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***Farias-Kovac C., Simbaña F., Reyes M., Ávila A.B., Carabaño R., Nicodemus N., García* J.**

EFFECT OF CELLOBIOSE SUPPLEMENTATION IN DRINKING WATER AND FEED RESTRICTION ON APPARENT FAECAL DIGESTIBILITY AND GROWTH PERFORMANCE IN RABBITS

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EFFECT OF CELLOBIOSE SUPPLEMENTATION IN DRINKING WATER AND FEED RESTRICTION ON APPARENT FAECAL DIGESTIBILITY AND GROWTH PERFORMANCE IN RABBITS

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ABSTRACT

The objective of this work was to evaluate the effect of cellobiose supplementation in water (CEL) and its potential synergy with feed restriction on fecal digestibility and growth performance. Four treatments in a factorial arrangement were used: 2 levels of CEL (0.0 and 7.5 g/L) × 2 feeding plans (*ad libitum* and restricted, from 32 to 47 d of age). A total of 236 32-d old rabbits weighing 700 ± 116 g were blocked by litter randomly assigned to the four treatments and caged individually until 60 d of age. The restricted group was fed with 50% the feed eaten by the *ad libitum* group at weaning and the daily feed supply increased linearly until 100% of intake of the *ad libitum* group at 47 d of age. Fecal digestibility was determined between 39 and 43 d (D1) and between 53 and 56 d of age (D2) (10/treatment). Cellobiose supplementation had no effect on faecal digestibility but tended to increase starch digestibility in D1 (P = 0.074). Feed restriction improved energy, protein (both by 5%), starch (+0.3%) and total dietary fibre digestibility by 11% (P ≤ 0.026) in D1, with no effect in D2. Feed efficiency improved in the whole experimental period with cellobiose supplementation (+3%. P = 0.003), due to the trend to increase the growth rate (P = 0.11), with no effect on feed intake and mortality. During the restriction period feed intake of restricted rabbits was a 72% of that of the *ad libitum* group, while in the whole experimental period accounted for a 90% of the *ad libitum* group. As expected, it decreased growth rate (-3.5%; P = 0.015) and improved feed efficiency (+7%. P < 0.001) in the whole period, resulting in a lower final liveweight (2287 *vs*. 2231d g; P = 0.015). Feed restriction, tended to reduce mortality rate (18.6 *vs*. 10.1%; P = 0.067), and curiously no differences in mortality were observed during the restriction period, and this tendency was explained by reduction of the mortality during the refeeding period (P = 0.041). There was no relevant interaction between CEL and feed restriction. In conclusion, CEL supplementation improved growth traits but had no influence on mortality, while gradual feed restriction, tended to decrease mortality with a minor reduction of growth traits.

Keywords: Cellobiose, Feed restriction, Digestibility, Growth performance, Rabbit.

INTRODUCTION

Cellobiose supplementation in drinking water (CEL, 7-7.5 g/L) reduced mortality rate when it was combined with a low soluble fibre diet (8.4%, on DM basis) in a context of epizootic rabbit enteropathy, but a higher dose (15 g/L) increased mortality (Ocasio-Vega *et al*., 2018a, 2019). The positive effect of cellobiose might be related to the increase of the proportion of butyrate in the ileal digesta, effect also observed in the caecal *in vitro* fermentation (Ocasio-Vega *et al*., 2018a,b). Feed restriction programs have proven to be effective in reducing mortality and optimizing feed efficiency after weaning in rabbits, although has the disadvantage of the impairment of growth traits when the fattening period is short like in Spain (Romero *et al*., 2010; Gidenne *et al*., 2012). An alternative might be a progressive feed restriction plan (Duperray and Guyonvarch, 2009; Birolo *et al*., 2016), that helps to control mortality but still impaired growth traits (Farias-Kovac, 2021). The aim of this study is to evaluate the possible synergic effect of cellobiose supplementation with feed restriction on faecal digestibility and growth traits.

MATERIALS AND METHODS

Experimental design

Four treatments in a 2 x 2 factorial arrangement were used with two levels of CEL (0, and 7.5 g/L. Savanna Ingredients GmbH, Elsdorf, Germany) along the whole fattening period, and two feeding plans (*ad libitum*, AL; restricted, Rest.). Restriction started with a 50% of the AL group and increased progressively until 100% at 47 d of age inspired in the feeding plan studied by Duperray and Guyonvarch (2009) and Birolo *et al*. (2016). From 47 to 51 d restricted rabbits were offered the same feed eaten by the AL group, and from 51 to 60 d they were fed *ad libitum*. A control diet was formulated to meet the nutrient requirements for growing rabbits with 21.1% crude protein, 35.5% neutral detergent fibre, 9.1% soluble fibre, 13.4% starch and 19.0 MJ/kg gross energy (on DM basis). A total of 236 rabbits weighing 700 ± 116 g were weaned at 32 d of age, blocked by litter, randomly assigned to the four treatments and individually caged. Due to the design of the farm, treatments were not balanced (AL-CEL− =56 rabbits, R-CEL− =56, AL-CEL+ =62, R-CEL+ =62). No antibiotic was supplied. Rabbits had *ad libitum* access to water. Faecal digestibility was determined (9/treatment) from 39 to 43 d age (D1), and from 53 to 56 d of age (D2). Growth traits were recorded at 32, 47 and 60 d of age.

Statistical Analysis

Data were analyzed as a completely randomized block design with level of CEL, feed restriction and their interaction as the main sources of variation and litter as a block, weaning weight was used as a covariate for growth traits by using a mixed model. Mortality was analyzed using a logistic regression considering a binomial distribution including the same variables in the model, and the results were transformed from the logit scale. All data were presented as least-squares means.

Chemical Analyses

Procedures of the AOAC (2000) were used to determine DM (method 934.01), crude protein (968.06), starch (amyloglucosidase-α-amylase method; method 996.11), and total dietary fibre (985.29), with no mucin correction for faeces. Gross energy was measured by adiabatic bomb.

RESULTS AND DISCUSSION

Cellobiose supplementation had no effect on faecal digestibility (D1 and D2), but a trend to improve starch digestibility in D1 (P = 0.074. Table 1). The lack of effect on the total dietary fibre digestibility contrast with the modification of *in vivo* and *in vitro* fermentation observed previously with CEL supplementation (Ocasio-Vega *et al*., 2018a,b). Cellobiose supplementation improved feed efficiency in the whole experimental period by 3% (0.404 *vs*. 0.416; P = 0.003), due to the trend to increase the growth rate (P = 0.11. Table 2) with no effect on feed intake, which is similar to the results obtained by Ocasio-Vega *et al*. (2018a, 2019). Nevertheless, these authors reported a positive influence of CEL supplementation (combined with a low dietary soluble fibre level) on mortality, but in this study no effect of CEL on mortality was observed. Feed restriction improved in D1 faecal digestibility of energy (63.8 *vs*. 60.9%; P = 0.036), protein (78.3 *vs*. 75.1%; P = 0.002), starch (98.9 *vs*. 99.2%. P = 0.026) as well as the total dietary fibre digestibility (39.8 *vs*. 35.7%; P = 0.011) as expected according to most data reviewed by Gidenne *et al*. (2012) and Birolo *et al*. (2016).

Table 1*:* Effect of cellobiose (CEL) supplementation and feeding plan (*Ad libitum vs*. Restricted - Rest.-) on apparent faecal digestibility at two different periods (39-43 d and 53-56 d of age) in growing rabbits.

	CEL –		**CEL+**		**SEM[1]**		***P*-value[2]**	
Feeding plan	***Ad libitum***	**Rest.**	***Ad libitum***	**Rest.**	***Ad libitum***	**Rest.**	**CEL**	**Feeding plan**
n[3]	10	10	9	8				
n[4]	9	8	8	9				
39-43 d of age (restriction period)								
Body weight, g	940	895	1121	937	40.6	33.5	0.006	0.005
DM intake, g/d	104	85.7	102	83.7	3.40	1.08	0.702	<0.001
Faecal digestibility, %								
Gross energy	60.9	62.8	60.8	64.7	1.05	0.87	0.359	0.006
Crude protein	75.5	77.2	74.6	79.4	1.65	1.04	0.648	0.026
Total dietary fibre[5]	35.8	38.3	35.6	41.3	1.41	1.60	0.377	0.011
Starch	98.8	99.1	99.0	99.2	0.10	0.07	0.074	0.012
53-56 d of age								
Body weight, g	2037	1949	2087	1904	81.3	52.4	0.970	0.058
DM intake, g/d	116	132	112	131	2.65	4.63	0.528	<0.001
Faecal digestibility, %								
Gross energy	63.3	62.4	61.8	62.5	0.78	1.00	0.486	0.919
Crude protein	73.9	73.7	72.4	74.7	0.87	0.88	0.830	0.246
Total dietary fibre[5]	41.9	39.3	38.2	38.9	1.64	1.90	0.253	0.599
Starch	99.1	99.2	99.3	99.0	0.05	0.06	0.894	0.145

[1] Due to variance heterogeneity, a SEM value was included for each mean of the ad libitum groups, and another one for the means of restricted groups. [2] The interactions Cellobiose × Feeding plan were not significant ($P > 0.10$). [3,4] Number of rabbits used per treatment for digestibility from 39 to 43 d, and from 53 to 56 d of age, respectively. [5] All values corrected for ash and protein.

Table 2: Effect of cellobiose supplementation (CEL) and feeding plan (*Ad libitum vs*. Restricted - Rest.-) on growth performance from 32 to 60 d of age of rabbits.

	CEL –		**CEL+**		**SEM[1]**		***P*-value[2]**	
Feeding plan	***Ad libitum***	**Rest.**	***Ad libitum***	**Rest.**	***Ad libitum***	**Rest.**	**CEL**	**Rest.**
n[3]	44	50	42	51				
32-47 d of age (restriction period)								
Body weight 32 d, g	726	683	713	712	18.6	14.6	0.632	0.193
Growth rate, g/d	61.3	49.5	62.3	48.9	1.32	0.63	0.867	<0.001
Feed intake, g/d	127	90.8	124	89.2	2.72	0.78	0.299	<0.001
Feed efficiency, g/g	0.488	0.546	0.504	0.548	0.0	0.006	0.200	<0.001
Mortality	8.93	7.14	11.3	8.06	-	-	0.67	0.51
47-60 d of age								
Body weight 51 d, g	1627	1451	1642	1441	19.8	9.40	0.863	<0.001
Growth rate, g/d	48.3	59.7	52.2	61.0	1.25	1.47	0.057	<0.001
Feed intake, g/d	162	173	163	171	2.27	2.37	0.946	<0.001
Feed efficiency, g/g	0.299	0.345	0.320	0.353	0.007	0.006	0.023	<0.001
Mortality	7.14	1.79	9.68	3.23	-	-	0.50	0.041
32-60 d of age								
Body weight 59 d	2254	2230	2320	2233	21.1	22.7	0.110	0.015
Growth rate, g/d	55.2	54.4	57.6	54.5	0.76	0.81	0.109	0.015
Feed intake, g/d	143	129	142	127	2.00	1.19	0.507	<0.001
Feed efficiency, g/g	0.388	0.421	0.406	0.427	0.004	0.004	0.003	<0.001
Mortality	16.1	8.93	21.0	11.3	-	-	0.45	0.067

[1] Due to variance heterogeneity, a SEM value was included for each mean of the Ad libitum groups, and another one for the means of Restricted groups. [2] No interaction was found between Cellobiose × Feeding plan ($P \geq 0.133$). [3] Number of rabbits at the end of the fattening period and used to calculate growth traits. For mortality values the initial number of rabbits was 56, 56, 62, and 62, respectively. Morbid rabbits were not considered in the growth traits data: 3, 1, 7, and 4, respectively.

It might seem to be accounted for an increase in the mean retention time of the digesta (Gidenne *et al.*, 2012), although it might also depend on the type of diet. During the restriction period feed intake of restricted rabbits was a 72% of that of the *ad libitum* group, while in the whole experimental period accounted for a 90% of the *ad libitum* group. As expected, it decreased growth rate (56.4 *vs*. 54.5 g/d; P = 0.015) and improved feed efficiency (0.424 *vs*. 0.397; P < 0.001) in the whole period, reaching a lower final liveweight (P = 0.015) but optimal for the market. Feed restriction, tended to reduce mortality rate (18.6 *vs*. 10.1%; P = 0.067), and curiously no differences in mortality were observed during the restriction period, and this trend was due to the mortality reduction during the refeeding period (P = 0.041). This result is similar to that reported by Farias-Kovac (2021), although this author reported a reduction in the mortality rate during the restriction period, and larger differences in the final liveweight between the *ad libitum* and restricted groups. It might be probably due to the shorter duration of the restriction period (-4 d) in this study. These results are mostly in agreement with those associated with feed restriction reviewed by Gidenne *et al*. (2012) and reported by Birolo *et al*. (2016). There was no relevant interaction between CEL and feed restriction.

CONCLUSIONS

Cellobiose supplementation improved growth traits but had no influence on mortality. Gradual feed restriction, tended to decrease mortality with a minor influence of growth traits. No synergism was found was found between feed restriction and cellobiose supplementation.

ACKNOWLEDGEMENTS

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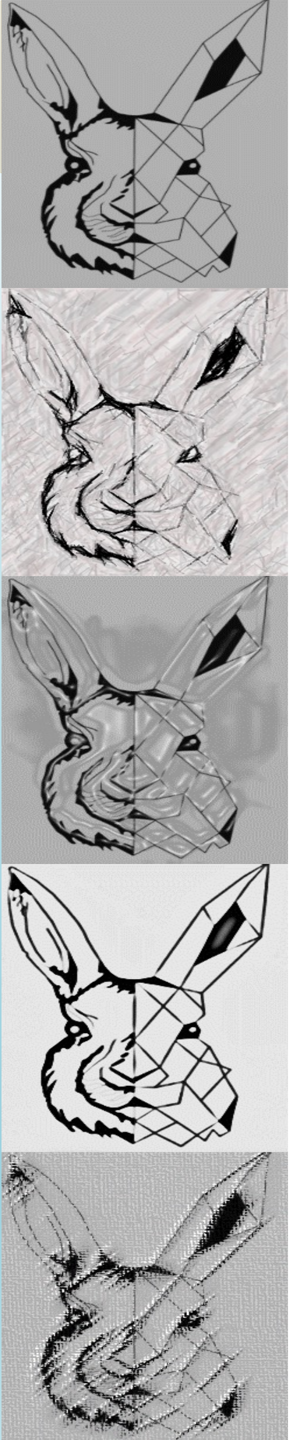
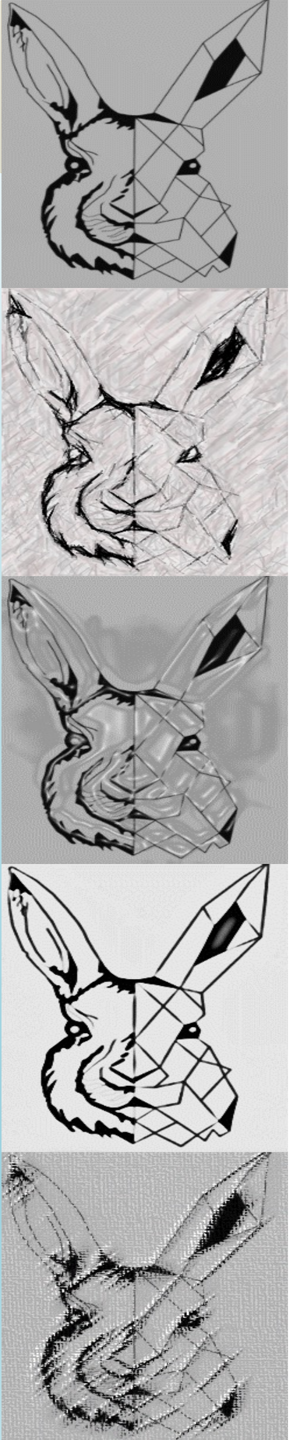


Effect of cellobiose supplementation in drinking water and feed restriction on apparent faecal digestibility and growth performance in rabbits

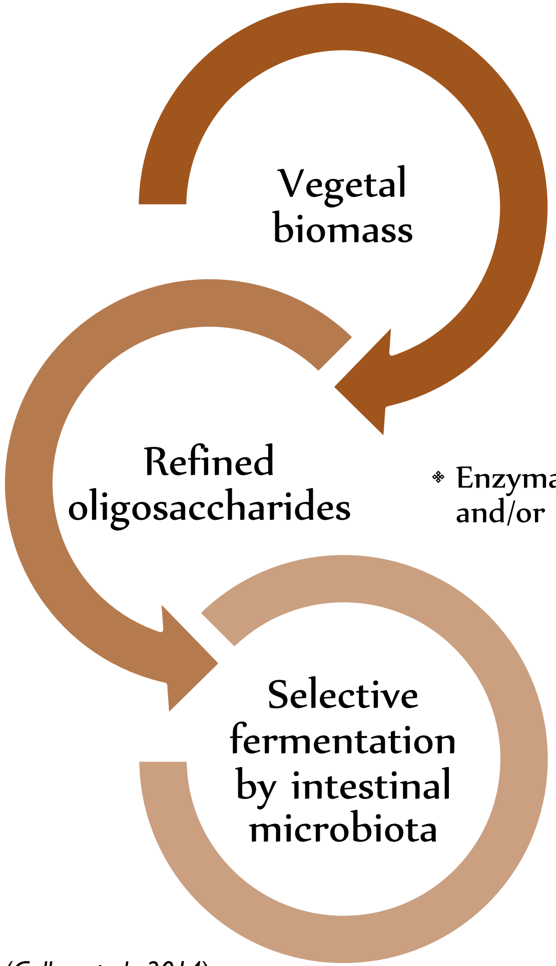
Carlos E. Farías Kovac

12th World Rabbit Science Congress.
3-5 November, 2021.
Nantes, France



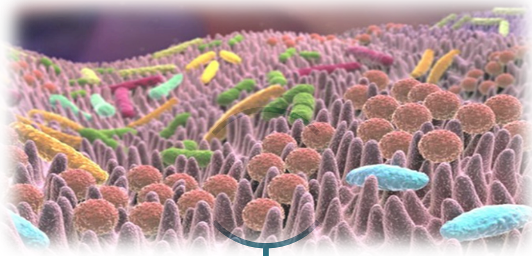
Oligosaccharides (NDO's)

- Some considered dietary fibre, different from NSP *(Englyst et al.,2002)*
- Selective effect on beneficial microbiota *(Bielecka et al.,2002)*



- Enzymatically and/or chemically
- Inclusion in the diet

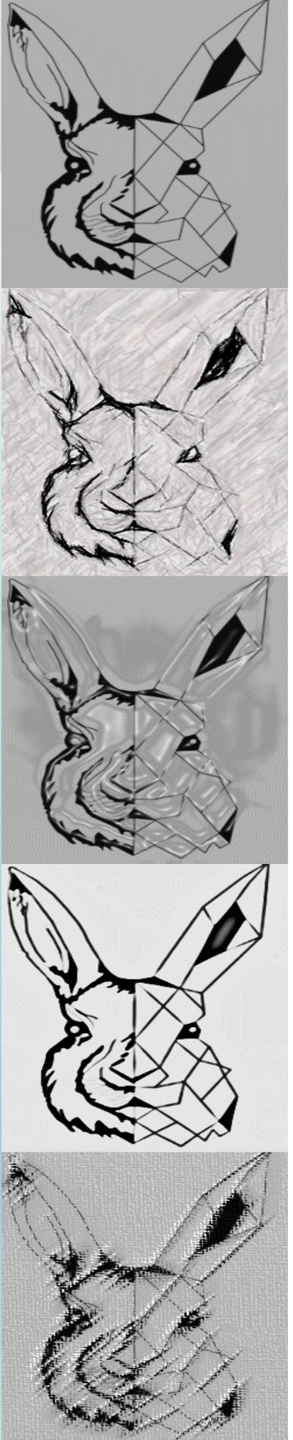
(Gullon et al., 2014)



VFA production | Shift to benfecial bacteria | Direct effect on mucosa

Among 7 principal NDO's used as prebiotics (Falcao-e Cunha et al., 2008)

- <u>Cello-oligosaccharides (COS)</u>
- <u>Xilo-oligosaccharides (XOS)</u>



Cellobiose use

Cello-oligosaccharides

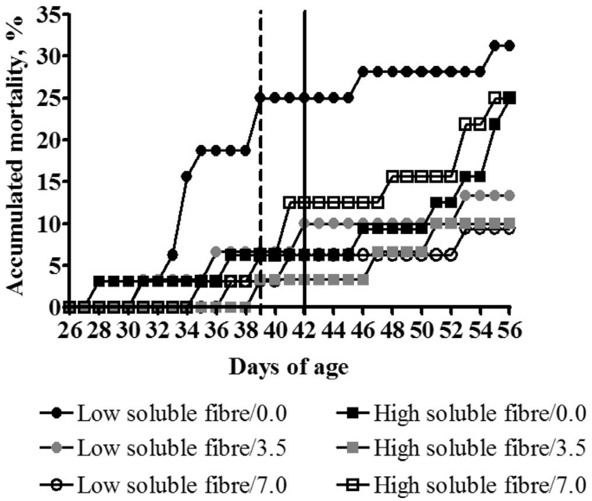
Derived from cellulose

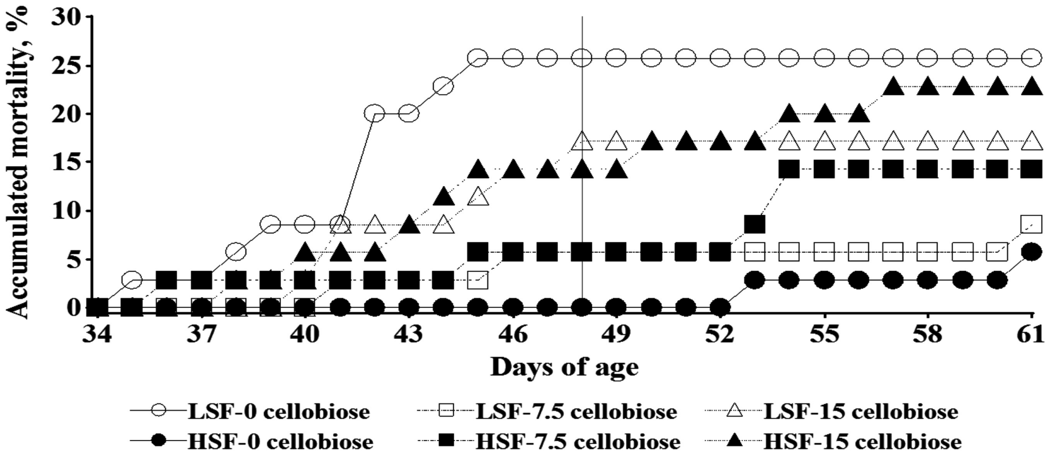
2 glucose molecules (ß-1,4)

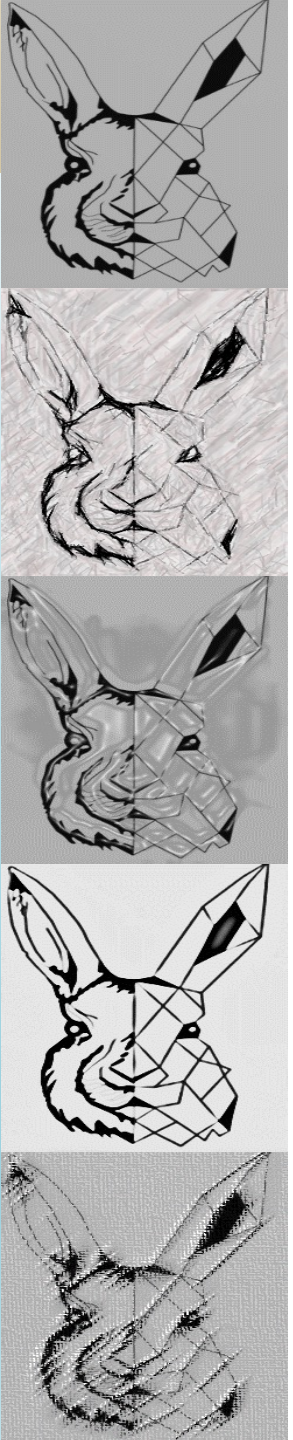
Cellobiosidase

Cellulase

7,0 g/L and 7,5 g/L x Low SF reduced mortality (*Ocasio-Vega et al., 2018[a]; 2019*)







Feed Restriction

Feed Restriction

- ↓ Mortality
- ↑ Feed efficiency

(*Gidenne et al., 2012*)

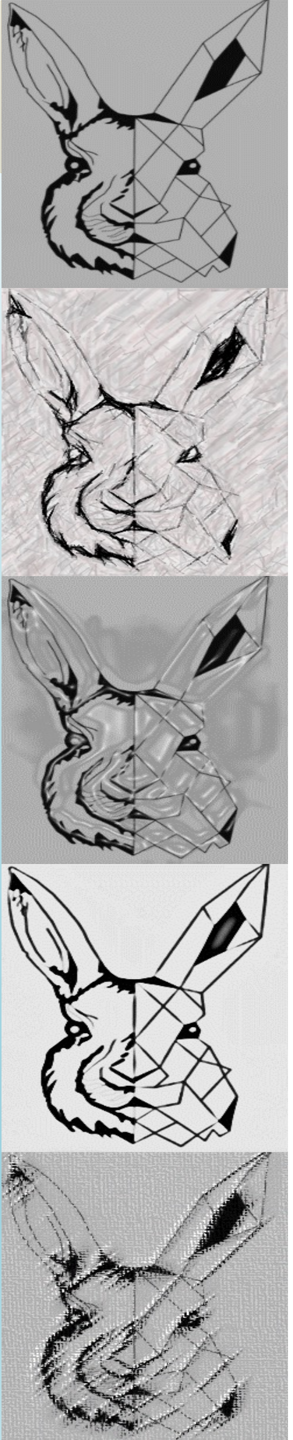
- ↓ Growth performance

(*Gidenne et al., 2009; Romero et al., 2010*)

Non-consistent results or lack of effects(*Crespo et al., 2020*)

Progessive feed restriction??

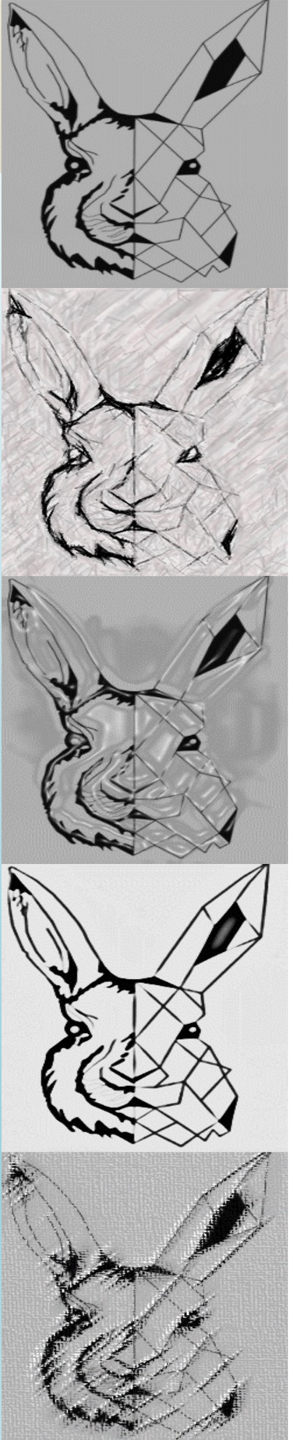
(*Duperray and Guyonvarch 2009; Birolo et al., 2016;*)



Objective

To evaluate the possible synergic effect of cellobiose supplementation with feed restriction on faecal digestibility and growth traits.

MATERIAL Y METHODS



Factorial
2 x 2

Diet: 21,1% CP; 35,5% NDF; 9,1% SF;13,4% Starch

2 Cellobiose in drinking water (g/L)

0,0 (Ocasio-Vega, 2018) 7,5

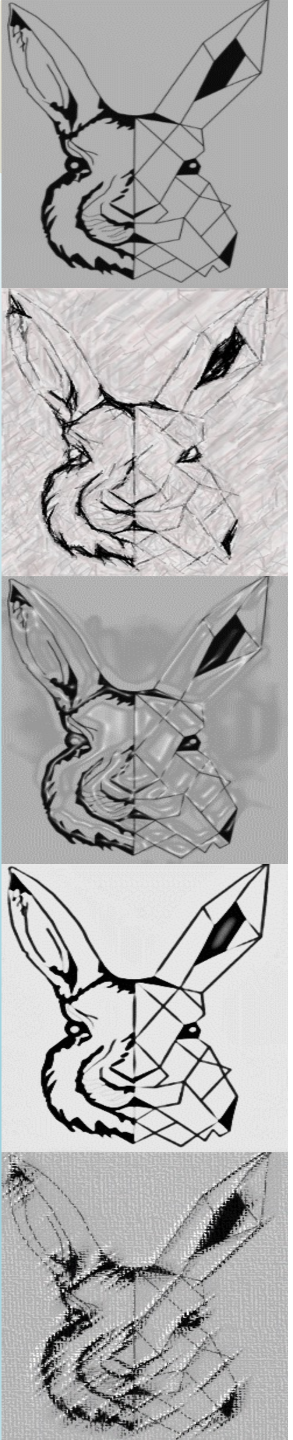
- Growth performance (32-60)
- Carcass yield 10 /TTO
- Digestive organs.

Progessive feed restriction period

F.A.D

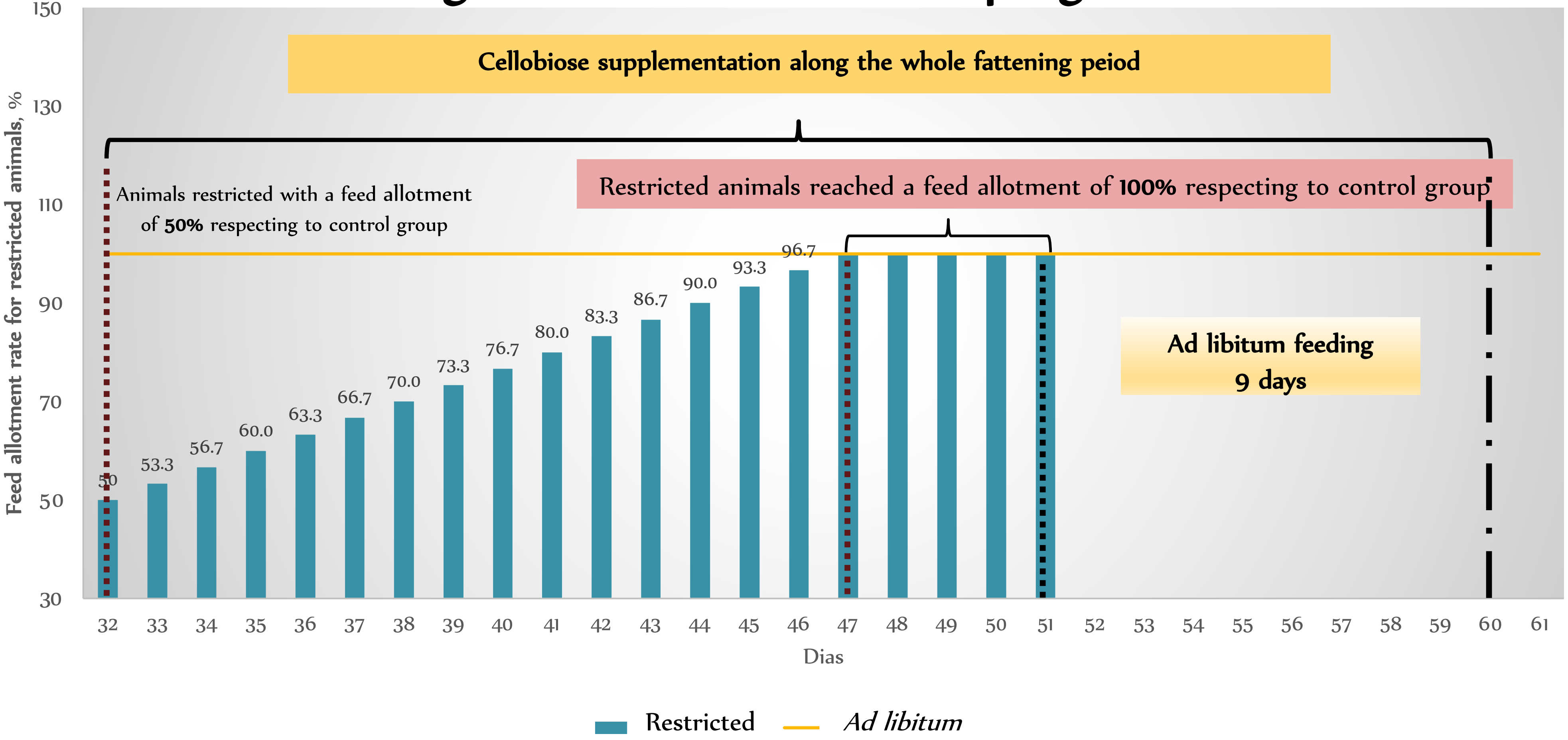
32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

Faecal apparent digestibility (F.A.D)

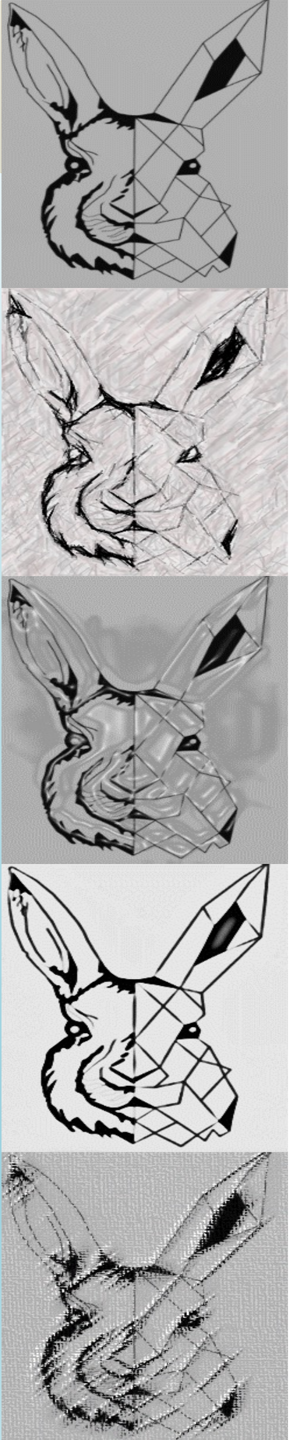


MATERIAL Y MÉTHODS

Progressive feed restriction program

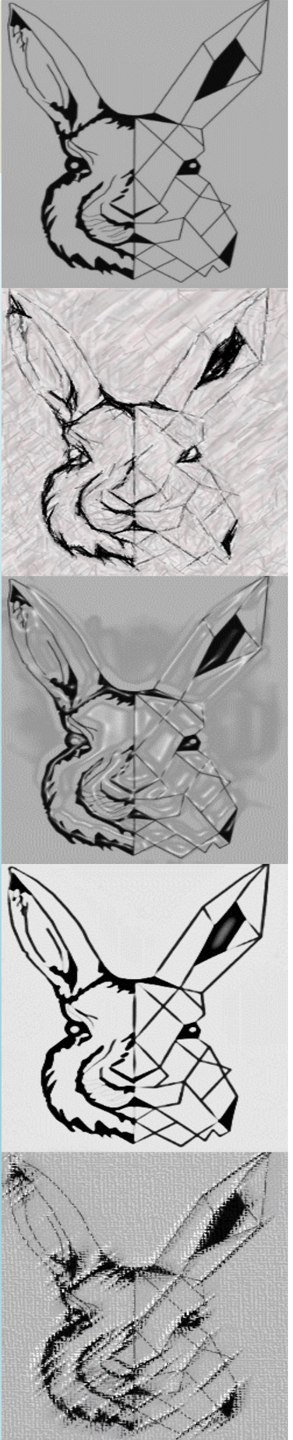


(Duperray and Guyonvarch, 2009; Birolo et al., 2016)

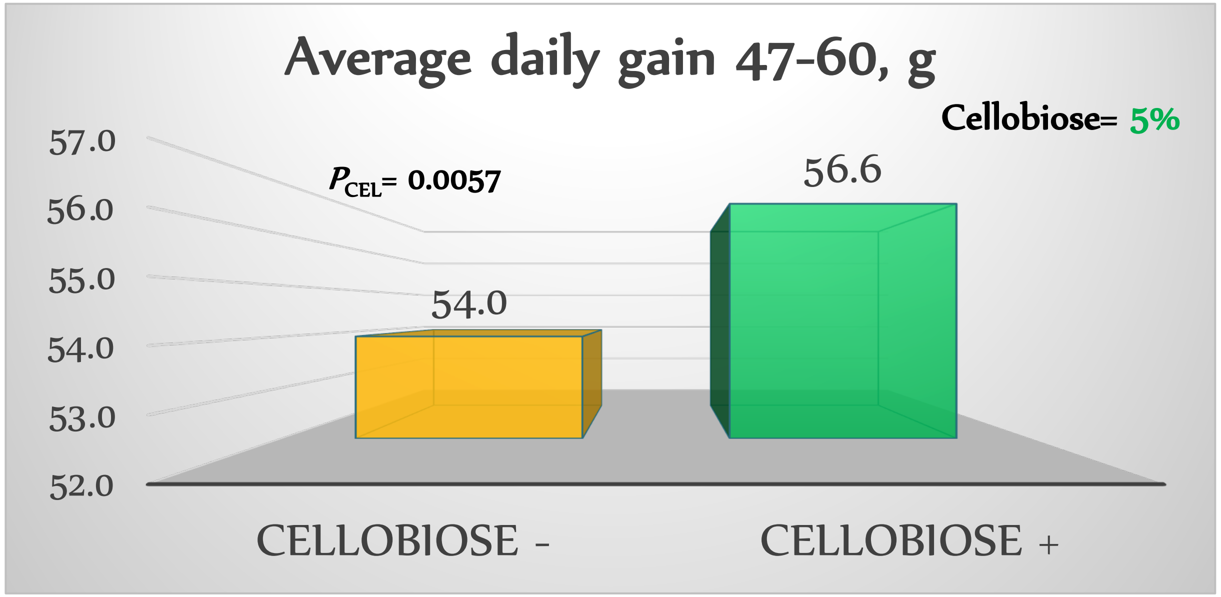


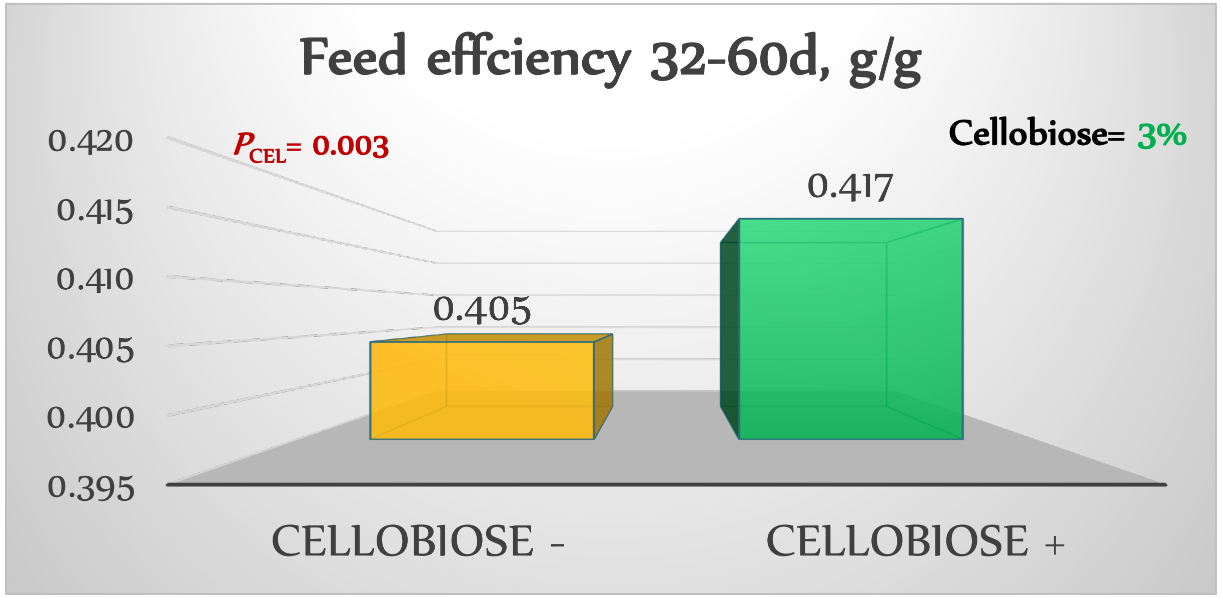
No effect of Cellobiose was oberved on mortality

- ❖ Why the lack of positive effect ?
- ❖ Cellobiose intake in drinking water was equivalent to an inclusión in the diet of 1,5%.
- ❖ Levels of 2.2% increased mortality (Ocasio-Vega et al., 2018b)

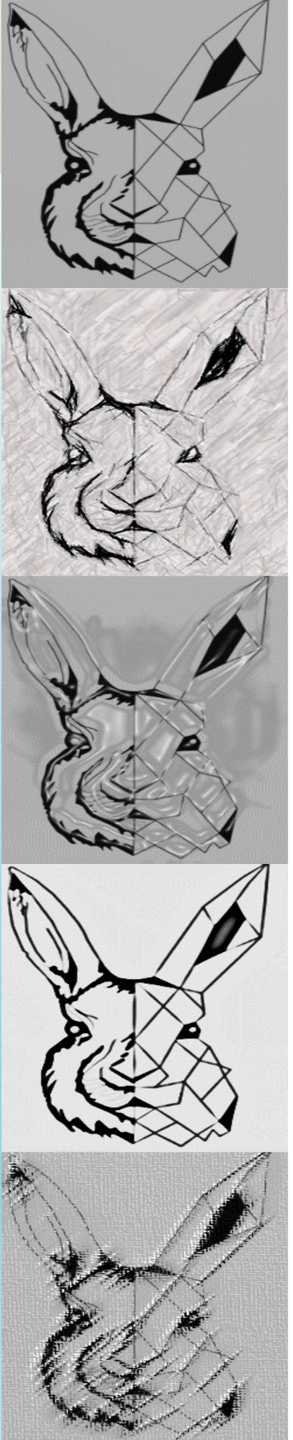


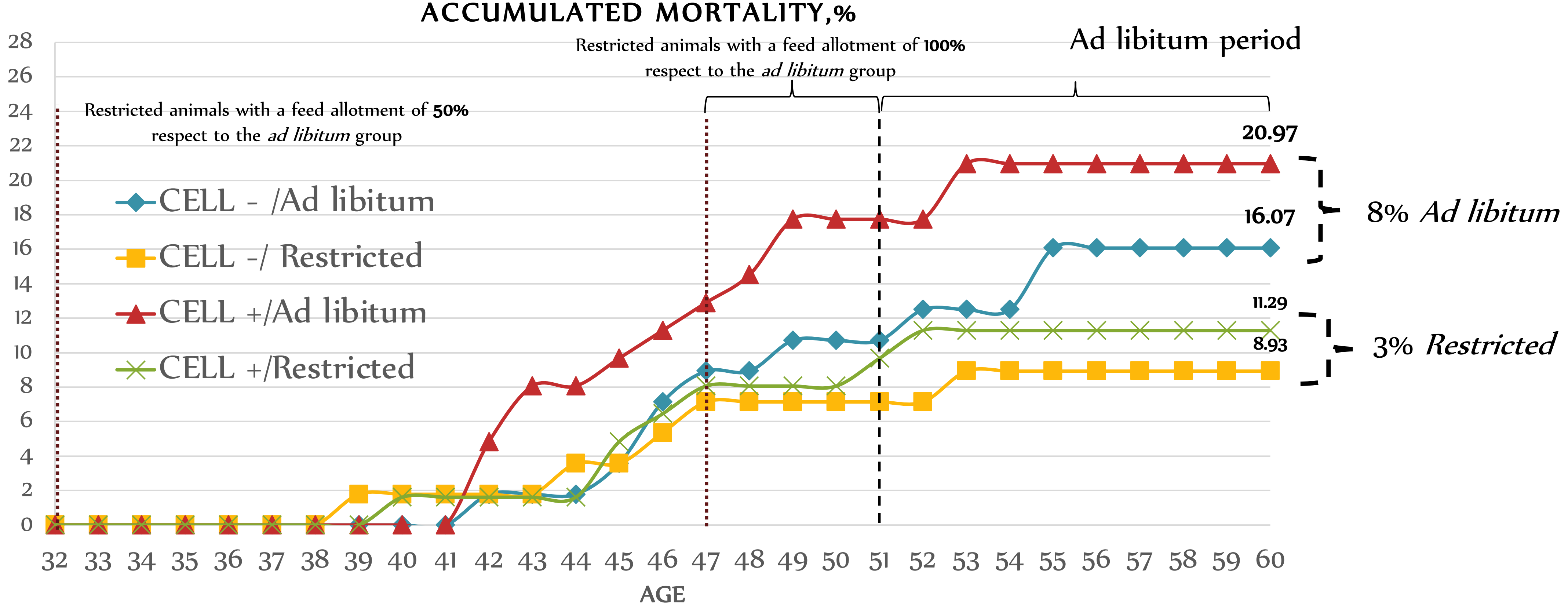
❖Cellobiose did not influence average feed intake.

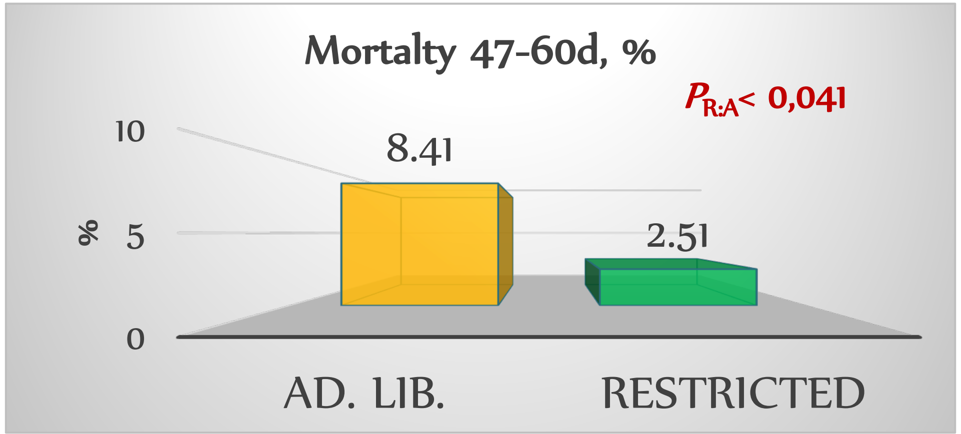


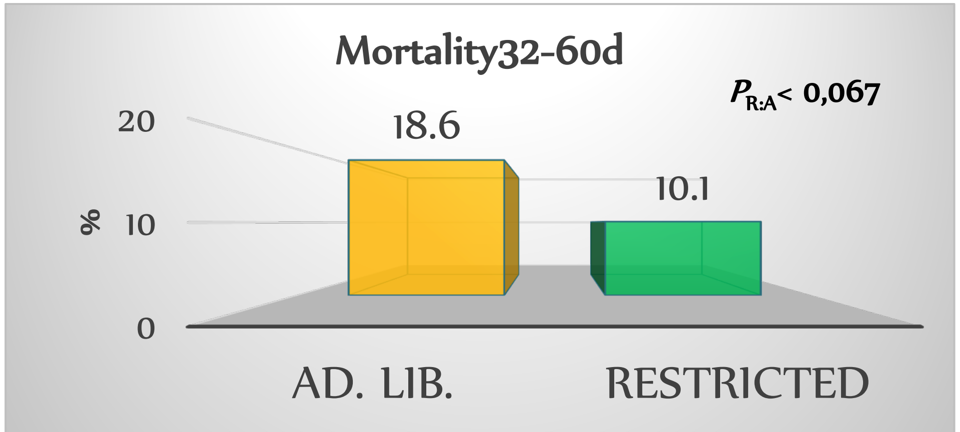


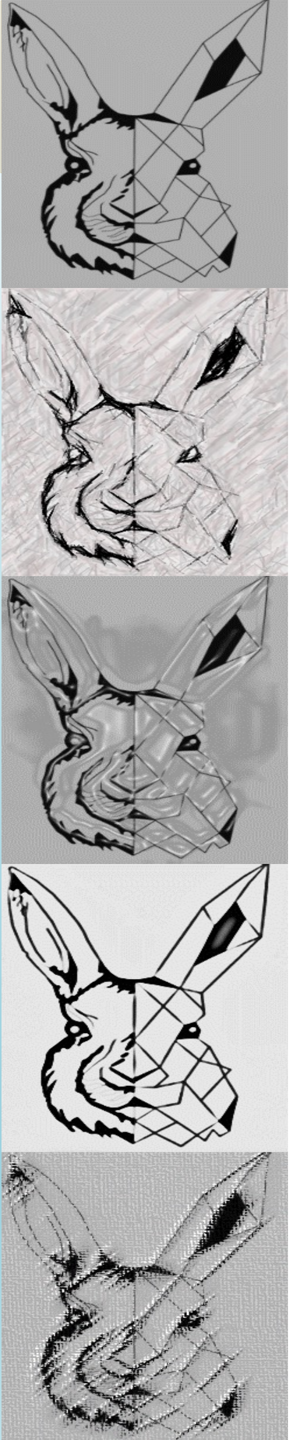
RESULTS



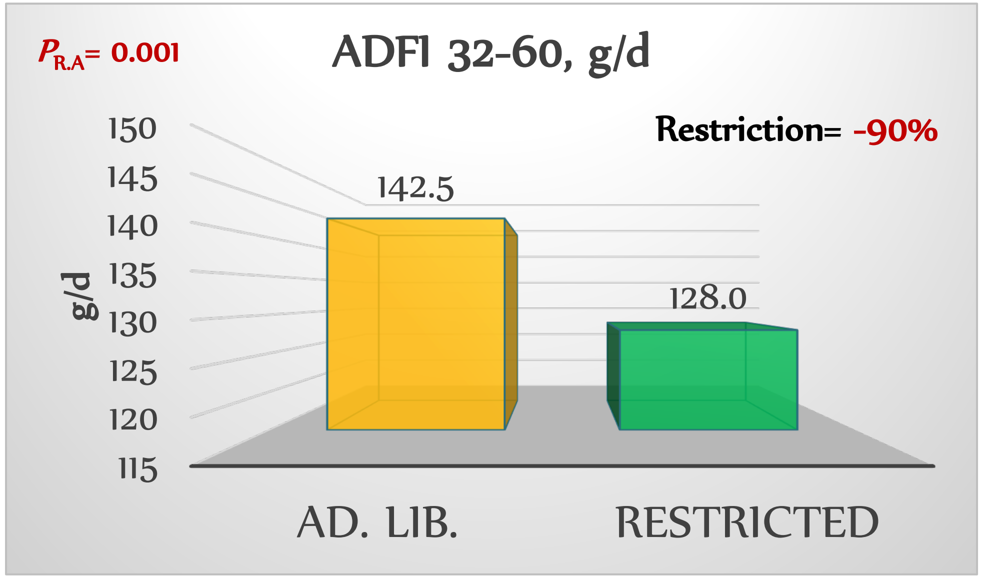

ACCUMULATED MORTALITY,%
Restricted animals with a feed allotment of **50%** respect to the *ad libitum* group
Restricted animals with a feed allotment of **100%** respect to the *ad libitum* group
Ad libitum period
CELL - /Ad libitum
CELL -/ Restricted
CELL +/Ad libitum
CELL +/Restricted
20.97
16.07
11.29
8.93
8% *Ad libitum*
3% *Restricted*
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
AGE

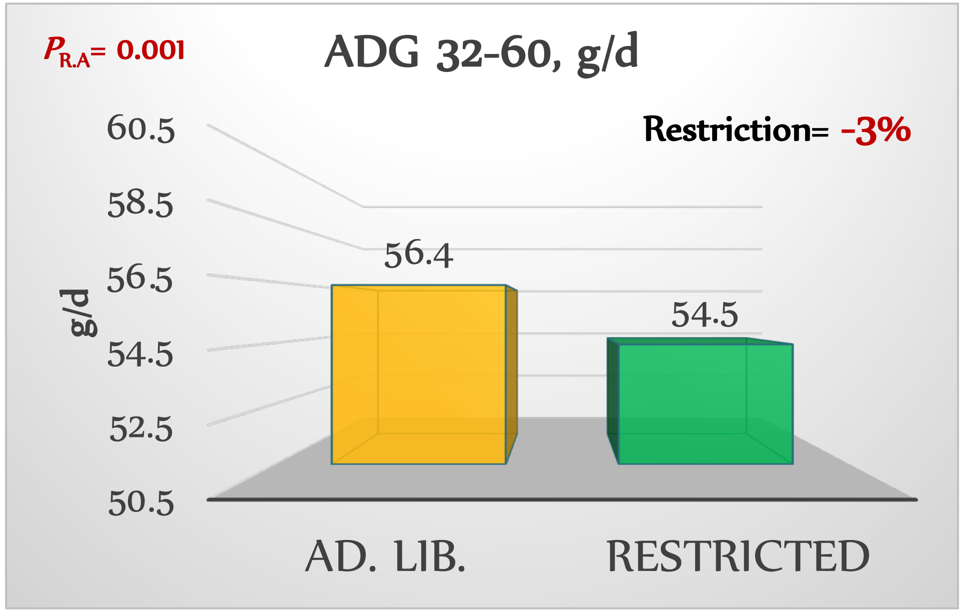

Mortalty 47-60d, %
$P_{R:A}$< 0,041
10
5
0
%
8.41
2.51
AD. LIB.
RESTRICTED

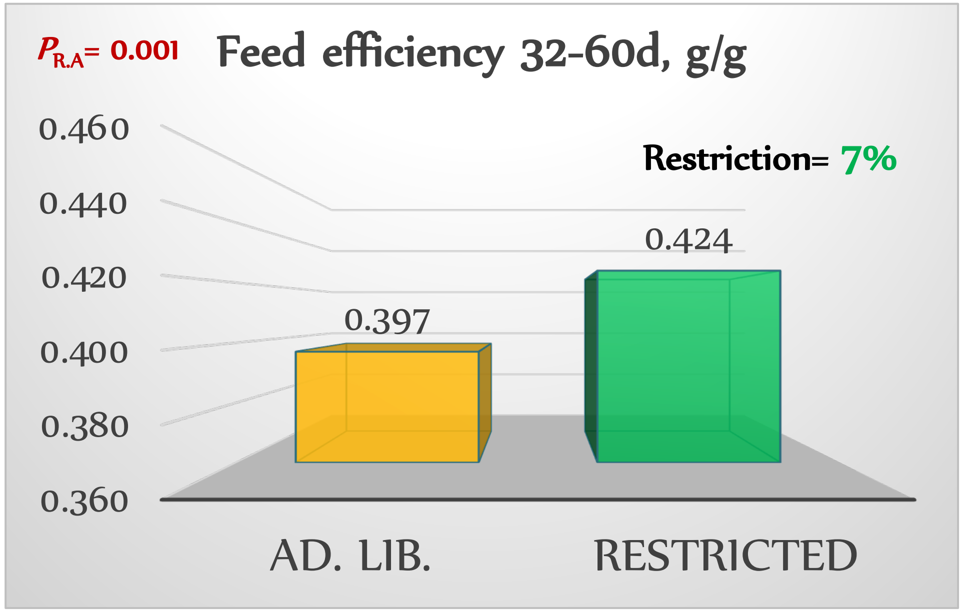

Mortality32-60d
$P_{R:A}$< 0,067
20
10
0
%
18.6
10.1
AD. LIB.
RESTRICTED

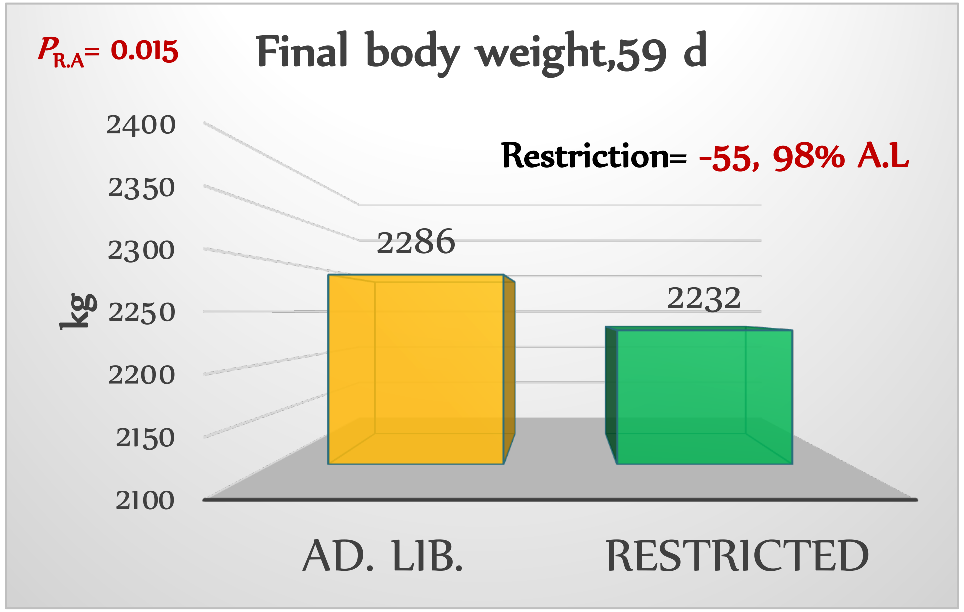


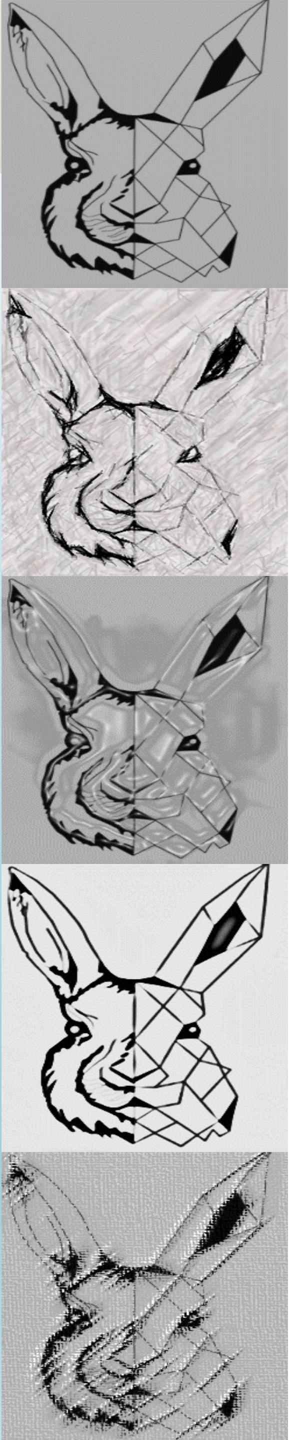
RESULTS


$P_{R.A}$= 0.001
ADFI 32-60, g/d
Restriction= -90%
g/d
150
145
140
135
130
125
120
115
142.5
128.0
AD. LIB.
RESTRICTED


$P_{R.A}$= 0.001
ADG 32-60, g/d
Restriction= -3%
g/d
60.5
58.5
56.5
54.5
52.5
50.5
56.4
54.5
AD. LIB.
RESTRICTED


$P_{R.A}$= 0.001
Feed efficiency 32-60d, g/g
Restriction= 7%
0.460
0.440
0.420
0.400
0.380
0.360
0.397
0.424
AD. LIB.
RESTRICTED


$P_{R.A}$= 0.015
Final body weight,59 d
Restriction= -55, 98% A.L
kg
2400
2350
2300
2250
2200
2150
2100
2286
2232
AD. LIB.
RESTRICTED



CONCLUSIONS

Cellobiose

N.S effect
Mortality

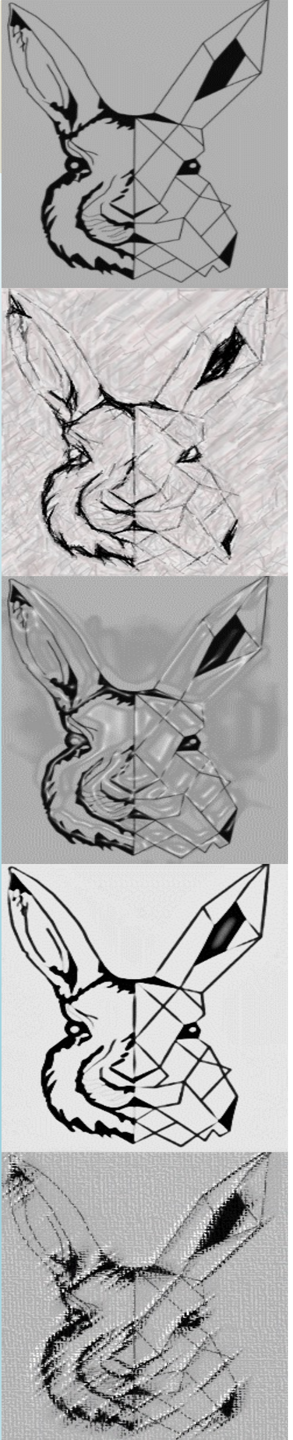
Feed restriction

$\downarrow$ Mortality

$\uparrow$ Feed effciency

Interation

No effect



Thank you.

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POLITÉCNICA

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