Contribution des mécanismes digestifs à la variabilité individuelle de l'efficience alimentaire chez le jeune bovin en engraissement. Rapport de Stage ingénieur, Toulouse INP- ENSAT, 30 pages.

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Rapport de stage de deuxième année

Contribution des mécanismes digestifs à la variabilité individuelle de l'efficience alimentaire chez le jeune bovin en engraissement

Contribution of digestive determinants to individual variability in feed efficiency in young fattening cattle

CONFIDENTIEL

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Résumé

Le contexte agricole actuel, produire plus pour nourrir une population croissante tout en protégeant l'environnement, amène à optimiser l'utilisation des ressources alimentaires. Bien que les ruminants soient la seule espèce capable de valoriser les ressources végétales riches en cellulose, ils ont un faible rendement de transformation de ces ressources en protéines animales comparés aux autres espèces animales. Il existe cependant de la variabilité entre individus au niveau de cette efficience d'utilisation des ressources qu'il est intéressant d'étudier pour sélectionner des animaux efficients. De plus la compétition entre l'alimentation animale et humaine encourage l'étude des régimes correspondants aux pratiques actuelles (maïs) avec des régimes qui s'inscrivent dans une évolution durable de l'élevage (herbe). Ce projet a pour objectif de comprendre les mécanismes digestifs impliqués dans la variabilité individuelle de l'efficience alimentaire de jeunes bovins à l'engraissement. Nous avons testé 2 régimes différents (ensilage de maïs/ensilage d'herbe) sur 25 taurillons Charolais pour chaque régime. Suite à une période de test de 70 jours pour classer les animaux sur leur ingestion résiduelle (RFI), 16 animaux extrêmes (8 RFI+ et 8 RFI-) ont été sélectionnés pour des mesures supplémentaires de digestion. Les mesures d'ingestion sur les 50 animaux ont montré des quantités de matière sèche (MS) ingérée plus importante pour les RFI+ ($P<0.001$) pour un gain moyen quotidien (GMQ) et un poids final similaire. Les animaux nourris au régime herbe ont également ingéré plus de MS que ceux nourris avec le régime herbe ($P=0.001$). L'étude de la digestibilité de la MS n'a pas apporté de résultats significatifs mais une corrélation négative entre le niveau d'ingestion et la digestibilité a été observée. Des différences de pH et de pH normalisé (NpH) ont été observées entre RFI et régime. Les animaux RFI- ont un pH moyen plus faible ($P=0.041$) et passent plus de temps sous pH 5.8 et 6 ($P=0.026$ and $P=0.021$). Les taurillons nourris avec la ration maïs ont une plus grande amplitude de NpH ($P=0.004$) et passent plus de temps sous NpH= -0.3 ($P=0.005$). Les données collectées à l'abattoir montrent des différences dans le poids des compartiments digestifs notamment pour le rumen qui est moins lourd chez les animaux RFI- au sein du régime maïs ($P=0.072$). Les animaux nourris avec du maïs ont un abomasum et des intestins plus lourds ($P=0.033$, $P=0.039$). Les mesures de diamètre de l'orifice réticulo-omasal ne montrent pas de différences significatives liées au RFI ou au régime. Enfin, l'étude du transit des digestats montre des résultats prometteurs qu'il reste à développer et approfondir.

Mots clés : efficience alimentaire, digestion, variabilité individuelle

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List of common abbreviations

ADG: average daily gain

ADF: acid detergent fiber

AUC: area under curve

BCS: body condition score

BW: body weight

DM: dry matter

DMI: dry matter intake

DT: digestive tract

FI: feed intake

INRA: Institut National de la Recherche Agronomique – French National Institute for Agricultural Research

LI: level of intake

NpH: normalized pH

RFI: residual feed intake

ROO: reticulo-omasal orifice

TiO_2: titanium dioxide

Introduction

As part of my gap year and my second year internship, I spent six month at INRA site in Theix (Puy de Dôme-63). The main subject I worked on was the contribution of the digestive mechanisms involved in the individual variability of feed efficiency of growing cattle. This question arises in a context where beef production is criticized because it has high environmental impacts (Tuomisto and Teixeira de Mattos, 2011), mainly because of greenhouse gases emissions and particularly methane emissions (FAO, 2019). Animal productions are also competing with human food production, which is a big concern with the growing population that is expected to rise to 9 billion people by 2050 (Barnosky, Ehrlich and Hadly, 2016). Even if we don't have to forget that ruminants plays a major role in food production because they are able to value non-consumable fibrous plants into animal products (milk, meat) for consumption by humans, ruminants are also criticized due to their low feed processing efficiency.

However, some individuals are more efficient than other ones, bred on the same conditions, to transform vegetal feed resources into food for human consumption. Given this, it is necessary to understand what determines the individual variability of feed efficiency. This is what we have been trying to do during this internship, focusing on digestives aspects in growing cattle.

Thus, I will in this report, begin with the presentation of the host organism (INRA) and the research unit (UMR Herbivores). Then, I will introduce the origins and the context of the study before to produce the scientific presentation of the project. In other words, I will detail the experimental strategy with the protocol and the analysis methods. In a second part, I will comment the results obtained and try to interpret them. Finally, I will discuss the results and the opportunities of this study for the next years.

I. Presentation of the host institution

A. The National Institute for Agricultural Research (INRA)

INRA is a public research institute specialised in the area of agriculture, food and environment. Created in 1946, its reputation is known all over the word as it is ranked second world institute for its publications in agricultural sciences (INRA, 2016). A large number of partnerships exist with European and international universities, research institutes and veterinary schools.

The institute also plays an important role in France as it employs 13000 person located in thirteen scientific divisions and in two hundred and fifty research units in different regions of France. A large part of the studies conducted on farming animals is comprised in the Phase division (animal physiology and livestock system) which intends to understand and to develop mechanisms and technics in 4 subjects: feed resources, animal's performance, animal products and livestock systems (INRA, 2018b)

B. UMRH, the Herbivores Joint Research Unit

This unit is part of the Inra Auvergne-Rhône-Alpes centre located in Theix (63122, Saint-Genès-Champanelle). In this group, 75 scientists conduct research on cattle and sheep where they try to understand mechanisms, to model processes and to foster innovation. The research is organised in five topics (UMRH, n.d.):

- Adaptive capacities and robustness of animals,
- Feed efficiency of animals and farms,
- The construction and prediction of sensorial and nutritional qualities of meat, milk and cheese,
- The valuation of complementarities to increase farm systems' sustainability and resilience,
- Ecosystem services and dys-services provided by farming systems.

The research activities are structured among 5 team (Figure 1) operating in parallel and in interactions. To pursue their experiments, the researchers of the UMRH benefit performant equipment, workshops and an experimental platform on herbivores and grassland in mountain areas (UE Herbipôle).

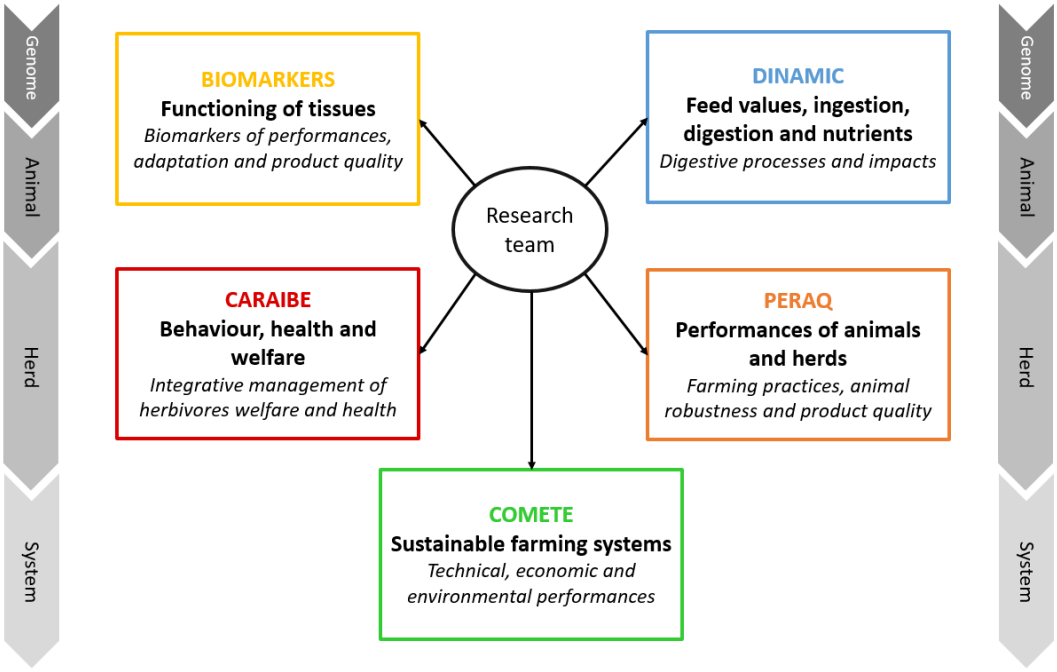

Figure 1 Organizational chart of the research unit (personal source)

The study about the understanding of the feed efficiency mechanism is part of the Dinamic team. The aim of this group is to understand the intake, the digestive and metabolic mechanisms involved in ruminant's nutrition and their impacts on several dimensions: production, efficiency, wastes, digestive welfare and quality of products. Dinamic uses a diversity of approaches (biochemistry, in vivo experiments, analytical predictive methods…) to pursue its research lines (UMRH, n.d):

- Characterisation of ruminant feeds (including new resources) and diets (including mixed diets), and development of prediction methods, to assess the dimensions listed above;
- Quantification of intake, digestive and metabolic fluxes of nutrients to understand and predict the responses of digestion and metabolism to variations of intake and diet composition, and to develop indicators of digestive and metabolic functions;
- Characterisation of the digestive microbial ecosystem and its interactions with the diet and the host, to understand and control the metabolism of the holobiont (i.e. microbiota & host as an entity).

My internship took place in this team and it is integrated to an important project: Beefalim 2020.

II. The project of feed efficiency

A. Beefalim 2020

This program began in 2015 when researchers notice that at equivalent weight and average daily gain (ADG), there is a significant difference in food intake between animal (Griffon, 2015).

That is how the Institut de l'élevage, INRA, Allice, APIS-GENE, chambers of agriculture and cattle breeding companies gathered in a program to conceive a strategy and a tool to select the feed efficiency genetics' traits. Different projects have been launched on different themes and are summarised on figure 2.

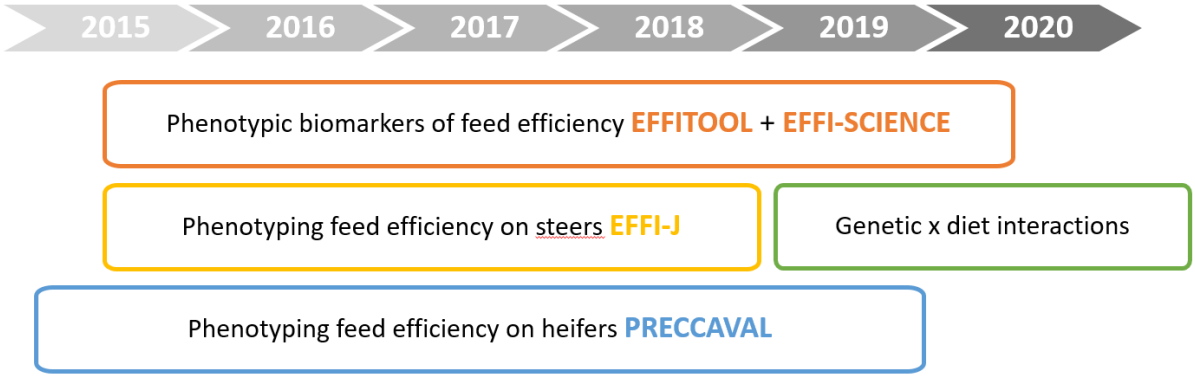

Figure 2 : General organisation of the Beefalim 2020 project (adapted from Griffon, 2015)

Four years after the creation of Beefalim 2020, we can highlight some results. The phenotyping of 400 young Charolais revealed between-animal variation on feed efficiency (Cantalapiedra-Hijar, 2018). The project Effi-tool also found a model with 9 targets and identified molecules that explain 58% of the residual feed intake (RFI) (Idele, 2018). Following these remarks, the Effi-science project found its place to understand the mechanisms of this variability.

B. Effi-science

Effi-science is based on the idea that feed efficiency, as estimated through the RFI is an heritable trait (h^2=0.4) (Herd book Charolais, 2015) when the animal is fed with concentrate. This heritability related with the variability shows a potential interest in the improvement of feed efficiency through genetic selection. However, it would be a multiple selection on different physiological traits and it could have negative interactions in other biological functions. Several studies demonstrate negative effects on immunity (Gondret et al., 2017), on methane emissions (Flay et al., 2019) and on the quality of meat products (McDonagh et al., 2001). In that case, is it better to select on the character of feed efficiency or on physiological

determinants of feed efficiency? Gonzalo Cantalapiedra-Hijar, a researcher of the Dinamic team, and his collaborators published in 2018 a review gathering the biological determinants of variation in feed efficiency; “such as feeding behaviour, digestion and methane production, rumen microbiome structure and functioning, energy metabolism at the whole body and cellular levels, protein turnover, hormone regulation and body composition”. Thus, the objectives of the Effi-Science project are to understand the digestive and metabolic determinants of the between-animal variation in feed efficiency, and to rank the major determinants of feed efficiency variation according to diet (starch or fiber).

Pablo Guardino-Lopez, a PhD student is currently studying the metabolism determinants (Protein turnover, energy spending and body composition). Following my internship, I am probably going to pursue it with my end of school internship then a PhD, where I will investigate the digestive determinants (digestibility, the methane emissions, the feeding behaviour, the rate of the digestive transit, characteristics of the digestive tissues and contents and the rumen microbiota). I will present in this report my work on the DM (dry matter) digestibility, the rate of the digestive transit, the rumen pH and anatomical measures of the digestive tract in bulls.

C. Experimental strategy

In France, before any animal study, an application for animal experiment must be submitted to an ethical committee (Menesr, 2014). The Effi-Science project received a positive opinion from the ethical committee and the ministry of agriculture (APAFIS #16194-2016101016361277 v6).

1. Animals and feeding

The experiment take place in the experimental farm of the Intrabois (INRA, UE 1414 Herbipôle, Theix), during two consecutive years with two different bands of growing bulls (October 2018-May 2019 and October 2019-May 2020). The results in this report belonged to the 1st band. Next year, we will extend the same scheme on 50 new young bulls. In each band, 50 young Charolais were previously selected according to their phenotype in feed efficiency. Half of them received a diet based on maize silage and the other part on grass silage. Both diet were made with silage (60%), concentrate (35%) and straw (5%) and are detailed in table 1.

Table 1 Ingredients and chemical composition of diets

	Grass diet	Maize diet
Ingredients %		
Grass silage	60	
Maize silage		60
Wheat	7	23
Beet pulp	23	
Soymeal	5	12
Wheat straw	5	5
Chemical composition, % DM		
Crude protein	14.2	14.3
NDF	49.6	33.1
Starch	4.4	39.9
Feed value[1] INRA 2018		
UFV, / kg DM	0.81	0.94
Rumen Protein Balance, g/kg DM	11	9.0
PDI, g/kg DM	75	83
PDI/UFV	93	93

[1] Calculated according to INRA, 2018a

The animals were housed in barn separated in two parts according to their diet. Each part is equipped with 9 biocontrol weighing troughs to offer an individual and ad libitum food. To ensure that, the ration was readjusted every week to have on average 10% of refusal. The same feeding conditions were applied for the animal in digestibility stalls.

During the first part of the experiment (October 2018-January 2019), a feed efficiency test was made during 70 days to determine the individual RFI of the 50 animals. This period enabled to select 4 efficient (RFI-) and 4 inefficient (RFI+) bulls for each diet, i.e 16 ‘extreme’ animals retained for digestion and metabolism measurements in digestibility stalls. The selection was made from the RFI ranking describe as the difference between actual feed intake and the expected feed intake to cover requirements for both maintenance and body weight gain (Sainz & Paulino, 2004) (annex 1). To avoid any potential effect on this measure, the bull-calves had similar age and body weight (BW) (Jarrige et al, 1995).

2. Measurements and samplings

a. Measurement of the feed intake and the ADG

During the seven month of the experiment, the feed intake was measured every day for each of the 50 bulls thanks to the weighing troughs, and particularly during the first twelve weeks (RFI test). The animals were weighed every two weeks during the whole trial period.

b. Measurement of the individual digestibility

The measures took place during two different 10 days periods. On the experimental farm, there are only eight digestibility stalls, so we separated the digestibility measurements on two periods (A and B) beginning February 6th for the first one and February 27th for the other one. Diets (Grass vs Maize) and RFI groups (RFI+ vs RFI-) were balanced between the 2 periods.

During the 10 days periods, the individual total amount of fresh offer, refusal, and emitted faeces, were weighed, and their DM content were measured daily. Faeces were separated from urine thanks to harness fixed around the chest of the animals. The DM content was determined by drying collected fresh samples during 24h at 103°C in a ventilated oven (DM content (%) = dry weight/fresh weight x 100). Apparent digestibility of DM (%) was calculated as (DM intake – DM faecal flow) / DM intake x 100, with DM intake = DM offered – DM refused (all terms in g/d) (Jarrige and al, 1995).

During the 10 days periods, the DM evaluation was made every day for the diets and the refusals. We also collected faeces that we were able to separate from urine thanks to harness fixed around the chest of the animals to determine the %DM. Each day, we acquired the quantities of refusal and faeces produced, that able us to measure the total tract digestibility.

It is important to notice that the results obtained do not represent the real digestibility of the diet but the apparent digestibility. Actually we collected in the faeces an animal-related endogenous fraction (dead cells, digestive fluids) and microbial flora from the digestive tract (living/dead bacteria, protozoa, etc) (Jarrige and al, 1995) and not only the undigestible fraction of diet intake. In this report, we will simplify this concept of apparent digestibility by the term digestibility.

c. Rumen passage kinetics of particles based on titanium dioxide (TiO_2) excretion

The study of the passage kinetic was inspired by the passage kinetics measured with (Yb) and (Sm)-labelled forages (Krämer and al., 2013). We assessed a simplified way, also based on the decrease rate of marker in faeces following the stopping of its administration. In our case,

we used TiO2 as an external marker because previous publications described TiO2 as an inert marker to estimate faecal excretion and digestibility (Titgemeyer and al., 2001, Glindemann and al., 2009). To begin, we gave 12g/d of TiO2 during 10 days. It was given as solution in water during animal's weighing (the 6 days before animals were in stalls) or given as powder mixed with the diet (the 4 first days animals were in stalls).

After the last TiO2 administration, faeces spot samples were taken sequentially for 6 days. We forecast to collect 6 samples during the first day (every 2 hours between 8 am and 6 pm), then twice a day (at 8 am and 4 pm) during the other 5 days. To avoid invasive rectal search, we made a round to be present near the animals at all times to collect the faeces just after the excretion, and the actual excretion time was noted. According to this schedule, we obtained an average of 15 samples for each bull.

Once collected, samples were weighted (to account for their contribution to total faeces excretion for digestibility calculation), then prepared for further analysis. They were stored at -20° until they were dried in heating chamber at 60°C for 72h. After that, the faeces were chronologically ground by animal inside a 1 mm grinder (BJL 8500-2) to simplify the cleaning process. The TiO2 kinetic analysis requires a specific procedure detailed in Annex 2. With the aim to collaborate with international research team, our analysis were made in the Department of Animal Science of Aarhus University in Denmark. Peter Lund and Martin Weisbjerg, scientists of this department, already completed studies in rumen passage kinetics using marker techniques (Krämer and al., 2013, Guinguina and al., 2019) and backed us with their knowledge.

d. pH and temperature of the rumen

The extreme animals (n=16) received orally pH bolus (eBolus, eCow, Exeter, UK) after the RFI test (January 2019). The sensors were introduced in the rumen thanks to a dedicated balling gun. Twice a month, the data were collected during the weighing with an electronic sensor (eCow handset: smartphone + antenna) until the beginning of the slaughtering period in May.

e. Tissue sampling and collection of rumen content

After the digestibility experiment, the 16 extreme bulls were bred until an average weight of 700 kg. They were killed at the experimental slaughterhouse in Theix. Just after the death of the animal, we collected the rumen, reticulum, omasum, abomasum, small intestine and large intestine and they were weighed all together. We particularly took care in keeping the digestas

inside the digestive tract. Thereafter, all the compartments were separated, emptied and washed with water and individually weighed.

The total fresh rumen content was weighed, then we sampled approximately 2kg of it in trays to determine its DM content (24h in ventiled oven at 103°C).

In parallel, we measured the size of the reticulo-omasal orifice (ROO). As we had no experience on this measurement, and there was no method described in the literature, we investigated three different methods. The first one consists in taking a picture of the ROO with a scale (a ruler in our case) and to further determines the area of the orifice with an image-processing program, Imagej. In the second one, we uses a calliper (accuracy = ±0.02mm) to measure the diameter of the ROO. Then we uses a range of tube from 30 to 54 mm considering that the biggest tube entering the orifice corresponds to its diameter. The 3 methods were applied by manipulating the samples in order to avoid apparent tension of the tissue. Moreover, the same people made the measurements during the experiment, as far as possible, and they were repeated at least 3 times for each animal to limit measurement errors.

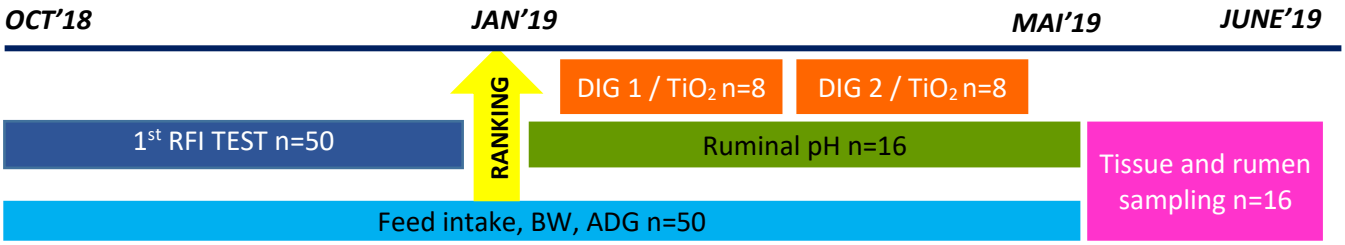

Figure 3 Summary of the measurements and samplings for each group of animal

3. Data analysis

Digestibility

We compared two different approaches to calculate the total-tract digestibility, based on the following formulas:

$$(1) Digestibility\ (\%DM) = \frac{DMI - DM\ faeces}{DMI}$$

$$(2) Digestibility_{\ D/D+1}\ (\%DM) = \frac{DMI_D - DM\ faeces_{D+1}}{DMI_D}$$

The first method (1) consists in using the DMI and the DM faecal excretion (DMf) measured the same day. On second thoughts (2), based on a more physiological basis, we considered

that the faeces excreted one day correspond to the DMI of the day before (D-1). This method (2) provides only 9 data due to the offset in comparison with the method (1) (10 data). We performed student test to compare means, having ascertained its conditions of application (Gaussian distribution, variance homogeneity). A significant difference was observed between the two calculation methods ($P<0.03$). At last, we decided to use the "D-D+1" method to get closer to the biological digestive cycle (formula 2).

pH data

The pH data were summarized with Excel Software and a Visual Basic for Application program previously developed as described in Villot et al. (2018) was used. A decomposition of the raw data was made to separate the interesting component from abnormal or random variation. The data were smoothed filtered and normalized. By the end of those modifications, we had the following indicators:

- mean pH
- time pH<5.8 and 6 (min/d)
- NpH range = NpH_{max}-NpH_{min} (Normalized pH)
- time NpH<-0.3 and -0.5 (min/d)

Statistical analysis

The statistical analysis were performed with R software (version 3.5.3). Before each analysis, we checked the conditions of application of the following tests: normality and homogeneity of variances. In the case of our data did not respect one of these assumptions, we made the following transformation: $Y = arcsin(\sqrt{x}) \times \frac{180}{\pi}$

Variance analysis were conducted by ANOVA. We considered as fixed effect the RFI group (RFI+ vs RFI-), the diet (maize vs grass). The animal effect was considered as a random effect. We used the following model: X ~ RFI * Diet to also consider the interaction between both effects. Differences were considered significant at $P<0.05$ and trends were discussed at $P< 0.1$.

III. Results

A. Measurement of the feed intake and the ADG over the whole trial

Through the use of weighing trough, we measured the feed intake for the 50 animals during 200 days. We have to notice that one animal died of liver disease during the trial, so the results of 49 animals will be presented. The values obtained are gathered in table 2. The DMI (g/d) is significantly higher for RFI+ whatever the diet (P=0.001) and with the maize diet (P<0.001). Expressed in g/kg BW of g/kg $BW^{0.75}$ the difference between RFI is more notable for the maize diet (RFI x diet: P=0.067 and P=0.021 respectively).

Table 2 Effects of RFI and diet on feed intake for all animals of the study (n=49)

Items	Grass		Maize		RSE[1]	P-values		
	RFI- n=12	RFI+ n=13	RFI- n=13	RFI+ n=11		RFI	Diet	RFI x Diet
DMI (g/d)	8258	8671	9248	10223	0.2	0.001	<0.001	0.116
DMI (g/kg BW)	15.6	16.5	16.3	18.1	0.32	<0.0001	<0.001	0.067
DMI (g/kg $BW^{0.75}$)	74.7	78.8	79.3	88.2	1.22	<0.001	<0.001	0.021

[1]Residual Standard Error

The body weight measured over the trial and the ADG for each effect are presented in table 3. There is no difference between groups in the initial BW of bulls at the beginning of the experiment. The bulls fed maize diet are significantly heavier (P=0.007, P=0.007, P=0.001) when compared to grass diet through mean metabolisable BW ($BW^{0.75}$), mean BW and final BW respectively. We observed the same trend with the ADG which is greater with the maize diet (P=0.0001). There was not effect of RFI on BW and ADG.

Table 3 Effect of RFI and diet on BW and ADG for all animals of the study (n=49)

Items	Grass		Maize		RSE[1]	P-values		
	RFI- n=12	RFI+ n=13	RFI- n=13	RFI+ n=11		RFI	Diet	RFI x Diet
Initial BW (kg)	409	416	427	423	16.5	0.99	0.313	0.75
Mean $BW^{0.75}$ (kg)	110	110	117	116	2.6	0.752	0.007	0.959
Mean BW (kg)	530	529	570	567	16.8	0.769	0.007	0.959
Final BW (kg)	656	647	708	710	19.8	0.652	0.001	0.726
ADG (kg/d)	1.24	1.18	1.46	1.48	0.04	0.515	0.0001	0.521

[1]Residual Standard Error

B. Digestibility measurement

The digestibility measurements, occur on the 16 extreme RFI bulls on two different 10 days periods. There is a significant effect of the feed efficiency (RFI) on the DMI (P=0.006) and on the level of intake (LI) (P=0.002) during this period of10 days. It shows that less efficient bulls (RFI+) ingested more DM than the efficient ones. We can also highlight the significant effect of RFI on the amount of faecal DM excreted during the trial (P=0.008), with more faeces in RFI+ animals. In contrast, there are no significant effects of RFI and diet on DM digestibility.

There is variability between animal in DM digestibility, with 5 points between the 2 extremes with the grass diet and 7 points with the maize diet (figure 4).

Table 4 Effects of RFI, diet and period on DM intake, DM digestibility and DM faecal excretion for the extreme RFI animals (n=16)

Items	Grass		Maize		RSE[1]	P-values		
	RFI- n=4	RFI+ n=4	RFI- n=4	RFI+ n=4		RFI	Diet	RFI x Diet
DMI (g/d)	7896	8459	7754	9387	224.9	0.006	0.258	0.131
LI (DMI%BW)	1.43	1.60	1.36	1.61	0.036	0.002	0.562	0.406
Faecal DM (g/d)	2226	2447	2287	2775	74.8	0.008	0.097	0.238
DM digestibility (%)	71.8	71.1	70.5	70.4	0.44	0.544	0.134	0.604

[1]Residual Standard Error

For each diet, we managed to study the correlation between the amount of DM ingested and the DM digestibility. To overcome the differences of BW between bulls, we also evaluated the correlations between DM digestibility and LI calculated as : LI (DMI%BW) = DMI / BW x 100. The trend lines are illustrated in figure 4. A negative correlation is observed for both diets but it is not significant (Maize: R^2=0.09, P=0.47; Grass: R^2=0.03, P=0.67). A third model was studied with both equations with an effect of the diet as: DM_Digestibility = 73.96 – 1.980 x LI (R^2=0.13, P=0.432). The differences between diets are not significant.

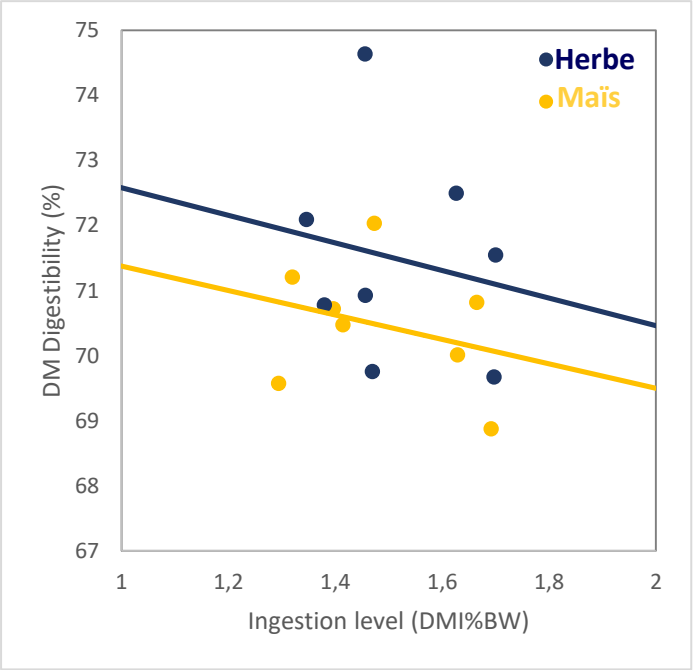

Figure 4: Relationship between ingestion level (DMI%BW) and DM digestibility (%) measured in young bulls in digestibility stalls

Grass diet: DM_Digestibility = 74.70 – 2.119 x LI, R^2=0.03, P=0.67
Maize diet: DM_Digestibility = 73.26 – 1.878 x LI, R^2=0.09, P=0.47

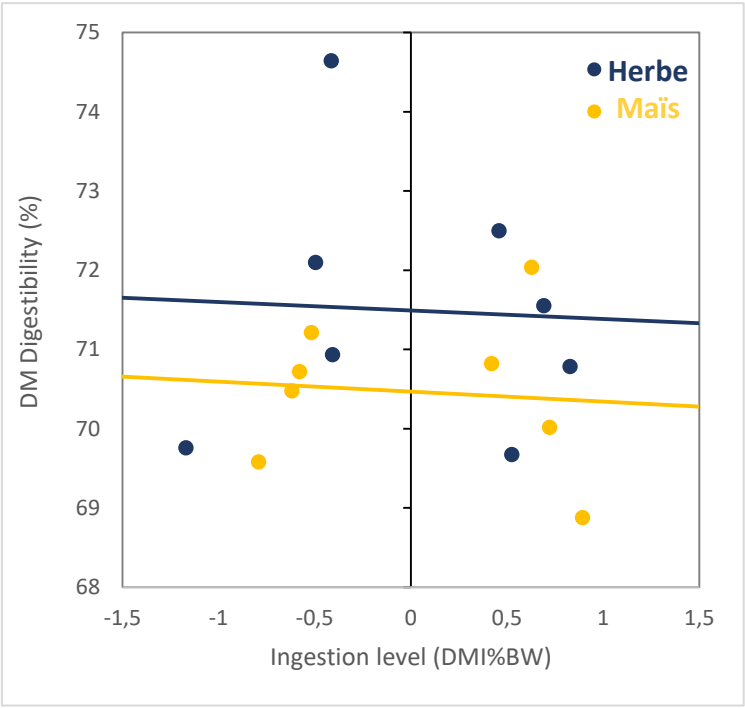

Figure 5: Relationship between the residual feed intake (kg/d) and the DM digestibility (%) for each diet measured during the period in digestibility stalls.

Grass diet: DM_Digestibility = 71.49 – 0.107 x RFI, R^2=0.002, P=0.911
Maize diet: DM_Digestibility = 70.47 – 0.127x RFI, R^2=0.008, P=0.830

Figure 5 shows the relationship between the RFI and the DM digestibility. There is a non-significant negative relationship for each diet: when the RFI increase, the DM digestibility tend to be less important. The model integrating both diet is DM_Digestibility = 70.98 – 0.117x RFI (R^2=0.15, P=0.830) with no significant difference between diet.

C. Rumen passage kinetics particles based on titanium dioxide (TiO_2) excretion

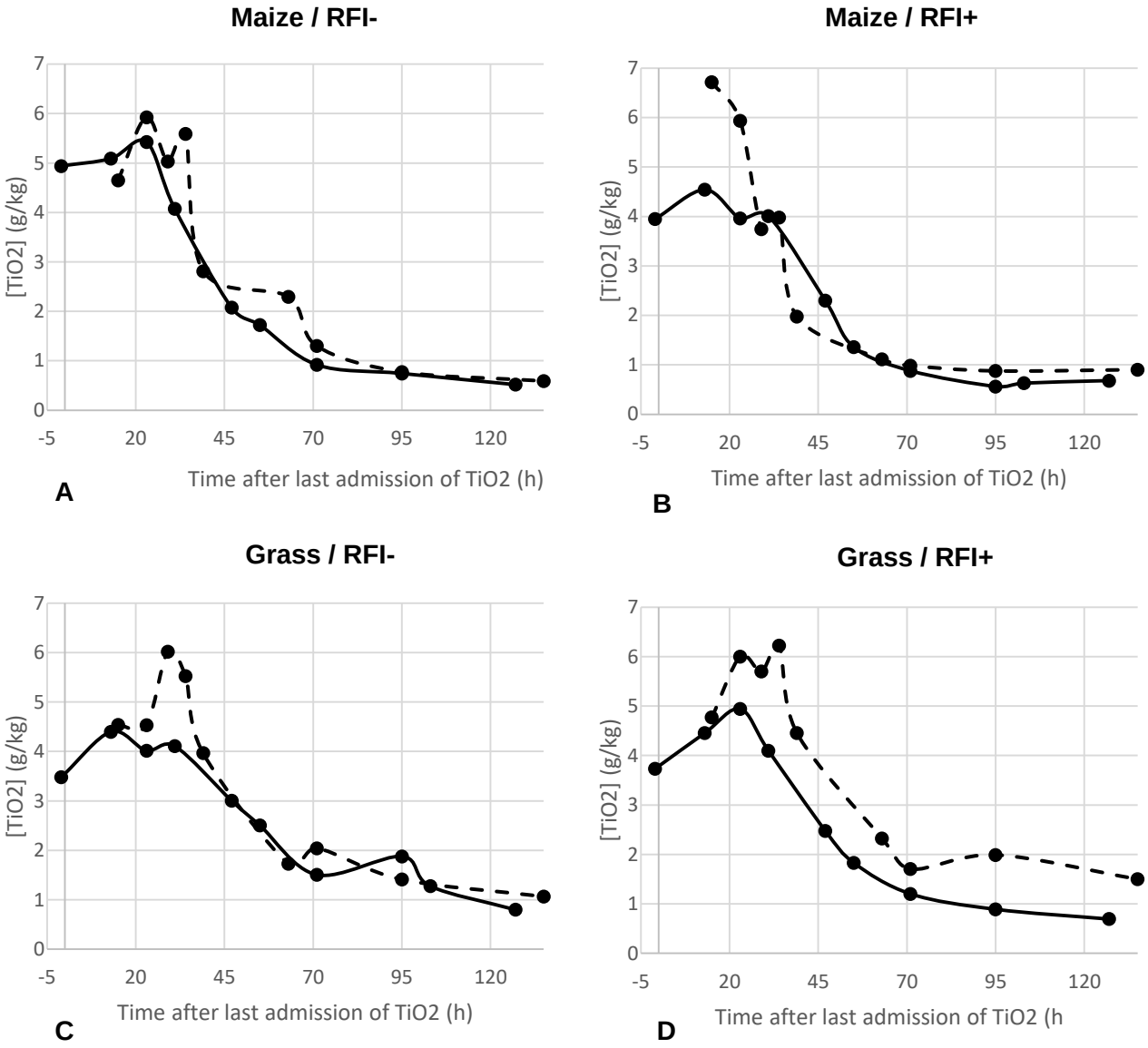

Figure 6 : Titan dioxide excretion kinetics for each diet and RFI measured on the extreme RFI animal (n=16)

Animals tested during period A are represented with dashed lines, animals from period B with full lines
Each curve is a mean of 2 animals.

Figure 6 shows the exponential decay kinetics of the concentration of TiO_2 in the faeces collected during the digestibility period.

The AUC (Area Under Curve) presented in table 5 is significantly higher for the animals fed with grass diet (P=0.020). The difference between RFI is not significant.

Table 5 Effects of RFI and diet on the area under the curve (AUC, arbitrary unit) for the extreme RFI animals (n=16)

Item	Grass		Maize		RSE[1]	P-values		
	RFI- n=4	RFI+ n=4	RFI- n=4	RFI+ n=4		RFI	Diet	RFI x Diet
AUC	590	628	521	459	89.0	0.795	0.020	0.284

[1]Residual Standard Error

D. pH and temperature of the rumen

The statistics results for the pH and temperature data are gathered in table 6. Mean pH is lower for the RFI- (P=0.041) irrespective of the diet. Animals ranked as RFI- spend more time per day under pH 5.8 and 6 (P=0.026 and P=0.021). When we consider the normalized pH, RFI- animals also stay the longest time under NpH<-0.3, in average 36.2 min more per day (P=0.002). The diet has an effect on the NpH range that is wider for animals fed the maize diet (P=0.004) which spend also more time under NpH= -0.3 (P=0.005). The diet and the RFI have no effect on the temperature of the rumen.

Table 6 Effects of RFI and diet on ruminal pH, NpH range, time under pH=5.8, 6, -.3 and -.5 and mean temperature for the extreme RFI animals (n=16)

Items	Grass		Maize		RSE[1]	P-values		
	RFI- n=4	RFI+ n=4	RFI- n=4	RFI+ n=4		RFI	Diet	RFI x Diet
Mean pH	6.24	6.37	6.08	6.35	0.174	0.041	0.340	0.429
NpH range	0.470	0.390	0.652	0.650	0.1265	0.514	0.004	0.564
Time pH<5.8 (min/d)	45.4	0.450	320	9.19	0.828	0.026	0.102	0.969
Time pH<6 (min/d)	198	47.1	583	106	0.68	0.021	0.175	0.711
Time NpH<-.3 (min/d)	39.8	12.4	81.12	36.11	18.954	0.002	0.005	0.373
Time NpH<-.5 (min/d)	5.63	0	7.73	0.975	6.856	0.661	0.096	0.872
Mean temperature (°C)	39.9	39.3	39.5	39.6	0.51	0.406	0.680	0.227

[1]Residual Standard Error

E. Tissue and rumen content sampling

Weight of the digestive contents

The weight of the rumen and total digestive tract contents were similar among RFI and diets (table 7). The DM of the rumen content was higher for the maize silage (P<0.0001). Looking at the interactions, there is a trend (P=0.06) for the DM of the rumen content. Within the maize diet, the DM of the rumen content is higher for RFI+ while it is RFI- with the grass diet. We observed the same trend for the weight of the DM rumen content (P=0.082)

Table 7 Effects of RFI and diet on the weight of the rumen and digestive tract content for the extreme RFI animals (n=16)

Items	Grass		Maize		RSE[1]	P-values		
	RFI- n=4	RFI+ n=4	RFI- n=4	RFI+ n=4		RFI	Diet	RFI x Diet
Rumen content weight (kg)	39.1	38.7	33.5	38.1	5.50	0.465	0.285	0.377
DM rumen content (%)	14.9	13.8	16.2	17.1	0.93	0.786	<0.0001	0.06
DM rumen content weight (kg DM)	6.34	5.78	5.99	7.11	0.888	0.541	0.289	0.082
Total digestive tract content weight (kg)	65.7	64.2	56.7	66.3	7.36	0.296	0.374	0.157

[1]Residual Standard Error

Weight of the digestive tissues

The weight of each compartment of the empty digestive tract (DT) are presented in table 8. Data were also expressed in percent of the BW of the animal before the slaughtering and of the weight of the whole digestive tract.

There is no effect of RFI and diet on the weight of the different digestive compartments.

There is a trend on the weight of the rumen which differs between RFI within a same diet (RFI x Diet: P=0.072) the rumen being heavier for RFI+ with the maize diet and similar between both RFI for the grass diet. There is also a trend of a simple effect of the diet with the intestines (P=0.065) that are heavier with the maize diet irrespective of the RFI group.

Those trends are confirmed when the weight of digestive compartments is expressed in proportion of BW or of the whole DT. The rumen (%BW and % total DT) has a similar weight between RFI for the grass diet and is heavier for the RFI+ for the maize diet (RFI x diet: P= 0.022 in %BW and in % total DT). For the same body weight, the digestive tract is heavier with the grass diet (P=0.001) and for RFI+ animals (P=0.034).

A significant effect of diet was observed for the proportion of abomasum (P=0.033) and intestine (P=0.039), proportionally to the digestive tract weight. Animals feed with the maize diet presents heavier abomasum and intestine in proportion of their digestive tract.

Table 8 Effects of RFI and diet on the weight of empty digestive tract and compartments for the extreme RFI animals (n=16)

Items	Grass		Maize		RSE[1]	P-values		
	RFI- n=4	RFI+ n=4	RFI- n=4	RFI+ n=4		RFI	Diet	RFIxDiet
Total digestive tract (kg)	27.5	28.4	25.8	28.3	1.91	0.114	0.377	0.418
Rumen (kg)	10.0	10.0	8.5	10.2	0.04	0.072	0.186	0.072
Omasum (kg)	5.3	5.4	4.2	4.5	0.15	0.73	0.133	0.939
Abomasum (kg)	1.9	2.0	1.8	2.0	1.27	0.118	0.742	0.203
Intestine (kg)	10.3	10.9	11.3	11.6	0.79	0.301	0.065	0.665
Digestive tract (%BW)	4.89	5.04	4.30	4.67	0.002	0.034	0.001	0.481
Rumen (%BW)	1.78	1.78	1.42	1.69	0.227	0.019	<0.0001	0.022
Omasum (%BW)	0.93	0.97	0.69	0.72	0.007	0.745	0.025	0.990
Abomasum (%BW)	0.34	0.35	0.30	0.33	0.002	0.96	0.843	0.705
Intestine (%BW)	1.84	1.95	1.88	1.93	0.002	0.454	0.902	0.733
Rumen (% total DT)	36.3	35.3	33.0	36.2	0.95	0.209	0.15	0.022
Omasum (% total DT)	19.1	19.1	16.1	15.5	0.14	0.873	0.102	0.879
Abomasum (% total DT)	7.06	6.94	6.99	7.15	0.038	0.200	0.032	0.272
Intestine (% total DT)	37.5	38.7	43.9	41.2	0.04	0.709	0.039	0.341

[1]Residual Standard Error

Size of the reticulo-omasal orifice

The three different methods used to measure the area of the ORO showed no significant differences between RFI and diet (table 9). On the following graph (figure 7 a, b, c), we compared 2 to 2 the results obtained with the 3 different techniques. The three significant linear regressions (P<0.0001 for the 3) evidenced that the 3 techniques allowed the same ranking of extreme animals, although they did not give the same absolute values (ImageJ < Calliper and Tube).

Table 9 Effects of RFI and diet on the area of the reticulo-omasal orifice (ROO) measured with 3 methods for the extreme RFI animals (n=16)

Area measured with :	Grass		Maize		RSE[1]	P-values		
	RFI- n=4	RFI+ n=4	RFI- n=4	RFI+ n=4		RFI	Diet	RFIxDiet
Calliper (cm²)	12.3	12.2	8.94	14.0	3.768	0.144	0.88	0.128
ImageJ (cm²)	6.99	7.19	5.29	8.49	2.296	0.163	0.865	0.216
Tube (cm²)	13.8	13.6	11.7	16.2	3.18	0.204	0.886	0.166

[1]Residual Standard Error

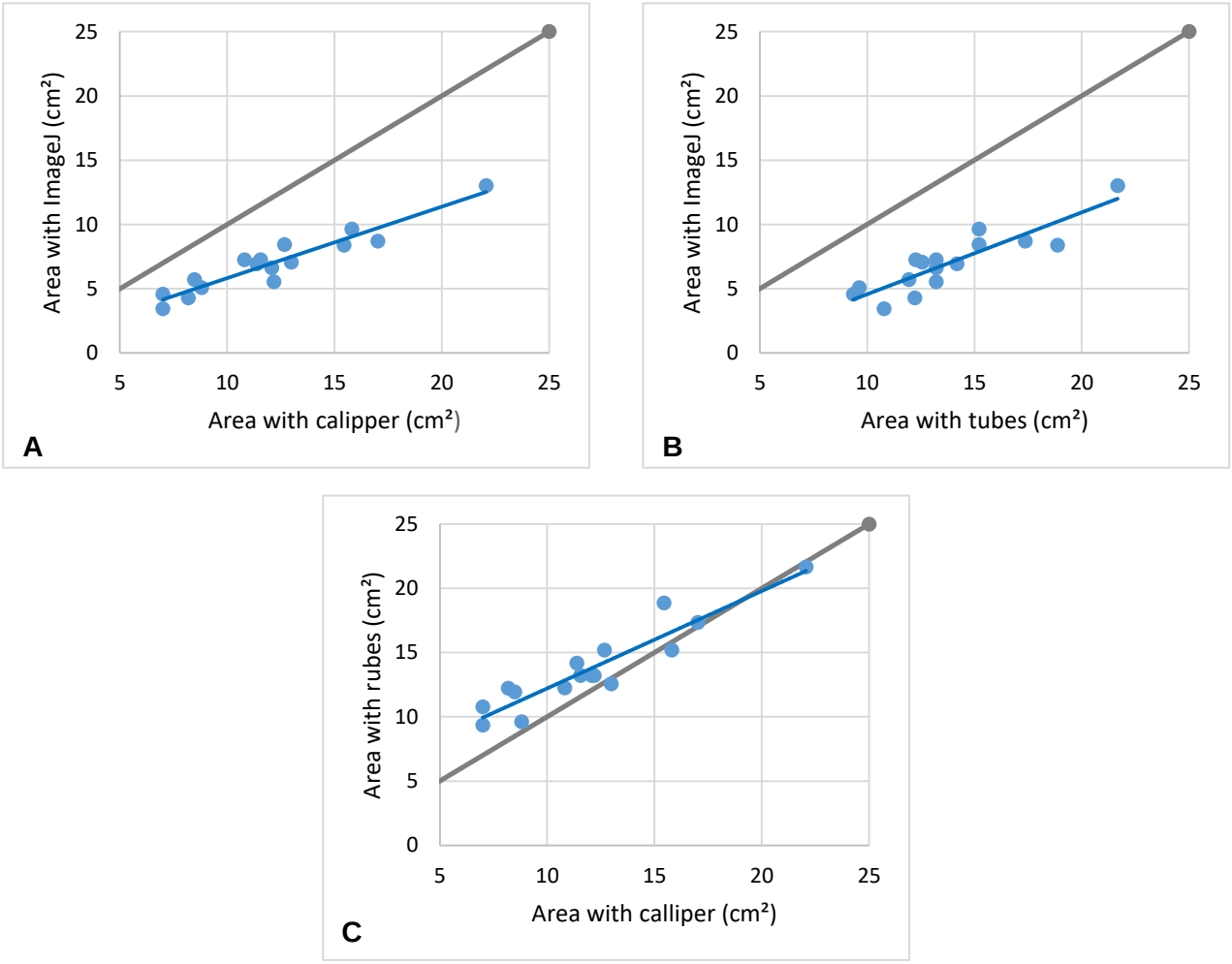

Figure 7: Relationship between the 3 methods used to measure the area (unite) of the ROO

A: area with calipper (X) vs ImageJ (Y): $Y = 0.557 \cdot X + 0.256$, R^2=0.90, P<0.0001
B: area with tubes (X) vs ImageJ (Y): $Y = 0.638 \cdot X - 1.81$, R^2=0.78, P<0.0001
C: area with calliper (X) vs tubes (Y): $Y = 0.758 \cdot X - 4.64$, R^2=0.87, P<0.0001
The line in black is the first bisector

IV. Discussions and perspectives

Feed intake and ADG

As expected, the DMI over the whole period is higher for animals classified as RFI+, whereas the body weight (as mean or final BW or $BW^{0.75}$, or ADG) is similar between RFI+ and RFI-. This agrees with the observations made in several publications (Cruz et al., 2010), and supports the idea of feed efficiency: “How to earn as much, or more by consuming less “(Herd book Charolais, 2015). The RFI- bulls, reach the same BW than RFI+ consuming on average 694 g/d of DM less or 138 kg of DM less during the whole period of measurements (October-18/Mai-19). When we consider the effect of the diet, bulls fed with maize silage are heavier than the ones fed with grass silage. This difference can be explained by the energetic level of the diets. Indeed, the UFV for the maize diet is 0.94 UFV/kg DM while it is 0.81 UFV/kg DM for the grass diet.

Measurements of intake during our digestibility trial contribute to confirm some observations already done. Indeed, during these 10 days measurements, we observed a higher ingestion for the RFI+ extreme animals. It confirms the results obtained during the RFI test allowing the selection of the extreme RFI and joins the conclusions obtained on beef heifers, dairy heifers and beef cattle (Kelly et al., 2010, Potts et al., 2017, Kenny et al., 2018), that RFI is a repeatable trait (h^2~0.23-0.43)(Basarab et al., 2013) in cattle irrespective of the diet.

Digestibility measurement

Digestibility take part in the understanding of physiological determinants of feed efficiency as it explains 10% of the variability between individuals fed the same diet (Herd and Arthur, 2009). The relation between RFI and DM digestibility would be diet dependant. Some studies reported a negative correlation between RFI and DM digestibility with animal fed high roughage diet (Jonhson et al., 2019, Rius et al., 2012). Others reported that RFI- beef heifers tended to have higher DM digestibility when consuming low-starch, but not when fed high-starch (McDonnell et al., 2016). In our study, we did not have significant RFI effect on DM digestibility whatever the diet. However, observed trends match with results already published. Actually, we observed a negative trend between digestibility and the level of intake (figure 4) across individuals for both diets. A meta-analysis handled by Cantalapiedra-Hijar et al. (2018) found the same correlation through the analysis of 15 data with various diet. Therefore, the variability in level of intake could explain one part of the variability in the digestibility across diets.

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pH of the rumen

The pH measurements are consistent with the ones made by Lam et al. (2017) on steers and bulls with ruminal bolus. Indeed, with continuous pH monitoring, efficient animals spent more time under pH=5.8 and pH=6 and had a lower mean pH for both diets. In contrast, other studies, working with spot samples revealed a lower mean pH for less efficient animals (grass silage diet) (Fitzsimons et al., 2014) or no differences (grass silage, pasture and corn silage diet) (McDonnel et al., 2016). Our observations made on the ruminal pH should be linked with the characterization of the microorganisms and the fermentative parameters (volatile fatty acids, VFA) that is currently under way. Given that, Lam et al. (2017) characterized the rumen parameters of efficient cattle as animals with more bacteria, less methanogens at slaughter and a lower pH. Meale et al (2016) reported that the bacterial diversity (number of species, evenly distributed) is higher in more efficient animals. For the VFA, results between studies are inconsistent (Johnson et al., 2019) but this subject need to be deepened.

Weight of the digestive compartments

The weighing of the digestive compartments during the slaughtering reveals differences between both diet and RFI. Firstly, the empty digestive tract is heavier for RFI+ with both diets, and the rumen is heavier for the RFI+ with maize diet. We can assume an effect of the intake on the development of the digestive tissues. RFI+ animals ingested more, that may induce mechanical and chemical stimuli to develop the rumen and the DT. A higher chemical stimulus may explain the higher effect on the rumen with the high-energy maize diet. In consequence, RFI+ animals have a higher weight of digestive tissues than RFI- animals which may require more energy and protein for maintenance, making nutrients less available for growth with less efficient (RFI+) than with more efficient (RFI-) animals.

Another point is that, total digestive tract, rumen (for RFI-) and omasum, are heavier with the grass diet, and that could be explained by the effect of a more fibrous diet. Fibrous diet may mechanically stimulate the digestive tract and its tissues more than maize diet.

Finally, bulls fed with the maize diet have heavier intestines than the ones fed with grass silage diet. The enzymatic digestion of the “by pass” starch in the intestines, and its subsequent glucose active absorption, may stimulate the intestinal tissues and could explain this observation.

Size of the ROO

We began our studies on ROO with the hypothesis that a faster transit rate could be link to a larger ROO. This measurement was innovative so we had no technical references on the best way to measure its size. We developed three measurement methods. With these measurements, we did not wanted to have the precise diameter/area of the ROO but a ranking of the animals. Thus, we can put forward the fact that the surface of the ROO is highly variable between individuals, and that the 3 methods used kept a similar ranking between animals (figure 7 a, b, c). Whatever the method used, we observed no significant differences in the ROO surface between RFI groups and diets, but numerical differences suggest that the ROO surface is higher in less efficient animals with the maize diet suggesting a higher passage rate of particles outside the rumen. This is in line with our initial hypothesis.

Rumen passage kinetics

Another approach was developed to study the potential impact of the digestive transit on feed efficiency, the excretion kinetic of TiO_2. As a reminder, the TiO_2 analysis were made in Denmark at Aarhus University Data were available at the end of my internship and this is why I had only time for simple analysis.

These preliminary results show an effect of the diet on the transit rate when we use the AUC as an indicator of transit rate. The AUC was simply calculated from the decreasing curve of the TiO_2 concentration in faeces versus time following the last TiO_2 administration. The AUC was higher with the grass silage diet that with the maize diet. This may reflect a longer retention time for grass silage diet than with corn silage diet but will need to be compare to results already reported (i.e. Krämer et al, 2013). However, the interpretation of the shape of the curves as indexes of transit rate requires more work than this simple calculation of AUC.

Indeed, if we have a look at the figure 6 a, b, c, we observed the exponential decay kinetics of TiO_2 as expected, but the initial concentration at t_0 differed between curves, that highly impacts the value of the calculated AUC. Data transformation is required to compare the AUC of the different animals with the same initial conditions as normalisation. An adjustment of the curves to a decreasing exponential model will also be needed.

Perspectives

The results obtained during this first year of experiment are very promising. We observed effects of the diet and the RFI on several digestive aspects that are physiologically related to feed efficiency. The young bulls band which will be experimented (n=50) next year will allow to increase the strength of our analysis and to confirm or counterbalance the observations of the first band of animals.

We will have the opportunity next year to make further chemical (constituent analysis) and statistical analysis that will bring additional information on subjects already investigated. Other digestive aspects will be added (CH_4 emissions, digestive tissues histology …) and will allow us to study the potential links and consequences between the different aspects.

Afterwards, when all the data will be integrated, we will have the possibility to study and to try to understand the digestive mechanisms and their contribution in the individual variability in feed efficiency.

V. Conclusion

The experimentation of this year went well and we have interesting results to explore. In fact, we observed individual variability between diets and RFI. We noticed effects or trends following our expectations between DMI and digestibility for example. The data collected at the slaughterhouse show differences in digestive compartments weight according to the study modalities.

We had the opportunity to develop methodology for the measurement of the size of the ROO and the digestive tract passage kinetics of particles based on titanium dioxide (TiO2) excretion. Those new methods gave us promising results that will need to be deepened and compiled with next year results.

On my side, I discovered the research system during this internship and I have been fully integrated to the Effi-Science project. I got the chance to participate to the whole project from the sampling and the processing of the data to the monitoring of the experiment. I have also seen the usefulness of the knowledge acquired during my two years of engineering studies in agronomy, such as the use of computer tools for statistical analysis, the animal nutrition and physiology basis.

It allowed me to understand the world of cattle and animal nutrition and to apprehend its challenges for the next years. This comes to the crossing with the mentored project realised with Interbev in 2017 that told me about the issues of the beef industry and the solutions

implemented by the professionals (farmers, slaughtermen, retailers, restaurateurs…). Now I have seen the solutions at work on the research side.

On a professional side, this internship drawn my interest and opened a professional way for me as I am going to pursue my engineering scholarship by a PhD thesis on this project. So I am considering working in research for at least the next 3 years.

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VI. Annex

Annex 1

Adapted from Meale et al., 2017

Calculation of Feed Efficiency Traits.

Residual feed intake (RFI) was calculated for each animal as the difference between actual dry matter intake (DMI) and expected DMI. The expected DMI was determined for each animal using a multiple regression model, regressing observed DMI on calculated MBW and ADG, with the period included as a blocking factor.

The base model used was: $Yj = \beta_0 + \tau_i + \beta_1 MBW_j + \beta_2 ADG + e_j$

Yj is the observed DMI of the jth animal, β_0 is the regression intercept, τ_i is the fixed effect of the ith period, β_1 is the regression coefficient for MBW, β_2 is the regression coefficient for ADG, and e_j is the random error associated with the jth animal.

The actual DMI minus the predicted DMI corresponds to the RFI. This means that a more efficient animal has a negative RFI (observed feed intake is less than predicted feed intake), and a less efficient animal has a positive RFI (observed feed intake is greater than predicted feed intake).

Annex 2

TITANIUM DIOXIDE ANALYSIS PROCEDURE
Updated November 2007

03796 - IEA
F. Titanium atomic spectroscopy standard concentrate 1.00g Ti ampoule 1,00g/L for 1L standard solution. MW: 189,68 g/mol

I. Reagents

A. Concentrated Sulfuric Acid (H_2SO_4)
ACS grade, from Chemical Stores

B. 30% Hydrogen Peroxide (H_2O_2)
From Chemical Stores

C. Kjeldahl digestion catalyst tablets (containing 3.5 K_2SO_4 + 0.4 $CuSO_4$)
PRO-PAC Tablets (Alfie Packers, Inc., Omaha, NE)

D. Titanium Dioxide (TiO_2)
Titanium (IV) oxide, rutile, 99.5% min (Alfa Aesar #43047)

E. Distilled water

II. Standards

= 1000 ppm ampoule

Standards (mg TiO_2/100 g) are prepared in the same manner as samples. Begin by exactly weighing (in duplicate) titanium dioxide in the amounts shown below using a 5 decimal-place balance (in AB 116). Carefully transfer the weighed titanium dioxide into a pre-weighed and acid-washed 250 mL macro-kjeldahl digestion tube, and continue with procedure at Step #2 as described for sample analysis.

Standard (mg TiO_2/100 g)	g TiO_2 added per tube
0	0.00000
2	0.00200
4	0.00400
6	0.00600
8	0.00800
10	0.01000

0 - 360 - 840 - 1200 - 3600 - 4800 - 6000 - 8400 µl into 100ml volum flasks add some dem. H_2O. Then add 5ml H_2SO_4 cool down add 15ml H_2O_2 fill up with destil. water.

III. Procedure

1. Weigh duplicate 0.5 g samples into pre-weighed and acid-washed 250 mL macro-kjeldahl digestion tubes. For each set of 20 tubes (max number for digestion block), include 2 baseline samples of feces (or duodenal, ileal digesta) collected from an animal that was not fed titanium dioxide.
2. Add 1 catalyst tablet to each digestion tube.
3. Carefully pipette 13 ml of concentrated H_2SO_4 to each tube. Place tubes into Kjeldahl digestion block preheated to 420°C under the hood. Carefully place aspirator onto tubes and turn water on. Visualize smoke being drawn from tubes. Wait 10 minutes for block to return to temperature. Digest samples at 420°C for 2 hours.
4. Remove tubes from heat and allow 10 minutes before removing aspirator. Then allow tubes to cool for a minimum of 30 minutes.
5.** Add 15 ml of 30% H_2O_2 to each digestion tube, and allow to cool for 30 minutes. Samples should turn an intense greenish-orange/yellow color and sometimes form a gel-like appearance upon addition of peroxide.

(3ml 5gauge)

6. Add distilled water to each digestion tube to bring the total liquid weight up to 100 g. Swirl or vortex each tube, and filter contents to remove any precipitate using an acid-washed plastic funnel through Whatman 541 filter paper into an acid-washed 250 mL Erlenmeyer flask (or other suitable acid-washed beaker, etc.).

7.** Add 5 drops of 30% H_2O_2 to each Erlenmeyer flask, and swirl.

8. Read samples on the UV/Vis spectrophotometer at 410 nm. Calibrate spectrophotometer with working standards as described above. Use the zero standard to zero the instrument.

**Denotes modification to the Myers et al. (2004) procedure.

IV. Calculations

Subtract the baseline sample from the TiO_2 samples for each run of samples (20 tubes).

$$\text{mg } TiO_2/g = \frac{\text{mg } TiO_2{}^{\text{sample}}}{\text{sample wt (g)}} - \frac{\text{mg } TiO_2{}^{\text{baseline}}}{\text{sample wt (g)}}$$

$$\% \text{ } TiO_2 = \text{mg } TiO_2/g \div 10$$

$$\text{ppm } TiO_2 = \% \text{ } TiO_2 \times 10000$$

EXAMPLE: If a sample read 5 mg/100 g TiO_2 and your baseline sample read 0.25 mg/100 g TiO_2 (both with sample weight of 0.500 g), your calculations would be:

$$\frac{5 \text{ mg } TiO_2}{0.5 \text{ g}} - \frac{0.25 \text{ mg } TiO_2}{0.5 \text{ g}} = 9.5 \text{ mg } TiO_2/g$$

$$9.5 \text{ mg } TiO_2/g = 0.95\% \text{ } TiO_2 = 9500 \text{ ppm } TiO_2$$

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