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1.3.2. Laying house

Laying house is equipped with 4 batteries that are numbered 1 to 5 from left to right.
Test is placed in 2nd level of batteries #1 (T1) and #2 (T2).
Model of cage is FACCO furnished type (10 hens/cage).

1.4. MANAGEMENT OF THE BIRDS DURING THE TEST

1.4.1. Criteria for inclusion

Pullets are reared and vaccined according to flock owner protocol.

1.4.2. Criteria for no inclusion

Are totally excluded from the test all birds that, before the laying period, die or show pathological signs that could affect egg production.

Are partially excluded from the test all the birds whose visual condition degrades during laying period.

1.4.3. Housing

1.4.3.1. Rearing house

Vaccine program and lighting program will be attached to the final report.

1.4.3.2. Laying house

Laying house, located in Saint-Gilles-Pligeaux city, is equipped with batteries.
Model of cage is FACCO furnished type.
Lighting program is attached to the experiment report.

1.4.4. Transfer

Pullets are moved from rearing to laying house at 17 weeks of age.

1.4.5. Feeding and watering

The experimental diets will be manufactured by LE GOUESSANT. The diet will be supplied as crumbs.

1.5. PRODUCTS

1.5.1. Experimental product

GREENFEED CHLORELLA is used as water supplementation (0.2 %).

1.5.2. Other treatments during laying period

No other treatment was applied during the trial period.

1.6. EXPERIMENTAL PROCEDURE and TRAITS MEASURED

1.6.1. Calendar of the test

Data collection takes place between 77 and 81 weeks of age. The main events are listed in the table below:

Age (weeks)	Date (weeks)	Events
Between 77 et 81 weeks	22	- Egg production recording - Mortality recording
77	22	- **Start of water supplementation for T2 treatment** - Egg quality measurement (160 eggs/treatment) (egg collection = 180 eggs)
81	28	- Egg quality measurement (160 eggs/treatment) (egg collection = 180 eggs) - **End of water supplementation**

1.6.2. Egg quality measurements

Egg quality of one day of production (160 eggs/treatment/age ⇔ 5 eggs/cage x 32 cages) is precisely estimated per treatment at 2 different ages (77 and 81-wk-old).
Eggs are analysed in ZOOTESTS laboratory (Parc Technologique du Zoopôle - 5 rue Gabriel Calloet-Kerbrat – Ploufragan).

1.6.2.1. Individual egg weight

For each repeat, the eggs are individually weighted using a Sartorius scale (0.1 g precision).

1.6.2.2. Shell strength

Shell strength is evaluated using a compression machine MTS Synergie 200. Compression speed is 30 mm/mn. 2 different traits characterized shell strength:

- Shell stiffness that presents deformation of the shell under a constant force of 15 Newton. The less the shell deforms, the stronger it is,
- Fracture force (0,1 Newton precision) that is the maximum force applied on the egg before fracture of the shell occurs.

1.7. CRITERIA TO ESTIMATE EFFECT OF THE EXPERIMENTAL PRODUCT

1.7.1. Zootechnical criteria

- Mortality: Cumulative mortality rates are compared during experimental period.
- Egg production: Weekly salable egg production per repeat is estimated between 77 and 81 weeks of age. Egg production is recorded 5 days a week, so weekly production is estimated applying rule of three. Then, cumulative egg numbers can be compared during experimental period.

1.7.2. Egg quality criteria

Averages and variances of individual values are compared between the 2 treatments at 2 different ages (77 and 81 weeks of age).

Traits concerned are: egg weight, shell stiffness, fracture force and Haugh Units.

1.8. STATISTICAL ANALYSIS

1.8.1. Experimental unit / Statistical unit

Experimental and statistical units are the egg.

1.8.2. Comparability of initial groups

77-wk-old egg quality measurements inform us about comparability of initial groups for the different traits.

1.8.3. Statistical tests

Alpha risk will be 5%.
SAS software will be used to run statistical tests.

1.8.4. Quantitative traits analysis

Quantitative traits are egg weight, shell stiffness and fracture force.

For 81-wk-old egg quality traits, following statistical model is used

$$X_{ijk} = \mu + \alpha_i + \Omega_j + \varepsilon_{ijk}$$

with X_{ijk} = measured trait
μ = average
α_i = fixed effect of i^{th} **treatment**
Ω_j = 77-wk-old egg quality trait **as covariate**
ε_{ijk} = residual

Constraints of above mathematical model are:

- data from each population have to be normally distributed and to get same variance:
  - Normality of the traits is tested with residuals of Shapiro test.
  - Evenness of variances is tested with Bartlett test.
- recordings are supposed to be independent.

If one of these above constraints is not accepted, Kruskal-Wallis test is used.

Fixed effects are tested using variance analysis.

1.8.5. Qualitative traits analysis

In our study, qualitative trait is mortality and Laying rates.
Proportions will be compared using 2 different statistical analyses:

- χ^2 test.

1.9. DATA COLLECTION

Data will be collected either on data sheet, or directly on handheld.

1.10. PROTOCOL AMENDMENT or DEVIATION

1.10.1. Amendments

The sponsor or the director for experiment is able to propose changes in protocol during the test communicating an amendment document. This amendment has to be approved by the different parts involved in the test before being applied.

1.10.2. Protocol deviations

All deviations identified by staff involved in the test have to be recorded by the director for experiment. They will be listed and commented in the experiment report.

1.11. EXPERIMENT REPORT

A draft report is prepared by the Director of experiment.
Then, following possible corrections, a final report is communicated.

1.12. RESULTS PROPERTY

According to the present contract, ZOOTESTS give up all the property rights of the test to ALGAE. The sponsors will be able to complete, modify or correct test information by their own.

2.RESULTS

2.1. MORTALITY DATA

Table 1. Mortality rate according to batch

Batch	Mortality (%)	Number of hens housed
T1	0.3%	1319
T2	0.5%	1320
χ^2 test Batch	p = 0.527	
Whole sample	0.4%	2639

Mean
Differences in same row are significant or trending (a/b: P ≤ 0.05; x/y 0.05 <P≤ 0.10) means separated by Khi² test
Significant codes of p-value: 0 '***' (very highly significant difference), 0.001 '**' (highly significant difference), 0.01 '*' (significant difference), 0.05 '.' (no significant difference)

Zootechnical comments:

Overall, **Mortality** is 0.4 %.

There is no significant batch effect on **Mortality** (p_{Khi^2} **= 0.527**).

2.2. LAYING RATE

Table 2. Laying rate according to batch

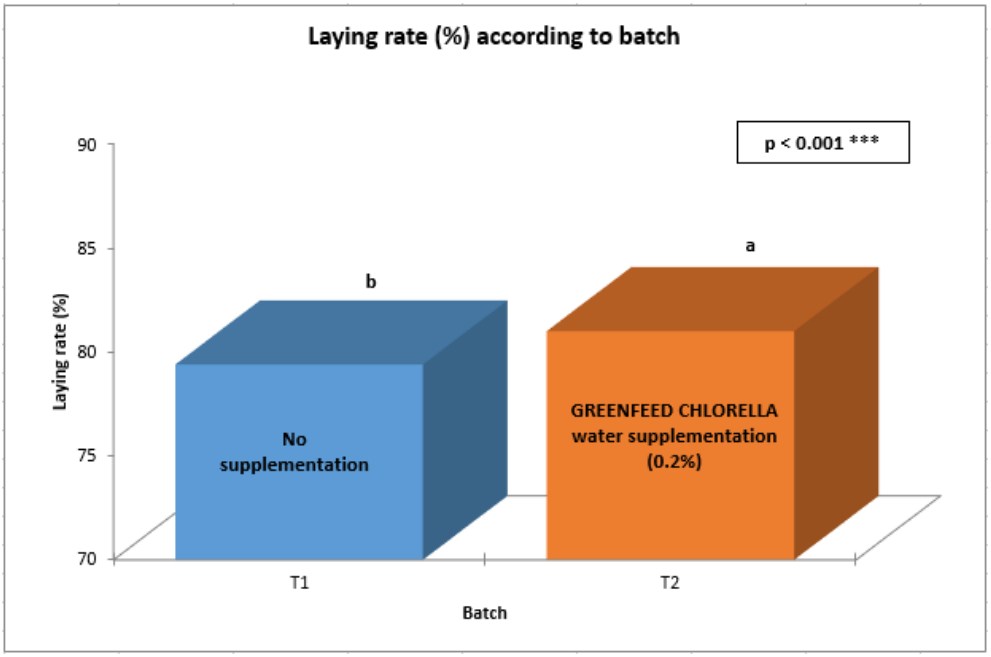
Batch	Laying rate (%)	Number of observations (Hen number*Days number)
T1	79.4 % **b**	32943
T2	81.0 % **a**	32955
Whole sample	80.2 %	65898
χ^2 test Batch	**p < 0.001*** **	

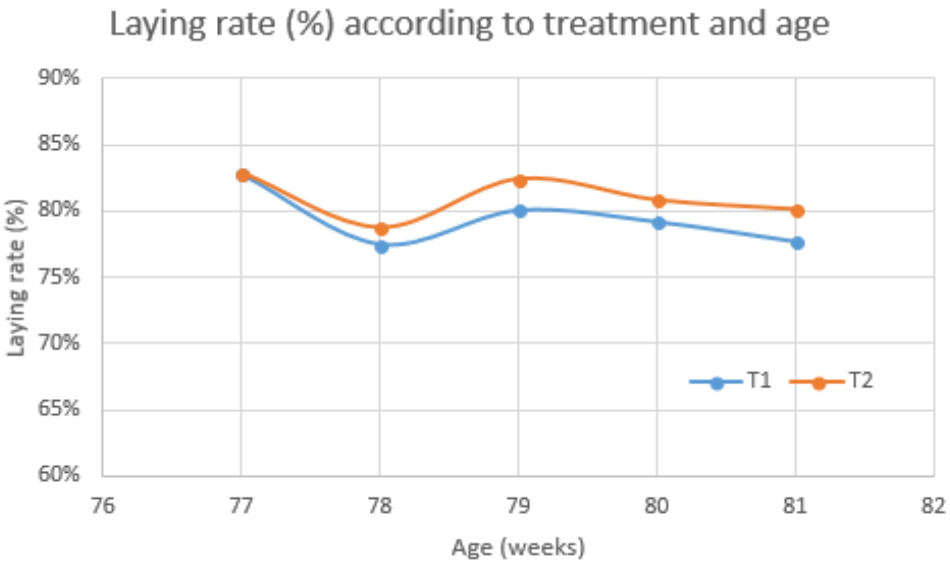
Mean
Differences in same row are significant or trending (a/b: P ≤ 0.05; x/y 0.05 <P≤ 0.10) means separated by Khi² test
(1) Model with all interactions, (2) Restricted model (⇔ without interaction)
Significant codes of p-value: 0 '***' (very highly significant difference), 0.001 '**' (highly significant difference), 0.01 '*' (significant difference), 0.05 '.' (no significant difference)

Zootechnical comments:

There is a significant batch effect on **Laying rate** with higher egg production for T2 (⇔ GREENFEED CHLORELLA water supplementation (0.2 %)) group:

- T2 (81.0%) **>** T1 (79.4 %) (**p_{Khi^2} < 0.001*****).




Laying rate (%) according to treatment and age
90%
85%
80%
75%
70%
65%
60%
Laying rate (%)
76
77
78
79
80
81
82
Age (weeks)
T1
T2

2.3. Egg Quality

Table 3. 77-wk-old Egg Quality measurements according to treatment

Treatment	Egg weight (g) (CV %)	Static Stiffness (N/mm) (CV %)	Fracture Force (N) (CV %)	Number of eggs
T1	64.2 ± 1.7 (2.7)	180.9 ± 11.6 (6.4)	39.1 ± 3.1 (7.8)	36
T2	63.9 ± 2.1 (3.2)	175.7 ± 13.2 (7.5)	37.4 ± 2.6 (6.9)	36
Whole sample	64.0 ± 1.9 (3.0)	178.3 ± 12.6 (7.1)	38.3 ± 2.9 (7.7)	72

Mean ± Standard deviation (CV%)

Table 4. 81-wk-old Egg Quality measurements according to treatment

Treatment	Egg weight (g) (CV %)	Static Stiffness (N/mm) (CV %)	Fracture Force (N) (CV %)	Number of eggs
T1	64.5 ± 2.2 (3.4)	175.4 ± 13.6 (7.8)	38.5 ± 3.2 (8.4)	36
T2	64.2 ± 1.8 (2.7)	174.3 ± 14.2 (8.1)	37.8 ± 3.2 (8.5)	36
Whole sample	64.3 ± 2.0 (3.1)	174.9 ± 13.8 (7.9)	38.2 ± 3.2 (8.4)	72

Mean ± Standard deviation (CV%)

Table 5. 81-wk-old Egg Quality statistical analyses (with 77-wk-old Egg quality traits as covariate)

Treatment	Egg weight (g) (Ls means)	Static Stiffness (N/mm) (Ls means)	81-wk-old - Static Stiffness (N/mm) (Ls means)	Number of eggs
T1	64.4	175.2	38.5	36
T2	64.3	174.5	37.8	36
Bartlett test	p = 0.099	p = 0.878	p = 0.982	
Shapiro test	p = 0.112	p = 0.739	p = 0.352	
Treatment (2)	p = 0.727	p = 0.825	p = 0.376	
77-wk-old covariate	p = 0.061	p = 0.577	p = 0.896	

Ls mean
Differences in same row are significant or trending (a/b: P ≤ 0.05; x/y 0.05 <P≤ 0.10) means separated by LSD test
Significant codes of p-value: 0 '***' (very highly significant difference). 0.001 '**' (highly significant difference). 0.01 '*' (significant difference). 0.05 '.' (no significant difference)
(1)Model with all interactions, (2) Restricted model (⇔ without interaction)

Zootechnical comments:
There is no significant Treatment effect on 81-wk-old **Egg Quality** measurements:

- Egg weight: **p = 0.727**,
- Static Stiffness: **p = 0.825**,
- Fracture Force: **p = 0.376**.