UNIVERSITÀ DEGLI STUDI DI MILANO

Feed design applying circular economy principles: the case of former food products

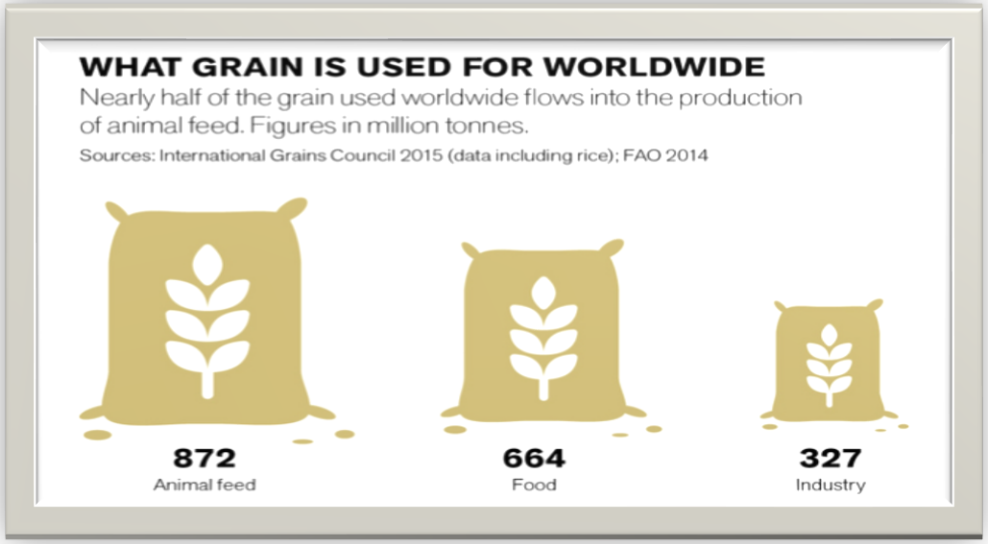
L. Pinotti, M. Tretola, A. Luciano and M. Ottoboni

[1] *Dipartimento di Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare "Carlo Cantoni", University of Milano, Italy*

Do more with less!

- Today agriculture is faced with a wide range of complex challenges.
- The task is to meet the growing demand for food, feed, fibre, fuel, and industrial products using fewer resources.
- We need to find alternative protein/energy sources.....

i.e. non-conventional feed ingredients like former food products (FFPs)



International Grains Council 2015. FAO

Pinotti et al., 2019

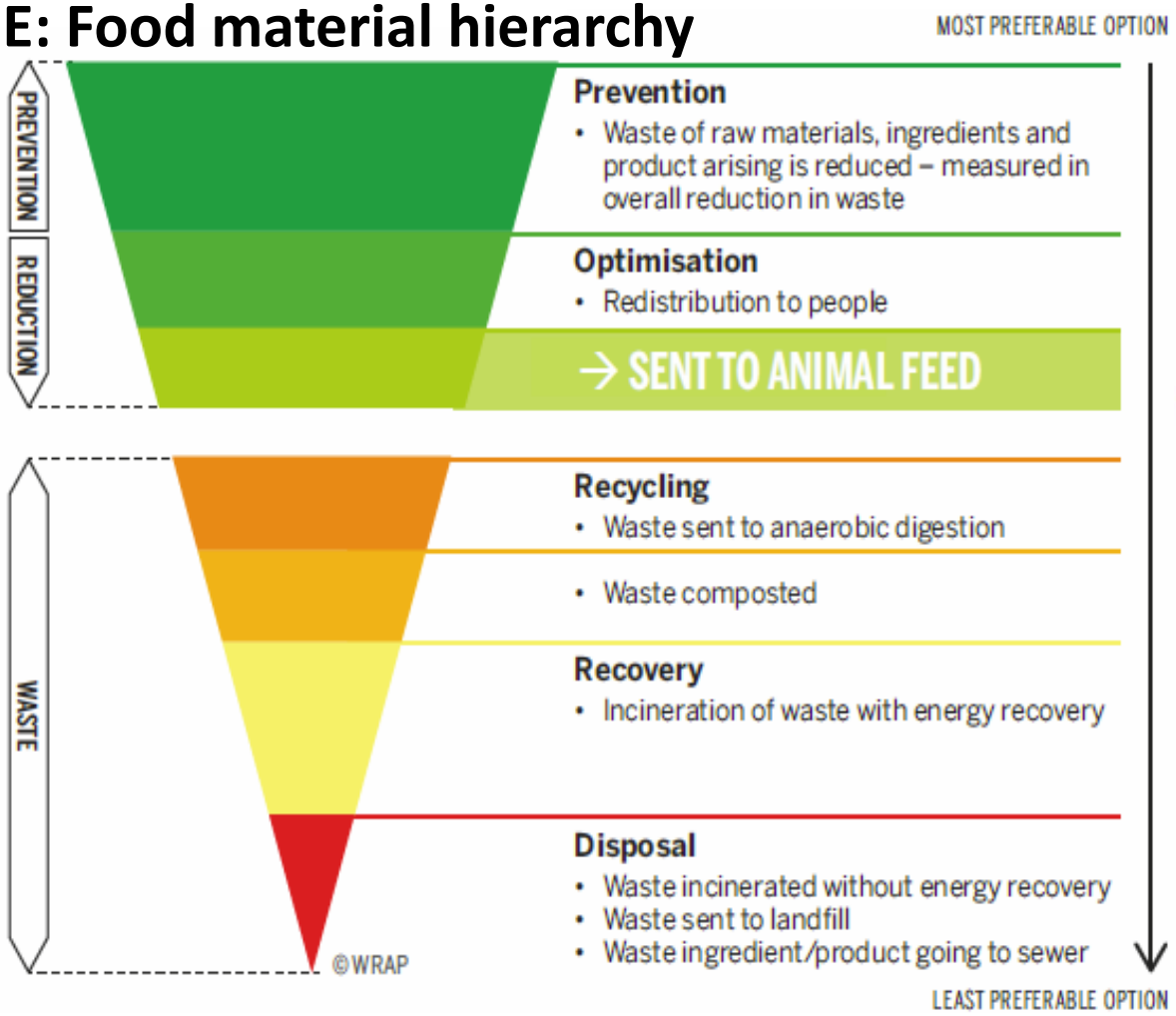
WHAT ARE FORMER FOODSTUFFS PRODUCTS?

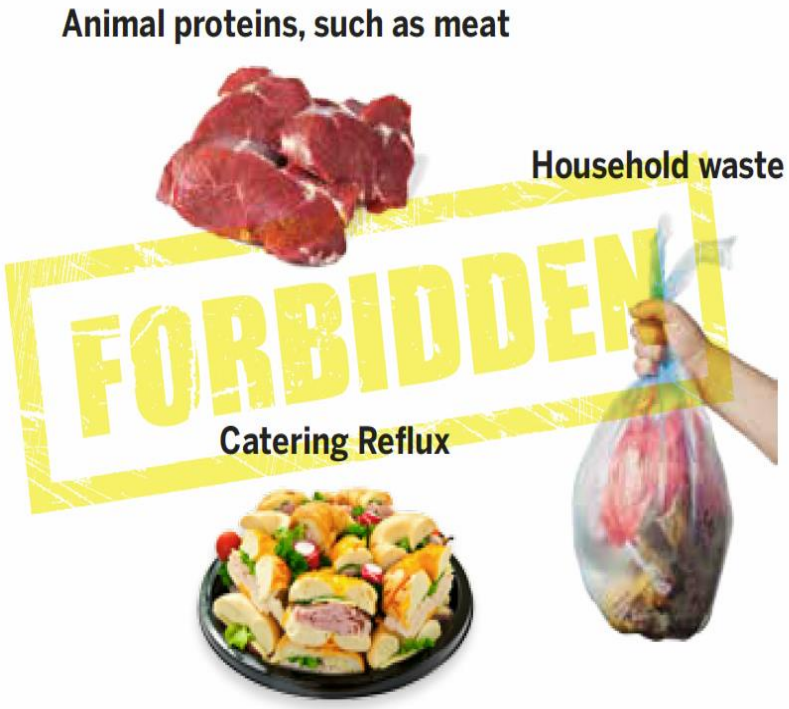
- Foodstuffs manufactured for human consumption
- In full compliance with the EU food law
- No longer intended for human consumption for practical or logistical reasons (problems of manufacturing or packaging defects or other defects)

Regulation (EC) No 1069/2009
Regulation EC 68/2013

WHAT ARE NOT FORMER FOODSTUFFS PRODUCTS?

UE: Food material hierarchy





An exception for these animal proteins is made

- ☑ milk
- ☑ eggs
- ☑ honey
- ☑ non-ruminant gelatin

EU Regulation 1069/2009
DIRECTIVE (EU) 2018/851

FFPs/ex-food

- Typical former foodstuffs are represented by leftover of the food industry such as biscuits, bread, breakfast cereals, chocolate bars, pasta, savoury snacks and sweets
- Different than Ecofeed in Japan!!!

Nutritional Evaluation

- Chemical composition
- *in vitro* digestibility
- Glycemic and Hydrolisis indexes

Safety Evaluation

- Microbiological quality
- Presence of packaging remnants

Functional Evaluation

- Effects on piglet's growth performance
- *in vivo* digestibility
- Effects on piglet's gut microbiota

FFPS NUTRITIONAL COMPOSITION

Food Additives & Contaminants: Part A

ISSN: 1944-0049 (Print) 1944-0057 (Online) Journal homepage: http://www.tandfonline.com/loi/tfac20

Nutritional evaluation of former food products (ex-food) intended for pig nutrition

C. Giromini, M. Ottoboni, M. Tretola, D. Marchis, D. Gottardo, V. Caprarulo, A. Baldi & L. Pinotti

ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 838–849
https://doi.org/10.1080/1828051X.2019.1596758

Taylor & Francis
Taylor & Francis Group

PAPER

OPEN ACCESS Check for updates

Carbohydrate digestion and predicted glycemic index of bakery/confectionary ex-food intended for pig nutrition

Matteo Ottoboni[a], Marco Tretola[a], Alice Luciano[a], Gianluca Giuberti[b], Antonio Gallo[c] and Luciano Pinotti[a]

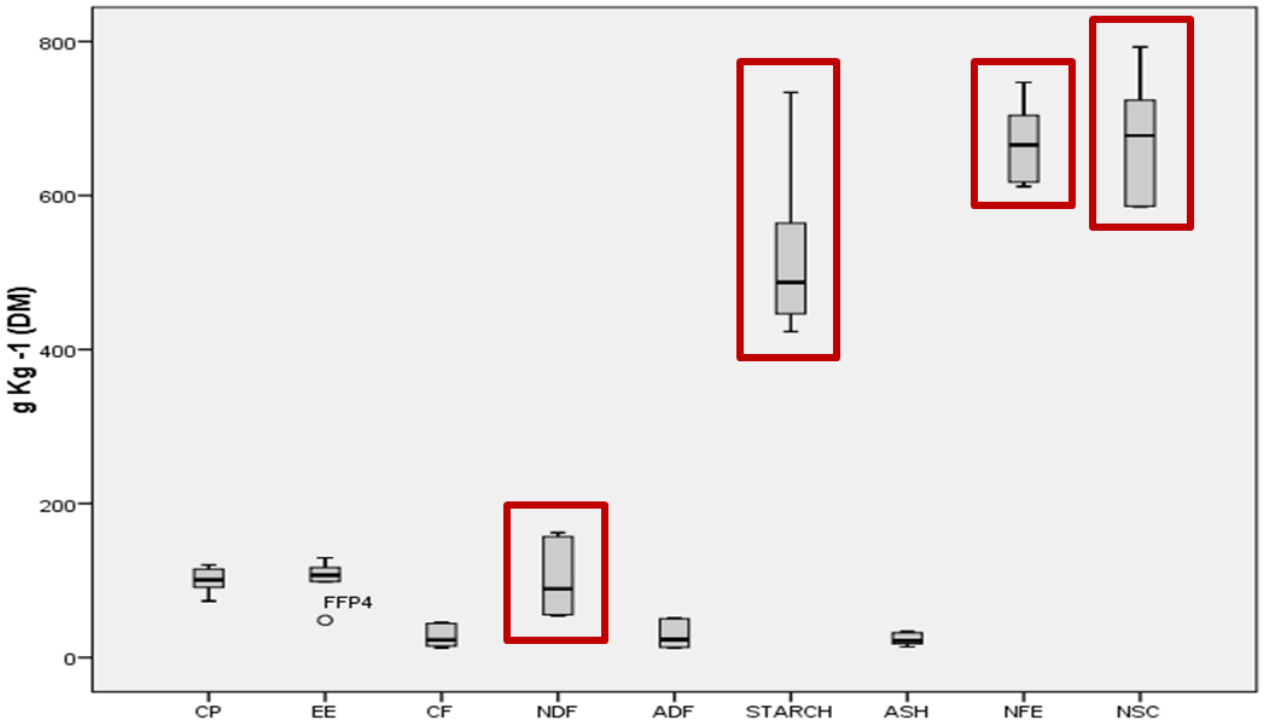
Animal (2019), 13:7, pp 1365–1375 © The Animal Consortium 2019
doi:10.1017/S1751731118003622

animal

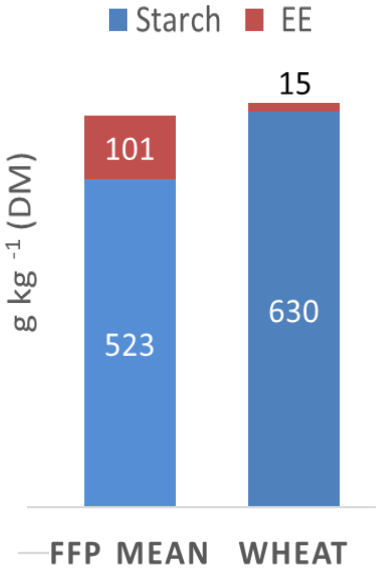
Review: Insects and former foodstuffs for upgrading food waste biomasses/streams to feed ingredients for farm animals

L. Pinotti[1†], C. Giromini[1], M. Ottoboni[1], M. Tretola[1] and D. Marchis[2]

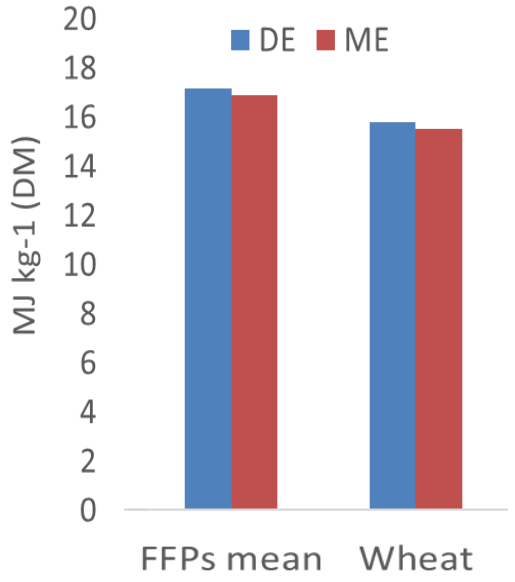
FFPS NUTRITIONAL COMPOSITION



Fats 6 times higher than cereals



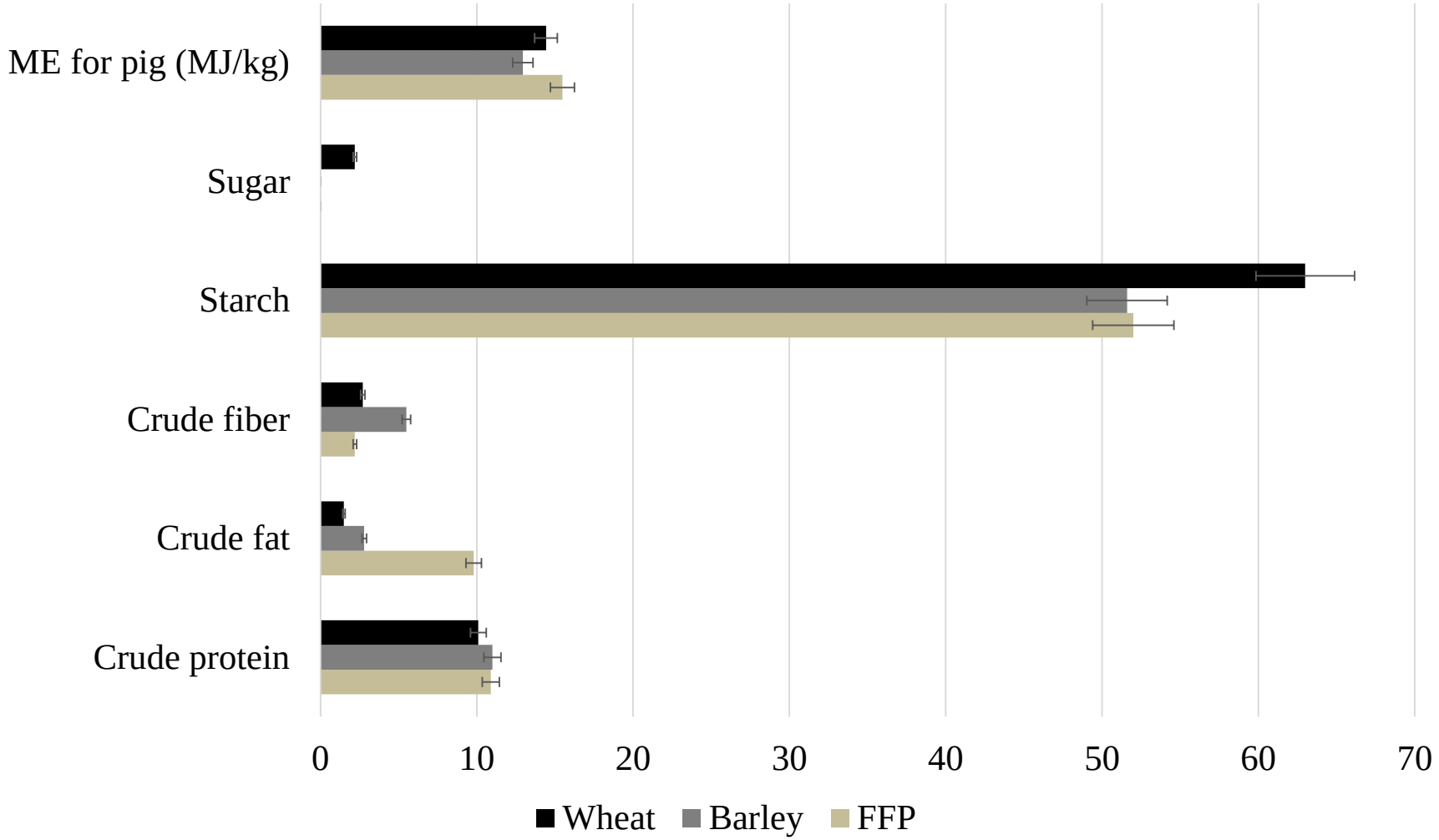
FFPS Energy Evaluation



FFPs mean **DE**: 4175,155 (kcal/kg DM)
FFPs mean **ME**: 4107,078 (kcal/kg DM)

UNIVERSITÀ DEGLI STUDI DI MILANO

In comparison with cereals

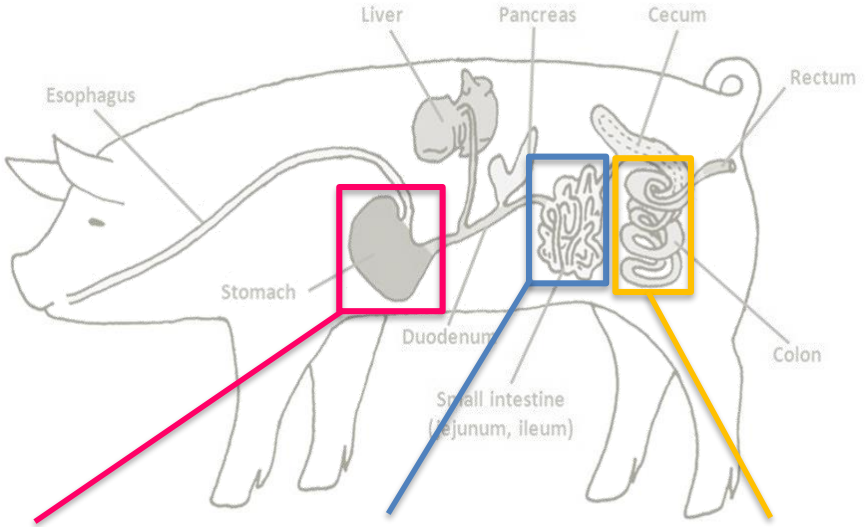


IN-VITRO DIGESTIBILITY

METHODS

$$IVD\ (\%\ DM) = \frac{(sample\ \ DM - sample\ \ UF\ DM)}{sample\ \ DM} * 100$$

Nutritional Evaluation



FILTRATION

DM determination on residue

WEIGHING & MILLING → **PEPSIN** → **PANCREATIN** → **VISCOZYME** → **PROTEIN SOLUBILIZATION**

0.5g ± 0.1 FFPs (milled 1-mm) | 25mg Pepsin pH 2, 39°C, 2h | 100mg Pancreatin pH 6.8, 4h | 0.5mL Viscozyme pH 4.8, 18h | Sulphosalicylic acid

Adapted from Boisen & Fernández (1997).
https://doi.org/10.1016/S0377-8401(97)00058-8

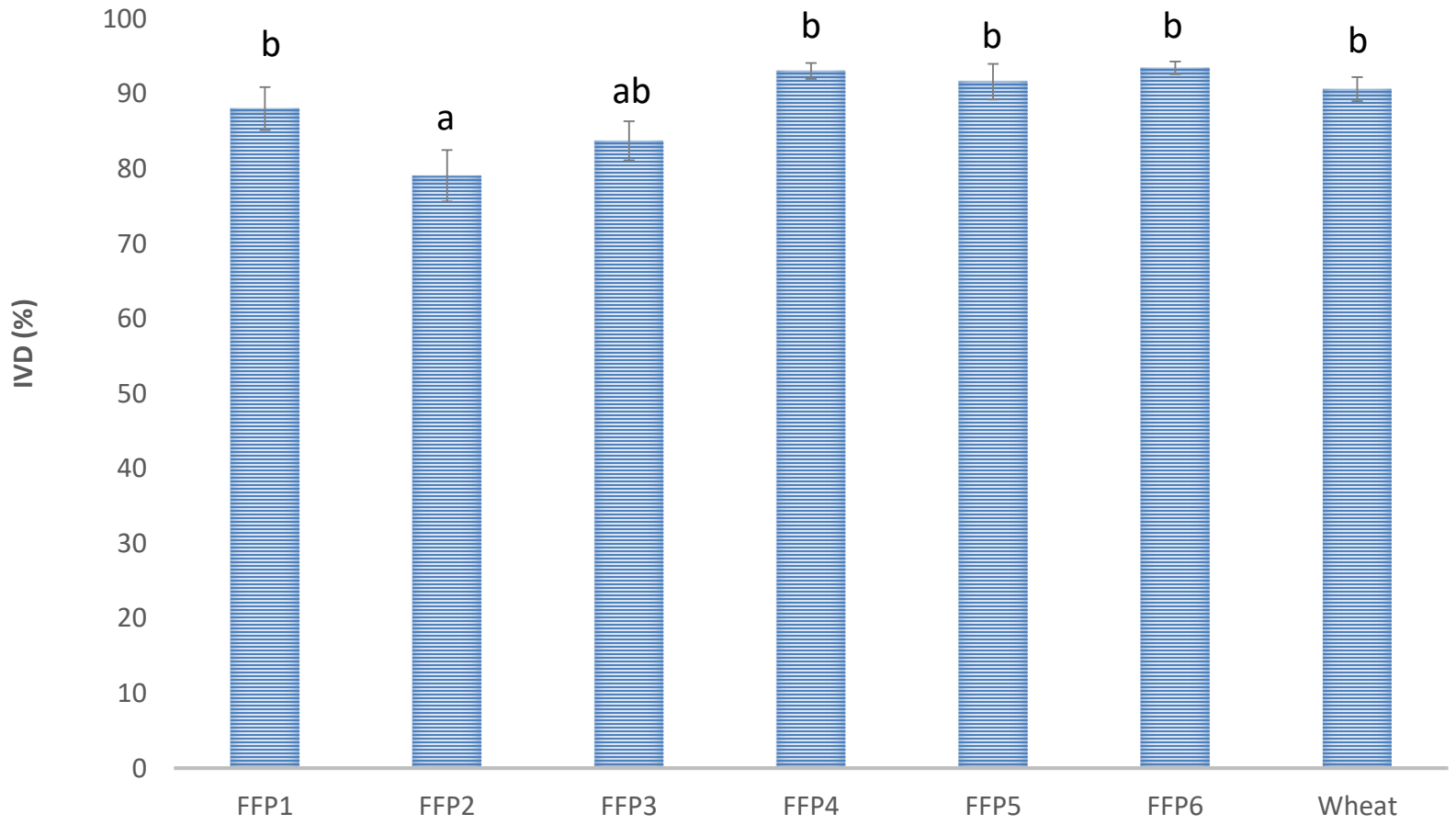
UNIVERSITÀ DEGLI STUDI DI MILANO

IN-VITRO DIGESTIBILITY

RESULTS

Nutritional Evaluation

In vitro Digestibility (IVD, %DM) of FFPs samples from three independent experiments and presented as least square means ± SEM. Samples identified with different letters are significantly different ($p < 0,05$)



UNIVERSITÀ DEGLI STUDI DI MILANO

Carbohydrates sources can affect FI

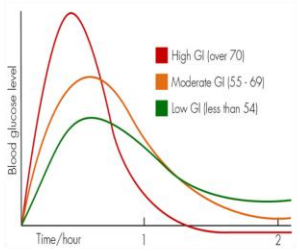
FEED INTAKE

GLYCEMIC INDEX

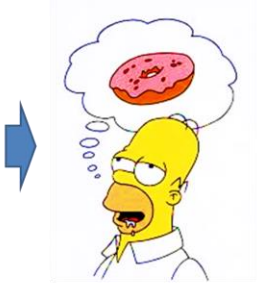
CARBOHYDRATES DIGESTIBILITY

CEREAL SOURCE

MEAL



DIGESTION



RETURN IN HUNGER STATE

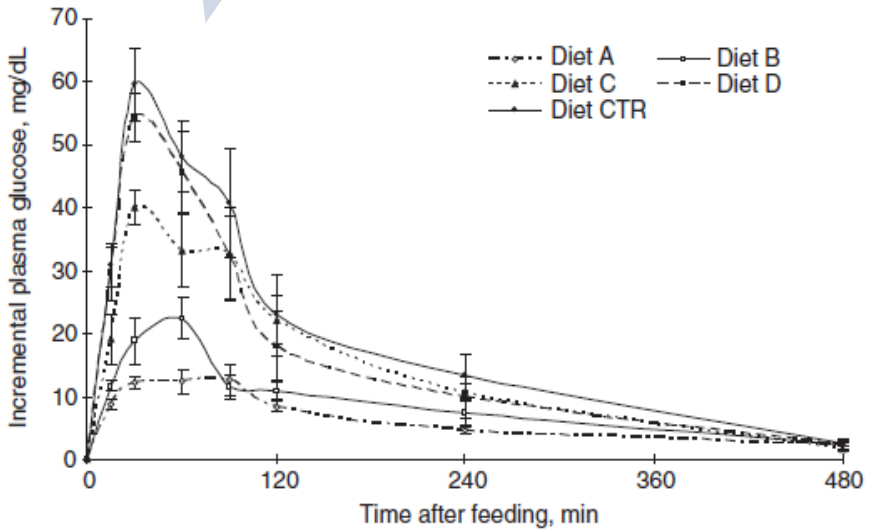
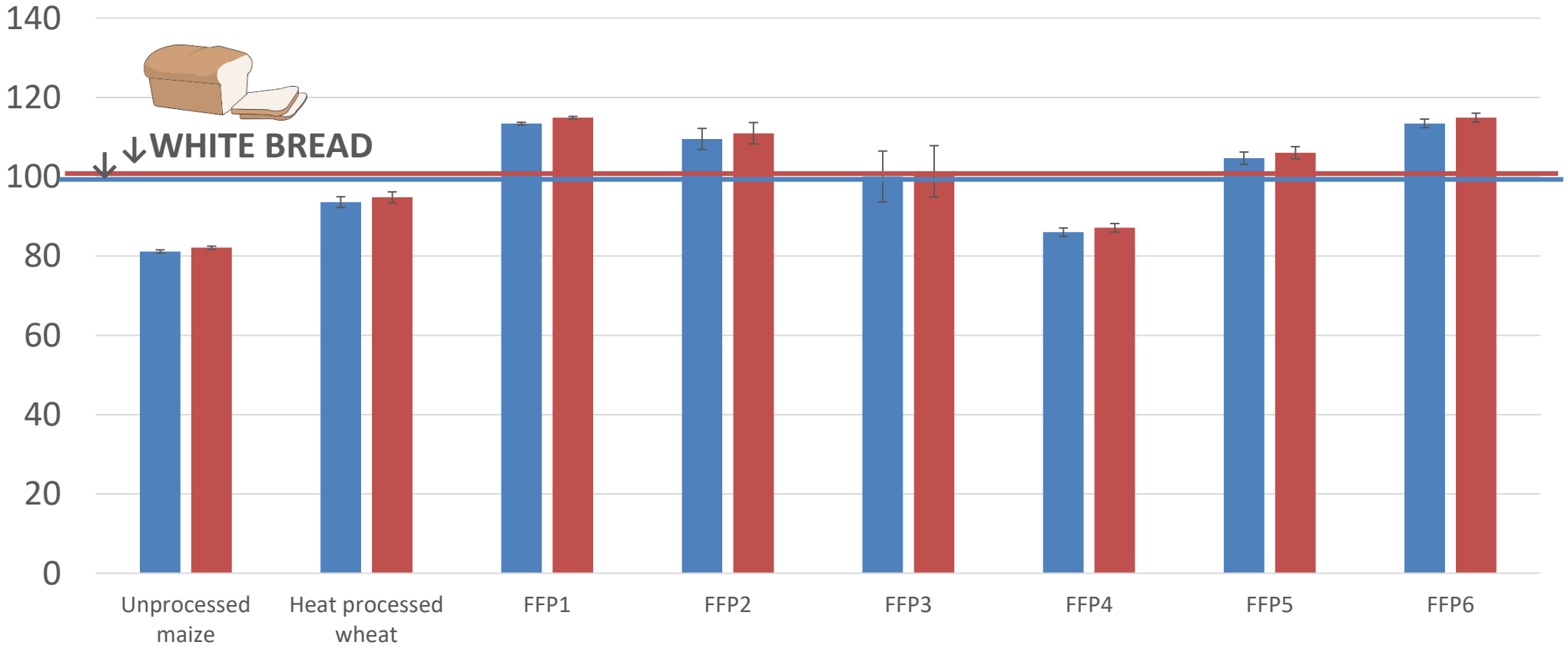


Figure 2 Effect of the five experimental diets on the incremental postprandial plasma glucose concentration in jugular-catheterized pigs. Values are means ($n = 5 \pm$ s.e.). CTR = corresponding area for the reference diet.

(van Kempen 2007; Menoyo et al. 2011; **Giuberti et al., 2012a;** Giuberti et al., 2012b; Doti et al., 2014;

Hydrolysis index & predicted Glycemic Index

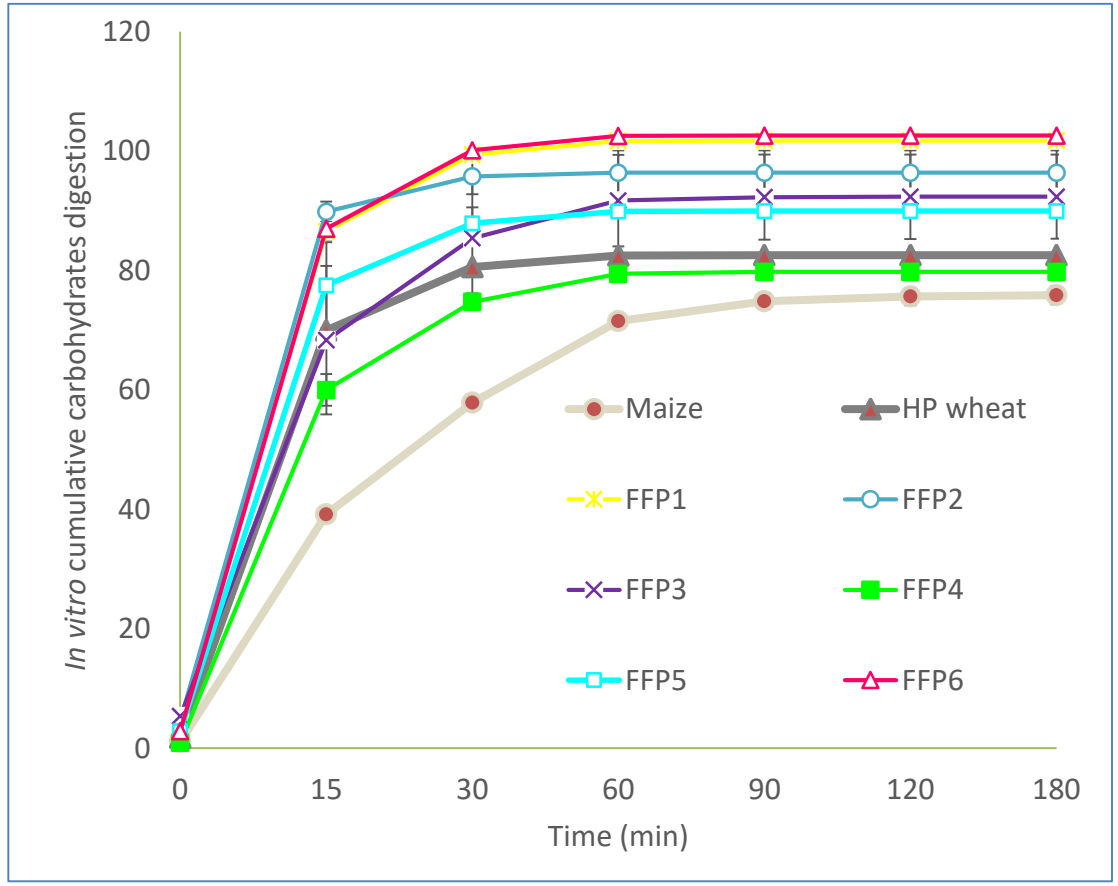


Ottoboni et al., 2019

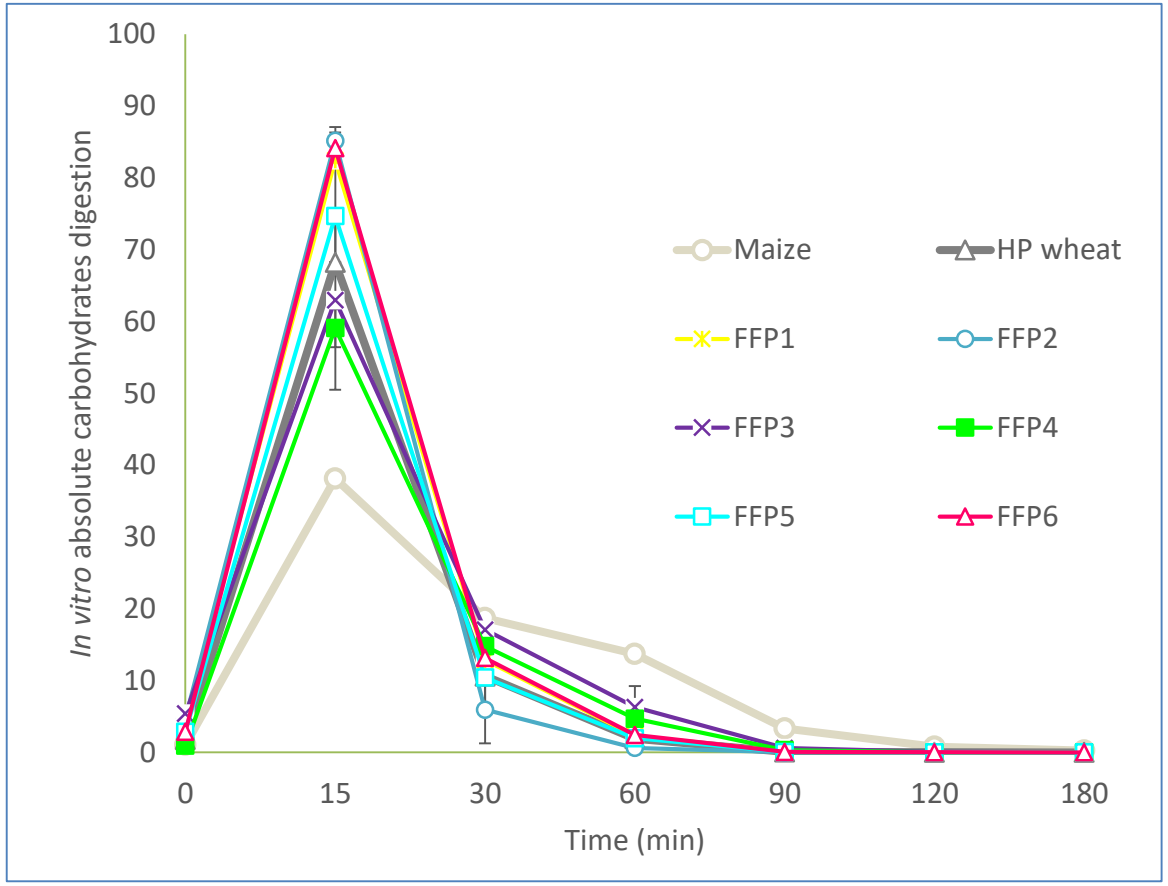
ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 838–849
https://doi.org/10.1080/1828051X.2019.1596758

Results: glucose release over the time

Cumulative



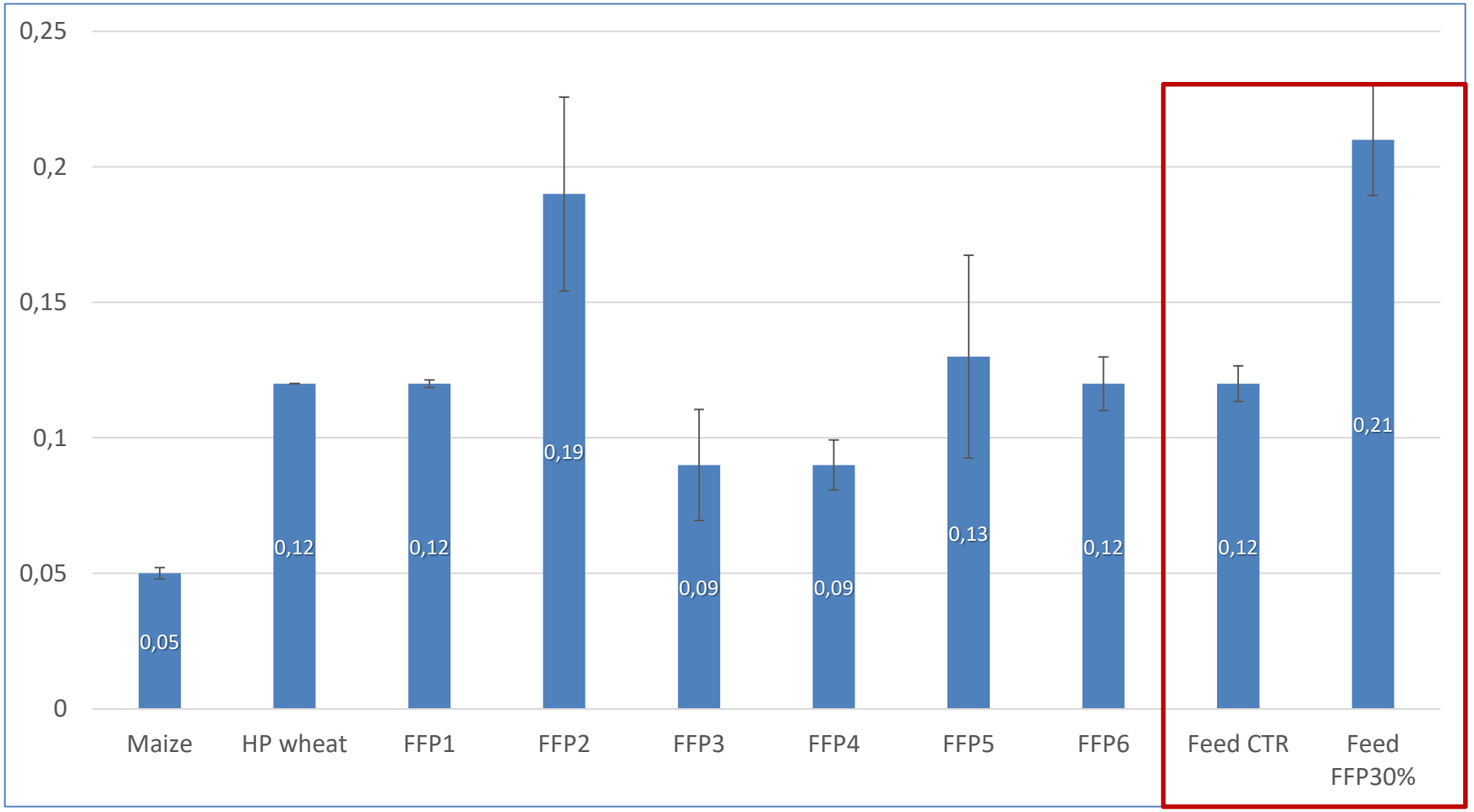
Absolute



Ottoboni et al., 2019

ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 838–849
https://doi.org/10.1080/1828051X.2019.1596758

k – rate of carbohydrates digestion (rate/min)



Ottoboni et al., 2019

ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 838–849
https://doi.org/10.1080/1828051X.2019.1596758

Summary : 1/4
(FFP as ingredient)

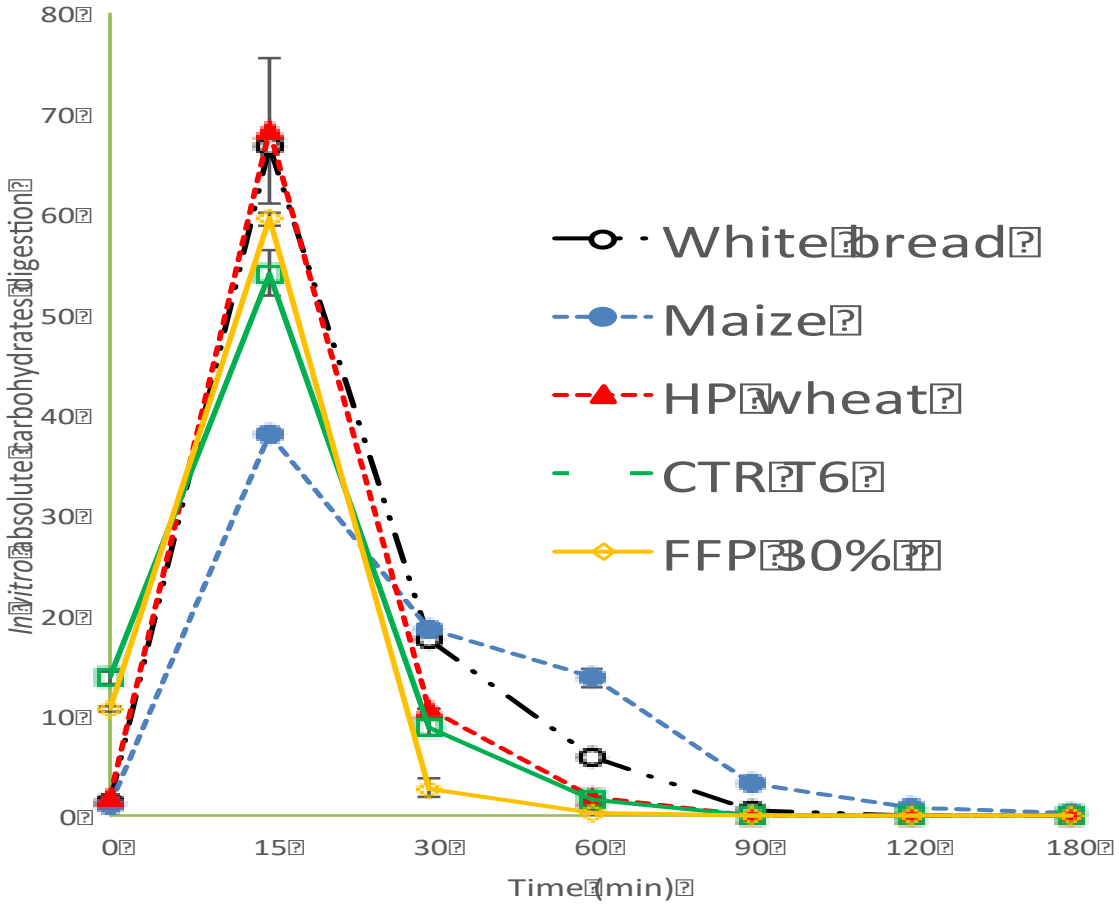
- FFPs are Nutritious novel feed
- High digestible
- Energy dense feedstuffs (high starch+Adequate fat content)in line with Liu et al. 2018 (J. Anim. Sci. 2018.96:4685–4692)
- Indexes like pGI and HI indicates
  - –FFPs are Rich in “ready to use” carbohydrates,

Summary : 2/4
(FFP in a pig compound feed)

CTR vs. FFPs

-At similar pGI

-*K* – **rate of carbohydrates** digestion has been **increased by 75%** in the FFP diet, indicating a quick digestion potential of the carbohydrate fraction



Nutritional Evaluation

- Chemical composition
- *in vitro* digestibility
- Glycemic and Hydrolisis indexes

Safety Evaluation

- Microbiological quality
- Presence of packaging remnants

Functional Evaluation

- Effects on piglet's growth performance
- *in vivo* digestibility
- Effects on piglet's gut microbiota

Safety Evaluation

MICROBIOLOGICAL QUALITY OF FFPS

	Contaminant/threshold limit (log CFU g^{-1})									
Sample	TVC/6[a]	Enterobacteriaceae/10[b]	*Escherichia coli*/2[a]	Coagulase-positive Staphylococci/4[a]	*Bacillus cereus*/5[a]	*B. cereus* spores/5[a]	Clostridia/4[a]	Yeasts/7[b]	Moulds/7[b]	*Salmonella* spp./absent[b]
FFP1	4.98	3.65	< 2	< 2	3.20	< 2	< 1	4.38	3.40	Absent
FFP2	5.11	3.67	< 2	2	2.00	< 2	1.70	4.12	3.26	Absent
FFP3	4.90	< 2	< 2	< 2	2.00	2	1	3.00	3.88	Absent
FFP4	4.81	4.20	< 2	< 2	3.86	3.70	< 1	4.21	3.30	Absent
FFP5	4.51	3.81	< 2	< 2	3.08	2	< 1	4.60	3.40	Absent
FFP6	5.20	2.70	< 2	< 2	2.90	< 2	< 1	3.88	2.60	Absent

Sources: [a]Health Protection Agency (2009).
[b]Regulation (EC) No. 142/2011.

Hindawi
Journal of Food Quality
Volume 2017, Article ID 1064580, 6 pages
https://doi.org/10.1155/2017/1064580

WILEY | Hindawi

Review Article

Former Food Products Safety Evaluation: Computer Vision as an Innovative Approach for the Packaging Remnants Detection

Marco Tretola,[1] Matteo Ottoboni,[1] Ambra Rita Di Rosa,[2]
Carlotta Giromini,[1] Eleonora Fusi,[1] Raffaella Rebucci,[1] Francesco Leone,[2]
Vittorio Dell'Orto,[1] Vincenzo Chiofalo,[2] and Luciano Pinotti[1]

UNIVERSITÀ DEGLI STUDI DI MILANO

PACKAGING REMNANTS

Safety Evaluation

Paper, Plastic, Aluminium

Bar in lower left corner is 1 cm

European Union Member States

"a zero tolerance for these traces is neither practical, nor proportionate to the risk"

German authorities tolerate the presence of packaging up to a level of **0.15%**

L. v. Raamsdonk, et al., 2011. RIKILT Report 2011.002

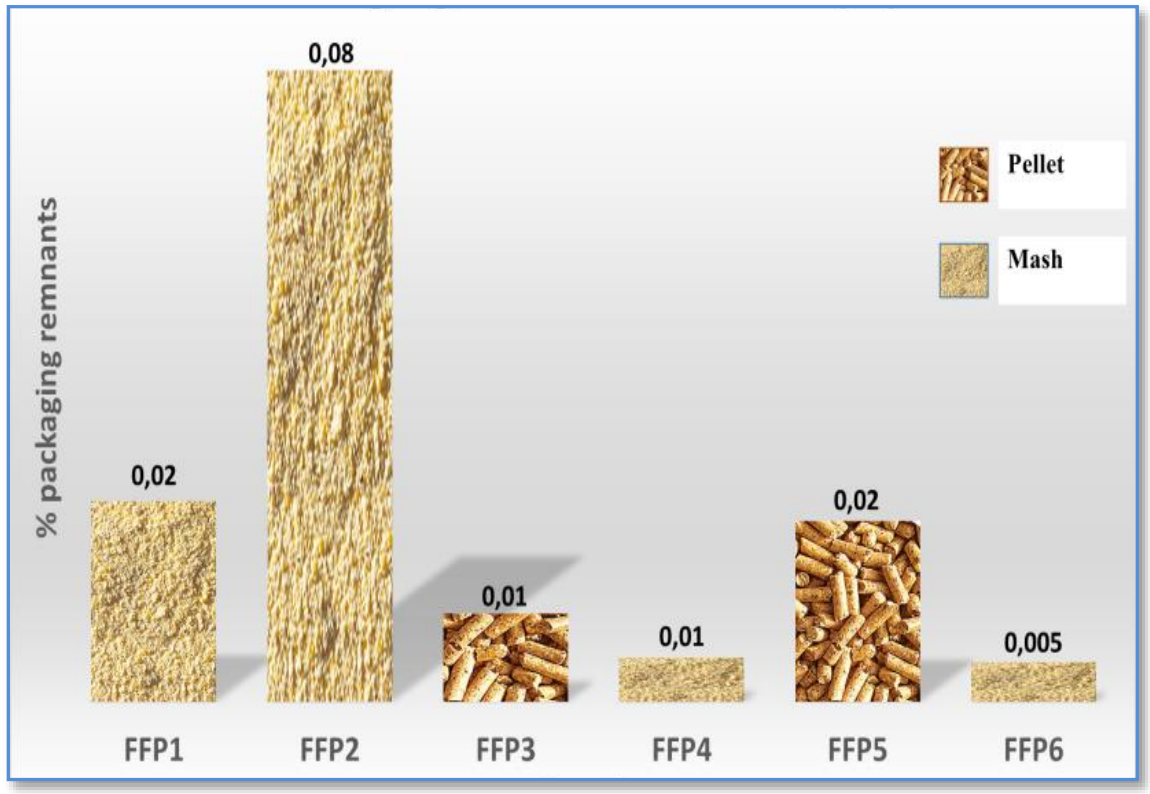
UNIVERSITÀ DEGLI STUDI DI MILANO

PACKAGING REMNANTS DETECTION

RESULTS (VALIDATED METHOD)

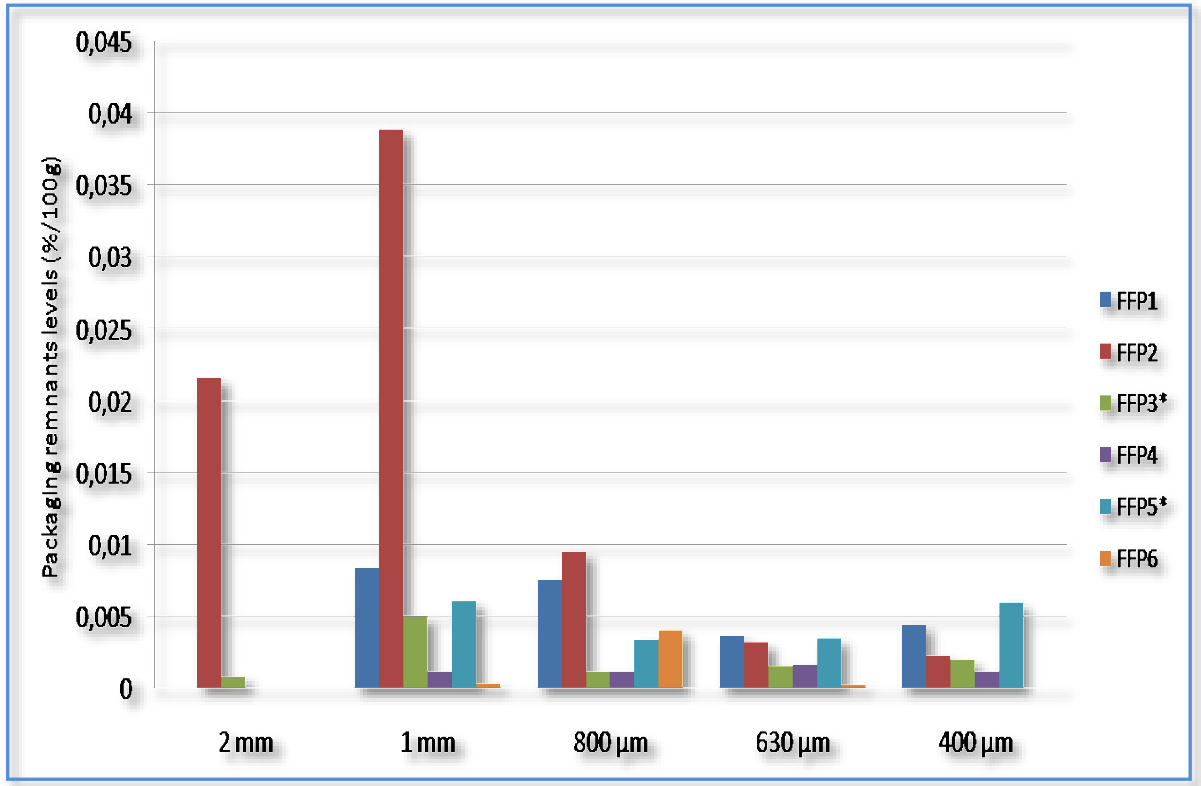
Safety Evaluation

Packaging Remnants Levels (%)



Samples

Packaging Remnants Levels distribution



Sieve mesh fractions

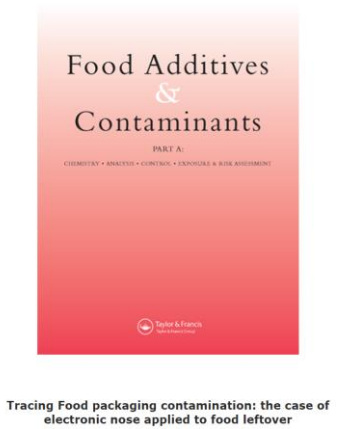
Tretola et al., 2017

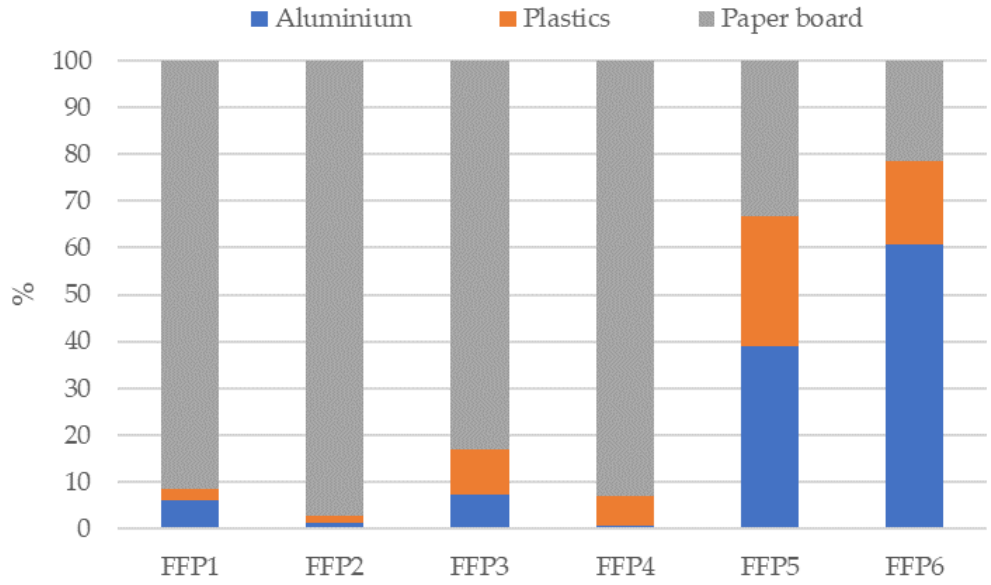
UNIVERSITÀ DEGLI STUDI DI MILANO

Tracing Food packaging remnants by electronic nose

Safety Evaluation

The packaging quantification ranged from a minimum of 0.017% w/w to a maximum of 0.214 % w/w. Paperboard was the most detected contaminant in all the samples from FFP processor 1 (from FFP1 to FFP4), while plastics were often the least abundant. (Tretola et al. 2019).





Tretola et al., 2019 in press

Cleaned samples

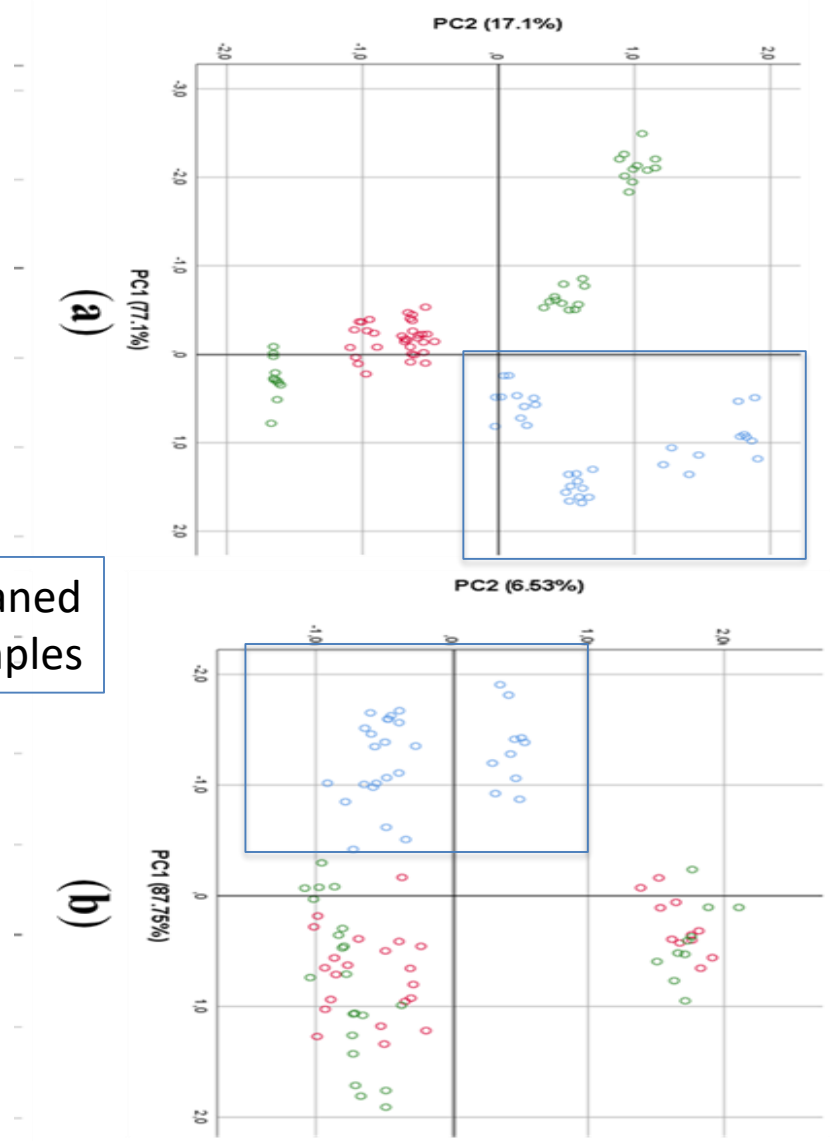
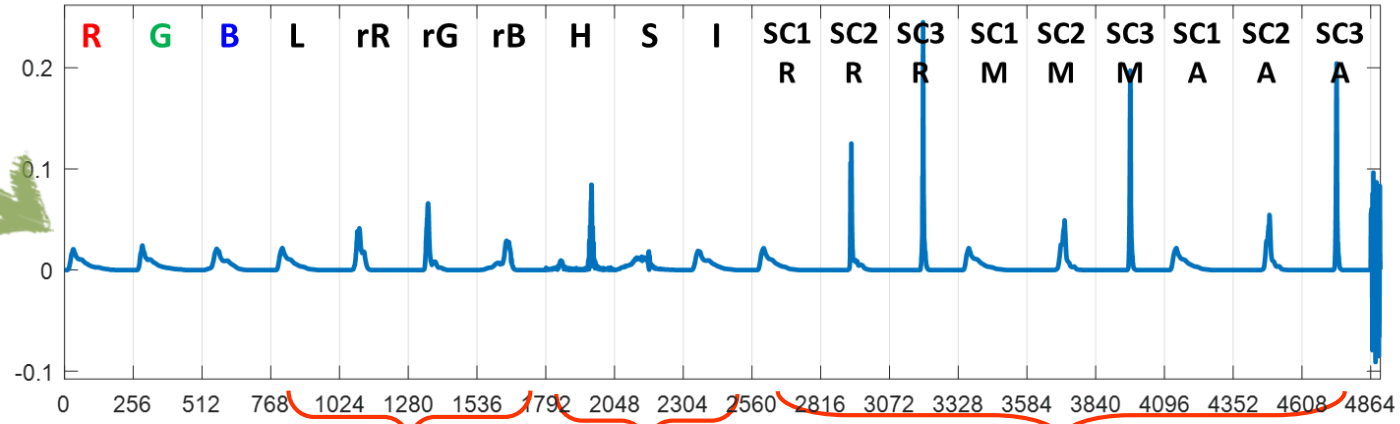
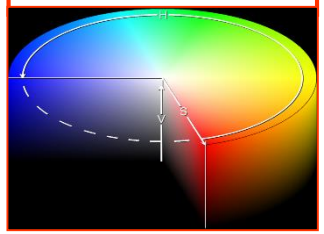


IMAGE ANALYSIS- COLORGRAM

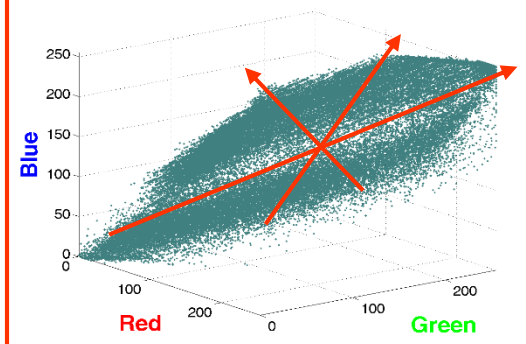


Lightness (L) = R + G + B
Relative red (rR) = R / L
Relative green (rG) = G / L
Relative blue (rB) = B / L

Hue
Saturation
Value
colour space

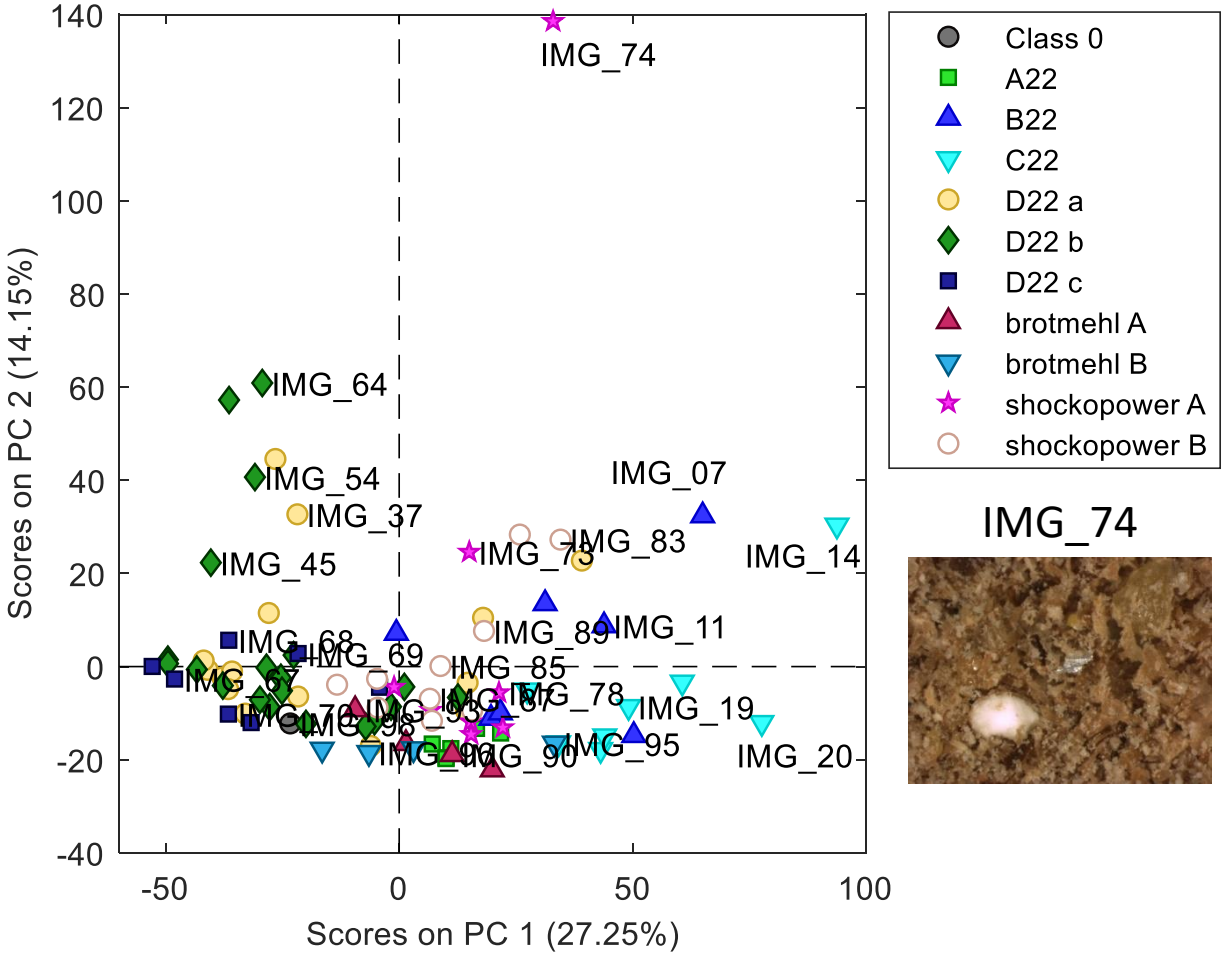


PCA of RGB matrix

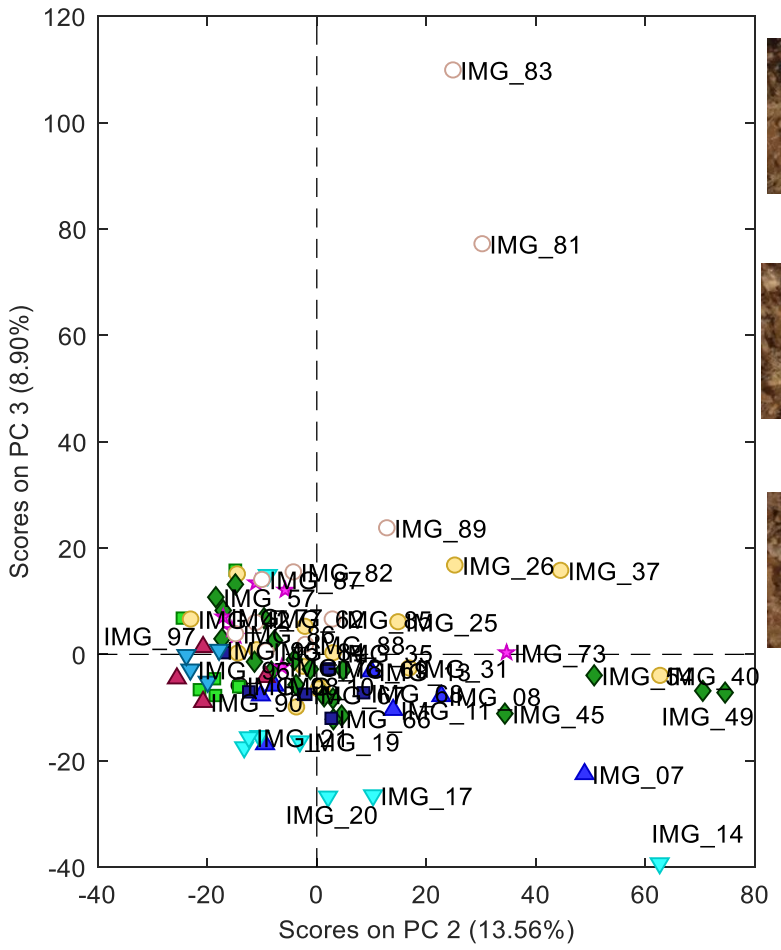


Ulrici & Pinotti, subm.

RESULTS



IMG_74



IMG_83

IMG_81

IMG_89

Ulrici & Pinotti, subm.

Results - Advantages and drawbacks of existing and innovative methods

Safety Evaluation

Method	Advantages	Drawbacks
Stereomicroscopy	-Quantification -Evaluation of heterogeneous distribution - Determination of packaging remnants nature	-Underestimation -Laborious/time consuming -Operator-dependent
Computer Vision	-Rapidity -Objectivity -Sensibility -Remote sample image analysis	-No quantification -No determination of packaging remnants nature
Eletronic nose	-method promising ability to discriminate experimentally cleaned samples from the standard and spiked samples	-necessary to clarify the nature of the VOCs released by the packaging remnants

UNIVERSITÀ DEGLI STUDI DI MILANO

Summary : 3/4

Safety Evaluation

Safety

FFPs showed:

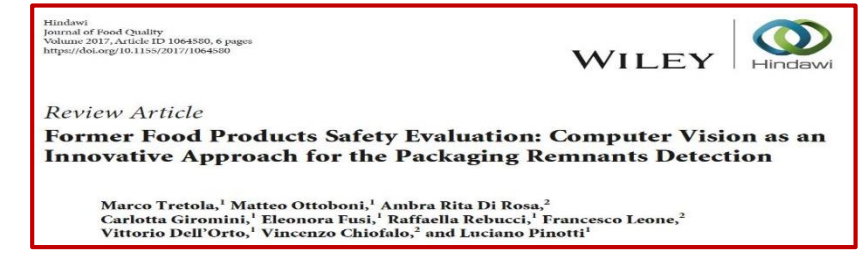
- limited microbial load that can be improved/ salmonella absence;
- limited contamination by packaging remnants.
- High safety standards


Food Additives & Contaminants: Part A

ISSN: 1944-0049 (Print) 1944-0057 (Online) Journal homepage: http://www.tandfonline.com/loi/tfac20

Former food products safety: microbiological quality and computer vision evaluation of packaging remnants contamination

M Tretola, A. R Di Rosa, E Tirloni, M Ottoboni, C Giromini, F Leone, C. E. M. Bernardi, V Dell'Orto, V Chiofalo & L Pinotti


Hindawi
Journal of Food Quality
Volume 2017, Article ID 1064580, 6 pages
https://doi.org/10.1155/2017/1064580

WILEY | Hindawi

Review Article

Former Food Products Safety Evaluation: Computer Vision as an Innovative Approach for the Packaging Remnants Detection

Marco Tretola,[1] Matteo Ottoboni,[1] Ambra Rita Di Rosa,[2] Carlotta Giromini,[1] Eleonora Fusi,[1] Raffaella Rebucci,[1] Francesco Leone,[2] Vittorio Dell'Orto,[1] Vincenzo Chiofalo,[2] and Luciano Pinotti[1]


Food Additives & Contaminants: Part A

ISSN: 1944-0049 (Print) 1944-0057 (Online) Journal homepage: http://www.tandfonline.com/loi/tfac20

Gravimetric quantitative determination of packaging residues in feed from former food

Giuseppina Amato, Rosanna Desiato, Tiziana Giovannini, Luciano Pinotti , Marco Tretola , Marilena Gili & Daniela Marchis


FOOD ADDITIVES & CONTAMINANTS: PART A
https://doi.org/10.1080/19440049.2019.1653498

Taylor & Francis
Taylor & Francis Group

Check for updates

Tracing food packaging contamination: an electronic nose applied to leftover food

Marco Tretola [a*], Matteo Ottoboni [a], Alice Luciano[a], Vittorio Dell'Orto [a], Federica Cheli [a] and Luciano Pinotti [a]

UNIVERSITÀ DEGLI STUDI DI MILANO

Nutritional Evaluation

- Chemical composition
- *in vitro* digestibility
- Glycemic and Hydrolisis indexes

Safety Evaluation

- Microbiological quality
- Presence of packaging remnants

Functional Evaluation

- Effects on piglet's growth performance
- *in vivo* digestibility
- Effects on piglet's gut microbiota

AIMS

Pig performance
Gut microbiota

Increase knowledge > Increase use > Increase sustainability

Partially replacing common cereal grains with 30% of FFPs in post weaning diets to evaluate effects on:

Feed Intake

Growth Performance

Feed Efficiency

ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 987–996
https://doi.org/10.1080/1828051X.2019.1607784

Taylor & Francis
Taylor & Francis Group

PAPER

OPEN ACCESS Check for updates

Former food products have no detrimental effects on diet digestibility, growth performance and selected plasma variables in post-weaning piglets

Marco Tretola, Matteo Ottoboni, Alice Luciano, Luciana Rossi, Antonella Baldi and Luciano Pinotti

animals

MDPI

Article

Influence of Traditional vs Alternative Dietary Carbohydrates Sources on the Large Intestinal Microbiota in Post-Weaning Piglets

Marco Tretola *, Alice Luciano, Matteo Ottoboni, Antonella Baldi and Luciano Pinotti

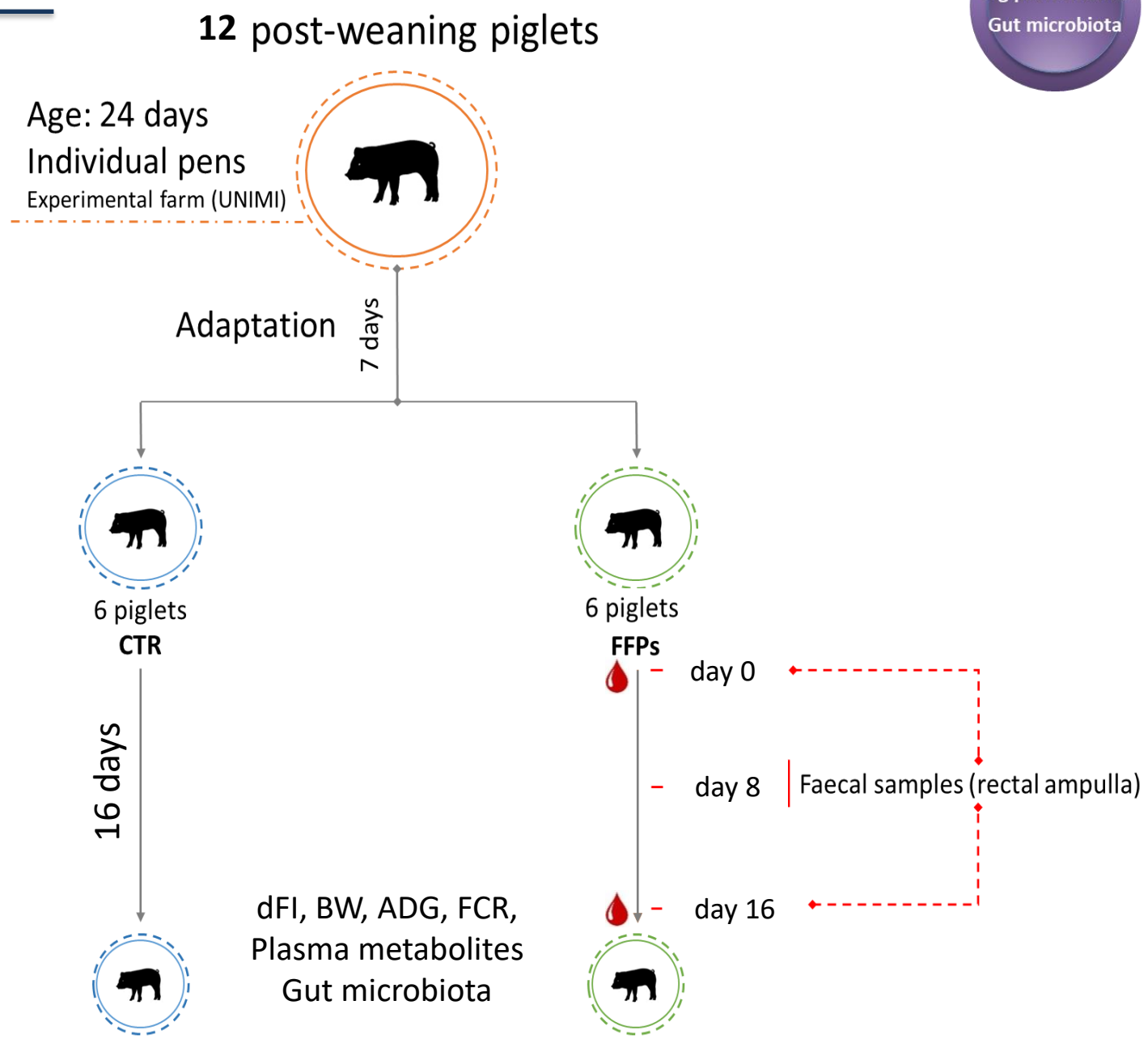
Faecal gut microbiota composition

UNIVERSITÀ DEGLI STUDI DI MILANO

MATHERIALS & METHODS

EXPERIMENTAL DESIGN

Pig performance
Gut microbiota



All pigs had *ad libitum* access to the feed and water.

Tretola et al., 2019 ITALIAN JOURNAL OF ANIMAL SCIENCE 2019, VOL. 18, NO. 1, 987–996 https://doi.org/10.1080/1828051X.2019.1607784

UNIVERSITÀ DEGLI STUDI DI MILANO

MATHERIALS & METHODS

EXPERIMENTAL DIETS

Diets were:

- Iso-nitrogenous,
- Iso-energetics.

Pig performance
Gut microbiota

Ingredients	CTR	FFPs
Former Foodstuffs	-	30
Barley	22.8	22.1
Dextrose	5	4,5
Flaked decorticated barley	4	0
Corn	6,5	4
Flaked corn	6,5	1
Vegetable fibres	1	1
Wheat	12,33	10,1
Flaked wheat	6	1
Wheat bran	3	2,48
Vegetable oil	1,5	0,5
Soy oil	1,5	0,5
Fish meal (65% protein)	2,5	2,6
Plasma powder	3,5	3,8
Whey powder	11	4,5
Soy f.e. 50	3,5	3,5
Soycomil R	5,5	4,55
L-lysine HCl	0,55	0,55
DL-methionine	0,23	0,23
L-threonine	0,25	0,25
L-tryptophan	0,08	0,08
Vitamin-mineral premix[1]	2,76	2,76
Total	100	100

(g/100g DM)

Tretola et al., 2019 ITALIAN JOURNAL OF ANIMAL SCIENCE 2019, VOL. 18, NO. 1, 987–996 https://doi.org/10.1080/1828051X.2019.1607784

UNIVERSITÀ DEGLI STUDI DI MILANO

RESULTS: *GROWTH PERFORMANCE*

Pig performance
Gut microbiota

Item*	CTR	FFPS	P values
Initial Body Weight (kg)	9.20 ± 1.4	8.76 ± 1.8	0.68
Final Body Weight (kg)	14.1 ± 1.5	13.6 ± 1.1	0.61
Average Daily Gain (kg)	0.29 ± 0.1	0.31 ± 0.1	0.52
Average daily Feed Intake (kg)	0.45 ± 0.1	0.43 ± 0.1	0.81
Feed convertion ratio (kg/kg)	1.55^{A} ± 0.1	1.39^{B} ±0.1	**0.002**
Initial ATTD (%)	78.0 ± 1.3	81.2 ± 1.8	0.06
Final ATTD (%)	78.6^{a} ± 1.2	83.3^{b} ± 2.4	**0.02**

ATTD = Apparent total tract digestibility

*Value for each item is the mean ± SD (standard deviations)

^{A,B} Values within a row with different superscripts differ significantly at $P<0.01$.

^{a,b} Values within a row with different superscripts differ significantly at $P<0.05$.

Tretola et al., 2019 ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 987–996
https://doi.org/10.1080/1828051X.2019.1607784

UNIVERSITÀ DEGLI STUDI DI MILANO

RESULTS: *PLASMA METABOLITES*

Pig performance
Gut microbiota

Item	CTR		FFPs		P values
	d 0	d 16	d 0	d 16	
Total Proteins (g/L)	49.8 ± 4.2	51.5 ± 4.2	49.7 ± 2.7	49.6 ± 4.1	0.45
Albumin (g/L)	27.7 ± 2.3	26.9 ± 3.1	26.5 ± 2.8	24.8 ± 2.1	0.22
Globulins (g/L)	22.1 ± 4.7	24.6 ± 5.4	23.2 ± 2.4	24.7 ± 2.3	0.96
Urea (mmol/L)	2.03^{a} ± 0.9	1.58^{a} ± 0.4	**1.51^{a} ± 0.8**	**1.03^{b} ± 0.2**	**0.01**
Glucose (mmol/L)	4.82^{a} ± 0.3	5.08^{a} ± 0.7	**5.65^{a} ± 1.1**	**6.18^{b} ± 0.9**	**0.04**
Colesterol (mmol/L)	2.05 ± 0.3	2.33 ± 0.2	1.71 ± 0.4	1.97 ± 0.4	0.10

^{a,b} Values within a row with different superscripts differ significantly at $P<0.05$.

Tretola et al., 2019 ITALIAN JOURNAL OF ANIMAL SCIENCE
2019, VOL. 18, NO. 1, 987–996
https://doi.org/10.1080/1828051X.2019.1607784

UNIVERSITÀ DEGLI STUDI DI MILANO

RESULTS: *FAECAL MICROBIOTA*

Pig performance
Gut microbiota

Alpha-diversity indexes (richness and evenness)

	D0		D8		D16		P values[1]		
	CTR	FFPs	CTR	FFPs	CTR	FFPs	T	G	TxG
Shannon's	6.19±0.5^{AB}	6.39±0.4^{AB}	6.46±0.12^{A}	5.96±0.49^{B}	6.25±0.27^{A}	5.76±0.31^{B}	0.57	0.001	0.71
Chao1	549.71±53.2	556.7±92.7	603.9±43	581.2±56.3	609.3±28.6	541.3±55.7	0.67	0.10	0.55
OTUs	470.6±51.3^{ab}	489.±86.4^{ab}	529.3±37.4^{ab}	500.0±62.5^{ab}	534.1±31.7^{a}	435.1±46.6^{b}	0.78	0.03	0.16
PD-whole tree	35.3±2.82	36.5±4.1	38.1±2.45	36.4±2.62	37.8±1.9	33.2±2.6	0.70	0.10	0.28

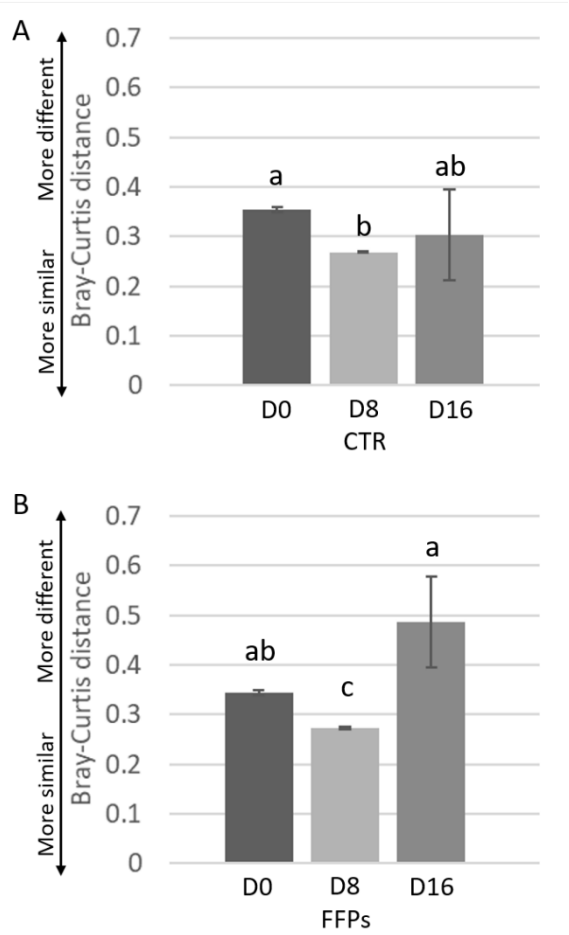
[1] Probability values for the effects of Time (T), Group (G) and T X G.; ^{a,b} Values within a row with different superscripts differ significantly at P<0.05. ^{A,B} Values within a row with different superscripts differ significantly at P<0.01.

i.e: bacterial abundance (OTU number) and its biodiversity (Shannon's index) , decreased in the post-weaning piglets fed FFPs, compared to the CTR group

UNIVERSITÀ DEGLI STUDI DI MILANO

RESULTS: *FAECAL MICROBIOTA (DAY 16)*

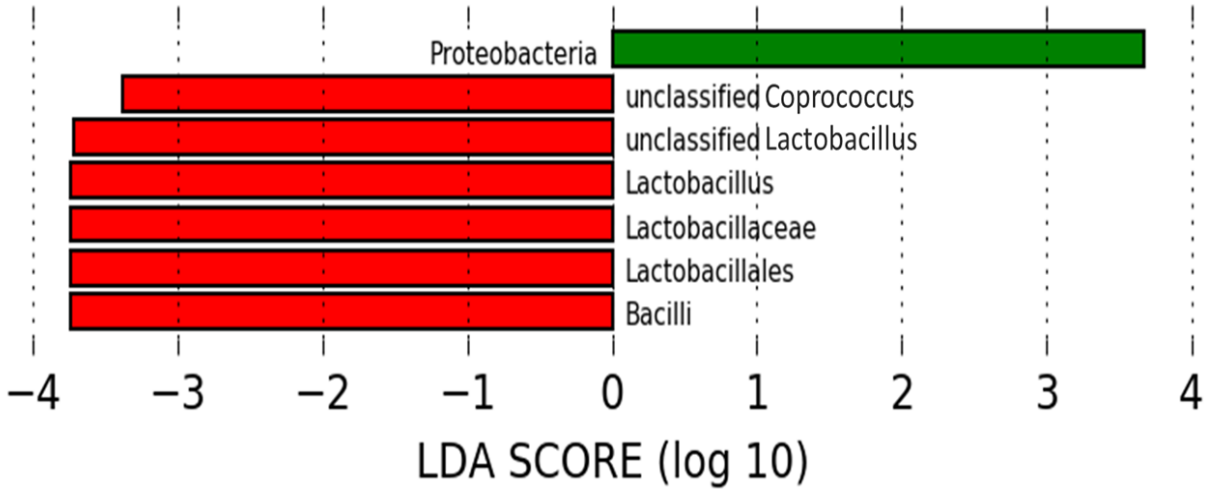
- Pigs microbiota was
- D0 –uniform
- D8- no changes were observed
- D16 the unweighted UniFrac β-diversity analysis showed a slight clusterization in the microbial community between the two dietary groups
  - FFPs led to a qualitative modification in the gut microbial community over time.



Tretola et al., 2019 *Animals* **2019**, *9*, 516; doi:10.3390/ani9080516

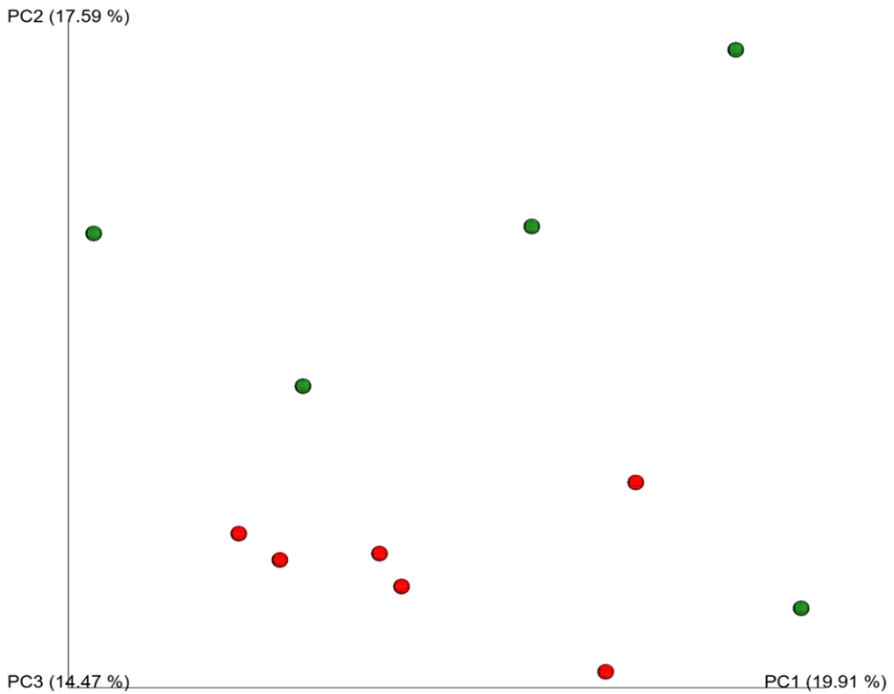
RESULTS: *FAECAL MICROBIOTA*

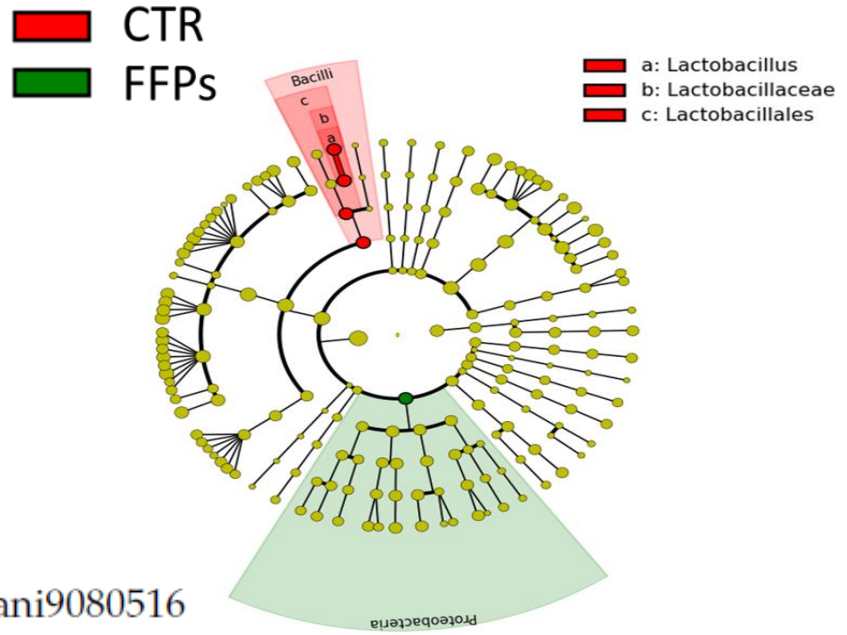
LefSe: Linear discriminant analysis Effect Size **(D16)**



Unweighted UniFrac beta-diversity (D16)

(P < 0.05, R= 0.2)





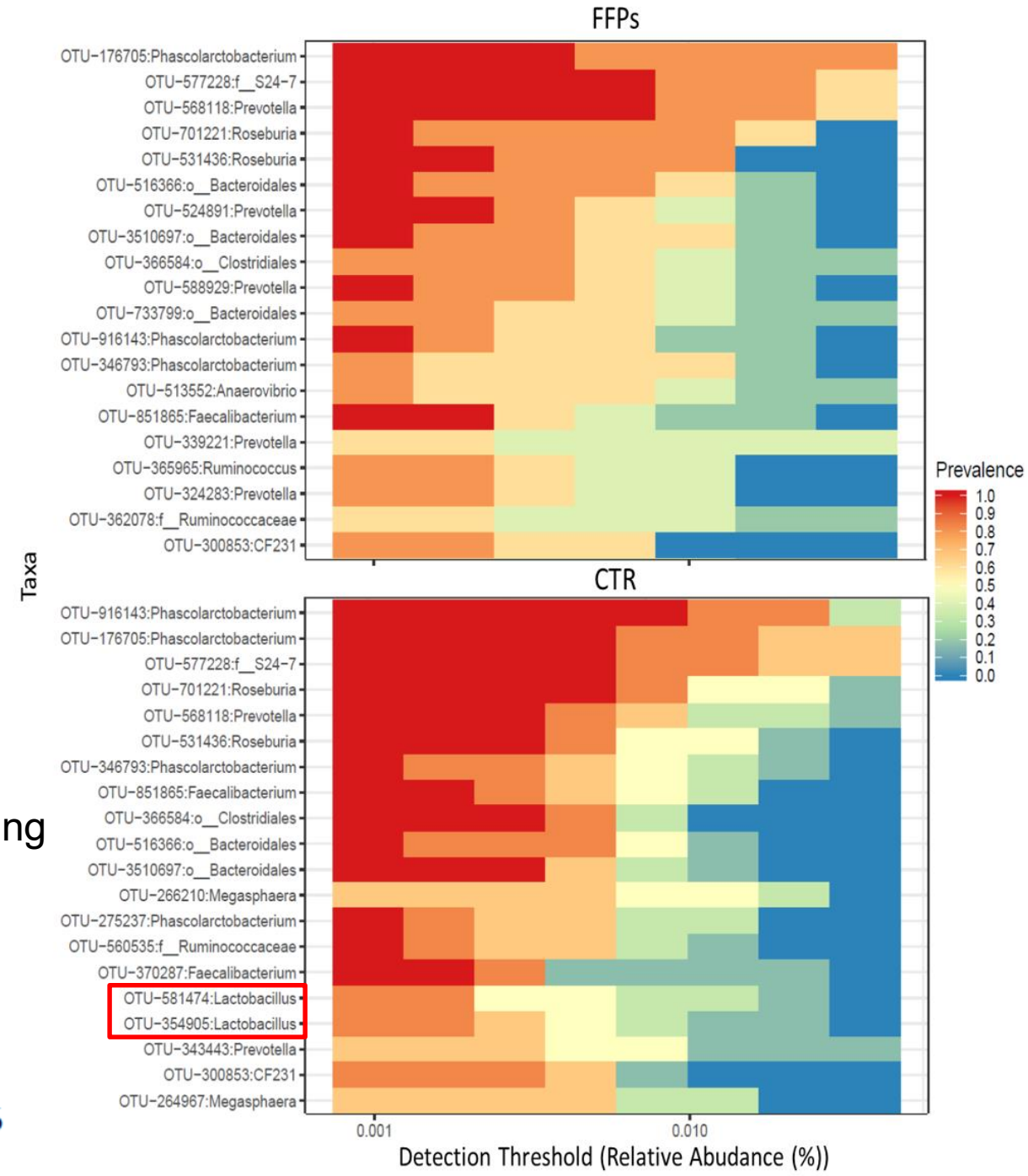
Tretola et al., 2019 *Animals* **2019**, *9*, 516; doi:10.3390/ani9080516

UNIVERSITÀ DEGLI STUDI DI MILANO

RESULTS: *FAECAL MICROBIOTA*

Core Microbiota (D16)

In FFP's core microbiota there are no OTUs belonging to lactobacillus genus



Tretola et al., 2019 *Animals* **2019**, *9*, 516; doi:10.3390/ani9080516

UNIVERSITÀ DEGLI STUDI DI MILANO

Summary : 4/4

Diet

- FFPs good alternative of cereal grains
- Highly digestible
- Excellent energy/carbohydrates source

Growth Performance

- No detrimental effects on growth
- No gastrointestinal disorders
- Improved FCR (?) and feed digestibility (ATTD)

Faecal Microbiota

- FFPs decreased richness and evenness of gut microbiota
- ↑ Proteobacteria ↓ Lactobacillales

FFPS can be included up to 30% in piglets diets without detrimental effects on growth performance.

Further studies are necessary to evaluate faecal microbiota composition in growing/finisher pigs

Advice-recommendation

- *These results should be interpreted with care since they are case sensitive- i.e., they represent just some examples of different former food products that can be present on the feed market (more than 60 samples from 4 UE countries….)*

General conclusions

Nutritional Evaluation

- FFPs as fortified version of cereal grains
- Highly digestible (depending on the composition)
- Excellent energy/carbohydrates source

Safety Evaluation

- Low Microbial load
- Low Presence of packaging remnants
- Methods for rapid packaging quantification need to be implemented

Functional evalution (*In vivo*)

- No detrimental effects on growth performance
- FFPs decreased richness and evenness of gut microbiota.
- ↑ Proteobacteria ↓ Lactobacillales

(**Further investigations are needed**)

UNIVERSITÀ DEGLI STUDI
DI MILANO

Thank you for your attention

Contact: luciano.pinotti@unimi.it