The 41th Animal Nutrition Research Forum

Wageningen, April 15 2016

LIVESTOCK RESEARCH
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41th Animal Nutrition Research Forum

Wageningen UR Livestock Research

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Presentations Session 1

Thermal and shear processing of pea protein concentrate: Effects on secondary structure and protein hydrolysis

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Introduction

Processing of ingredients for feed manufacturing involves the use of heat and shear. Heat can induce changes in the proteins with positive (e.g. denaturation and random coil formation) or negative (e.g. protein aggregation and Maillard reactions) effects on protein digestibility (Gerrard et al., 2012). Shear is the dissipation of mechanical energy through friction of particles. Effects of shear on protein nutritional value can be similar to those of heat (Lei et al., 2007).
The disruption of the secondary or tertiary structure of the proteins due to heat or shear can facilitate the access of the enzymes for protein hydrolysis. Nevertheless, only limited effects of increasing screw speeds during extrusion of soybean meal were reported (Marsman et al., 1993).
The aim of the present study was to test the effects of thermal and shear processing on the secondary structure and hydrolysis of the proteins in a pea protein concentrate (PPC).

Materials and methods

The experiment consisted of 3 treatments: native PPC, heat-processed PPC (H) and heat and shear-processed PPC (HS). Shear processing was performed at 90 °C for 20 min in the shear cell (Laboratory of Food Processing Engineering, Wageningen University, The Netherlands) with (HS) or without (H) the input of mechanical energy. Before processing the PPC was mixed with water in a 1:3 (w/w) ratio. The shear cell is capable of simulating the conditions used during extrusion (Draganovic et al., 2014). This device consists of a stationary cone and a rotating plate, which have a grooved surface in order to avoid slippage. Temperature in the jacketed cone and plate can be controlled through an oil bath. Temperature and torque values during processing can be monitored online (Thermo drive unit, Thermo Scientific, Staffordshire, UK). Processing in the shear cell was performed in duplicate. Following processing, the samples were freeze-dried and ground through a 1 mm sieve (ZM200, Retsch, Haan, Germany).
Hydrolysis was performed using the pH-STAT method after the addition of trypsin. Briefly, 10 mL of a protein suspension in water containing 1 mg N/mL were equilibrated to pH 8 using a 0.1 M NaOH solution. After equilibration, 1 mL of a trypsin solution (1.6 mg trypsin/ml water, T8253, Sigma-Aldrich, St. Louis, MO, USA) was added. Hydrolysis was allowed to proceed for 60 min at 39 °C. Degree of hydrolysis (DH) and fractional rate of hydrolysis (k) were calculated according to the equations described by Butré et al. (2012). The PROC MODEL procedure from SAS (SAS Institute, 2011) was used to model k.
Samples were re-ground with a ball mill (MM2000, Retsch) for 3 min for secondary structure measurement. Amide I region (1600 – 1700 cm^{-1}) was measured using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR, Tensor 27, Bruker, MA, USA). The spectra was deconvoluted according to the procedure described by Hu et al. (2006) and analysed using the OPUS software Version 7.2 (Bruker). Identification of the resulting peaks was performed according to literature (Carbonaro et al., 2012).
Statistical analysis was performed using the PROC GLM procedure from SAS with treatment as fixed factor. *Post-hoc* testing was performed using the Bonferroni adjustment.

Results and discussion

Heat and heat-shear processing increased the formation of intermolecular □-sheet hydrogen-bonded aggregates (A2) and random coils (Fig. 1a). The proportion of random coils was higher in the H compared to HS processed PPC. In contrast, formation of A2 was higher in the HS processed compared to H processing only. Whilst random coil formation facilitates the access of enzymes for cleaving of peptide bonds, the formation of aggregates can reduce it. Although the formation of A2 and random coils originated from the more stable conformations in the secondary structure (e.g. □-sheets, □-helices and T2-turns), there was no difference ($P>0.05$) in the proportion of these structures between the processing treatments.

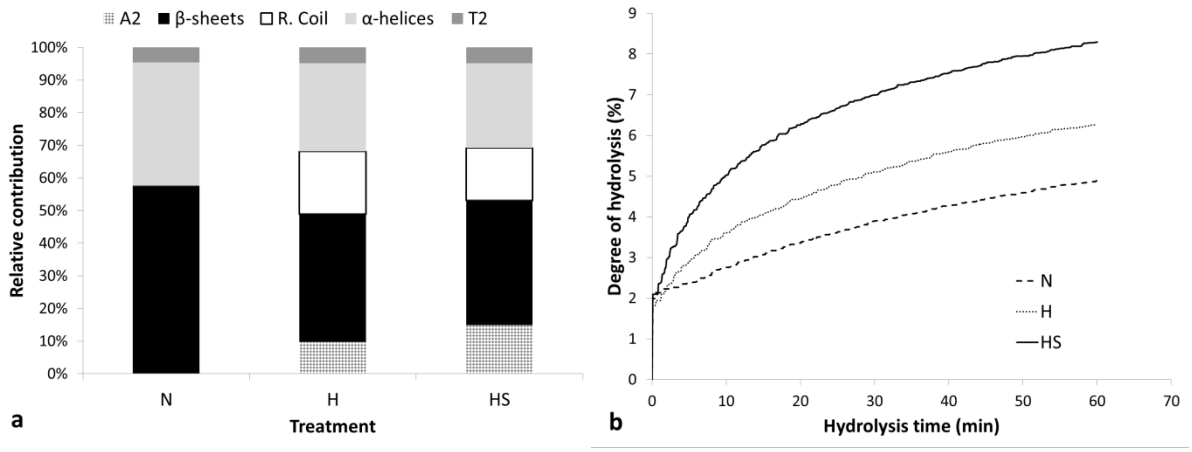

Figure 1. Proportion of secondary structure (a) and degree of hydrolysis (b) of native (N), heat (H), and heat and shear (HS) processed pea protein concentrate.

The DH was higher in the HS processed PPC compared to H processing, and higher in both of these treatments compared to the native PPC (Fig. 2b). The DH of H processed PPC was 28% higher than the native PPC, whilst that of HS processed PPC was 69% higher than native PPC. A higher DH probably indicates that the proteins are more digestible. In contrast, the *k* was higher ($P<0.05$) in the native PPC (0.100 s^{-1}) compared to either processing treatments (0.057 and 0.060 s^{-1} for H and HS treatments, respectively). The rate of hydrolysis in the processed PPC could be reduced due to the formation of aggregates (A2) in the secondary structure. However, changes in the secondary structure do not completely explain the observed changes in enzymatic hydrolysis. It could be possible that the tertiary structure of the proteins was more affected by HS processing than H processing only. This could facilitate the access of trypsin for enzymatic hydrolysis.
In conclusion, heat and heat-shear processing of PPC induces the formation of aggregates and random coils in the secondary structure of pea proteins and increases the degree of enzymatic hydrolysis.

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Surplus dietary tryptophan reduces plasma urea concentrations in endotoxaemic pigs

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Introduction

The essential amino acid tryptophan not only serves as building block for protein synthesis but also affects many physiological pathways in the body through modulation of serotonin, a neurotransmitter involved in organ and tissue functionality. Tryptophan is the immediate precursor for serotonin synthesis, and as such, dietary tryptophan is able to affect whole body serotonin production by mass action (Koopmans et al, 2006; Lallès et al, 2009). Previously we have shown that surplus dietary tryptophan exerts positive effects on stress, behavior, endocrinology, immunology and intestinal morphology in post-weaning and/or juvenile pigs (Koopmans et al, 2005 and 2012). Infection and inflammation lead to increased degradation of serotonin and increased sickness-behaviour and catabolism (Richard et al, 2009). We hypothesized that supplementation of surplus dietary tryptophan may alleviate impaired life functions during inflammation. The objective of the present study was to compare the effects of suboptimal versus surplus dietary tryptophan on performance, gut robustness and clinical health in a pig model of intraperitoneal endotoxaemia.

Materials and methods

In total, 44 crossbred piglets were weaned at 4 weeks of age (ca 8 kg BW) and housed individually over a period of 3 weeks. Three dietary L-tryptophan levels were compared: 1) sub-optimal (basal diet containing ~1.8 g TRP/kg diet), n=14; 2) optimal (basal diet plus 0.3 g L-TRP), n=16; 3) surplus (basal diet plus 5 g L-TRP), n=14 ; in control and endotoxaemic piglets. LPS from E Coli was injected in half of the pigs on days 4, 7, 11 and 14 at doses of 2, 16, 32 and 64 µg/kg, respectively and subsequently blood and gut tissue was collected from all pigs on days 18-22.

Results

LPS caused a transient fever response (+1.5 ^{o}C), reduced feed intake by ~15% and increased plasma C-reactive protein (CRP) concentrations by ~40% (all $p<0.05$) but TRP did not affect the fever response, feed intake, body weight gain nor CRP. However, TRP reduced plasma urea concentrations by ~30% ($p<0.01$) in LPS-treated but not in control piglets at identical feed intake. Furthermore, TRP increased the weight of chyme in the ileum ~2.5-fold, increased the empty weight of the duodenum by ~17%, and increased baseline body temperature by +0.6 ^{o}C (all $p<0.05$) in all piglets.

Conclusion

TRP is a bioactive amino acid which protects from whole body protein catabolism during inflammation, modulates passage time of digesta in the small intestine, increases the anabolism of the duodenum, and increases baseline body temperature.

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Effects of new potential prebiotics on *Salmonella* Typhimurium in pigs

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Introduction

S. enterica subsp. *enterica,* serovar Typhimurium is one of the most important zoonotic pathogens in pigs (Methner et al., 2011). Prebiotics are nowadays under scrutiny to limit infections. The effect of two novel carbohydrates on performance, transmission and colonization of *Salmonella*, intestinal eco-physiology and immunity in weaning piglets were evaluated in a Trojan challenge model (Pieper et al., 2012).

Material and methods

Sixty-four 28 d-old weaned pigs were distributed between 4 dietary treatments (2 pens/treatment): semi-synthetic diet supplemented with 3% isomaltooligosaccharides (IMO), pecticoligosaccharides (POS), inulin or saccharose (control). After 11 days of adaptation, two pigs per pen were orally challenged with 2 ml of a culture (10^9 cfu/ml) of *S*. Typhimurium (Trojan pigs). Then, they returned into their original pen with 6 remaining pigs (Contact pigs). Rectal swabs were taken every day from each individual piglet after morning feeding for 5 days to test for the presence of *S*. Typhimurium. Body weights were recorded every 2 days to calculate daily weight gains (DWG). Six to seven days after challenge, 2 Trojan and 2 Contact pigs per pen were slaughtered to collect tissue (mesenteric lymph nodes (MLN), spleen, and gallbladder) for *Salmonella* colonization test. Blood was collected into EDTA tube for Immunoglobulin A, G, M ELISA tests. Ileal, caecal and rectal digesta samples were collected for short-chain fatty acids (SCFA) analyses. The data were assessed using a one or two-ways ANOVA model in the R 3.1.0 software. The Student–Newman–Keuls post-hoc test was applied for comparisons of means

Results and discussion

Only one piglet displayed clinical signs of illness and was removed for a diagnosed pneumonia from the IMO group before challenge. Trojan and contact pigs showed nor differences in DWG different treatments according to the diet during pre- and post-challenged periods (data not shown). However, there was a difference in DWG between Trojan (46 $g.day^{-1}$) and Contact pigs (143 $g.day^{-1}$) ($P = 0.006$). Almost all Trojans from each dietary treatment were found positive with *S*. Typhimurium in feces during the post challenge period (Table 1) while the total number of fecal shedding contact pigs was lower, between 50% to 92%.

Table 1. Presence of *S*. Typhimurium in feces of Trojan (T; N=4) and Contact pigs (C; N=12, except for IMO where N=11)

	Time post infection [d]										**Number of pigs shedding**	
Diets	**1**		**2**		**3**		**4**		**5**		**at least once over 5 d**	
	T	C	T	C	T	C	T	C	T	C	T	C
IMO	2	5	4	6	4	10	4	4	4	5	3	2
Inulin	4	5	4	7	3	7	4	7	4	6	2	3
POS	4	10	4	7	4	10	4	11	3	7	3	5
Control	4	9	4	9	4	8	4	6	4	6	4	3

Fifty to seventy percent of the Trojans were colonized by *S*. Typhimurium in mesenteric lymph nodes and spleen but almost none in the gallbladder (Table 2). Although not significant, it seemed that IMO could prevent to some extent the colonization by *S*. Typhimurium.

In Trojans, POS-fed pigs had higher levels of plasma IgM than control ones ($P = 0.04$) (Table 3). Nonetheless, plasma IgA and IgG levels were not affected by different diets.

SCFA concentrations were not consistently affected by the diet in both Trojan and Contact pigs. However, butyrate in AC of IMO-fed Contact pigs (11.2%) was higher than control (7.6%).

Table 2. Detection frequency of *S.* Typhimurium in mesenteric lymph nodes (MLN), spleen, and gallbladders

	Trojan pigs (N=4)			**Contact pigs** (N=4)		
Diets	**MLN**	**Spleen**	**Gallbladder**	**MLN**	**Spleen**	**Gallbladder**
Inulin	2	2	1	1	0	0
POS	3	2	0	0	0	0
IMO	0	1	1	1	0	0
Control	2	3	0	1	0	0

Table 3. Serum immunoglobulins (log ng.ml^{-1}) (N=4)

	Trojan pigs			**Contact pigs**		
Diets	**IgA**	**IgG**	**IgM**	**IgA**	**IgG**	**IgM**
Inulin	5.6	6.6	6.3^{ab}	5.6	6.8	6.3
IMO	5.5	6.6	6.4^{ab}	5.6	6.6	6.2
POS	5.6	6.7	6.5^{a}	5.6	6.6	6.2
Control	5.6	6.7	6.1^{b}	5.6	6.7	6.3
SEM	0.17	0.16	0.25	0.11	0.15	0.12
P values	0.84	0.84	0.04	0.85	0.34	0.51

Table 4. Total short-chain fatty acid (SCFA) and acetate (C2), propionate (C3), n-butyrate (C4), and lactate (LA) molar ratios in intestinal contents (N=4)

		Trojan pigs					**Contact pigs**				
Diets	**Segment**	**SCFA**	**C2**	**C3**	**C4**	**LA**	**SCFA**	**C2**	**C3**	**C4**	**LA**
		(mg.g^{-1})	(%)	(%)	(%)	(%)	(mg.g^{-1})	(%)	(%)	(%)	(%)
Inulin	Ileum	15.9	3.7^{b1}	4.3^{b}	2.4^{c}	79.6	7.5^{bc}	10.3^{d}	2.7^{d}	2.4^{c}	52.2
	Caecum	17.3	34.5^{a}	18.8^{a}	11.1^{a}	ND[3]	16.0^{abc}	36.3^{ab}	22.6^{a}	10.1^{ab}	ND
	AC[2]	16.9	35.3^{a}	16.3^{a}	9.0^{ab}	ND	22.2^{ab}	32.8^{ab}	14.2^{b}	8.6^{ab}	ND
IMO	Ileum	8.9	13.6^{b}	3.2^{b}	2.3^{c}	62.4	6.1^{c}	18.2^{bcd}	5.0^{cd}	4.0^{c}	51.9
	Caecum	22.6	30.2^{a}	12.9^{a}	8.2^{ab}	ND	16.8^{abc}	39.3^{a}	15.3^{b}	9.3^{ab}	ND
	AC	16.0	40.4^{a}	15.3^{a}	8.6^{ab}	ND	27.4^{a}	32.1^{ab}	14.7^{b}	11.2^{a}	ND
POS	Ileum	11.2	5.1^{b}	5.2^{b}	3.3^{c}	71.0	7.3^{bc}	14.4^{cd}	5.6^{cd}	2.7^{c}	48.7
	Caecum	21.9	35.8^{a}	18.1^{a}	7.7^{ab}	ND	16.9^{abc}	39.4^{a}	17.1^{b}	9.1^{ab}	ND
	AC	20.9	35.6^{a}	14.8^{a}	7.2^{b}	ND	27.0^{a}	31.9^{ab}	11.2^{bc}	7.7^{b}	ND
Control	Ileum	12.4	5.2^{b}	3.2^{b}	1.2^{c}	80.3	9.7^{bc}	6.6^{d}	4.0^{d}	2.7^{c}	68.2
	Caecum	15.5	38.8^{a}	18.6^{a}	9.7^{ab}	ND	14.0^{abc}	41.9^{a}	17.5^{b}	8.4^{ab}	ND
	AC	22.8	31.3^{a}	13.4^{a}	7.1^{b}	ND	26.5^{a}	27.9^{abc}	10.6^{bc}	7.6^{b}	ND
SEM		6.8	5.5	6.7	3.5	17.2	79.6	13.6	6.1	3.1	21.5
	P-value Diets	0.84	0.76	0.16	0.16	0.45	0.85	0.35	0.85	0.02	0.62
	Segment	<0.01	<0.01	<0.01	<0.01	-	<0.01	<0.01	<0.01	<0.01	-
Diets x Segment		0.16	0.24	0.34	0.08	-	0.69	0.79	0.03	0.08	-

[1]For one column, means followed by a different letter differ for $P < 0.05$; [2]AC, ascending colon. [3]ND, not detectable

It can be concluded that compared to POS, IMO seems potentially more interesting and possibly comparable to inulin since it displays a consistent, although not always significant, beneficial effect on pathogen transmission and colonization, and butyrate production. However, results from this experiment should be taken with caution because of a low numbers of pigs regarding rather high inter-individual variation.

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Impact of wheat bran supplementation to sows on their milk quality, their performances and their progeny's.

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Introduction

To improve sustainability in pig production, there is a need to find alternatives to antibiotics that were used as growth promoters. These have been commonly used in Europe (Lallès et al. 2004, Zanello et al. 2013) especially in piglets feed acting not only as a growth promoter but as well in order to prevent the recurrent post-weaning diarrhoea. In 2006, the European Union definitely banned the use of antibiotics as growth promoters in the diets of pigs and poultry (EU IP/05/1687). A strategy to prevent the post-weaning diarrhoea is the use of feed additives, consisting i.e. of organic acids, probiotics, prebiotics, trace minerals and protein sources (Heo et al., 2013). More and more research now focusses on the impact of dietary fibres on health and microbiota of pigs. Wheat bran (WB), a source of insoluble non-starch polysaccharide (NSP) rich in arabinoxylans (Kamal-Eldin et al., 2009) is considered as a dietary fibre source and is commonly used in sows' diets i.e. for its bulking properties. The interest of this study mostly relies on the fact that piglets' health is closely related to the sow's condition, as highlighted by Paßlack et al. (2015), as offspring depend on sows for nutrients via milk and microbiota acquirement by contact with maternal faeces. As WB (140g/kg DM) has been shown to impact faecal microbiota and increase butyrate production on growing pigs (Ivarsson et al., 2014), the hypothesis is that giving a high-WB diet to sows will promote a healthy microbiota, and that this will impact piglets' health by a different composition of milk and the early establishment of a beneficial intestinal microbiota that could protect them from post-weaning diarrhoea.

Materials and methods

Sows used in this study were housed at the Walloon Agricultural Research Centre. The breed used was Landrace, parity 1 to 3, except for one sow (parity 5). The insemination was performed with Piétrain semen. Eight sows were fed an enriched WB diet while seven sows received a control diet (CON) containing no wheat bran. The dietary treatment extended from day 43 of gestation (WB 240g/kg DM) until the end of the lactation period (WB 140g/kg DM). Milk was sampled weekly from all functional tits, from birth until day 20 of lactation. Immunoglobulin contents (IgG and IgA) were quantified by *ELISA* using specific antibodies (Bethyl Laboratories Inc.), and lactose, fat and protein contents were determined using mid-infrared technology calibrated for sows' milk. Zootechnical performances of sows and piglets were recorded: weight and backfat changes of the sows between insemination and day 39, between d39 and d109 (diet change), between d109 and weaning (lactation period), ingestion from day3 to day26 of the lactation period (expressed as the percentage of the target ingestion curve) and weight of piglets each week from birth to weaning. Statistical analyses were performed using the MIXED procedure of SAS with repeated measurements.

Results and discussion

Zootechnical performances of sows and piglets

Backfat changes of sows after the diet change and at the end of the lactation period were not affected by the dietary treatment ($p=0.60$); neither were the bodyweight changes ($p=0.77$) (data not shown). Ingestion of sows was split into 4-days periods from day 3 to day 26 of the lactation period. The ingestion was similar between treatments during most of the lactation period but dropped significantly ($p<0.001$) for WB sows during the 6th period of time (66% of their planned feed intake-curve for the WB group vs 89% for the CON group). Concerning the litters' bodyweights, there was no effect of maternal treatment on piglets' bodyweights ($p=0.51$) from birth until weaning (data not shown).

Milk composition

IgA and IgG concentrations were not impacted by the treatment in general ($p=0.88$ and $p=0.47$, respectively). There was a significant time effect ($p<0.0001$) on the milk immunoglobulins of milk. Indeed, colostrum is very rich in IgA (13.6mg/ml on average) and particularly in IgG (66.2 mg/ml on average), and from the first week of lactation onwards, the concentrations of IgG were nearly 0 (0.40mg/ml on average). This highlights the importance of colostrum intake, especially because of the lack of maternal immunoglobulin transfer during gestation in pig (Ye et al., 2008).

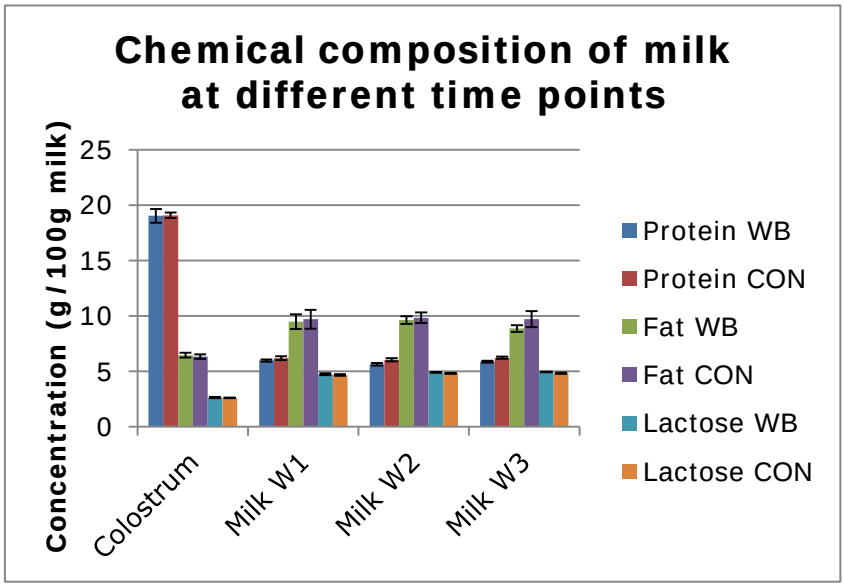

Figure 2. Chemical composition of milk at different time points (g/100g milk).

Concerning the chemical composition of milk, protein and fat were never affected by maternal treatment ($p=0.14$ and $p=0.46$, respectively). Interestingly, research on mannan incorporation in sows' diets didn't show any differences in fat, lactose, IgA or IgM but showed a significant increase in IgG for supplemented sows (Graugnard et al., 2014). Colostrum was always much more concentrated in protein than milk as shown in Figure 1. There was a time effect ($p<0.001$) and a treatment effect ($p=0.03$) without interaction for milk lactose concentration; however no treatment effect was observed per time point. Lactose concentrations were lower for colostrum than for milk.

In conclusion, the maternal dietary treatment did not impact the performances of the sow, neither the litters mean bodyweight. Colostrum and milk composition were not affected by the dietary treatment. In the future, the composition of the microbiota of the sows' feces and piglets' colon will be determined to investigate if dietary fibres provided to the maternal diet can prime the digestive tract of the progeny.

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Effects of neonatal microbial association and early life diet composition on systemic immunology and on in-situ gut functionality of caesarean derived piglets in later life

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Introduction

Little is known about the effect of early microbial colonization of the gut and subsequent early life feeding on the further development of the intestinal microbiota and on the development of the host immune system and functionality of the digestive tract (Bailey et al. 2005). Recently we have developed a model in which new-born piglets are subjected to either a simple (SA) or complex (CA) microbial exposure in a standardized way (Jansman et al, 2012).

Materials and methods

Twenty-four caesarean derived piglets were orally dosed on d 1-3 a simple microbiota consisting of 10^6 - 10^7 CFU from each *Lactobacillus amylovorus*, *Clostridium glycolium* and *Parabacteroides sp.* and, in addition, piglets received on d 3-4 a faecal inoculant obtained from a conventional adult sow (Complex Association, CA), or placebo (Simple Association, SA). Half of the CA and SA piglets received a moist diet with medium chain triglycerides (MCT) or a control (Con) diet with soya and palm oil. In-situ small intestinal segmented perfusion (SISP) experiments were conducted to investigate the functionality of the gut (Van der Meulen et al, 2010). In the SISP, the effects of pathogenic E.coli (ETEC)-induced intestinal fluid absorption (as a measure of gut functionality), gut wall permeability for fluorescein sodium salt (NaF, a measure of barrier function) and intestinal alkaline phosphatase concentration (IAP, a measure of intestinal detoxification capacity) were investigated.

Results and discussion

In the period between 14 and 21 days after birth, a higher incidence of diarrhoea was observed in SA compared to CA piglets. At 4-5 weeks of age, body weights were similar among treatment groups SA-Con, SA-MCT, CA-Con and CA-MCT (8.3 ± 1.2 kg) and the numbers of leukocytes and, in particular, neutrophil granulocytes in blood were higher in CA compared to SA piglets. These blood cells are part of the innate immune system and this may suggest that CA piglets are more prepared to withstand non-specific bacterial challenges. During the SISP, ETEC perfusion caused a 5-fold reduction ($P<0.001$) in net fluid absorption (µl/cm^2), a 50% increase ($P<0.001$) in gut permeability for NaF (ng/cm^2) and a 33% reduction ($P<0.02$) in IAP concentration (µg/mg protein). Neither neonatal microbiota association nor feeding diets containing either soya and palm oil or medium-chain triglycerides from day 5 after birth onwards significantly affected gut net fluid absorption or gut permeability. Piglets expressing a complex neonatal microbiota, however, showed 2-fold higher IAP concentrations in the perfusion fluid collected from the ileum ($P<0.02$) compared to piglets associated with a simple microbiota at young age. Association with a complex microbiota in the neonatal phase may therefore enhance the capacity of 4-5 week old piglets to detoxify bacterial lipopolysaccharides in the ileum.

Conclusion

Neonatal association with a complex microbiota (CA) compared to a simple microbiota (SA) reduced the incidence of diarrhoea and increased the resilience to withstand non-specific bacterial challenges, as based on the higher number of neutrophil granulocytes in blood of CA piglets. During an in-situ functionality test of the small intestine, neither CA nor early life feeding of an MCT diet was able to modulate the severe physiological effects of an ETEC challenge, however detoxification capacity of bacterial lipopolysaccharide in the ileum was increased in CA piglets.

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Feeding and health: the case of amphibians

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Introduction

Amphibian nutrition is one of the most critical aspects to consider in captive management programs. Health issues, reproductive output as well as success in breeding programs, are directly or indirectly linked with dietary management. Critical aspects, including metabolic pathways and specific functions of several nutrients, remain totally unknown in most amphibian species (Brenes & Dierenfeld 2011). Like insectivorous species maintained in captivity, anurans are prone to nutritional imbalances, for example, calcium and vitamin D deficiencies have been widely studied compared to other nutrients such as fat-soluble vitamins. Skin pigmentation, changes and health, specifically linked with dietary vitamin A concentrations, and their relationship with reproduction, have been studied in some amphibian species in captivity, and all are related to nutrition (Duellman and Trueb, 1994, Salazar 2000).
Likewise, environmental conditions have an important effect on body condition, and influence both food abundance and its utilization. Amphibians, like other species, are dependent on food quality including caloric content and digestibility, and impacted by behaviors such as prey capture efficiency and other energy expenditure(s) (Somsueb & Boonyaratpalin 2001, Pelegrin et al. 2004, Brenes & Dierenfeld 2014). The passage of food through the gastrointestinal tract and nutrient digestibility both have been investigated in several frog species; selective retention of food in the gut may serve to prolong the contact of the digesta with absorption sites and hence maximize nutrient assimilation; these features may vary widely, depending on activity and life stage (Gossling et al., 1980).
This presentation aims to showcase several recent studies of amphibian nutrition: a) the impact of dietary carotenoids as a potential source of vitamin A and/or coloration important for health, using the false tomato frog *Dyscophus guineti* as a model; b) summarization of blood parameters, body condition and coloration in three species of tree frog *Agalychnis* sp. from the wild, to evaluate health, nutritional status, and variation over time, and c) investigations currently in progress using *Xenopus laevis* to examine passage rate and digestibility.

Materials and Methods

False tomato frog study: Twenty-four animals were used for a 9-week trial period. Frogs were randomly assigned to three treatments, eight animals per treatment. Feeder crickets *Acheta domestica* were injected with either: soy oil (Control) or known concentrations of ß-carotene or mixed carotenoids including ß-carotene, lutein, canthaxantin and xanthophylls. Morphometric measurements were taken weekly, and blood samples were drawn at the beginning and the end of the trial for vitamin A analysis. Skin colors were quantified using a hand-held spectrophotometer, which registered data from the frog´s back.

Tree frog study: Eighty four adult frogs (n=77 *A. callidryas,* n=7 *A. spurrelli,* and n=23 *A. annae*) were collected from the wild. Morphometric measurements were taken as well as blood samples to obtain values of glucose, hematocrit and plasma proteins. Colors (dorsal and ventral) were measured with the same instrument used in tomato frogs.

***Xenopus laevis* study:** Twenty adult animals distributed in 10 tanks (2 animals per tank) were randomly assigned two isocaloric diets with different protein:fat ratios (40:10 or40:15, 5 tanks per diet). The tanks from each diet were maintained at different temperatures (19 to 24°C), with 1°C of difference among each. Animals were fed a bolus containing TiO_2 on Day 1, then feces were collected for 17 consecutive days for marker recovery to measure passage as well as dry matter digestibility.

Results and Discussion

False tomato frogs showed a constant weight increase of all animals throughout the trial period. Treatment had a highly significant effect ($p<0.0001$); weight increase was significantly less in treatment 2 (ß-carotene) animals; meanwhile, weights of animals in the mixed carotenoids treatment group were higher. Vitamin A from feeder crickets was higher in insects supplemented with carotenoids ($p=0.0001$), and Pearson correlation coefficients showed that dry matter intake had an important effect on weight and length of the animals. Growth results suggest that carotenoid pigments have an effect on weight gain patterns in this frog species.
Frogs´ plasma retinol (vitamin A) concentrations were identical at the beginning of the experimental period. While retinol concentrations increased numerically in frogs fed crickets exposed to all experimental treatments, only the mixed carotenoids treatment effect was statistically significant ($p<0.02$). Skin coloration demonstrated that carotenoids added to frog´s diets had an important effect in

saturation level or color intensity of the skin ($p<0.0001$). The significant increase in plasma retinol concentrations in frogs fed with a mixture of carotenoids suggests that tomato frogs have the ability to utilize at least some of the carotenoids fed as precursors for retinol and/or ultimately vitamin A activity (Brenes & Dierenfeld 2014).

Preliminary results of the *Agalychnis* sp. study reveals significant differences in blood values between *A. callydrias* from the Atlantic population compared to the Pacific, as well as compared to *A. spurrelli* ($p<0.05$). All frogs displayed time-related blood parameter and skin color intensities. Data obtained are similar to those found in *Rana catesbeiana* and *R. tigrina* (Fioranelli et al., 2005). Regarding the *Xenopus laevis* trial, results from passage and digestibility studies are pending laboratory analysis.

Nutrition is one of the basic aspects to take into account in animal management and health. Assessing accurate feeding management and nutritional evaluation criteria of amphibians will have an important impact in conservation and breeding programs, both *ex situ* and for managed populations in zoos.

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Nutritional management in a horse after caecocolic intussusception **with almost total typhlectomy: a case report**

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Introduction

Caecocolic intussusception in the horse is a condition believed to result from abnormal intestinal motility, moving ingesta from the apex of the caecum into the caecal base and right ventral colon (Milne *et al*., 1989). Factors believed to predispose to this condition include infestation with parasites (Owen *et al*., 1989; Mair *et al*., 2000), caecal abscesses (Pearson *et al*., 1971) and motility-modifying drugs (Pearson *et al*., 1975). Clinical signs can be diverse. While certain horses suffering from caecocolic intussusception will develop acute colic, other will present with intermittent abdominal pain (Gaughan and Hackett, 1990). Treatment requires surgery, and, depending on caecal compromise and whether the intussusception can be manually reduced, partial or complete typhlectomy may be indicated (Hubert *et al*., 2000). Unfortunately, there is very little information available at this time about optimal nutritional management after typhlectomy in the horse. And as there is no indication of caecal regrowth in horses, as opposed to rabbits (Herndon and Hove, 1955), it is yet important to take some nutritional considerations into account after large partial caecum amputation.

Case history

A 492kg 17-year-old Haflinger gelding was presented at the Faculty of Veterinary Medicine of Ghent University with complaints of acute colic. Clinical examination resulted in a presumptive diagnosis of caecocolic intussusception, which was later on confirmed by exploratory laparotomy. The detected intussusception could be reduced manually during surgery, but the affected caecum was severely oedematous and showed significant irritation at serosal level. Therefore, a large partial typhlectomy was performed, only leaving the base of the caecum in situ. Postoperative recovery after the two-hour surgery was uneventful.

Nutritional management

Whereas immediate post-surgical support in typhlectomized horses aims to maintain current bodyweight, the long-term goal is to obtain and maintain an optimal body condition score. Since decreased digestibilities of energy, protein and dry matter can be a consequence of equine caecal amputation (Sauer *et al*., 1979), a 20% increase above maintenance energy requirements was provided post-op to try and maintain current body weight, as well as a protein supply well above NRC (2007) requirement. Having almost lost its entire caecum, the horse was initially fed short-cut and pelleted roughage in order to reduce the physical and mechanical load on the remaining colon, as is common practice in diet formulations for horses suffering from right dorsal colitis (Cohen *et al*., 1995). A dry matter amount of 1% on ideal bodyweight of roughage (dried short-cut grass, non-molassed beet pulp and alfalfa pellets) was provided, as this is the minimum recommended value to maintain gastro-intestinal health (Geor and Harris, 2007). A high-energy/high-protein concentrate, specifically formulated to provide nutritional support to pregnant or lactating **mares** (Pavo PodoLac-stalseizoen) was advised to focus primarily on small intestinal digestion. The diet was divided in four meals a day to reduce starch intake below 1 g/kg bodyweight per meal, which is recommended in colic prone horses (Durham, 2013). Furthermore, feeding small but frequent meals may also aid better digestion and absorption (Harris, 2007). Corn oil was added to the diet to further increase energy intake, finally resulting in a total dietary crude fat of 5.5% (DM basis). This vegetal oil was gradually introduced to allow the horse to get used to its taste, and to monitor faeces for any sheen on the fecal balls, as this could be a sign of some fat escaping digestion (Kronfeld *et al*., 2004). Additionally, a Saccharomyces cerevisiae supplement (Yea-sacc^{1026}, 4x10^{9}CFU) was added to the ration in order to help support hindgut fermentation (Medina *et al*., 2002), along a general vitamin and mineral supplement to ensure NRC (2007) nutrient requirement minimums, including a safety factor of 1.2, were met. To prevent peroxidation, an additional requirement of 1 IU of vitamin E per ml of added vegetal oil was taken into account when calculating NRC vitamin E requirements (Harris, 1999). Furthermore, water and a salt lick were provided *ad libitum.* Finally, all straw was removed from the stable to prevent any long-stem roughage intake.

Follow-up

All diet components were introduced gradually over the course of five days, and were well tolerated. Ten days after the surgery, the horse was discharged from the hospital. As the horse continued to recover at home, the post-operative diet was maintained. After the horse remained doing well for a whole month on the new diet, a slow transition towards a more traditional diet containing long stem roughage was made. Afterwards, the post-operative diet ingredients were gradually decreased in order to remain on an all-grass hay diet only (supplemented with a forage balancer). Six months after the surgery, the horse remains doing well, and even presented slightly overweight. Evaluation of more horses with the same condition is however necessary to determine long-term success of this nutritional approach to large partial typhlectomy in the horse.

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PRELIMINARY RESULTS. Feeding supplemental concentrate during the dry period: can we prepare the rumen for the lactation ration?

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Introduction

The rumen adapts to changes in feed intake on a morphological and functional level. Increases in papilla surface area and absorption of volatile fatty acids (VFA) have been observed after increasing the fermentable OM (FOS) intake (Dirksen et al., 1984; Dieho et al., 2016). In dairy cattle, the transition from the dry period to lactation coincides with a rapid and large increase in FOS intake which is generally thought to be a risk of the development of sub-acute rumen acidosis (SARA). Liebich et al. (1987) showed that feeding a lactation ration before calving increases papilla size. They hypothesized that this increase may also increase the capacity to absorb VFA already before calving, thereby reducing the risk for SARA after calving. However, recent work indicated that an increase in papilla surface area may not coincide with an increase in VFA absorption capacity (Dieho et al., 2015). This challenges the idea that the rumen can be prepared for lactation during the dry period. Therefore, we examined the effect of feeding supplemental concentrate (increasing FOS intake) during the dry period on the morphological and functional adaptation of the rumen during the dry period as well as the early lactation period. It was expected that both papilla surface area and fractional VFA absorption rate would increase during the last weeks of the dry period and in early lactation in response to feeding supplemental concentrate.

Material and Methods

Ten dry rumen-cannulated Holstein-Friesian cows entered the experiment nine weeks before the expected calving date. Cows were randomly assigned to a control (CON**;** n = 5) or treatment group (SUP; n = 5). Treatment consisted of a daily supplement of 3.0 kg DM concentrate during the last 4 weeks of the dry period. All dry cows had free access to a ration consisting (DM basis) of 27% grass silage, 28% corn silage, 11% soybean meal, and 34% wheat straw, containing (/kg DM) 791 VEM, 56 g DVE, 3 g OEB, and 476 g FOS. On the day of calving, cows were switched to a basal lactation ration consisting of 42% grass silage, 42% corn silage, and 16% soybean meal, containing 985 VEM, 87 g DVE, 13 g OEB, and 547 g FOS. From 1 to 3 d pp, 0.9 kg DM/d of concentrate was fed which thereafter linearly increased to 8.9 kg DM/d on 11 days postpartum (d pp). Concentrate contained (/kg DM) 1086 VEM, 103 g DVE, 1 g OEB, and 685 g FOS. Daily feed intake was measured throughout the experiment. On measurement days (28, 18, and 8 d antepartum (ap), and 3, 17, 31, and 45 d postpartum (pp), rumen fluid was sampled and all rumen contents were evacuated. Subsequently, rumen papillae were collected from the ventral, caudo-dorsal, and caudo-ventral rumen sacs for measurement of the surface area (Dieho et al., 2016). Fifty liters (L) of standardized buffer fluid (modified from Dijkstra et al., 1993), containing 120 m*M* of VFA (60% acetic (Ac), 25% propionic (Pr), and 15% butyric (Bu) acid) and 0.17 m*M* Co-EDTA as fluid passage marker was prepared at 39 °C with pH 5.9. After rinsing the rumen wall using 5 L of buffer fluid, and complete removal of all fluid, the remaining 45 L of buffer fluid was infused. After 60 min remaining buffer fluid was recovered from the rumen. Buffer fluid samples were taken immediately before and 60 min after infusion into the rumen for measurement of pH, and VFA and Co concentration. Fractional rate of absorption averaged for Ac, Pr, and Bu (k_aVFA), and fluid passage rate (k_l) were calculated according to Dijkstra et al. (1993). Data were analyzed with a mixed model (Littell et al., 2006) for repeated measurements.

Results and Discussion

Neither intake of DM (Figure 1), nor intake of VEM and FOS was affected by the supplemental concentrate during the dry period and lactation ($P \geq 0.305$). This indicates a (partial) substitution of the dry period ration intake by the concentrate without a carry-over effect on feed intake pp. Rumen papilla surface area (Figure 1) differed between groups before treatment started and no differences in surface area between SUP and CON were found during the experiment. An interaction between treatment and day was observed on surface area ($P = 0.038$). Surface area of SUP increased 29% from 28 to 8 d ap ($P = 0.003$), whereas it remained similar for CON ($P = 0.301$). Unexpectedly, a reduction in surface area was observed for SUP ($P = 0.018$) between 8 d ap and 3 d pp, in contrast to CON ($P = 0.368$). This decrease in surface area negated most of the effect of the supplemental concentrate ap. Such a decrease may be explained by a larger peri-parturient reduction of DM intake in SUP compared with CON. During lactation surface area increased ($P < 0.001$) similarly in CON and SUP, indicating no carry-over effect of the supplemental concentrate on pp papilla development. The surface area of the papillae found during

the dry period and lactation, and their development in time, correspond with earlier measurements (Dirksen et al., 1984; Reynolds et al., 2004; Dieho et al., 2016). The effect of supplemental concentrate on papilla development ap supports the findings of Liebich et al. (1987), but they did not observe a reduction in papilla size around calving. The results seem to contradict Reynolds et al. (2004) who did not find an effect of supplemental barley fed ap. However, they fed 1 kg/d of barley and used an end-point measurement instead of the present repeated measurement approach. In contrast to surface area, no interaction was observed between treatment and day (P = 0.910) for k_aVFA (Figure 1). It was expected that k_aVFA would increase for SUP during the dry period, but no increase was observed ($P \geq$ 0.478). During the dry period k_aVFA remained similar (P = 0.591), but during lactation an increase was observed (P = 0.003) for both CON and SUP. The values for k_aVFA found in this study agree with those reported by Dijkstra et al. (1993), and Dieho et al. (2015). The apparent lack of response in k_aVFA to supplemental concentrate during the dry period suggests factors besides surface area influence k_aVFA measured using a standardized buffer. Similarly, Dieho et al. (2015) reported that a rapid increase in concentrate allowance increased surface area but not k_aVFA, compared with a gradual increase of concentrate allowance.

In conclusion, feeding supplemental concentrate during the dry period increased papilla surface area without increasing k_aVFA. This increase in papilla surface area did not carry-over into lactation. The present findings do not support the notion that the rumen can be adequately prepared for lactation by feeding supplemental concentrate during the dry period.

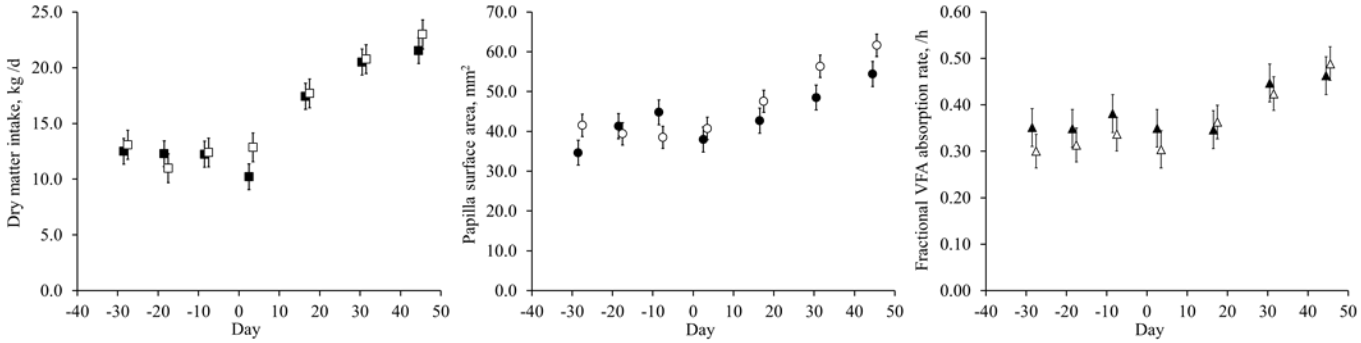

Figure 1. Dry matter intake (□■), papilla surface area (○●), and fractional VFA absorption rate (△▲) for a control dry period (□○△) and dry period with supplemental concentrate (■●▲), and in the subsequent lactation. Values represent LS-means (± SE) and are slightly offset for clarity.

Acknowledgements

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Available on request.

Inter-animal variation in milk fat content and milk fat C18:1 trans-10 concentration in early lactating dairy cows

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Introduction

Subacute rumen acidosis (SARA) represents one of the most important metabolic disorders in intensive dairy farms with a suggested incidence between 19% and 26% in early and mid-lactation in the dairy cattle (Plaizier et al., 2008).

However, besides dietary factors playing a role in SARA development, inter-animal variation has been reported during experimental SARA challenges (Brown et al., 2000; Schlau et al., 2012). Gao and Oba (2014) found that cows with less sorting behavior were more tolerant to a SARA challenge, whereas weakness in VFA absorption capacity also could partly explain why some cows were more susceptible for SARA (Schlau et al., 2012).

Milk fat depression as well as milk fat C18:1 *trans*-10 concentration has been associated with a decrease in rumen pH in SARA induction trials (Colman et al., 2010; Fievez et al., 2012). If these parameters effectively are appropriate SARA markers, they also should allow monitoring inter-animal variation in SARA susceptibility within a cohort of dairy cows. As SARA challenge trials typically are performed with a limited number of animals, which do not allow to assess inter-animal variation, the latter was monitored at herd scale in the first 4 weeks in lactation during which the amount of compound feed was gradually increased and which was considered a risky period for SARA development.

Methods and Material

At the Schothorst Feed Research, 150 Holstein cows were monitored from calving until 4 weeks in milk (WIM), when cows were subjected to a gradual increase in concentrate (from 3.7 to 4.0 kg at day 1 to 9.0 to 10.0 kg at day 22 after calving for heifers respectively older animals. The basal diet consisted of grass silage/maize silage (50/50) and soybeanmeal, pressed beetpulp, rolled barley and wheat straw. Ration was optimized to fulfill dietary SFR-recommendations of Net Energy (VEM), metabolisable protein (TMP), Met and Lys (TMMet and TMLys) and rumen fermentation parameters. The calculated level of rapidly fermentable carbohydrates (RFCH) was below maximum recommendation resulting in a low risk for SARA. About half of the samples have been analyzed until now.

Milk samples were collected once every Wednesday evening. The FA profile was obtained after milk fat extraction (mini Röse-Gottlieb method, adapted from Chouinard et al., 1997), methylation (Stefanov et al., 2010), and gas chromatographic analysis of FAME (Agilent Technologies 7890A GC System equipped with a flame ionization detector, Agilent Technologies, Santa Clara, CA). Fatty acids were expressed as grams per 100 g of FAME.

Results and Discussion

Increasing proportions of dietary concentrate coincided with increases in milk fat C18:1 *trans*-10 concentration which was consistent with previous SARA induction trials. Most C18:1 *trans*-10 outliers were observed at 2 and 3 WIM (13.51% = 10 out of 74 and 11.11% = 7 out of 63, respectively), with variation in C18:1 *trans*-10 being highest (stdev = 0.095) at 3 WIM. The frequency analysis of milk fat C18:1 *trans*-10 concentration, revealed two “separate” subhistograms (Figure 1). Nevertheless, such ‘dual picture’ could not be seen for milk fat and at maximum 1 outlier with low milk fat syndrome could be identified in each of the 4 weeks. Accordingly, the milk fat C18:1 *trans*-10 concentration showed more inter-animal variation when compared with the milk fat content, which also could be concluded by the larger coefficient of variation of C18:1 *trans*-10 as compared with milk fat: 30.77% *vs.* 17.07% at 2 WIM and 35.81% *vs.* 15.17% at 3 WIM. The decrease of milk fat content always coincided with an increase of C18:1 *trans*-10 but not vice versa.

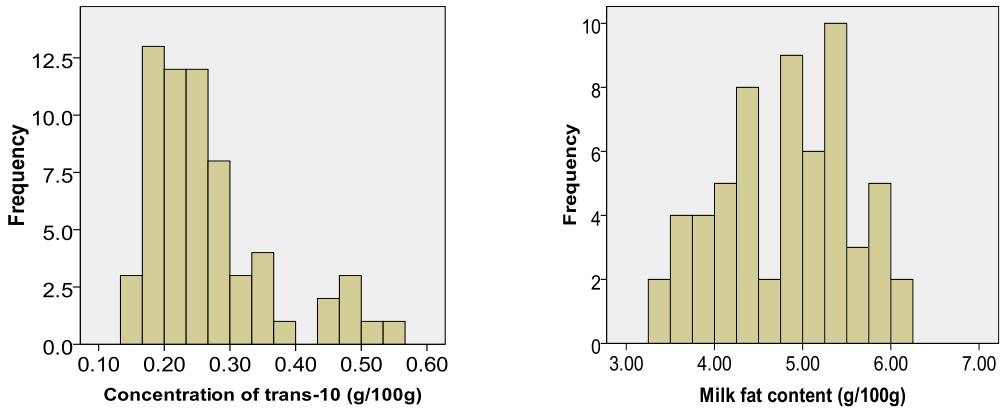

Figure 1 Frequency analysis of milk fat C18:1 *trans*-10 concentration (n=63) and milk fat content (n=60) at 3 WIM

Conclusion

Milk fat C18:1 *trans*-10 concentration could be a better marker than milk fat content in terms of studying inter-animal variation during early lactation.

Acknowledgements

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Presentations session 2

Effect of pH, glucose and Na-lactate on the biohydrogenation of 18:2*n*-6 by *Butyrivibrio fibrisolvens* and *Propionibacterium acnes*

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Introduction

Highly fermentable carbohydrates have been increasingly introduced in ruminant livestock production systems to support higher production rates and increased demand for animal products. Such feeding practices increased the incidence of milk fat depression (MFD) in dairy cattle (Bauman and Griinari, 2003). It is well established that MFD involves an inter-relationship between rumen digestive processes and mammary tissue metabolism. The basis for MFD involves alterations in rumen biohydrogenation (BH) of dietary polyunsaturated fatty acids. This BH theory proposes that under certain dietary conditions, typical pathways of rumen BH are altered to produce unique fatty acid intermediates that inhibit milk fat synthesis (Bauman and Griinari, 2003; Harvatine *et al.*, 2009). Under normal conditions, linoleic acid (LA; 18:2*n*-6) is converted to *cis-9, trans-11* CLA (conjugated linoleic acid; *c9, t11* CLA) followed by the formation of *trans-11* 18:1 (*t11* 18:1. Under conditions of MFD, LA is converted by an alternative pathway and *trans-10, cis-12* CLA (*t10, c12* CLA) and *trans-10* 18:1 (*t10* 18:1) are produced, referred to as the *trans-11* to *trans-10* shift. This shift is described to occur when high starch diets are fed, when rumen pH is low or when marine oils are fed (Bauman and Griinari, 2003; Harvatine *et al.*, 2009). However, the microbial etiology of this condition is not well understood. Possible candidates for the formation of *c9, t11* CLA and *t10, c12* CLA are *Butyrivibrio fibrisolvens* and *Propionibacterium acnes*, respectively (Wallace *et al.*, 2007). This study aimed at investigating the formation rate of both CLA isomers by these two bacteria under different rumen conditions *in vitro* to give more insight in the microbiology of the *trans-11* to *trans-10* shift.

Material and methods

Four different growth media containing 20% (v/v) rumen fluid were used: (1) control medium, (2) low pH medium, (3) glucose medium and (4) lactate medium (as a reference to the different diet types that induce the *trans-11* to *trans-10* shift). The rumen fluid was collected from three adult sheep, combined and centrifuged twice (15 min at 12000 g). The control medium (1) contained 75 mL mineral solution (6 g KH_2PO_4, 12 g NaCl, 6 g $(NH_4)_2SO_4$, 1.6 g $CaCl_2.2H_2O$, 2.5 g $MgSO_4.7H_2O$ per liter of distilled water), 0.3 g K_2HPO_4, 2.0 g trypticase peptone, 2.0 g yeast extract, 1.0 g glucose, 1.0 g maltose, 1.0 g cellobiose, 0.5 g soluble starch, 4 g Na_2CO_3, 5 drops resazurin (0.1% w/v), 0.5 g cysteine-HCl and 200 mL rumen fluid per liter of distilled water. The low pH medium (2) was the control medium in which pH was reduced from 6.4 to 5.5 by means of addition of 2 M HCl solution. The glucose medium (3) was the control medium without maltose, cellobiose and soluble starch, supplemented with glucose (1 mg/mL). The lactate medium (4) was the control medium supplemented with Na-lactate (1 mg/mL). These media were transferred to Hungate-type tubes (9.5 mL per tube), LA was added to a final concentration of 40 µg/mL and then the tubes were autoclaved (121°C, 20 min) prior to inoculum addition. All transfers were carried out under continuous flushing of CO_2. Two bacteria species were used: *Butyrivibrio fibrisolvens* D1 as a producer of *c9, t11* CLA and *Propionibacterium acnes* DSM 1897 as a producer of *t10, c12* CLA (Deutsche Sammlung von Mikroorganismn und Zellkulturen GmbH, Braunschweig, Germany). Inoculum size was 0.5 mL for *B. fibrisolvens* and 1.0 mL for *P. acnes*. Then the tubes were maintained under anaerobic conditions at 39°C, with intermittent shaking in a batch culture incubator (Edmund Bühler GmbH, Hechingen, Germany). The incubations were stopped at different time points (0 h, 2 h, 4 h, 8 h and 24 h) by removing the tubes from the incubator and cooling the tubes in an ice bath. Optical density at 600 nm (Amersham Biosciences corp., Piscataway, NJ, USA) and pH (Hanna Instruments, Temse, Belgium) were measured and subsamples were collected for analysis of volatile fatty acids (VFA: 2 mL) and long-chain fatty acids (LCFA: 8 mL or 8.5 mL). Analysis of VFA and LCFA was done as described by Escobar *et al*. (2016). All treatment combinations (bacterium × growth medium × incubation period) were performed in duplicate. Data were analyzed separately for *B. fibrisolvens* and *P. acnes* for each incubation period using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA) with growth medium as a fixed factor. Differences among means at $P < 0.05$ were considered significant and evaluated by the Tukey-Kramer multiple comparison test.

Results and discussion

The concentration of LA, CLA and *t11* 18:1 (µg/mL) after 24 h of incubation and the rate of LA disappearance by *B. fibrisolvens* and *P. acnes* are presented in **Table 1**. LA was almost completely converted by *B. fibrisolvens* after 24 h of incubation except with the low pH medium. With the control and glucose treatment, disappearance of LA was accompanied with a transient accumulation of *c9, t11* CLA which was further transformed to *t11* 18:1. No *t10, c12* CLA or 18:0 was formed by *B. fibrisolvens* in

accordance with previous reports (McIntosh *et al*., 2009; McKain *et al*., 2010). The concentration of *c9, t11* CLA after 24 h of incubation was significantly greater with the low pH medium and when Na-lactate was added indicating inhibition of the hydrogenation of *c9, t11* CLA to *t11* 18:1. LA was converted more slowly by *P. acnes* compared with the conversion by *B. fibrisolvens*, irrespective of growth condition. Growth media did not affect the concentration of LA and *t10, c12* CLA after 24 h of incubation. The rate of LA disappearance was reduced with the low pH and the lactate medium compared with the control medium. The lack of *t10* 18:1 and 18:0 formation might indicate that *t10, c12* CLA is the end product of LA metabolism with *P. acnes* which is in accordance with previous reports (McKain *et al*., 2010). The results of the current experiment show that a low pH reduced the *in vitro* formation of CLA by *B. fibrisolvens* and that both a low pH and Na-lactate reduced the *in vitro* transformation of *c9, t11* CLA to *t11* 18:1. These conditions also affect the *in vitro* formation of CLA by *P. acnes* but to a lesser extent.

Table 1. Effect of *in vitro* growth medium on the concentration of LA, CLA and *trans-11* 18:1 (µg/mL) after 24 h of incubation and the rate of LA disappearance (µg/mL/h) by *Butyrivibrio fibrisolvens* and *Propionibacterium acnes*

		Growth medium					
Bacterium	Parameter	Control	Low pH	Glucose	Lactate	SEM	P-value
B. fibrisolvens	LA	1.05	15.81*	0.52	0.58	3.261	0.017[1]
	c9,t11 CLA	0.00	27.44*	0.00	40.17*	2.088	<0.001
	t11 18:1	37.92	0.28*	35.22	0.36*	2.950	<0.001[1]
P. acnes	LA	19.26	30.96	16.56	24.45	3.691	0.161
	t10,c12 CLA	19.22	8.60	17.06	14.76	1.861	0.057
Rate of LA disappearance							
B. fibrisolvens		5.42±0.376	1.17*±0.166	5.09±0.730	4.78±0.576		
P. acnes		1.00±0.106	0.45*±0.065	0.92±0.155	0.74*±0.067		

[1] P-value of the logarithm of the concentration of LA or *trans-11* 18:1 (µg/mL).
* Means differ (P < 0.05) from the control growth medium.

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Addition of uncentrifuged-autoclaved rumen fluid allows microbial biohydrogenation of 22:6*n*-3 in highly diluted rumen inoculum.

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Introduction

Docosahexaenoic acid (DHA, 22:6n-3) is a poly-unsaturated fatty acid that has been associated with physiological benefits in humans as well as in ruminants. Supplementation of DHA in the diet of ruminants is possible but the amount of DHA available for adsorption in the small intestine is limited due to extensive microbial biohydrogenation in the rumen. The bacterial species responsible for DHA biohydrogenation in the rumen remain unknown. Identification of the bacteria involved in DHA biohydrogenation is however important to be able to provide a more fundamental understanding of the mechanisms involved in DHA biohydrogenation in the rumen. Conventional techniques used for isolation of pure cultures are complicated and time consuming and only a small fraction of existing microbes in a natural microflora can be cultivated[1]. Isolation of pure cultures of biohydrogenating bacteria is further complicated as these are shown to have the greatest sensitivity towards poly-unsaturated fatty acids. An alternative approach is the dilution-to-extinction technique[2], in which the simplest biological consortium that exhibits the target function of a mixed culture (i.e. biohydrogenation of DHA) is selected by serially dilution. In previous experiments, we observed the loss of DHA biohydrogenation capacity at a low dilution (20 fold), most probably due to sensitivity of hydrogenating bacteria towards DHA[3,4]. Hence, to be able to apply the dilution-to- extinction technique, growth media needs adjustments in order to reduce the toxicity problems observed before. Here we hypothesize that addition of uncentrifuged-autoclaved rumen fluid (uRF), containing undigested food particles and microorganisms, stimulates biohydrogenation of DHA. Indeed, particles in the rumen fluid have shown before to stimulate biohydrogenation[5] and this might be the main reason of the stimulating effect of increasing the amounts of rumen fluid on the disappearance of DHA in one of our earlier studies. Hence, in this study we first evaluated the effect of addition of uRF on the disappearance of DHA (experiment 1). As we show that addition of uRF stimulated the disappearance of DHA, we then used this procedure to test the disappearance of DHA with highly diluted rumen inoculum (experiment 2).

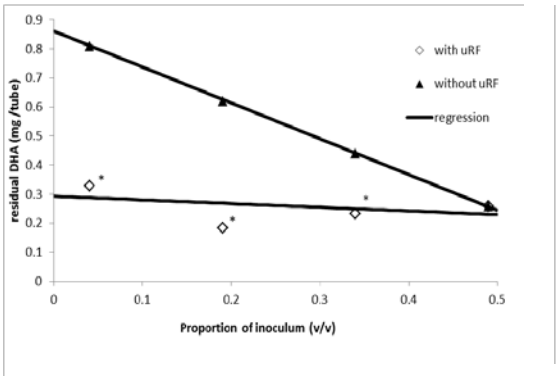
Material and methods

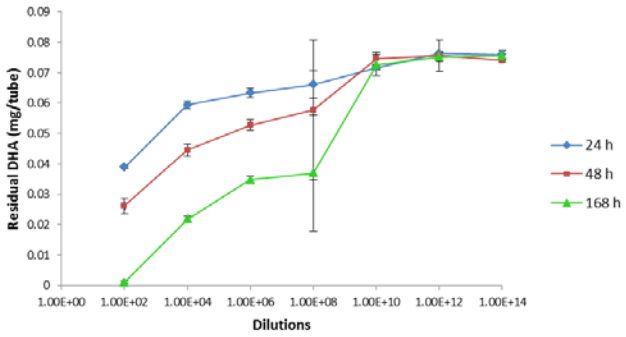
In experiment 1, cultures containing 5, 20, 35 or 50% (v/v) of rumen inoculum were incubated during 24h either with water or uRF in presence of DHA (1 mg/tube). Rumen inoculum was taken from three fistulated sheep. uRF was prepared by collecting rumen content from three fistulated sheep, autoclaved and stored at -20°C separately until use. All cultures contained 5 mL of a phosphate/bicarbonate[6] and rumen inoculum (0.5, 2, 3.5 or 5 mL). Water or uRF was added to reach a volume of 10mL. After 24h, incubations were stopped by placing the tubes in ice water. Residual DHA was determined by gas chromatography after conversion to their methyl ester derivatives[6]. Data were treated to estimate the effect of uRF on the residual DHA according to the following model: $y = \beta 0 + \beta 1X1 + \beta 2X2 + \beta 3X1X2$ where Y is the residual DHA, β_0 is the initial amount of DHA, β_1, β_2 and β_3 are the regression coefficients, X_1 is the inoculum size and X_2 is the presence or absence of uRF. Biological replicate (sheep inoculum was included as a random effect, and its interactions with inoculum and uRF.

In experiment 2, rumen fluid from three fistulated sheep was combined, mixed with a kitchen blender and strained through four layers of cheesecloth. Rumen fluid was serially diluted to produce cultures with different amounts of rumen fluid (dilution ranged from 10^2 to 10^{14}) and incubated in presence of DHA (0.1 mg/tube) for 24h, 48h and 168h. uRF was prepared as described before and combined prior to preparation of the media. The standard growth media for growth of rumen bacteria (DSMZ: medium 330) was used with inclusion of uRF (0.5, v/v). At the end of the incubation period, incubations were stopped by placing the tubes in ice water. Residual DHA was determined as described above.

Results and discussion

When no uRF was present, the decrease in inoculum size reduced the disappearance of DHA (experiment 1, **Figure 1**), in agreement with previous studies [7,6]. When uRF was added, the disappearance of DHA increased leaving less DHA in the tubes after 24 h of incubation (**Figure 1)**. Interestingly, the disappearance of DHA was independent of the amount of inoculum added when uRF was present. The experimental set-up does not allow to explain the reason for this effects but might relate to the presence of undigested food particles or certain metabolites present in the uRF.





Left: **Figure 1**. The effect of inoculum size in absence (▲) or presence (◇) of uRF on residual DHA after 24h of incubation as estimated from the regression model. Initial DHA concentration was 1 mg/tube. Each point represents the mean of three replicates (n=3; SEM= 0.0743). P<0.05*; indicate mean is different from the control (i.e without uRF). Right: **Figure 2**. Residual DHA in different enrichment tubes incubated with diluted inoculums in the medium containing initial DHA 0.1 mg/tube.

Given the stimulating effect of uRF on metabolism of DHA, we included uRF in the media which we used in the dilution-to-extinction experiment (**Figure 2**, experiment 2). Metabolism of DHA was observed at low dilutions and the biohydrogenation capacity was still present at a 10^6 dilution. At the 10^8 dilution, metabolism of DHA was highly variable between replicate tubes. With further dilutions of the inoculum, no metabolism of DHA was observed suggesting bacteria able to perform biohydrogenation of DHA were not present any more at these dilutions.
In conclusion, addition of uRF to the growth media allows microbial biohydrogenation of DHA in highly diluted rumen inoculum.

Acknowledgements

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Dry period length but not concentrate level affects energy balance and metabolic health in early lactation in dairy cattle

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Introduction

Previous studies showed that cows without a dry period (0 days; **DP**) have a better energy balance (**EB**) and improved metabolic health, compared with cows with a short DP (30 days) (Rastani et al., 2005; De Feu et al., 2009; Van Knegsel et al., 2014). The plasma concentration of free fatty acids (**FFA**) and β-hydroxybutyrate (**BHBA**), that are a measure for body fat mobilization, were, for instance, lower in cows with a 0-d DP (Van Knegsel et al., 2014). Van Knegsel et al. (2014) observed an improved EB due to a 16% lower milk production between 1 and 14 weeks in lactation in cows with a 0-d DP compared with cows with a 30-d DP. In these studies the improvement in energy balance was due to a reduced milk production in the subsequent lactation. The lower milk production in cows with a 0-d DP results in lower energy demands, and potentially requires less energy intake. Lower energy intake, through reduction of concentrates offered, reduces feed costs and is potentially beneficial for net herd returns, environmental impact of the herd, and reduces the risk of fattening of cows with a 0-d DP in mid and late lactation. To our knowledge, there are no studies available that evaluated reduced dietary energy intake in the subsequent lactation for cows with a 0-d DP. Feeding less energy may results in either an even lower milk production or a decreased EB in early lactation for cows with a 0-d DP, compared with cows with a 30-d DP. The aim of this study was to evaluate the effects of a 0-d DP, with a 84% or 100% level of concentrates postpartum or a 30-d DP with a 100% level of concentrates postpartum on milk production, energy balance and plasma metabolites between week -4 and week 7 relative to calving.

Materials and methods

Holstein-Friesian dairy cows (n=123) were randomly assigned to three groups with two DP lengths (0 or 30 days). Cows with a 0-d DP were fed either 84% (0-d DP – **C84%**) or 100% (0-d DP – **C100%**) of the concentrate level of cows with a 30-d DP (30-d DP – C100%).

Prepartum, cows with a 0-d DP received the lactation ration that consisted of grass silage, corn silage, soybean meal, sugar beet pulp, wheat straw, and vitamins and minerals (6.4 MJ net energy (NE_L)/kg dry matter (DM)). Dry cows, with a 30-d DP, received a ration that consisted of grass silage, corn silage, wheat straw, rapeseed meal, and vitamins and minerals (5.4 MJ NE_L/kg DM). Lactating cows (cows with a 0-d DP) received 1 kg/d of standard concentrate in the milking parlour (7.7 MJ NE_L/kg DM). All cows received 1 kg/d of experimental concentrate from 10 days prepartum till calving.

Postpartum, all cows received the same lactation ration up to 49 days in milk (**DIM**) as provided to the prepartum lactating cows. All cows received 1 kg/d of experimental concentrate till 4 days in lactation. The experimental concentrate increased stepwise by 0.3 kg/d from 4 DIM up to 8.5 kg/day at 28 DIM for cows receiving 100% of the concentrate, or stepwise by 0.3 kg/day from 4 DIM up to 6.7 kg/day at 22 DIM /d for cows receiving 84% of the concentrate.

Concentrate and forage were supplied separately. Cows had free access to the dry cow ration or the lactation ration throughout the experiment. Forage samples were taken weekly and were stored at −20°C pending analyses. Before analyses (near infrared spectrometry; BLGG AgroXpertus BV, Oosterbeek, the Netherlands), forage samples were pooled per batch. The DM content of forages was measured daily. Forage and concentrate samples were pooled per 6 months period for analysis of dry matter content, crude protein, crude fat, neutral detergent fiber, acid detergent fiber, and acid detergent lignin, starch, sugar, crude ash (Masterlab, Boxmeer, the Netherlands). Daily intake of the dry cow ration or the lactation ration was recorded from 4 weeks prepartum till 7 weeks postpartum using roughage intake control troughs (Insentec). Experimental concentrate was fed using a concentrate dispenser and the actual quantity dispensed (kg/d) was recorded (Manus VC5, DeLaval, Steenwijk, the Netherlands). Milk production was measured daily and milk composition (fat, protein, lactose) was measured weekly. Data were analysed, with week relative to calving as repeated measures and cow as subject, using a mixed linear model (SAS Institute Inc., 2011) including treatment (prepartum: 0-d DP, 30-d DP; postpartum: 0-d DP – C84%, 0-d DP – 100%, 30-d DP – 100%) and prepartum parity (1, ≥2) as fixed effects and calving season as random effect. Data are expressed as LSmeans ± SE.

Results

Preliminary results indicate that prepartum, DM intake, intake of concentrates, intake of the basal ration, and energy intake was lower in cows with a 30-d DP, than in cows with a 0-d DP ($P<0.01$). Postpartum, milk yield, FPCM yield, and yield of lactose, fat, and protein was greater in cows with a 30-d DP-C100%, compared with cows with a 0-d DP – C84% or 0-d DP – C100% ($P<0.05$). Concentrate level did not affect milk yield and milk component yield. Total DM intake, intake of the basal ration, and the plasma concentration of glucose was greater in cows with a 0-d DP – C84% or a 0-d DP – C100%, compared with cows with a 30-d DP – C100% ($P<0.05$). Intake of concentrates was lower in cows with a 0-d DP – C84%, than in cows with a 0-d DP – C100% or a 30-d DP – C100% ($P<0.01$). Energy balance was lower and plasma FFA and BHBA concentrations were greater in cows with a 30-d DP – C100%, than in cows with a 0-d DP – C84% or a 0-d DP – C100% ($P<0.01$), whereas concentrate level did not affect EB and concentrations of FFA and BHBA.

Discussion and conclusion

The NE_L consumed by cows with a 0-d DP and C84% was only 3% lower than in cows with a 0-d DP and C100%, because C84% cows consumed more of the basal diet (numerical only). This compensation of intake of concentrate with intake of basal ration shows that cows with a 0-d DP can consume sufficient energy through the basal ration and a limited amount of concentrate to provide for their milk production, without detrimental effects on EB. Feeding a reduced level of concentrates after a 0-d DP may be beneficial for roughage intake and rumination, and may be relevant to prevent subacute ruminal acidosis in early lactation. In conclusion, 0-d DP improved the EB and metabolic status of cows in early lactation, compared with a 30-d DP. Reducing the level of concentrates did not affect FPCM yield, energy balance or concentrations of FFA and BHBA in early lactation.

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Does cutting grass a later growth stage for silage decrease phosphorus excretion on dairy farms?

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Introduction

In the Netherlands excessive use of phosphorus (P) in the past in agriculture has led to a high P content in the soil, thus threatening the surface water quality by run-off and leaching. To protect the water quality legal limits have been established in application standards for P and N in animal manure and mineral fertilizer. The amount of P and N in animal manure is estimated by excretion standards. When excretion is higher than allowed, manure has to be removed from the farm. Due to the surplus of manure on national scale a farmer has to pay for removal. Dairy farmers can prove by measuring the feed intake and analysing P and N content of roughage if their livestock have a lower excretion. In grass based dairy production P in grass silage is an important source of P excretion and decreasing P content in grass can prevent manure removal costs. It was found that P content of grass decreases when cutting at a later growth stage (Fleming and Murphy, 1968; Whitehead, 2000; Wilson and McCarric, 1967). However, feeding values like energy and protein content also decrease with a later growth stage. The consequence on farm level is that milk production decreases or use of concentrates and/or by-products increases. Because milk production and ration influence P intake and thus excretion, the final effect of growth stage of grass at cutting on excretion is not easily foreseen at farm level. The objective of this study was to quantify the relationship between growth stage and P content of grass and if P-excretion decreases at farm level by cutting grass at a later growth stage than usual.

Materials and methods

Between 1999 and 2007 three experiments, nine experimental years, were performed on permanent grassland. One experiment was performed on sandy soil and clay, two experiments on peat soil. On all experiments the sward was dominated by *Lolium Perenne.* The treatments were N fertilization and growth stage. On the experiment on sandy soil and clay also P fertilization and on one experiment on peat soil also groundwater table was varied. Dry matter (DM) yield and P content of grass were determined every two weeks. For every harvest new plots were available within a treatment. Data were statistically analyzed with a linear model, comprising a random and a fixed part, using Restricted Maximum Likelihood (ReML) prediction modelling (Harville, 1977), provided by the Genstat package (15th ed.). The initial model included soil type, number of growing days, N and P fertilization, starting day of growth and all interactions. Random model included experiment and year. Non-significant factors and interactions ($P \leq 0,05$) were deleted step by step. The developed statistical model was included in farm model DairyWise (Schils et al., 2007) which simulates technical aspects and economics of dairy farms. In the model the relationship between feeding values of grass and growth stage and the reduction of growth after heavy cuts was already quantified. Three model farms were simulated (Table 1) cutting at two growth stages: normal (N, 3 ton DM ha^{-1}) and late (L, 4 ton DM ha^{-1}) cuts for silage. Cuts for grazing were not varied. Milk production per cow was maintained at 8600 kg yr^{-1}.

Table 1 Characteristics of three model farms

Farm	Farm area, ha	Grazing method	Indoor roughage feeding during grazing period	Feeding during housing period	Milkproduction, ton ha^{-1}
1	60.0	Day and night	0	100% grass silage	14.3
2	55.7	Day	8 kg DM/day maize silage	50% grass silage – 50% maize silage	15.4
3	60.0	Zero	---	100% grass silage	14.3

Soil type: sandy soil. Milk production per cow: 8600 kg yr^{-1}

Results and conclusions

The range of DM yields in the experiments were 60 to 10,000 kg DM ha^{-1} in a single cut. Cutting grass at a later growth stage resulted in a lower P content. The P content on a certain harvest date was higher if N fertilisation was higher. Calculation with the model using estimated factors quantified the decrease over time. For instance on sandy soil in a cut that started to grow at March 1st and fertilised with 120 kg N ha^{-1} the decrease of P content in time was 0.028 g P kg^{-1} DM day^{-1}. In the same situation the decrease on peat soil was 0.035 g P kg^{-1} DM day^{-1} and on clay 0.014 g P kg^{-1} DM day^{-1}. On sandy soil when fertilised with 80 kg N ha^{-1} the decrease was 0.025 g P kg^{-1} DM day^{-1}.
Simulations with the model DairyWise (Figure 1) showed that P intake of dairy cows in roughages decreased on all farms, when grass was cut at a later growth stage. Part of this decrease, however, was compensated with an increase of intake of P from manufactured feed. The largest decrease of total P

intake was reached on farm 3 (zero grazing, 100% grass silage): 1.2 kg P yr^{-1} cow^{-1}. On farm level this was equal to a decrease of 4.4 m^3 ha^{-1} manure export (≈ €88 ha^{-1}). On farm2 (50%-50% maize-grass in ration) the P excretion was already low in the normal situation and the effect of cutting at a later growth stage was small. On all farms cutting at a later growth stage decreased mowing costs, about €70 ha^{-1} on farm 1 and 3, and €115 ha^{-1} on farm 2. Another effect of cutting at a later growth stage on farm 3 was that grazing pressure was higher as mowing cuts took more time to grow.

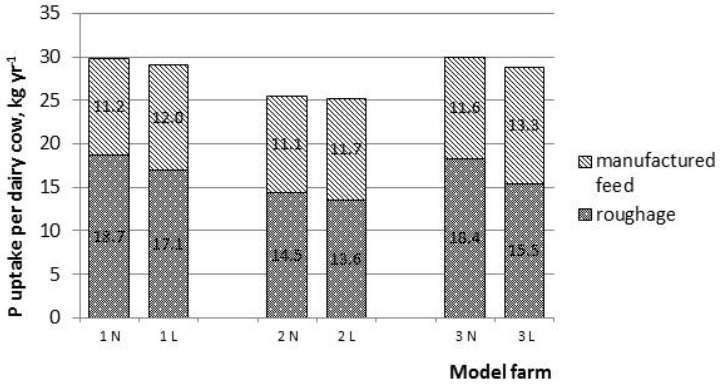

Figure 1 Phosphorus intake of dairy cattle (kg P cow^{-1} $year^{-1}$, milk production 8600 kg yr^{-1} cow^{-1}) on model farms at normal (N) and late (L) cuts.

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Management practices for double-muscled Belgian Blue heifers: comparison of permanent indoor feeding versus protein and energy supplementation on pasture

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Introduction

The average age of first calving for double-muscled Belgian Blue heifers (DMBB) is 29,9 months. As age at first calving is an important factor determining the net income, beef cattle farmers can increase profit by improving this parameter. The objective of first calving at the age of 24 months and a body weight of 600 kg before calving is achievable when BBDM heifers realize a daily growth of at least 0.75 kg (Fiems and De Brabander, 2009). To realize this growth rate, grazing heifers younger than 1 year need energy and protein supplements on pasture. Fiems et al. (2013) showed that the best animal performances on pasture were realized when heifers were fed 3 kg of 80% beet pulp (BP) and 20% of soybean meal protein (SBM). However, whether permanent indoor feeding during the first year of life would be a better alternative still needs to be investigated.
Therefore, the performances of DMBB heifers kept on pasture and fed a daily supplement of BP and SBM during the grazing season were compared with the performance of heifers receiving permanent indoor feeding during the first year.

Material and methods

Thirty DMBB heifers, were homogenously assigned to 2 groups (pasture group: PG and stable group: SG), based on age, live weight and daily gain from birth to the start of the trial. Initial age at start of the trial was 194 ± 45 days and average start weight was 212 ± 37 kg.
The trial started with the grazing period from May 2nd till October 10th 2013. During the grazing period, PG received a daily supplement of 3 kg per animal per day of 80/20 BP/SBM (on FM basis). Group intake of fresh grass was estimated from a 3-days period of indoor zero grazing in the middle of the grazing season. Daily amount of supplements fed was recorded on group level and refusals were weighted back on a weekly basis. SG was confined in a straw-bedded loose house during the grazing season and was fed a ration of maize silage (MS) and a vitaminated soybean meal concentrate at 85/15 ratio (on DM basis). Intake of the stable ration was recorded weekly on group level.
During the following winter period from October 10th till April 9th , both groups were fed the same indoor ration consisting of 60/40 MS /prewilted grass silage, supplemented with rumen protected SBM and a mineral-vitamin premix. Intakes were recorded weekly on group level.
Throughout the whole trial animals were weighted on 4-weekly intervals. Blood samples were taken from all heifers at the end of the grazing season and at the end of the winter period to determine the serum selenium (Se) levels.
Data were statistically analyzed using the REML approach in SAS. Analyses were based on individual data for body weight, growth rate and blood selenium levels. Age and initial weight were used as covariates. No statistical analyses were possible for feed intake results as feed intake was measured on group level.

Results and discussion

The results of the daily average feed intake per group and per period are shown in Table 1.
Supplementing DMBB heifers at pasture resulted in a daily growth rate of 0.632 kg/day (Table 2) which was in line with previous results (Fiems et al., 2013). With indoor feeding during the grazing period, a 22% higher daily growth rate was realized (0.812 kg/day; P= 0.070).
In the two weeks of the adaptation period between the grazing and winter period, the pasture group realized a daily weight gain of more than 1 kg, whilst the stable group had a drop in daily weight gain (0.404 kg; P<0.0001), resulting in a partial compensation for the difference in live weight between the groups. Over the total winter period, the daily weight gain for both groups was similar (Table 2).
Selenium analyses in serum showed a sever deficit in Se for heifers at pasture. The mean Se serum concentration of heifers at pasture was 0.33 ppm; all individual concentrations were below the reference value of 0.50 ppm (De Bleecker et al., 2010). SG heifers were fed a concentrate containing 1.1 mg Se/kg during the grazing period, resulting in significantly higher serum selenium levels (average 0.589 ppm – P < 0.0001). Nevertheless, the majority of the stable group was marginal in blood selenium (0.50 – 0.69 ppm). Only 3 heifers had sufficiently high Se serum concentrations (≥0.70 ppm). The Se levels in the concentrate fed during the winter period were raised to 3.4 mg Se/kg (Guyot et al., 2007). Half of this Se was in organic form. At the end of the winter period, the average Se serum concentration in both groups was above 0.70 ppm (P=0.940). The Se deficiencies found in the SG heifers during the grazing period, were all compensated for by the end of the winter period.
The results of this study show that heifers kept at pasture during the grazing season, in spite of the energy and protein supplement they were fed, performed worse than heifers receiving permanent indoor

feeding. During the following winter period the SG heifers showed a partial compensatory growth, what made that at the end of the winter period the difference between both groups was negligible. Over the entire trial period, both groups realized a daily weight gain higher than the recommended 0.750 kg/day, so even for heifers kept at pasture, a sufficient daily growth is achievable, on condition that they receive the necessitate extra energy, protein and vitamin-mineral supplements.

Table 1: Average daily feed and nutrient intake per animal per group and per period

	Grazing period		Winter period	
	PG	SG	PG	SG
kg FM/animal/day	15.68	13.94	20.08	20.25
kg DM/animal/day	4.30	5.37	7.58	7.56
CP (g/day)	801	744	1133	1111
DVE (g/day)	526	505	602	591
OEB (g/day)	45	-51	20	55
NEL (MJ/day)	32.47	37.57	51.16	50.92
FC (kg DM/kg BW gain)	6.73	7.00	9.05	8.69

FM: fresh matter; DM: dry matter; CP: crude protein; DVE: truly digestible protein in the intestine; OEB: rumen degradable protein balance; NEL: net energy lactation; FC: feed conversion

Table 2: Animal performances and selenium serum concentration
$^{\$}$: tendency (P<0.1); *: significant (P<0.05); ***: highly significant (P<0.001)

	PG	SG	P-value
Initial weight grazing period	211.1	212.6	0.941
Final weight grazing period	310.5	340.8	0.126
Daily gain grazing period (kg/day)	0.632	0.812	0.070$^{\$}$
Final weight winter period	465.3	490.6	0.200
Daily gain start winter (kg/day)	1.082	0.404	<0.0001***
Daily gain winter period (kg/day)	0.838	0.860	0.819
Daily gain grazing + winter (kg/day)	0.755	0.826	0.021*
Serum Se end grazing period (ppm)	0.330	0.589	< 0.0001***
Serum Se end winter period (ppm)	0.778	0.780	0.940

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Cross-validation of milk fat C18:1 *cis*-9 as biomarker for negative energy status in early lactating cows: comparison of fixed vs. experiment-corrected cut-off values

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Introduction

Most cows in early lactation inevitably encounter a period of negative energy balance (NEB) since dry matter intake (energy input) and milk production (energy output) are imbalanced, which is reflected in elevated blood non-esterified fatty acids (NEFA) and beta-hydroxybutyrate. Increased plasma NEFA concentrations during the pre- and postpartum period have been associated with reduced milk production and reproductive performance[1]. Released NEFA are partially transferred to the mammary gland and are particularly rich in long-chain fatty acids such as C18:1 *cis*-9. A milk fat C18:1 *cis*-9 concentration greater than 24.0 g/100 g milk fat (absolute cut-off) in week two of lactation has been identified as a potential indicator of elevated plasma NEFA (≥0.6 mmol/L)[2]. Moreover, other factors such as diet composition, stage of lactation, breed and parity or their interactions also might affect the C18:1 *cis*-9 content in milk fat[3,4,5]. Additionally, variation in Δ^9 - desaturase activity of the mammary gland, resulting in the conversion of C18:0 to C18:1 *cis*-9, also might influence milk fat C18:1 *cis*-9 concentrations. These potentially confounding factors might limit the transfer of formerly determined absolute cut-off values and correction might be needed. The current study aimed to cross validate formerly determined C18:1 *cis*-9 cut-off values to assess elevated blood NEFA and to propose corrections for confounding factors.

Material and Methods

A cross-dataset approach was made using samples and data from two consecutive lactations, i.e. experiment 1[2] (n=92) and experiment 2[6] (n=93) in early lactation from Wageningen University Research Centre, the Netherlands. Cows were attributed to varying dry period lengths (0, 30 or 60 days) and fed diets rich in glucogenic or lipogenic nutrients. Milk and plasma samples were collected once in the second week of lactation (between 8 and 14 DIM). Plasma NEFA concentration was measured using a commercial kit no. FA115 (Randox Laboratories Ltd., Ibach, Switzerland). Milk samples were analysed for fatty acids (FA) and were expressed as grams per 100 g of fatty acid methyl esters.
An absolute cut-off value of 24 g/100 g milk fat C18:1 *cis*-9 (experiment 1) was reported in our previous study[2] for diagnosis of elevated plasma NEFA (≥0.6 mmol/L) with specificity (Sp) and sensitivity (Se) values of 90 and 50%, respectively. A cut-off value of 27 g/100 g milk fat C18:1 *cis*-9, was obtained from dataset of experiment 2, resulting in the same classification performance. To test the robustness of these absolute cut-offs, cross-validation was done using the alternative experiment. Furthermore, the average milk fat C18:1 *cis*-9 concentration of cows with plasma NEFA concentrations below 0.6 mmol/L, which were considered 'healthy' was calculated for both experiments separately and was considered an experimental-dependent basal level. Each C18:1 *cis*-9 observation was then corrected for this basal level by subtracting the latter. Similarly, new cut-off values, corrected for the experiment-dependent basal level were calculated by subtracting basal level from the absolute cut-off values. Alternatively, an experiment-dependent basal level was calculated from the 80% lowest C18:1 *cis*-9 values within each experiment. The latter was an attempt to determine an experiment-dependent basal level under practical circumstances where information on the blood plasma NEFA level is lacking.

Results and Discussion

Cross-validation with the absolute cut-off of milk fat C18:1 *cis*-9 of 24g/100g from experiment 1 was performed using data from experiment 2, resulting in a Se of 70% and Sp of 55%. Similarly, the cut-off value of milk fat C18:1 *cis*-9 of 27g/100g obtained based on experiment 2 data was validated based on the dataset of experiment 1, resulting in an extremely low Se of 18%. For both cross-validations more than three-fourths of the negative test results were truly negative (PV- > 0.75). Moreover, predictive values of the positive tests were 46 and 67%, which indicated that almost half and two-thirds of all cows having a positive test were truly positive. Odds ratios were 2.8 and 7.6 for the first and second cross-validation. These values indicated how much more risk cow had for elevated plasma NEFA concentrations when their milk fat contained C18:1 *cis*-9 concentration above 24 or 27 g/100 g, respectively, as compared with animals having milk fat C18:1 *cis*-9 below these cut-offs.

Cross-validation was then performed using the corrected observations. Cut-offs using this approach were 3.85 (24.00-20.15) and 4.11 (27.00-22.89) and were then used for validation based on data of experiments 2 and 1, respectively. Overall, cross-dataset validation showed high specificities of at least

90% associated with sensitivities of about 50% and test accuracies of more than 75%. Additionally, PV+, PV-, OR and LR+ for both experiments performed better than when using absolute cut-offs. Finally, the experiment-dependent basal level was calculated by using the 80% lowest milk fat C18:1 *cis*-9 concentrations to mimic practical circumstances, resulting in similar overall results to the former experiment-corrected approach with Se levels reaching 55% at the minimum and Sp of about 90%.

Table 1. Validation tests using absolute cut-offs of milk fat C18:1 *cis*-9 (g/100g FA) for diagnosis of elevated plasma NEFA (≥0.6 mmol/L) as well as cut-offs obtained by subtracting an experiment-dependent basal level from these absolute cut-offs. Cut-offs were obtained based on datasets from experiments 1 and 2 and validated cross-experiment, using the dataset of experiments 2 and 1, respectively.

Exp.	Cut-off	Basal level	Prevalence	Se	Sp	Accuracy	PV+	PV-	OR	LR+
Absolute approach										
						%				
1	24.0*	-	35	70	55	60	46	77	2.8	1.6
2	27.0	-	24	18	97	78	67	79	7.6	6.4
Experiment-dependent correction using a basal level based on milk fat C18:1 *cis*-9 concentrations of healthy cows (NEFA<0.6 mmol/L)										
						%				
1	3.85	22.89	35	52	92	77	77	77	12	6.2
2	4.11	20.15	24	45	90	79	59	84	7.5	4.6
Experiment-dependent correction using a basal level based on the 80% lowest milk fat C18:1 *cis*-9 concentrations										
						%				
1	3.85	22.63	35	58	90	78	76	79	12	5.8
2	4.11	19.73	24	55	89	80	60	86	9.3	4.8

*milk fat C18:1 *cis*-9 cut-off based on a previous study of Jorjong et al. (2014) as an indicator of elevated plasma NEFA in week two of lactation, Se=sensitivity, Sp=specificity, PV+=positive predictive value, PV-=negative predictive value, OR=odds ratio, LR+=likelihood ratio

Conclusions

Cross-validation using two consecutive lactations (experiments 1 and 2) indicated that experiment-dependent correction resulted in a better classification performance as compared with classifications based on an absolute cut-off. Milk fat C18:1 *cis*-9 concentrations corrected using as experiment-dependent basal level would be useful as a method to predict elevated plasma NEFA. The need for this correction might be related to confounding factors influencing the milk fat C18:1 *cis*-9, such as dietary composition or parity.

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The role of condensed tannins in browse species preference by goats

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Introduction

Browse species sustain grazing and browsing animals during the driest period in arid and semi-arid areas. Browse possesses plant secondary metabolites (PSM) such as condensed tannins which can have beneficial roles for ruminants (Aerts et al., 1999) at optimal intake. In free ranging systems, goats select a variety of browse species for nutritive and non-nutritive characteristics (e.g. PSM). There is thus a trade-off between ingesting nutritious feed and increasing the potential risk from PSM ingestion. It is thus important to understand the relationship between browse tannin composition and its nutritive value to develop a strategy for efficient utilization. The present study examined the role of condensed tannins in browse preference (measured in terms of dry matter intake) by local goats in Ethiopia.

Material and methods

Leaves of browse species, *Acacia etbaica, Cadaba farinosa, Capparis tomentosa, Dichrostchys cinerea, Dodonea angustifolia, Euclea racemosa, Maerua angolensis, Maytenus senegalensis, Rhus natalensis and Senna singueana* were collected from the Tigray region of Ethiopia and air dried. Samples were analysed for dry matter, ash and crude protein (CP, N×6.25) (AOAC, 1990) as well as, neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (Van Soest and Robertson, 1985). The NDF, ADF and ADL fractions were also used to calculate cellulose (CELL) and hemicellulose (HEMI) contents. Total tannins (TT) and total phenol (TP) analyses were conducted according to Makkar (2003) and condensed tannins by the method of Grabber et al. (2013) (Table 1).

Four mature male goats were used in two subsequent preference trials each lasting 10 d measurement after 10d adaptation. In the two trials, goats received grass hay (4% body weight) and wheat bran (200g) daily. In both trials goats were provided with each browse (25g) simultaneously for 10 min. In trial 2 polyethylene glycol (PEG, 25g) was added to the wheat bran to counteract potential effects of tannins.

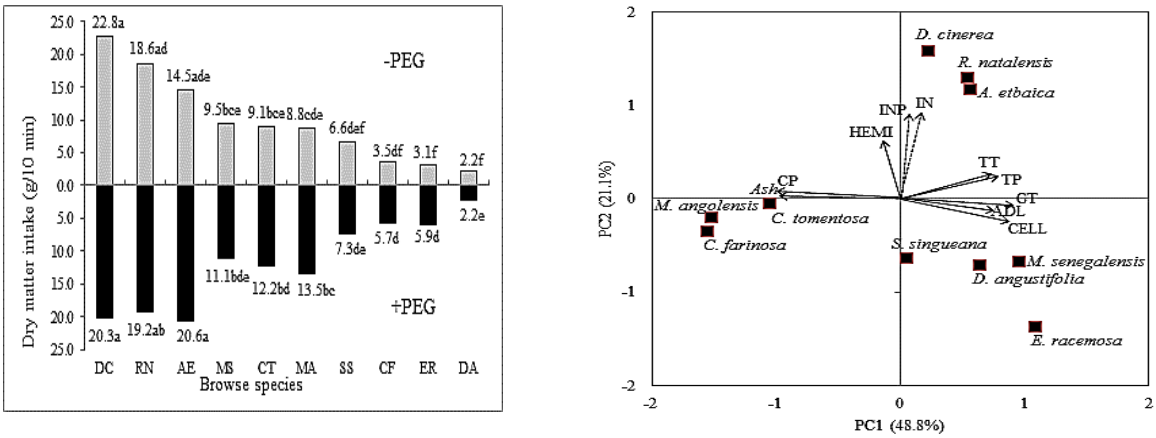
Data were analysed with the PROC MIXED procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC, USA) with browse, PEG and day as fixed factors and goat as random factor. Principal component analysis was used to explore browse species clustering and relationships between browse chemical components and intake.

Table 1. Browse species phenol and tannin composition.

Browse species	Total Phenol[a]	Tannins	
		Total[a]	Condensed[b]
E. racemosa	29.7	28.6	18.4
R. natalensis	44.9	34.5	17.7
M. senegalensis	46.5	32.7	16.9
D. angustifolia	66.2	61.1	16.8
A. etbaica	70.6	68.1	11.2
D. cinerea	41.3	38.9	9.3
S. singueana	41.6	38.5	6.9
C. tomentosa	10.2	8.1	6.8
M. angolensis	7.2	4.6	3.3
C. farinosa	3.7	2.5	1.7

[a]Calculated as mg tannin acid equivalent/g DM.
[b]Expressed as Abs $_{550nm}$/g DM.

Results and discussion



(A) (B)

Figure 1. Browse intake in the absence (-) and presence (+) of polyethylene glycol (PEG) (**A**); Principal component analysis displaying browse species position in relation to tannins, phenols and other chemical components (**B**). AE: *A. etbaica,* CF:*C. farinosa,* CT:*C. tomentosa*, DC:*D. cinerea,* DA: *D. angustifolia,* ER:*E. racemosa,* MA:*M. angolensis,* MS:*M. senegalensis,* RN:*R. natalensis and* SS:*S. singueana*, IN: intake in the absence of PEG, INP: Intake in the presence of PEG

There was a significant difference among browse species in intake ($P<0.0001$), PEG inclusion ($P<0.0001$) and interaction between browse and PEG ($P=0008$). Goats visited all the 10 browse species during the 10 min time and predominantly preferred *D. cinerea*, *R. natalensis* and *A. etbaica* Fig. 1 (A). In short-term tests, diet discrimination by goats is likely related to diet chemical composition (Morand-Fehr, 2003). However, the ability of herbivores to associate diet sensory property with post-ingestive consequences is limited when diets are offered simultaneously (Duncan and Young, 2002). In the present study, goats were obtained from the same habitat where the browse species grew naturally, and familiarity is likely to have influenced the preference by the goats. The most preferred species contained significant levels of tannins and phenols and this preference was contrary to expectations. It suggests that tannin concentrations at the observed level of intake were tolerated by goats. In Fig. 1 (B), the first two principal components explained 69.9% of the variation. The CP was negatively correlated with the fiber and phenolic components. However, the association of condensed tannins or phenols to intake/preference was not evident under the conditions of the present experiment. It appeared that intake was more associated with the hemicellulose fraction.

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Inter-animal variation in milk fat content and milk fat C18:1 *trans*-10 concentration in early lactating dairy cows

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Introduction

Subacute rumen acidosis (SARA) represents one of the most important metabolic disorders in intensive dairy farms with a suggested incidence between 19% and 26% in early and mid-lactation in the dairy cattle (Plaizier et al., 2008).

However, besides dietary factors playing a role in SARA development, inter-animal variation has been reported during experimental SARA challenges (Brown et al., 2000; Schlau et al., 2012). Gao and Oba (2014) found that cows with less sorting behavior were more tolerant to a SARA challenge, whereas weakness in VFA absorption capacity also could partly explain why some cows were more susceptible for SARA (Schlau et al., 2012).

Milk fat depression as well as milk fat C18:1 *trans*-10 concentration has been associated with a decrease in rumen pH in SARA induction trials (Colman et al., 2010; Fievez et al., 2012). If these parameters effectively are appropriate SARA markers, they also should allow monitoring inter-animal variation in SARA susceptibility within a cohort of dairy cows. As SARA challenge trials typically are performed with a limited number of animals, which do not allow to assess inter-animal variation, the latter was monitored at herd scale in the first 4 weeks in lactation during which the amount of compound feed was gradually increased and which was considered a risky period for SARA development.

Methods and Material

At the Schothorst Feed Research, 150 Holstein cows were monitored from calving until 4 weeks in milk (WIM), when cows were subjected to a gradual increase in concentrate (from 3.7 to 4.0 kg at day 1 to 9.0 to 10.0 kg at day 22 after calving for heifers respectively older animals. The basal diet consisted of grass silage/maize silage (50/50) and soybeanmeal, pressed beetpulp, rolled barley and wheat straw. Ration was optimized to fulfill dietary SFR-recommendations of Net Energy (VEM), metabolisable protein (TMP), Met and Lys (TMMet and TMLys) and rumen fermentation parameters. The calculated level of rapidly fermentable carbohydrates (RFCH) was below maximum recommendation resulting in a low risk for SARA. About half of the samples have been analyzed until now.

Milk samples were collected once every Wednesday evening. The FA profile was obtained after milk fat extraction (mini Röse-Gottlieb method, adapted from Chouinard et al., 1997), methylation (Stefanov et al., 2010), and gas chromatographic analysis of FAME (Agilent Technologies 7890A GC System equipped with a flame ionization detector, Agilent Technologies, Santa Clara, CA). Fatty acids were expressed as grams per 100 g of FAME.

Results and Discussion

Increasing proportions of dietary concentrate coincided with increases in milk fat C18:1 *trans*-10 concentration which was consistent with previous SARA induction trials. Most C18:1 *trans*-10 outliers were observed at 2 and 3 WIM (13.51% = 10 out of 74 and 11.11% = 7 out of 63, respectively), with variation in C18:1 *trans*-10 being highest (stdev = 0.095) at 3 WIM. The frequency analysis of milk fat C18:1 *trans*-10 concentration, revealed two “separate” subhistograms (Figure 1). Nevertheless, such ‘dual picture’ could not be seen for milk fat and at maximum 1 outlier with low milk fat syndrome could be identified in each of the 4 weeks. Accordingly, the milk fat C18:1 *trans*-10 concentration showed more inter-animal variation when compared with the milk fat content, which also could be concluded by the larger coefficient of variation of C18:1 *trans*-10 as compared with milk fat: 30.77% *vs.* 17.07% at 2 WIM and 35.81% *vs.* 15.17% at 3 WIM. The decrease of milk fat content always coincided with an increase of C18:1 *trans*-10 but not vice versa.

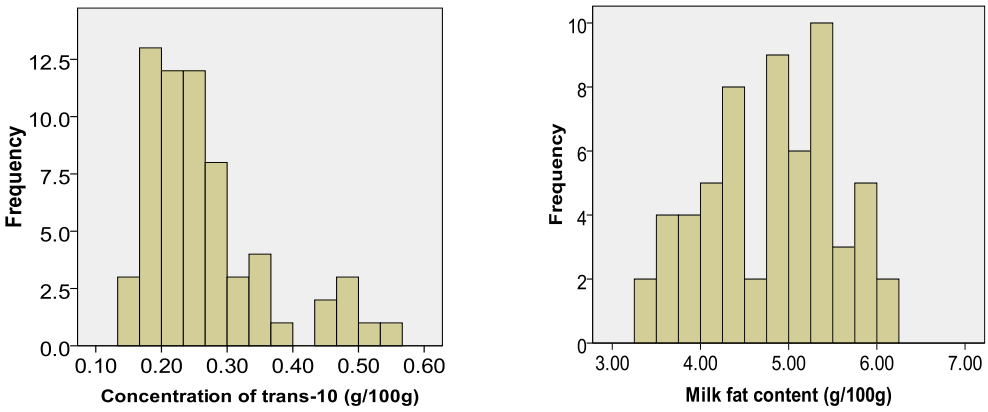

Figure 1 Frequency analysis of milk fat C18:1 *trans*-10 concentration (n=63) and milk fat content (n=60) at 3 WIM

Conclusion

Milk fat C18:1 *trans*-10 concentration could be a better marker than milk fat content in terms of studying inter-animal variation during early lactation.

Acknowledgements

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Presentations session 3

Diurnal dynamics of gaseous and dissolved metabolites in the bovine rumen in relation to control of fermentation pathways

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Introduction

Concentrations of gaseous and dissolved metabolites in the bovine rumen are elevated following feeding (Brask et al. 2015). Partial pressure of hydrogen (P_{H_2}) dictates the redox state of coenzyme NAD and thermodynamically controls the yield of individual VFA and CH_4 (Van Lingen et al., submitted). Increased understanding of these rumen fermentation dynamics may improve model predictions of type of VFA formed and CH_4 emitted. Nonetheless, studies reporting diurnal patterns of gaseous and dissolved metabolites in the rumen together with gaseous emissions from the rumen are limited. Furthermore, rumen fermentation and yield of CH_4 are affected by feeds supplemented with fats and oils sources, particularly by products high in C18:3 such as linseed oil (Patra, 2013). The aim of this study is to monitor daily patterns of gaseous and dissolved metabolite concentrations in the rumen, H_2 and CH_4 emitted from the rumen, and the effect of dietary inclusion of linseed oil on these patterns.

Materials and methods

Four multiparous rumen fistulated HF cows in late-lactation were used in a cross-over design with two 17-d experimental periods and two dietary treatments. A control diet (40% corn silage, 30% grass silage and 30% concentrates on DM basis; fat content 2.9% of DM) was compared with a diet supplemented with linseed oil (fat content 5.4% of DM). On day 11, rumen headspace gas and fluid were sampled at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8 and 10h after morning feeding using a custom fistula lid. Gas samples were analyzed for partial pressure of H_2 and CH_4, fluid samples for concentrations of dissolved metabolites. From day 13 to 17 cows were housed in respiration chambers to relate instantaneous rumen headspace pressure to emissions of H_2 and CH_4. Profiles of metabolites in the rumen and gaseous emissions were analyzed using a linear and a non-linear mixed model, respectively.

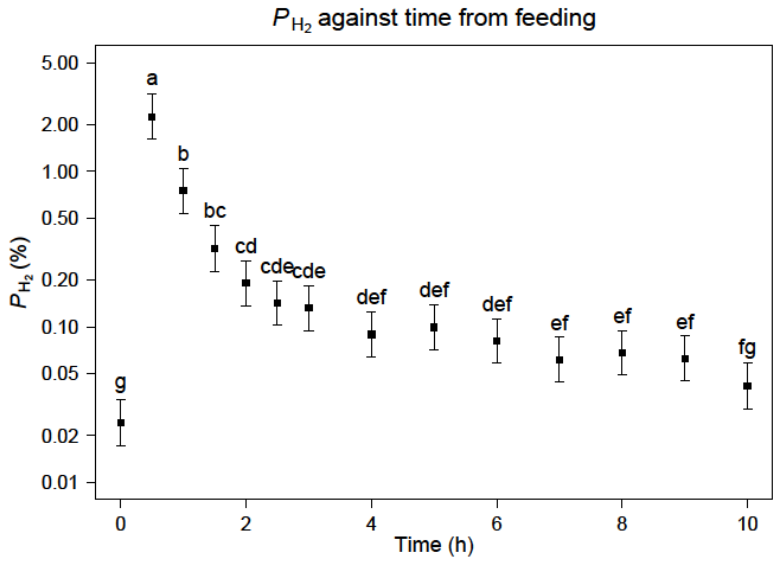

Figure 3 – Rumen H_2 partial pressure (P_{H_2}) over the first 10h after feeding. Values represent mean ± standard error, different letters indicate significant differences (P > 0.05), no diet effect was observed.

Results and discussion

A 100-fold increase in P_{H_2} was observed at 0.5h after feeding, followed by a decline (Fig. 1). Similarly, increased H_2 and CH_4 emission, ethanol and lactate concentrations, and acetate, propionate and butyrate concentrations, and decreased acetate to propionate ratios were observed after feeding (see Fig. 2 for selection of dissolved metabolites). This finding is explained by H_2 partial pressure inhibited oxidation of reduced NAD, which shifts fermentation end products to ethanol, lactate, and propionate at the expense of acetate. Only partial pressure and emission of CH_4 were significantly decreased by linseed oil. The metabolite profiles obtained in the present study support the key role of the redox state of NAD in rumen fermentation. Representing the redox state of NAD in rumen fermentation models will likely improve prediction of type of VFA formed and enteric CH_4 emitted.

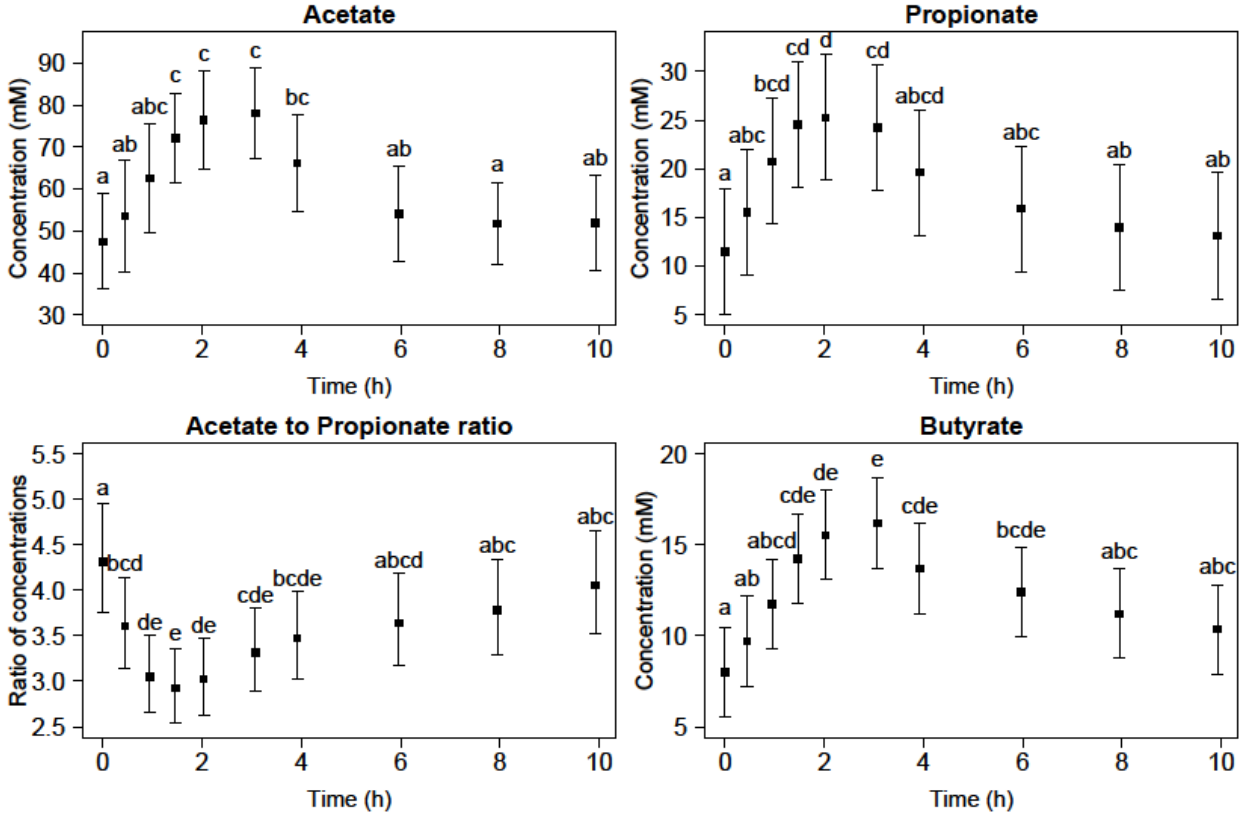

Figure 2 – Selected dissolved rumen metabolite concentrations over the first 10h after feeding. Values represent mean ± standard error, different letters indicate significant differences ($P < 0.05$), no diet effect was observed.

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Relationship between in vitro and in vivo methane production measured from donor cows fed maize silage, harvested at different stages of maturity

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Introduction

Ruminants are held responsible for 18 % of the total methane (CH_4) emission (Gibbs et al., 1989) and are therefore a relevant target to mitigate CH_4 emission. It is generally accepted that the ingredient composition of ruminant rations, has a major impact on rumen fermentation and thus on CH_4 production (Moss et al., 2000). Therefore, the effect of the various dietary ingredients on CH_4 production has to be quantified and reliable prediction on CH_4 production is needed. Roughage is a principal component of ruminant rations and both grass- and maize silage (MS) were widely tested in relation to CH_4 production. However, the simultaneous comparison of in vitro and in vivo experiments to derive a relationship on CH_4 production is lacking. This study aimed to determine the relationship between in vivo and in vitro CH_4 production, measured simultaneously using the same fistulated dairy cows in both experiments. We hypothesized that in vitro CH_4 measurements from maize silages with a wide range in maturity are related to the in vivo CH_4 production if both in vitro and in vivo CH_4 measurements are performed simultaneously, using the same animals as donor for rumen inoculum receiving same diet as used for in vitro incubation.

Materials and Methods

An in vitro experiment was conducted to evaluate the effect of increasing of harvest maturity of maize on gas and methane (CH_4) production during 48-h incubations, using rumen inoculum from adapted animals. Results obtained from the in vitro CH_4 measurements were compared with in vivo data (Hatew et al., 2016). Eight animals were fitted with a permanent rumen cannula were used in two periods. Within blocks (periods), animals were randomly assigned to one of four experimental diets. An experimental period consisted of 17 days, of which a 12-day adaptation period in a tie stall, followed by a 5-days measuring period in a respiration chamber. The animals were fed the total mixed ration (TMR) consisted of (DM) 75% MS, 20% concentrate and 5% wheat straw. The whole plant maize was harvested very early on September 20 in 2013 (25% DM; MS25), early (28% DM, harvested September 28; MS28), medium (32% DM, harvested October 9; MS32) and late (40% DM, harvested October 31; MS40). Gas production (GP) profiles of the dried and ground maize silages were determined using fully automated GP equipment (Cone et al., 1996). CH_4 production was determined as described by Pellikaan et al. (2011). Data from triplicate bottles for each substrate per period were averaged before statistical analysis. Data were analyzed using Proc GLM in SAS (SAS, 2010), with substrate as a main effect.

Results and Discussion

Gas production increased with increasing maturity of maize (Table 1), wich is due to the reduction in NDF and increase in starch content upon maturity. The calculated asymptotic of GP of the soluble and insoluble fraction (A1 and A2) was not affected by maturity (Table 1). However, for the half time of total gas or CH_4 production (B2 and B) numerically decreases with increasing maturity at harvest, indicating that the rate of fermentation increased, although not significantly. In vitro CH_4 24-h expressed in (g/kg OM incubated not correlated with in vivo CH_4 (g/kg DMI or g/kg OM digested) (R^2 = 0.04; P = 0.524; R^2 = 0.04; P = 0.537 data not shown) respectively. The in vitro (48-h) CH_4 production per unit of incubated OM showed a weak correlation but positive with the in vivo CH_4 prduction expressed per unit of DMI or OM digested (Figs 1 and 2) respectively. The main reason for this weak relationship could be due to the small differences in CH_4 production observed in vivo experiment.

Table 1. In vitro gas and methane production of maize silages differing in maturity at harvest

Parameters	Substrate				SEM	P-value
	TMRMS25	TMRMS28	TMRMS32	TMRMS40		substrate
Gas production (GP)						
48-h GP(ml/g OM)	320.6	311.6	358.0	367.3	8.83	0.027
A1(ml/g OM)	58.5	58.0	49.8	46.5	8.17	0.689
A2 (ml/g OM)	205.3	197.2	206.8	202.6	3.45	0.825
B2 (h)	7.7	6.7	6.7	5.5	0.58	0.204
CH_4 production						
48-h CH_4 (ml/g OM)	56.0	45.8	45.5	38.8	5.05	0.261
A (ml/g OM)	70.9	70.6	59.7	50.1	9.82	0.473
B (h)	14.8	14.9	12.4	11.5	1.97	0.533

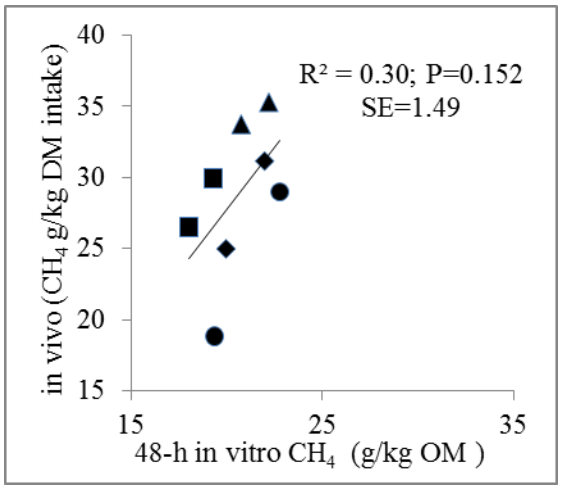

Fig.1. Relationship between in vivo CH_4 production (g/kg DMI) and 48-h in vitro CH_4 production (g/kg OM incubated)

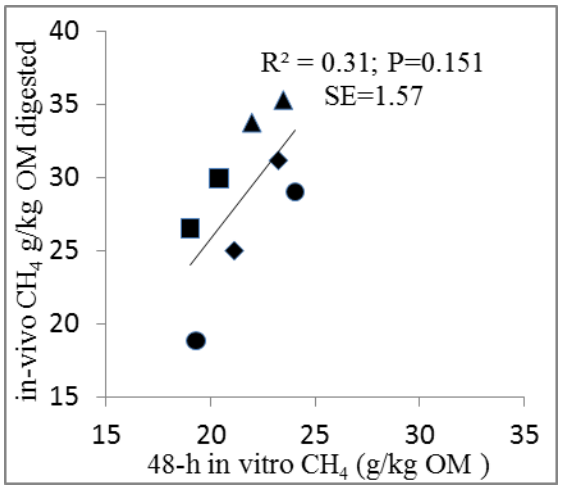

Fig.2 Relationship between in vivo CH_4 production (g/kg OM digested) and 48-h in vitro CH_4 production (g/kg OM incubated)

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In vitro effects of medium-chain fatty acids from coconut oil on methanogenesis from rumen inoculum of goats supplemented or not with coconut oil in early life

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Introduction

Methane (CH_4) is the second most prevalent greenhouse gas in the atmosphere, which has a significant impact on the global warming. Methanogenesis in ruminants takes place during microbial fermentation in the rumen and contributes considerably to anthropogenic CH_4 emissions and dietary energy losses for ruminants (Blaxter and Clapperton, 1965). Therefore, a considerable amount of research has focused on CH_4 mitigation strategies, particularly using nutritional additives e.g. medium chain fatty acids (MCFA). The latter such as lauric (C12:0) and myristic (C14:0) are known to modify ruminal fermentation (Henderson, 1973) and to mitigate greenhouse gas emissions (Machmüller, 2006) by direct inhibition of rumen methanogens, probably by changing their metabolic activity and abundance. Coconut oil containing 470 g/kg C12:0 and 180 g/kg C14:0 reduced *in vitro* methane production to half of the original production when supplemented (756 mg/day) to a Rusitec system (Dohme *et al*. 1999). Recent experiments have evaluated the persistency of nutritional interventions early in life to program rumen function, including rumen methanogenesis, after ceasing the treatment (Abecia *et al*. 2012). Although early life intervention might result in a certain persistency of the effect later in life, these permanent effects might be modest (Abecia *et al*. 2012) and repeated treatment later in life could be considered. However, the effect of repeated treatment on ruminant's methanogenesis is not known. This study aimed to evaluate whether the MCFA dose response on *in vitro* methane production is influenced by an earlier exposure of the ruminants to the same treatment (early in life).

Material and Methods

Saanen dairy goats (65±2 kg BW) pregnant of at least two kids were selected in the last 6 weeks of the dry period (DP), allocated in group pens (5m×25m) with *ad libitum* access to water, corn silage and concentrate (1 kg/goat/day) twice daily (0900 h and 1500 h). From the third week before lambing the goats were randomly divided into two experimental groups, which differed in type of concentrate, with the concentrate control group (D-) containing 4% of palm oil which was replaced by 4% coconut oil in the concentrate of MCFA group (D+), both were equal in net energy (VEM, Van Es, 1978) and protein digestible in the small intestine (DVE, Tammiya *et al*. 1994). After giving birth the kids were immediately separated from their mother and allocated to one of two experimental groups: non-treated (k−) or treated [daily 1 ml of MCFA, AVEVE, Merksem, Belgium] (k+), thus resulting overall in four experimental groups D+ k+ (n=10), D+ k− (n=10), D− k+ (n=8) and D− k− (n = 9). The treatment of the kids (k+) lasted for 2 months; during which kids were grouped per treatment in 20 separate pens (2 by 2). MCFA was administered orally after milk feeding using a syringe. Kids were given colostrum during the first three days after birth and from day 4 until weaning kids received goat milk powder (Milka®, Opwijk, Belgium). Kids' weights were registered each two weeks. Kids were gradually weaned from 6 to12 weeks, replacing the milk powder by hay and concentrate (a maximum of 1 kg/pen). Two weeks after weaning, goat kids were moved to the pasture for 3 months. The treatment was ceased at that time and all goats were housed together. At 6 months of age the goats were euthanized by a standard protocol (Release® Nembutal, 3 ml/10 kg body weight, injected intravenously). Total rumen content from each goat was sampled from which an aliquot of 200 ml was kept anaerobically and at 39° to perform *in vitro* incubations according to Castro-Montoya *et al*. (2012). Inoculum (5 ml) of each goat was incubated with five levels of MCFA (0, 15, 30, 60 and 120 mg/flask). Dried and ground grass silage (250 mg) was used as substrate. After 24 h of incubation, the temperature and pressure were measured and the gas composition was analyzed by GC (Hassim *et al*. 2010). After acidification and centrifugation, the supernatant was collected for VFA analysis (Castro-Montoya *et al*., 2012). Data were analyzed using the SAS PROC MIXED procedure (SAS Inst. Inc., Cary, NC) with early life treatment (N=4) and MCFA level (N=5) as fixed factors. Two analytical replicates were used per treatment.

Results and discussion

The CH_4/VFA ratio linearly decreased ($P<0.05$) with MCFA levels during the *in vitro* incubations (Table 1). Inoculum from kids of supplemented does with MCFA (D+) during the last three weeks of pregnancy resulted in a lower *in vitro* CH_4/VFA ratio as compared with the incubated inoculum from kids of non-supplemented does. MCFA are a powerful antimethanogenic source but also negatively affected the fermentation capacity of microbes (data not shown). This could suggest supplementing pregnant goats at the end of the pregnancy with coconut oil might program the offspring to produce less methane. Nevertheless, the CH_4 inhibitory effect of MCFA was smaller in incubations with inoculum from kids of treated does (D+) suggesting that their microbes may have adapted to the effect of MCFA.

Table 1 Effect of increasing *in vitro* doses of MCFA on inhibition of CH_4 and VFA production during 24 h *in vitro* incubation using inoculum of 6-month-old goats supplemented or not with MCFA during the first two months in life and/or during the last three weeks of the pregnancy of their mothers.

Parameter and EL treatment	Treatment (mg of MCFA/flask)							Statistical significance of the effect (P<0.05)
	0	15	30	60	120	Mean	SEM	Lin *P-value*
CH_4/VFA (µmol/µmol)								
D+K-	0.37^{a}	0.36^{b}	0.34^{c}	0.19^{d}	0.09^{e}	0.25^{B}	0.001	0.025
D+K+	0.36^{a}	0.35^{b}	0.32^{c}	0.20^{d}	0.12^{e}	0.25^{B}	0.009	0.032
D-K-	0.45^{a}	0.43^{b}	0.33^{c}	0.15^{d}	0.09^{e}	0.25^{B}	0.008	0.045
D-K+	0.50^{a}	0.43^{b}	0.38^{c}	0.16^{d}	0.10^{e}	0.27^{A}	0.005	0.029
***in vitro* CH_4 inhibition (%)**								
D+K-	0	3^{d}	8^{c}	49^{b}	76^{a}	34^{C}	2.21	0.026
D+K+	0	3^{d}	11^{c}	44^{b}	67^{a}	31^{C}	3.12	0.018
D-K-	0	4^{d}	27^{c}	67^{b}	80^{a}	44^{B}	2.56	0.015
D-K+	0	14^{d}	24^{c}	68^{b}	80^{a}	47^{A}	3.58	0.001

D+k+: treated kids from treated does; D+k−: untreated kids from treated does; D−k+: treated kids from untreated does; D−k−: untreated kids from untreated does. SEM: standard error of the mean. Statistical significance of the linear dose response on *in vitro* MCFA supplementation (P<0.05). (^{a,b,c,d}) Means of the same parameter annotated with a different letter in the same row differ significantly (P<0.05). Early life treatments (A, B) Overall means with different capital letter annotation for the same parameter differ significantly (P<0.05) between inoculum origin. Overall effect of CH_4/VFA ratio: D (p=0.023), K (p= 0.012) and DxK (p=0.035). Overall effect of CH_4 inhibition (%): D (p=0.031), K (p=0.053) and DxK (p=0.021).

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Effect of the feed additive 3-nitrooxypropanol on the CH_4/CO_2 ratio in an on-farm trial

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Introduction

Livestock is accounted as one of the most important sources of anthropogenic methane emissions. Especially ruminants are responsible for significant amounts of this potent greenhouse gas. In their rumen, micro-organisms breakdown the nutrients, like carbohydrates, of the feed. Methane is produced as a by-product of this process, called enteric fermentation, and is exhaled by the animals (Gerber et al., 2013). Because of the global (warming) importance of enteric methane (GWP_{100}: 34) (Myhre et al., 2013), various mitigation practices are widely investigated (Knapp et al., 2014). Nutritional intervention like the use of feed additives, is one of the main routes that can be followed for lowering methane emissions from ruminants (Gerber et al., 2013; Knapp et al., 2014). To assess the effectiveness of mitigation strategies different methane measurement techniques are available. Next to the respiration chambers, which are accepted as the golden standard of the experimental methods, other techniques like the SF_6-technique and the on-farm GreenFeed system (C-lock inc.) are widely used. The GreenFeed system is built as a concentrate feeder that measures the methane and carbon dioxide emissions of an individual animal when it visits the feeder. Typically short-term (3-7 min) measurements, several times per day, over several days are needed to obtain a 24h individual methane emission profile (Hammond et al., 2015). An on-farm evaluation of a feed additive was made using the GreenFeed system in a free stall with cubicles.

Material and methods

In an *in vivo* trial conducted at ILVO during Spring 2015, eight highly productive Holstein cows were treated with the methane inhibitor 3-nitrooxypropanol (3-NOP) to study the effectiveness of this feed additive in a Flemish ration. Two other high-producing Holstein cows were assigned as reference cows and received a placebo additive. Previous research showed a great potential of 3-NOP for reducing methane emissions in lactating dairy cows. When providing 40 to 80 mg/kg feed dry matter, methane emissions from high-producing dairy cows decreased with 30% (Hristov et al., 2015). In the current trial cows were supplemented 1,7g 3-NOP/day mixed in soybean meal via a standard concentrate feeder in the free stall with cubicles. The ration consisted of 50% maize silage, 40% pre-wilted grass silage and 10% pressed beet pulp, on a dry matter basis. The amount of concentrates (balanced concentrate, soybean meal and protected soybean meal) was calculated on an individual cow basis, to meet the needs of VEM and protein digestible in the small intestine. Methane and carbon dioxide emissions were measured with a GreenFeed system. A control of 18 days followed a two-week adaptation period. No feed additive or placebo substance was administered during both periods. During the treatment period eight animals received the feed additive and the two reference cows received the placebo. For both periods, emission data from 12 until 18 days were considered.

Results and discussion

To evaluate methane reduction due to the addition of 3-NOP the individual CH_4/CO_2 ratio was calculated for the control and the treatment period. This ratio is of particular interest for expressing the efficiency of the rumen micro-organisms fermenting the feed. Methane production is a loss of energy and this ratio describes the proportion of carbon that is not metabolized into CO_2 and in this way is lost as CH_4. The CH_4/CO_2 ratio can be used to identify the rations and/or the cows which give rise to a more efficient feed energy conversion or in other words which produce the least methane (Madsen et al., 2010). Figure 1 shows the CH_4/CO_2 ratio of all cows (n=10) during control period (CTRL - circles) and treatment period (TRTM - triangles). The ratio for the reference cows, which received a placebo additive, did not change in the treatment period compared to the control period ($p=0.92$). On the contrary, seven out of the eight treated cows showed a reduced ratio during the treatment period ($p<0.01$, N=8). Besides this it can be seen that the variation between the treated cows is larger in the treatment period (average ± stdev, 0.033±0.0034, N=8) than it is for the control period (average ± stdev, 0.039±0.0017, N=8). Variation between animals can be considerable and needs to be taken into account when testing mitigation strategies (Garnsworthy et al., 2012).

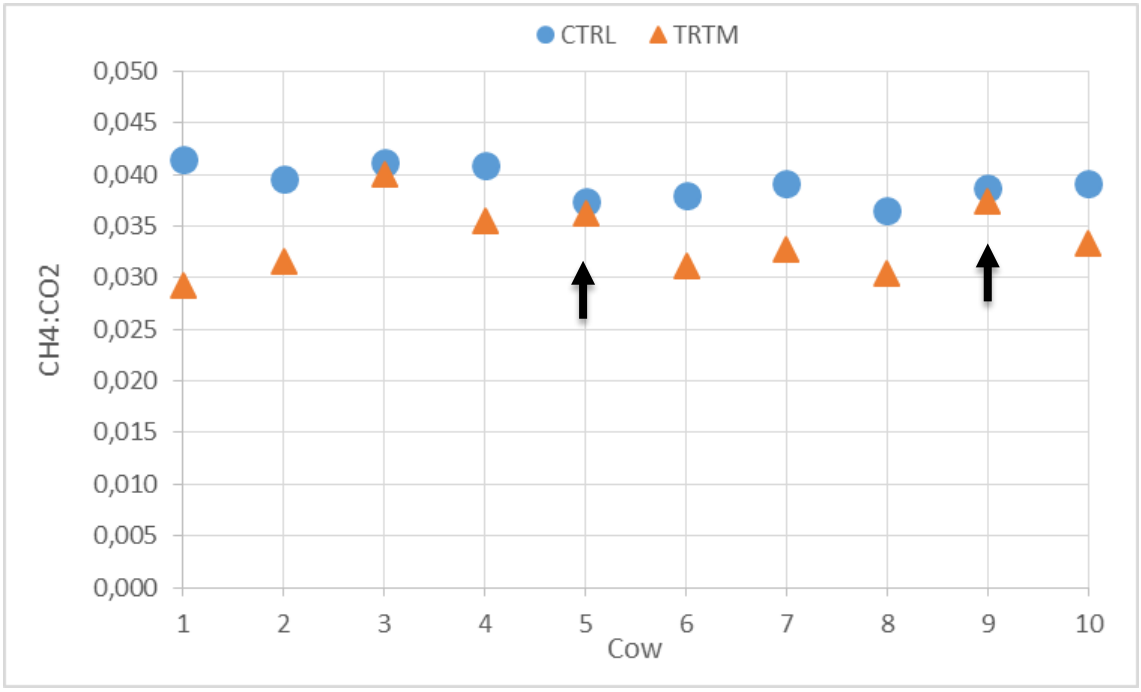

Figure 1: CH_4/CO_2 ratio of all cows (n=10) during control (CTRL) and treatment (TRTM) period, reference cows are indicated with an arrow.

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An *in vivo / in vitro* approach to investigate the potential and working mechanism of DHA Gold™ to reduce methane emissions in lactating dairy cows.

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Introduction

Methane (CH_4) is a potent greenhouse gas with a global warming potential 34-fold greater than that of carbon dioxide[1]. A substantial part of the CH_4 emissions of agriculture worldwide comes from livestock farming (61 Tg/year), which is largely the result of the anaerobic fermentation of plant material by the microbial community in the rumen[2]. Nutritional interventions, for example using poly-unsaturated fatty acids (PUFAs) as feed additives, are considered promising methods for CH_4 reduction in ruminants[3]. Because of environmental concerns about fish stocks, microalgae could serve as a valuable and more sustainable alternative for fish oil, which is known to reduce CH_4 production and increase rumen propionate concentrations *in vitro*[4]. Several studies showed the CH_4 reducing capacity *in vitro* of the product DHA Gold™ (DSM, Switzerland)[5,6], made from marine micro algae (*Schizochytrium* sp.) and containing ca. 18% of the omega-3 PUFA docosahexaenoic acid (DHA). However, the promising *in vitro* results could not yet be confirmed *in vivo*[7,8]. The aim of this experiment was to examine the potential of DHA Gold™ to reduce CH_4 in lactating dairy cattle. For this we used an *in vivo-in vitro* approach in which DHA Gold™ is supplemented *in vivo* to dairy cows, and from which fresh rumen fluid is obtained for an *in vitro* methane assessment.

Material and methods

Four fistulated lactating Holstein cows (193 ± 21 DIM) were subjected to a sequential dose-response experiment. The experiment consisted of 4 periods: 1) three control weeks without supplement, 2) two weeks of treatment with 180g DHA Gold™/d, 3) two weeks of treatment with 360g DHA Gold™/d and 4) three control weeks without supplement (recovery). DHA Gold™ was integrated into a concentrate and supplemented twice a day in equal portions. All cows were fed maize silage and pre-wilted grass silage in a ratio of 45/55 (w/w, DM basis) during the whole experiment. Concentrate was supplemented next to *ad libitum* roughage intake to cover 105% of the animals requirements for net energy (VEM) and digestible protein in the intestine (DVE). Rumen fluid and milk were sampled in the last week of all four experimental periods. Assessment of *in vitro* CH_4 production was performed by batch incubations with fresh rumen fluid, under either CO_2 or H_2 atmosphere. Incubations were repeated on two different days for each experimental period. Milk samples, taken during four consecutive milkings, were analyzed for fat, protein and lactose by near infrared spectroscopy. Feed samples of each period were analyzed for dry matter and chemical composition. Data was statistically analyzed by the MIXED procedure in SAS (Enterprise SAS Inst. Inc., Cary, USA) with dose as fixed effect and cow as random effect.

Results and discussion

An average reduction of 32% µmol CH_4/µmol total volatile fatty acids (VFA) was observed at the end of treatment period 1 (Figure 1). Daily milk yield was not affected. After treatment period 2, CH_4 mitigation was smaller (-25% µmol CH_4/µmol total VFA). This was accompanied with a sharp decrease in dry matter intake (DMI, -20%) and in daily milk yield (-32%). Also, concentration and yield of milk fat, protein and lactose were decreased in both treatment periods. At the end of control period 2, the recovery period, *in vitro* methane production was still reduced by 17% and DMI, daily milk yield, milk fat, protein and lactose levels did not yet recover to pre-treatment levels. Recovery of CH_4 production during the batch incubations under H_2 atmosphere (data not shown) suggested DHA Gold™ particularly affects the H_2-producing bacteria rather than the H_2-consuming methanogens. Total *in vitro* VFA production tended to increase among which a significant increase in propionate (Table 1). It has to be noted that doubling the DHA Gold™ dose in treatment period 2 was probably too harsh for the microbiota, and a smaller step-by-step increase could have been more appropriate to avoid DMI and milk production decreases.

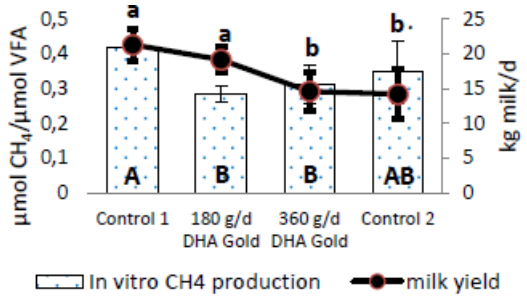

Table 1: Overview of most important *in vitro* VFA production data. (*= Acetate/Propionate)

Item (µmol)	Con 1	180g	360g	Con 2	p-value	SEM
Total VFA	1045[b]	1281[ab]	1268[ab]	1298[a]	0.020	52.06
Propionate	260.9[b]	385.2[a]	410.7[a]	304.4[b]	0.0004	18.11
Ac/Pr *	2.35[a]	1.81[b]	1.62[b]	2.22[a]	0.0064	0.121

Figure 1: *In vitro* methane productions (relative to total VFA produced) and daily milk yield for each experimental period. Averages with different letters differ significantly ($p<0.05$). Capital letters belong to methane production, small letters to daily milk yield.

Conclusion

In conclusion, a daily dose of 180g DHA Gold™ to lactating dairy cows established an *in vitro* molar reduction of 32% CH_4/total VFA and an increase in propionate, without negative effects on DMI and milk yield or composition. The mechanism by which this reduction is achieved, is probably through indirect inhibition of the methanogenesis process, by suppression of hydrogen producing rumen bacteria.

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Effect of split feeding on performance and egg quality of aged laying hens

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Introduction

Laying hens are kept in production on average until 80 wk of age. However, it is predicted that by 2020 genetic selection will make it possible to keep hens until 100 wk of age and produce 500 eggs per housed hen (Van Sambeek, 2010). One of the most important concerns however, is to maintain egg quality in longer production periods. In fact, not only genetic selection but also optimization of feeding systems is necessary to reach the goal of a 100 wk long production cycle. One of the possibilities is to apply an alternative feeding concept, the so called split feeding. This system is based on the varying nutrient requirements and calcium (Ca) utilization of hens during the day. In the first half of the day, after ovulation and during albumen formation hens need an energy- and protein rich diet, whereas during the afternoon and evening, when shell formation occurs, a diet with a higher Ca content is needed. However, not only the Ca content but also its form is important in the feed. Limestone (LS) is widely used as a Ca supplement in fine- (F) as well as coarse (C) form. It has been reported that C-LS particles (>0.8 mm) solubilize more slowly in the gizzard and intestine, allowing a more gradual Ca release to the plasma pool. In contrast, F-LS due to its powdery form is immediately available for absorption (Zhang & Coon, 1997).These properties make F-LS an ideal supplement during the first half of the day when medullary bone formation occurs and no eggshell is being formed. C-LS can be fed during the afternoon to supply Ca for the night when only the crop's content and the medullary bone reserves can support shell formation. Therefore, the aim of this experiment is to investigate the effect of split feeding treatments on performance and egg quality of aged laying hens (75-90 wk of age).

Materials and methods

The trial consisted of a control treatment and 5 split feeding treatments. Per treatment, there were 12 replications of individually housed Dekalb White hens that were fed twice a day a portion of 65 grams. The control treatment (T1) received the same feed in the morning (M) and in the afternoon (A), which contained F- and C-LS at ratio 50:50. For the split treatments, the ratio of F-LS and C-LS was 50:50 or 30:70 and time of administration (M/A) differed. Following treatments were given: T2=50CM:50FA, T3=30FA:70CA, T4=50FM:50CA, T5=30FM:20FA+50CA and T6=30FM:70CA. During the experiment individual records of egg production and feed intake (FI) were measured per hen. Egg quality (egg weight (EW) and shell thickness (ST)) was assessed on all eggs produced on three consecutive days of every second week. Data were analyzed with a linear mixed effects model in R 3.1.0 for Windows.

Results and discussion

FI and laying % were affected differently in the experimental treatments as hens aged ($P \leq 0.001$) (Table 1). FI of T1 was significantly lower (less than 100g/day) than all the split treatments. This strongly affected laying % as it dropped to 47% at 77 wk of age and stayed low until the 84th wk. This drop in FI and production could be a result of natural molting, therefore T1 could not be used as a reference group (control) for the split treatments. Laying% of the split treatments differed at 81-82 wk: T2 had a lower laying% (73.2%) compared to T4 (96.4%). At 89-90 wk of age not only T4 (89.5%) but also T5 (92.9%) had a higher laying % than T2 (66.1%). FI of hens in the split treatments differed throughout the experiment: FI of hens fed T2 was lower compared to the other split treatments. When comparing EW, an interacting effect of age and treatment was found ($P \leq 0.001$). EW in T3 and T6 was significantly higher than that in T2 at different periods throughout the experiment. In the morning, hens fed T3 and T6 received more protein during albumen formation which could result in a higher EW. This hypothesis was supported by the significant differences in albumen weight (AW) of the split treatments: at 82 and 86 wk eggs in T3 (41.1g) had higher AW compared to those in T2 (36.4g), whereas at 88 wk eggs in T6 (41.5g) had higher AW compared to those in T2 (36.6g). Shell thickness (ST) of eggs was affected by age ($P=0.016$) and treatment ($P=0.058$), however no interaction was found between the main effects. Highest ST was obtained in T4 and T5 at 90 wk.

These results indicate that timing of feeding F-LS and C-LS have an effect on production and egg shell quality. The lower FI and laying % in T2 were caused by the suboptimal LS provision, as hens fed this treatment received F-LS in the afternoon, therefore no Ca reserves were available in the crop for shell formation. Ca demand needed to be supported mainly by bone reserves during the night which resulted in low ST. In T3 an energy and protein rich diet was given in the first half of the day and all LS was fed in the afternoon, EW increased and ST decreased. Besides decreasing shell quality, increased EW is a problematic issue in aged laying hens as heavier eggs are more prone to break and more difficult to handle during collection and transport. Providing F-LS in the morning and C-LS in the afternoon in ratios of 50:50 (T4), 30:20+50 (T5) or 30:70 (T6) resulted in favorable performance and shell quality.

Therefore these feeding systems could be taken into consideration for aged hens in an extended laying cycle.

Table 1. Production and egg quality affected by split feeding in laying hens (75-90 wk of age)

	Age (wk)							
Treatments	75-76	77-78	79-80	81-82	83-84	85-86	87-88	89-90
				Laying %				
T2	93.2	82.7	73.2	73.2^{b}	76.8	70.2^{b}	70.2^{b}	66.1^{c}
T3	92.4	92.3	93.5	94.0^{a}	86.9	82.1^{ab}	79.2^{ab}	71.7^{bc}
T4	94.7	95.2	93.5	96.4^{a}	92.9	85.7^{ab}	87.5^{ab}	89.5^{ab}
T5	94.7	94.0	93.5	93.4^{ab}	93.5	91.7^{a}	91.7^{a}	92.9^{a}
T6	94.7	88.7	84.5	86.3^{ab}	79.8	89.2^{ab}	89.^{3ab}	87.2^{abc}
Treatment × Age				**0.0852**				
				Feed intake (g/day)				
T2	98.11	92.2^{b}	92.4^{b}	95.3^{b}	100.1	93.2^{b}	97.3^{b}	94.2^{b}
T3	106.5	109.1^{a}	112.3^{a}	110.0^{a}	110.7	105.9^{ab}	105.4^{ab}	102.8^{ab}
T4	105.6	110.9^{a}	113.1^{a}	109.6^{ab}	110.5	108.9^{a}	109.7^{ab}	109.8^{a}
T5	102.2	105.7^{ab}	106.2^{ab}	104.6^{ab}	107.6	106.9^{ab}	107.9^{ab}	104.8^{ab}
T6	103.4	105.5^{ab}	110.6^{a}	111.0^{a}	110.2	112.7^{a}	112.0^{a}	108.4^{ab}
Treatment × Age				**≤ 0.001**				
				Egg weight (g)				
T2	60.4	59.7^{b}	59.6^{b}	60.1^{b}	60.4	59.9^{b}	60.4^{b}	62.2
T3	65.3	65.9^{a}	66.4^{a}	66.1^{a}	64.3	67.9^{a}	66.3^{a}	66.0
T4	63.3	62.9^{ab}	63.4^{ab}	64.5^{ab}	64.5	63.8^{ab}	62.4^{ab}	63.8
T5	62.2	62.6^{ab}	62.7^{ab}	63.2^{ab}	63.1	63.3^{ab}	64.0^{ab}	63.4
T6	64.4	64.0^{ab}	65.5^{a}	65.4^{ab}	65.7	66.1^{a}	67.3^{a}	65.8
Treatment × Age				**≤ 0.001**				
				Shell thickness (µm)				
T2	386.1	395.3	392.7	397	398.7	378.1	383	383.6
T3	404.8	411.1	400.4	396.4	379.1	392.6	389.9	380.4
T4	412.1	419.5	412	409.5	401.6	398.6	408.4	402.7
T5	407.4	407.5	412.5	403.6	391.2	396.3	399.3	396.1
T6	405.6	404.7	400.9	404.3	386.5	393.8	390.8	388.2
Treatment × Age				**0.104**				

^{abc} Values without a common superscript differ significantly (P≤0.05).

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Nutritive value for ruminants of fungal treated wheat straw

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Introduction

In large parts of the world ruminant animals are fed with low quality feed, with a high lignin content. The low nutritive value results in a low performance, milk production and growth of the animals. Straw and other waste cellulosic products are potential good resources for ruminant production. The major limiting factor for the nutritive value of these waste and by-products is lignin. Biological (pre)treatments to degrade lignin, with the help of fungi, are cost-effective and environmentally friendly alternatives to improve the accessibility of polysaccharides for the microorganisms in the rumen (Sarnklong et al., 2010; Tuyen et al., 2012). The goal is to break the lignin seal and to disrupt the crystalline structure of cellulose (Mosier et al., 2005). Fungi colonize their substrate in such a way that they can utilize the "liberated" carbohydrates very fast when fruiting. They do this by modifying and/or degrading lignin in the plant cell walls to make cellulose and hemicellulose assessable for the moment energy is required for fruiting. Valuable feed will be obtained by feeding the material before the fruiting starts. In our experiments we used the white rot fungi *Lentinula edodes* and *Ceriporiopsis subvermispora.*

Material and Methods

Lentinula edodes (MES 11910) and *Ceriporiopsis subvermispora* (MES 13094) were taken from liquid nitrogen, and cultured on malt extract agar (10 g malt extract and 20 g bacteriological agar per liter of water), at 24°C until the mycelia colonized most of the agar dish surface. A piece of the agar culture (1.5 x 2.0 cm) was put into sterilized sorghum grains and incubated at 24°C until all grains were colonized by the mycelia and subsequently stored in a cold room at 5°C until be used for inoculation of the substrates (Tuyen et al., 2012).

Wheat straw was chopped into pieces of around 2 – 3 cm and water was added to approximately three times the weight of the straw and left overnight for the water to penetrate into the inner structures of the straw. The wet straw was weighted into 1.2-L plastic boxes with 200 g of straw and sterilized by autoclaving. The substrates were cooled in an aseptic flow cabinet at 20°C for 24 h. Inoculation was handled aseptically using 5 g (10% of dry straw) of previously prepared spawn for each straw in the box. Aerobic solid state fermentation was done in triplicate for 9 weeks at 24°C in an air-conditioned chamber. Straw samples were taken every week for chemical analyses and *in vitro* gas production measurement using rumen fluid of dairy cows (Cone et al., 1996).

Results and Discussion

The fungal treatment decreased the neutral detergent fiber (NDF) and lignin content, along increased the crude protein content of wheat straw during the 9 weeks of incubation. However, loss of hemicelluloses and celluloses happened as well, during the incubation. Hemicellulose is easier to degrade than the other components in lignocellulosic biomass.

The total gas production showed a gradual increase for both fungi from week 2 on. Tuyen et al. (2012) and Van Kuijk et al. (2015) stated that most fungal treatments showed a drop in *in vitro* gas production during the initial 2 weeks of incubation, due to the fact that also fungi need nutrients for their growth. In addition, the production of gas continuously until 9 weeks resulted in the degradation of lignin constantly by both *Lentinula edodes* and *Ceriporiopsis subvermispora*.

Table 1. Chemical composition of wheat straw subjected to treatment with *Lentinula edodes* for various incubation periods (g/kg DM)

Weeks of incubation	OM	CP	NDF	ADF	Hemicellulose	Lignin	Cellulose	Ash
0	923.1	26.7	784.4	503.5	280.9	69.2	434.4	23.4
2	928.3	34.1	739.3	483.8	255.6	72.8	410.9	20.9
3	916.8	30.9	699.3	477.9	221.4	60.7	417.2	28.7
4	909.4	28.4	663.6	468.7	194.9	46.6	422.1	32.9
6	924.4	36.3	687.5	470.9	216.6	53.1	417.8	23.3
9	929.3	37.4	642.0	486.5	155.6	36.1	450.4	31.9

Table 2. Chemical composition of wheat straw subjected to treatment with *Ceriporiopsis subvermispora* for various incubation periods (g/kg DM)

Weeks of incubation	OM	CP	NDF	ADF	Hemicellulose	Lignin	Cellulose	Ash
0	923.8	24.1	783.1	492.1	291.1	69.7	422.3	26.6
2	916.9	30.5	713.6	456.2	257.4	66.6	389.6	28.5
3	918.7	29.6	695.8	462.5	233.3	60.4	402.1	31.4
4	913.6	32.1	668.0	450.1	217.9	49.5	400.5	28.8
6	923.9	37.6	634.0	442.5	191.5	40.8	401.7	23.6
9	938.9	38.8	606.5	456.6	149.9	24.3	432.3	29.1

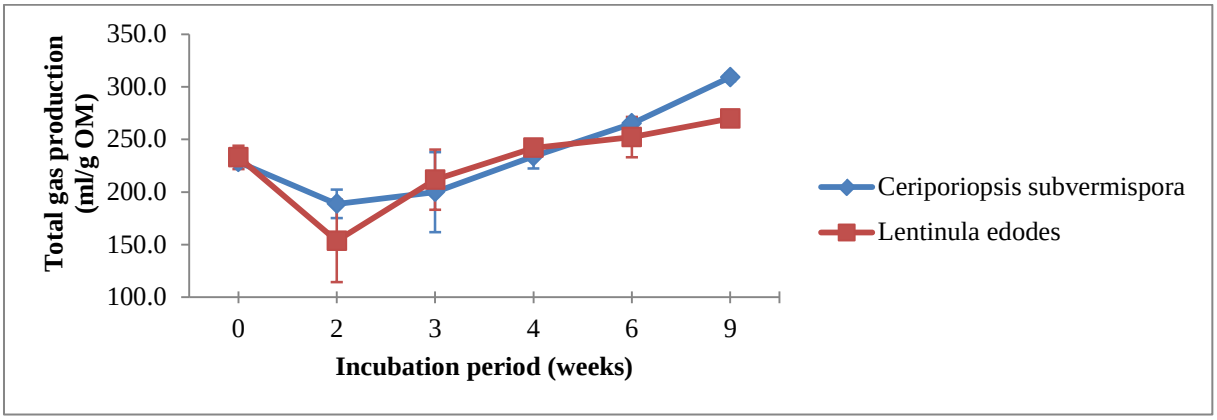

Figure 1. Total gas production (ml/g OM) after 72 hours of incubation in rumen fluid of dairy cows.

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Poster Session

Effect of dietary inclusion of palm date (*Phoenix dactylifera*) pits on performance of laying hens

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Introduction

In poultry industry, feed is the most important economical factor, because it contains 70% of cost of poultry production. Consequently, good quality, suitable price and availability of feed ingredients for poultry should be considered. Palm date is one of the major agricultural crops in most of the middle East countries, and it plays a key economic and sociological role. Iran is one of the largest producers of palm date with a production of about 1,000,000 tons, which is about 15% of the total world production. About 400 varieties are grown in Iran (Golshan Tafti and Fooladi, 2006). Date pits (known also as date stones, kernels, or seeds) availability and low cost is the reason for its usage in feeding livestock and poultry (Hussein *et al.* 1998). Due to the valuable nutrients in date kernel and its mass production in some parts of Iran, the present study aims to investigate effects of dietary inclusion of palm date pits on performance of laying hens.

Materials and Methods

A total number of 108 Lohman Breed LSL-lite laying hens (38 wk age) were randomly assigned to three dietary groups (36 birds per each) and housed with three birds per cage (12 replicate cages per treatment) for a 7 wk trial period. Three iso-caloric and iso-nitrogenous experimental diets (ME=2720 Kcal/Kg and CP=154.2 g/Kg) including a control group and two different levels of date pits (7, 14%) were formulated (Table 1) . Hens were fed approximately 120 gr/day and water was offered *ad libitum*. The metabolizable energy content (AME) of the used date pits (4.39±0.419) was measured in a study which has been already published (Zangiabadi and Torki, 2010). Feed intake (FI), egg production (EP), egg mass (EM) and calculated feed conversion ratio (FCR) were measured on a weekly basis. Data were tested with the Kolmogorow–Smirnow-test for normal distribution and subjected to ANOVA using the General Linear Models procedure of SAS 2003 software as a complete randomized design. Duncan's new multiple range test was used to compare the treatment means at $P<0.05$, following analysis of variance.

Results and discussion

The effects of dietary treatment on productive performance of laying hens are presented in Table 2. FCR, EP, EM and average egg weight were not affected by dietary date pits inclusion, but FI was significantly affected by treatment. FI of hens fed the diets including 14% date pits was lower compared to the 0% date pits group, where the 7% date pits group was in between. Based on the report by Najib and Al-Yousef (2012), feeding diets including date pits, regardless of supplemental enzyme, improved the performance of the laying hens. Hermes and Al-Homidan (2004) showed that feeding diets containing 10% date pits improved EP, EM, egg weight, and FCR of hens compared to control and diets included 5% or 15% date pits. Al-saffar *et al.* (2012) reported that dietary inclusion of 15 and 30% date pits decreased EP by 9.9 and 3.2%, respectively; however the EP decrease was only significant in hens fed 15% date pit included diet. Based on the results of the present experiment, it can be concluded that dietary inclusion of palm date pits up to 14% had no adverse effect on performance of laying hens, which might be economically beneficial.

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Table 1. Composition of experimental diets

Ingredients	g/ 100 g diet		
	Control	7% Date pits	14% Date pits
Corn	56.07	53.37	50.67
Fish meal	2.00	2.00	2.00
Soybean meal	16.78	18.10	19.41
Date pits	-	7.00	14.00
Wheat bran	12.10	6.60	1.10
Sunflower Oil	2.95	2.95	2.95
Limestone	7.87	7.70	7.53
DCP	1.26	1.30	1.34
Salt	0.25	0.26	0.27
Premix	0.50	0.50	0.50
DL- Met	0.21	0.22	0.23
ME	2720	2720	2720
CP	15.42	15.42	15.42
Ca	3.42	3.42	3.42
AP	0.35	0.35	0.35
Met	0.47	0.47	0.47

Table 2: Effects of dietary inclusion of date pits on performance parameters of laying hens

Treatments	**Feed intake (g/day/hen)**	**FCR**	**Egg production (%)**	**Egg mass (g/day/hen)**	**Average egg weight (g)**
0% Date pits	118.78[a]	2.03	94.16	58.57	62.20
7% Date pits	117.96[ab]	2.01	93.54	58.76	62.80
14% Date pits	116.11[b]	1.98	94.22	58.78	62.37
SEM	0.474	0.017	0.528	0.439	0.229
P values	0.052	0.569	0.859	0.981	0.571
CV	1.713	3.775	2.386	3.175	1.557

Means ± SD. Means within a column with different superscripts are significantly different.

Effect of dietary inclusion of two sources of selenium and *Satureja hortensis* essential oil on productive performance of laying hen

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Introduction

Due to consumer demand for safe and natural food and the potential development of antibiotic resistant to pathogenic bacteria, the European Union banned the use of antibiotics as performance-promoting agents in animal feeds [1]. Different additives such as probiotics, prebiotics, enzymes, organic acids and phytogenics are used to improve the poultry performance [2]. Medicinal herbs, and their associated essential oils or extracts are being considered as potentially growth promoters [3]. Recently, many researches have focused on antibacterial and antifungal activity of the essential oil or extracts of *Satureja* species. It has been showed that *Satureja* species have antimicrobial activity due to the presence of phenolic components such as thymol and carvacrol [4]. Selenium (Se) is an important nutritional trace element that is essential for vertebrates including birds, and is critical to their normal physiology [5]. Organic and inorganic selenium are two sources of selenium for poultry feed supplementation. The aim of the present study was to investigate the effect of essential oils extracted from *S. hortensis*, organic and inorganic selenium on growth performance of 66-week-old Lohmann laying hens.

Materials and Methods

A total number of 216 66-week old white Lohmann LSL-Lite laying hens were randomly distributed between 36 cages and hens in every 6 replicate cage allocated to feed one of the six experimental diets. The six iso-caloric and iso-nitrogenous corn-soybean meal-based diets (ME=2750 Kcal/Kg and CP=146.9 g/Kg) including the basal diet (control) or the basal diet with either 250 ppm *S. hortensis* essential oil, 0.3 mg/kg sodium selenite as inorganic and 0.3 mg/kg Selemax as organic Se source and two other diets including the basal diet with the mixed forms of the mentioned additives (*S. hortensis* essential oil + sodium selenite and *S. hortensis* essential oil + Selemax). During the 12-week experimental period, hens were fed approximately 120 (gr/day) and water was offered *ad libitum*. Feed intake (FI), egg production (EP), egg mass (EM) and calculated feed conversion ratio (FCR) were measured on a weekly basis. Data were tested with the Kolmogorow–Smirnow-test for normal distribution and subjected to ANOVA using the General Linear Models procedure of SAS software [6] as a complete randomized design. Duncan's new multiple range test was used to compare the treatment means at $P<0.05$, following analysis of variance.

Result and discussion

Table 1 shows the effects of diet supplementation by *S. hortensis* essential oil, sodium selenite and Selemax on productive performance parameters. No significant effect of dietary treatments was observed on the birds' performance ($P>0.05$). Jaderi et al [7] reported that performance of hens was not affected by *S. hortensis* treatment. Shaykhi et al [8] showed that savory, herbs of the genus *Satureja* , increased feed intake in comparison with a control group in Japanese quails. They suggested that due to palatability and ability of savory in stimulation of endogenous enzyme activity the quails fed diet added with savory had higher feed intake. Zoran et al [9] showed that the inclusion of 0.4 or 0.8 mg/kg selenium from sodium selenite and Se-enriched yeast in hen's diet had no significant effects on egg weights, feed intake, and conversion of feed. Also Utterback et al [10] indicated that supplementation of sodium selenite and Se-enriched yeast (0.3 mg/kg) in laying hens diets had no significant effect on egg weight and feed intake of birds. In brief, results from this research showed that diet supplementation by *S. hortensis* essential oil, sodium selenite and Selemax did not affect performance of laying hens for the age period of 66 to 78 weeks.

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Table 1: Productive performance

treatment	Feed intake (g/day/hen)	FCR	Egg production (%)	Average egg weight (g)	Egg mass (g/day/hen)
Control	109.43±0.26	1.93±0.03	89.45±4.33	63.45±0.41	56.76±1.58
Inorganic Se(In Se)	109.19±0.43	1.91±0.02	90.16±4.01	63.67±0.76	57.43±1.58
Satureja (Sat)	109.23±0.31	1.88±0.02	90.74±3.17	64.14±0.80	58.21±1.36
In Se+ Sat	109.61±0.15	1.92±0.02	89.52±3.84	63.96±0.38	57.28±1.49
Organic Se(O Se)	108.79±0.35	1.91±0.03	90.34±3.40	63.20±0.48	57.16±1.61
O Se+ Sat	108.63±0.46	1.88±0.02	89.18±3.08	64.59±0.41	57.61±1.10
P–value	0.359	0.982	0.994	0.586	0.989
SEM	0.143	0.010	0.793	0.230	0.560
CV	0.781	10.147	11.097	2.205	6.266

Influence of fiber type and content, and amino acids levels in the lactation diet on farrowing process, sow health and piglet vitality

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Introduction

Increasing litter size emphasized issues related to intense nutritional requirements of the lactating sow and to health and welfare related concerns like piglet vitality and post-partum dysgalactia syndrome (PDS). Questions remain about the need for increased supply of balanced amino acids (AA), or about the role of dietary fiber, either fermentable (fCHO) or inert (iCHO) carbohydrates (faecal digestibility), to improve farrowing process and growth of the offspring.

Material and methods

Hundred fifty sows entering farrowing room were allocated to one of 6 diets until weaning, in a factorial arrangement of 2 AA levels (basal 6.7 g SID Lys/kg vs. +40%) and 3 dietary fiber types (basal, +40% fCHO, +60% iCHO). Performance of sows and piglets was recorded until day of return to estrus. Farrowing process was studied on 12 sows per treatment, in addition to stress, PDS and faecal scores. Piglet birth time, individual weight and rectal temperature were recorded, and survival to 8 days was used to assess vitality.

Results and discussion

Increasing AA level reduced sow weight loss during lactation, but had no effect on vitality of the piglets or on sow health around farrowing. In the first 4 days after farrowing, fCHO and iCHO decreased feed intake, resulting in more back-fat loss. Farrowing process was not significantly affected by fiber type or content. PDS score worsened significantly with fCHO and iCHO. However, faecal consistency post-farrowing was improved with fCHO (Figure 1). Proportion of birth weight below 950g increased with fCHO, iCHO being intermediate. However, neither piglet vitality nor growth pre-weaning responded to treatments. Altogether, the current results didn't support the need for increased AA, fCHO or iCHO in lactation diets.

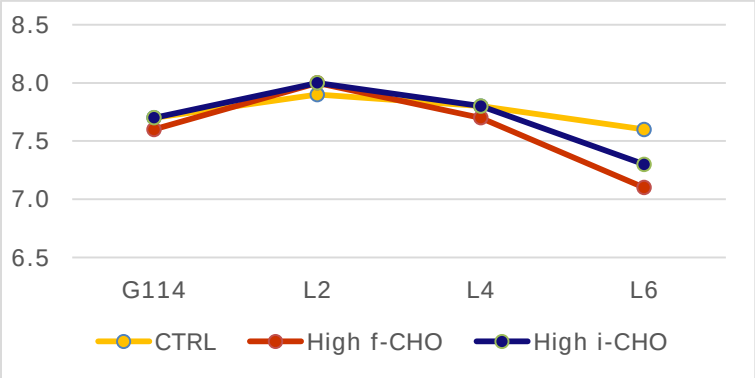

Figure 1. Fiber type and content improves fecal consistency post-farrowing.

Two techniques for viscosity measurements in poultry feedstuffs: does it render similar conclusions?

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Introduction

To date, the complete removal of solid particles by centrifugation has been applied in viscosity measurements of the intestinal contents in many poultry studies (Slominski, 2011). However, centrifugation may remove particles that contribute to digesta viscosity (Takahashi et al., 2004), hence potentially giving rise to an underestimation of digesta viscosity. Two viscosity measurement techniques, one including a centrifugation step and the other without, were compared to investigate whether both techniques result in similar conclusions regarding viscosity.

Material and methods

Two sets of feedstuff preparations were used. The first set was prepared with different combinations of milled (screen of 1 mm) feedstuffs: 100% corn, 25% corn + 75% wheat, 100% wheat, 90% wheat + 10% rye, all mixed with distilled water in order to have a wide range of viscosity. In the second set, barley was incubated with different beta-glucanases, and soybean and sunflower meal were incubated with different pectinases, all mixed with distilled water. Viscosity was assessed using both techniques (with and without centrifugation (at 3893 g for 5 minutes)) at 6 time points. In order to compare the two methods of viscosity measurement and to include all time points at which the viscosity was measured, the area under the curves (AUC) were assessed. The techniques use different units to measure viscosity: Pascal for the Haake viscometer (for whole suspensions) and centipoises for the Brookfield viscometer (for supernatants). Therefore, the relative percentage of change in viscosity was calculated within each method based on the AUC changes. In the first set, differences between feedstuffs were the target, whereas in the second set the addition of enzyme was evaluated.

Statistical analysis

To evaluate the extent of agreement between the two methods, linear regression was used to analyze the correlation between areas under the curves measured with the Brookfield and Haake viscometer. Furthermore, the Lin's Concordance Correlation Coefficient (CCC) was assessed using the percentage of change in viscosity (within each method), based on pairwise feedstuffs comparison (first set), or relative to the feedstuff without enzyme (second set).

Results and discussion

In Figure 1, a scatter plot of the correlation between the AUC of the viscosity measured with the Brookfield and Haake viscometers (with and without centrifugation step respectively) is shown. For the first set of feedstuffs (Figure 1A) and for barley incubated with glucanases (Figure 1B), the Pearson correlation coefficient (r) between the Haake and the Brookfield measurements were quite high ($r = 0.911$, and $r = 0.928$, for the first set of feedstuffs and barley incubated with glucanases respectively), opposite to the results obtained for soybean meal (Figure 1C) and sunflower meal (Figure 1D) incubated with pectinases. In the latter, these coefficients were <0.4. Based on CCC analysis, the rate of the agreement between the two methods, was substantial for the first set of feedstuffs (66%) and for the barley diets incubated with beta-glucanases (69%), whereas the CCC score for the soybean meal diets was very poor (2%) and fair for the sunflower meal diets, incubated with pectinases (32%). The latter can be explained by the lack of viscosity in these mixtures anyhow (Table 1). It can be concluded that results obtained with the common method, where centrifugation is used (Brookfield) agrees quite well with the method without centrifugation (Haake). Although the two techniques are considerably different (e.g. with or without preceding particle removal), they seem to render similar conclusions when applied to poultry feedstuffs unless the effect range of interest is really narrow.

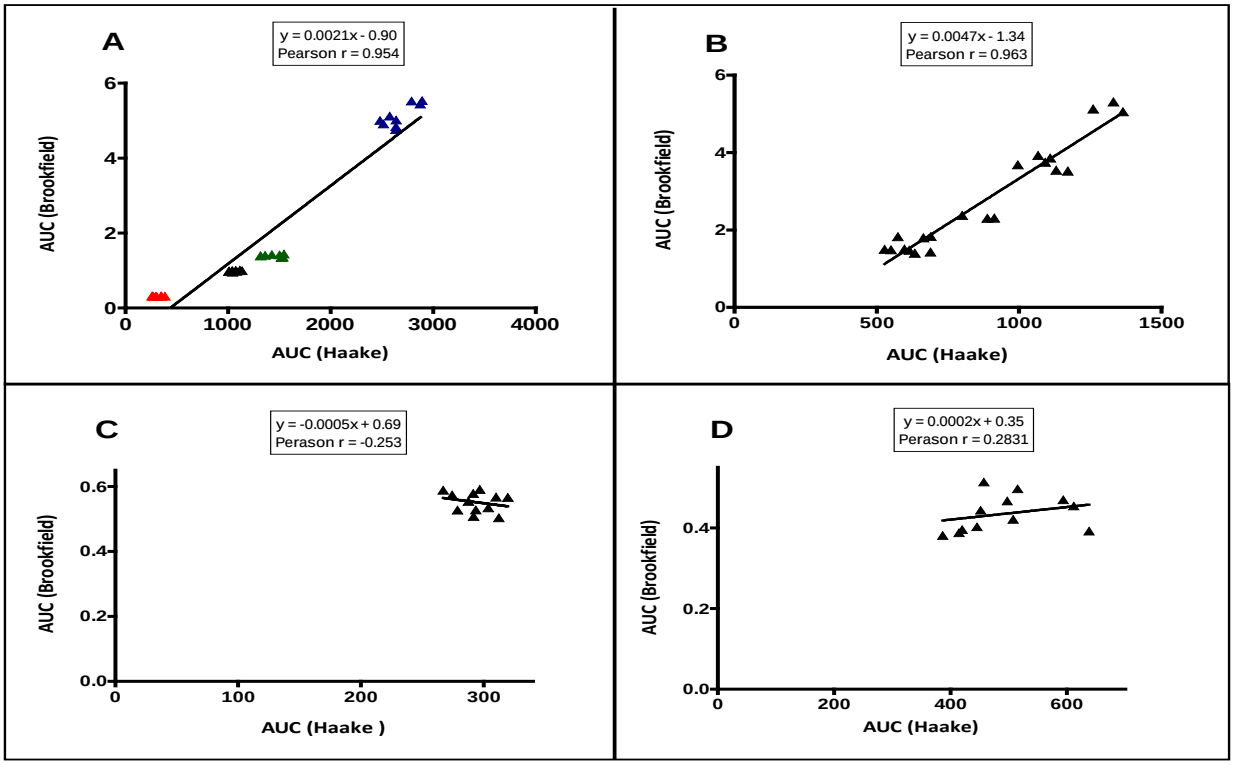

Figure 4. Scatter plot between area under the curve (AUC) using the Haake or the Brookfield viscometers with: A – the first set of feedstuffs (green – 100% corn, blue – 25% corn + 75% wheat, purple – 100% wheat, orange – 90% wheat + 10% rye); B – barley with or without glucanases added; C – soybean meal with or without pectinases added; and D – sunflower meal with or without pectinases added. The black line represents the linear regression line.

Table 1. Lin's Concordance Correlation Coefficient (ρc) and its 95% confidence interval (CI, with upper and lower limits (Upper lim and Lower lim, respectively)), Pearson correlation coefficient (ρ), bias correction factor (C_b) for the relative percentage of change in viscosity (based on AUC) for the first set and the second set of feedstuffs (using enzymes) measured with the Haake and the Brookfield viscometer

		First set of Feedstuffs	Barley (glucanases)	Sunflower meal (pectinases)	Soybean meal (pectinases)
ρc		0.660	0.690	0.320	0.022
95% CI	Upper lim	0.560	0.820	0.770	0.219
	Lower lim	0.740	0.480	-0.340	-0.176
ρ		0.900	0.950	0.340	0.083
C_b		0.730	0.730	0.930	0.270

References

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To explore the potential of nature to improve the quality of life

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