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Running head: DAIRY COW DIGESTIBILITY

Gastrointestinal tract size, total tract digestibility and rumen microflora in different dairy cow genotypes

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Interpretive Summary

DAIRY COW DIGESTIBILITY

This study aimed to measure if differences existed among dairy cow genotypes in gastrointestinal tract size, digestibility and selected rumen microbial populations. Jersey and Jersey×Holstein-Friesian cows had proportionally larger gastrointestinal tract weight than Holstein-Friesian cows. Jersey cows had a superior total tract digestibility and lower relative abundance of Ruminococcus flavefaciens in the rumen than Holstein-Friesian cows. These differences could contribute to the production efficiency differences among genotypes previously reported.

ABSTRACT

The superior milk production efficiency of Jersey (**JE**) and Jersey×Holstein-Friesian (**JE×HF**) cows compared with Holstein-Friesian (**HF**) has been widely published. The biological differences among dairy cow genotypes, which could contribute to the milk production efficiency differences, have not however been as widely studied. A series of component studies were conducted using cows sourced from a longer-term genotype comparison study (JE, JE×HF and HF). The objectives were to: (i) determine if differences exist among genotypes regarding gastrointestinal tract (**GIT**) weight, (ii) assess and quantify if the genotypes tested differ in their ability to digest perennial ryegrass, and (iii) examine the relative abundance of specific rumen microbial populations potentially relating to feed digestibility. Over 3 yr the GIT weight was obtained from 33 HF, 35 JE and 27 JE×HF non-lactating cows post-slaughter. During the dry period the cows were offered a perennial ryegrass silage diet at maintenance level. The unadjusted GIT weight was heavier for the HF than for the JE and JE×HF. When expressed as a proportion of bodyweight (BW) the JE and JE×HF had a heavier GIT weight than the HF. In vivo digestibility was evaluated on 16 each of JE, JE×HF and HF lactating dairy cows. Cows were individually stalled allowing for the total collection of feces and were offered freshly cut grass twice daily. During this time daily milk yield, BW and dry matter intake (**DMI**) were greater for HF and JE×HF than for JE. Milk fat and protein concentration ranked oppositely. Daily milk solids yield did not differ among the 3 genotypes. Intake capacity, expressed as DMI/BW, tended to be different among treatments, with the JE having the greatest DMI/BW, the HF the lowest and the JE×HF cows were intermediate. Production efficiency, expressed as milk solids/DMI, was higher for the JE than HF and JE×HF. Digestive efficiency, expressed as digestibility of dry matter, organic matter, N, neutral detergent fibre and acid detergent fibre, was higher for JE than HF. In grazing cows (n=15 per genotype) samples of rumen fluid, collected using a transesophageal

sampling device, were analyzed to determine the relative abundance of rumen microbial populations of cellulolytic bacteria, protozoa and fungi. These are critically important for fermentation of feed into short-chain fatty acids. There was a decrease in the relative abundance of *Ruminococcus flavenfaciens* in the JE rumen compared with HF and JE×HF. Deductions from this study are that the JE genotype has greater digestibility and a different rumen microbial population than the HF. Jersey and JE×HF cows had a proportionally greater GIT weight than HF. These differences are likely to contribute to the production efficiency differences among genotypes previously reported.

Key words: digestibility, breed, rumen microflora, production efficiency

INTRODUCTION

The topic of production efficiency, within the context of livestock production systems, has received renewed attention in recent years (Spurlock et al., 2012; Berry and Crowley, 2013; Connor et al., 2013). The great debate concerning land use for ruminant production versus production of human edible feed is a primary driver for this renewed interest in production efficiency (Wilkinson, 2011). Opportunities to directly select for improved efficiency are limited as dry matter intake (**DMI**) measurements from individual cows, required to generate breeding values for traits related to efficiency, are not routinely available. That said genetic diversity within feed efficiency has been demonstrated to exist. In a review of genetic parameters for the trait Berry and Crowley (2013) reported heritability estimates for feed efficiency related traits in cows (residual feed intake or feed conversion ratio) ranging from 0.00 (Svendsen et al., 1993) to 0.38 (Veerkamp et al., 1995). A review by Goddard and Grainger (2004) and more recently studies by Buckley et al. (2007) and Prendiville et al. (2011a) indicated genotype or strain within genotype variation for DMI capacity and milk

production efficiency. Milk production efficiency can be defined in many ways. The present study uses the definition of milk solids yield (kg of fat and protein) per unit of DMI (Lopez-Villalobos et al., 2008). While such variation in milk production efficiency has been demonstrated, the biological differences among dairy cow genotypes, which could contribute to the milk production efficiency differences measured, have not been as widely studied. Previous studies are either dated or use beef cattle (Smith and Baldwin, 1974; Richardson and Herd, 2004). Hence, further evidence of biological differences among the genotypes is warranted to enhance the understanding of the production efficiency differences and the potential to select for this increasingly important trait.

Prendiville et al. (2010) concluded that differences in grazing behavior contributed little to differences in DMI capacity among lactating dairy cow genotypes. They speculated that the higher DMI capacity expressed as DMI/BW observed with Jersey (**JE**) and Jersey×Holstein-Friesian (**JE×HF**) compared with Holstein-Friesian (**HF**) was likely a function of physical differences associated with gastrointestinal tract (**GIT**) size. Two studies exist which support this speculation but their relevance in the context of modern HF and JE genetics is questionable as the study of Smith and Baldwin (1974) is almost 40 years old, while the study of Nagel and Piatkowski (1988) compared JE to German Black-Pied cattle. Both studies are limited by very small numbers of lactating animals and neither compared the genotypes consuming a grass-based diet.

Differences in digestibility among beef steers are reported to account for 10% of the variation in feed efficiency with more efficient animals capable of digesting more of the diet (Richardson and Herd, 2004). Genetic variation among dairy cows in their ability to digest a predominately grazed grass diet ranges from 0.08 to 0.45, but digestibility was predicted

using the n-alkane method (Berry et al., 2007). The accuracy of the n-alkane method is questionable as errors can arise from estimation of the alkane concentration, herbage sampling errors, or analytical errors (Rymer, 2000). Digestibility measured in vivo is the most accepted method. Digestibility and DMI are related, as increasing DMI can result in a quicker passage rate (Thornton and Minson, 1972; Colucci et al., 1982) resulting in decreased digestibility (Tyrrell and Moe, 1975). Yet JE animals have been shown to have both a greater DMI capacity (Goddard and Grainger, 2004; Prendiville et al., 2009) and a higher NDF digestibility than HF cows, resulting in JE producing a greater milk energy output/kg DMI (Aikman et al., 2008). However, animals in the latter study were offered a TMR diet. Differences in digestibility among dairy cows offered a predominately grass-based diet warrants further research to accurately determine the digestibility differences in vivo among genotypes.

Approximately 65% of digestion occurs in the rumen (Hogan and Weston, 1967). Rumen microorganisms control rumen pH (Williams and Coleman, 1997) and the fermentation of cellulose, hemicellulose and fiber into short-chain fatty acids (Van Soest, 1994; Gordon and Phillips, 1998), which are utilized by the host for maintenance, growth and performance. Previous work has shown that diet has a large influence on the rumen microbial population, affecting the bacteria (e.g., *Bacteroidetes* and *Firmicutes*) and archaeal populations (de Menezes et al., 2011), while the celluolytic bacteria (e.g., *F. succinogenes, R. flavefaciens* and *R. albus*) are affected more by the individual host cow than by diet (Weimer et al., 1999). Data suggest that variation in feed efficiency in beef cattle may be explained by rumen microbial density and diversity (Guan et al., 2008; Carberry et al., 2012). There is, however, no information available comparing the rumen microbial populations among dairy cow genotypes consuming a grass diet.

The objectives of the present study were to: (i) determine if differences exist among dairy cow genotypes regarding GIT weight, (ii) assess if JE and by extension JE×HF differ from HF in their ability to digest perennial ryegrass, and (iii) examine the relative abundance of specific rumen microbial populations potentially relating to feed digestibility among dairy cow genotypes.

MATERIALS AND METHODS

All sampling procedures described as part of this experiment were executed in accordance with guidelines set by the Irish Minister for Health and Children under section 8 of the Cruelty to Animals Act (1876). This study comprised a series of component studies conducted using cows sourced from a longer term genotype comparison study (JE, JE×HF and HF) based at the Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland (52º 09'N; 8º16'W). The longer term study was established in 2006 to evaluate the performance and profit potential of JE, JE×HF and HF under an Irish grass-based production system (Prendiville et al., 2011b). Until 2009, the 3 genotypes grazed as a single herd, at which point the study was redesigned to implement treatments that would determine if performance differences existed at different stocking rates (genotype × environment; G×E) (Thackaberry et al., 2011). It was during this later stage in the research programme that the series of component studies presented were conducted.

Post-slaughter anatomical data

Over a 2 yr period, on dates approximating to the end of the 3 experimental seasons 2009 to 2011, a total of 95 non-pregnant cows were slaughtered and weights recorded for a range of tissues and organs associated with DMI and metabolic activity in the dairy cow: reticulo-

rumen, abomasum, omasum, intestines, heart, lungs, pancreas, liver, kidneys and weight of cold carcass. Cows were slaughtered over 2 d during February 2010, December 2010 and November 2011. During February 2010, December 2010 and November 2011, the cows slaughtered comprised 10 JE, 8 JE×HF and 12 HF; 14 JE, 8 JE×HF and 13 HF; and 11 JE, 11 JE×HF and 8 HF cows, respectively. The mean (**SD**) parity of JE, JE×HF and HF cows was 3.0 (1.21), 3.3 (1.22) and 3.3 (1.29), respectively. All cows were dry at time of slaughter and were managed similarly from dry-off to slaughter, offered a maintenance diet of perennial ryegrass silage. The average number of days dry (SD) within each genotype were as follows: JE 23 d (22.7), JE×HF 22 d (22.3) and HF 27 d (22.1). Animals were not fasted prior to slaughter (Dawn Meats, Charleville, Co. Cork, Ireland). Individual cow **BW** on arrival at the slaughter factory was recorded. Animals were stunned by captive bolt pistol, hung and bled. The slaughter of animals during this study complied with S.I. No. 328/1999 (Abattoirs Act, 1988 (Abattoirs) (Amendment) Regulations, 1999).

All organs/tissues were removed and weighed (CPWplus35M, P.J. Boner & Co. Ltd, Dublin 12, Ireland) within 60 min of slaughter. Adipose tissue was removed from the kidneys, liver, lungs and pancreas before weighing. The components of the GIT were separated and excess adipose tissue on the reticulo-rumen, omasum and abomasum was removed before weighing. The reticulo-rumen and abomasum were cleaned of digesta residues before weighing. The omasum and small and large intestines (hereafter referred to as intestines) were weighed as presented. Total GIT weight was calculated as the sum of the reticulo-rumen, omasum, abomasum and intestines weights.

In vivo digestibility trials

In vivo digestibility trials on 16 each of JE, JE×HF and HF lactating dairy cows were conducted over 4 consecutive time periods balanced for genotype. The study commenced on August 3 and finished on September 24 2010. At the beginning of the study, the mean (SD) BW of JE, JE×HF and HF cows were: 434 kg (39.3), 501 kg (40.6) and 576 kg (44.7), respectively. The mean parity of JE, JE×HF and HF cows was: 3.8 (1.06), 3.6 (1.31) and 3.3 (1.24), respectively. The mean (SD) DIM of JE, JE×H and HF cows was: 167 DIM (26.7), 180 DIM (21.0) and 170 DIM (22.7), respectively.

Each time period, consisting of 12 d, was conducted in a similar manner. Cow BW was measured the day before the animals entered the metabolism house and on the day they entered the metabolism house. Body weight was recorded using electronic portable weighing scales and the Winweigh software package (Tru-test Limited, Auckland, New Zealand).

During the study, the cows were individually stalled, were offered fresh cut perennial ryegrass twice daily at 08:00 h and 14:00 h and had ad libitum access to water. Grass was cut before each feeding time using a Pottinger Nova cat 266 F mower (Alois Pöttinger Maschinenfabrik GmBH, Grieskirchen, Germany) and transported using a Pottinger Europrofi 1 Euromatic self-loading wagon (Alois Pöttinger Maschinenfabrik GmBH, Grieskirchen, Germany). There were 2 herbage allowances: high and low. The HF and JE×HF cows on the high herbage allowance were offered 20 kg DM/cow per d and JE cows on the high herbage allowance were offered 17 kg DM/cow per d. The HF and JE×HF cows on the low herbage allowance were offered 16 kg DM/cow per d and JE cows on the low herbage allowance were offered 14 kg DM/cow per d. The low and high herbage allowances offered were to reflect treatments from which the animals were randomly selected, high and low stocking rates, described by Thackaberry et al. (2011). Pre- and post-cutting sward heights were determined daily using a

plate meter with a steel plate (diameter 355 mm and 3.2 kg/m^2; Jenquip, Fielding, New Zealand).

Following a 6-d acclimatization period, a 6-d measurement period began during which individual total DMI and feces production was recorded daily (Raymond et al., 1953). A representative sample of the grass offered was collected daily during the 6-d measurement period. Refused herbage was weighed back and recorded each morning for each cow during the measurement period. Total weight of feces produced by each cow was recorded daily and a 1% subsample retained.

Herbage Samples. Dry matter was determined by drying herbage at 95^{o}C for 15 hours. Further herbage samples were stored at -20^{o}C prior to being freeze-dried (LS40+Chamber, MechaTech Systems Ltd., Bristol, UK) at -55^{o}C for chemical analysis. The freeze-dried samples were milled through a 1-mm screen (Cyclotech 1093, Foss, DK-3400 Hillerød, Denmark). Samples were analyzed for ash content by placing samples into a Gallenkamp muffle furnace size 3 (Thermo Fisher Scientific INC., Waltham, MA) for 16 hours at 500°C (AOAC, 1995; method 942.05). The CP concentration of the samples was analyzed using a Leco N analyzer (Leco FP-528; Leco Corporation, St., Joseph, MI). The samples were analyzed for NDF and ADF with an Ankom Fiber Analyzer (Ankom Technology Corporation, NY) using the method of Van Soest et al. (1991). Amylase and sulfite were used in the NDF process. The NDF and ADF values are expressed excluding ash.

Fecal Samples. Fecal samples were frozen at -20^{o}C and stored until the end of the study. The frozen samples were thawed prior to drying at 60^{o}C for 48 h and subsequently milled through a 1-mm screen. Following milling the daily fecal samples were composited by

278 cow within measurement period. Fecal samples were analyzed for DM, ash, N, NDF and ADF
279 concentration using the methods for herbage samples described above.
280
281 ***Milk production.*** Cows were milked twice daily (08:00 h and 16:00 h) and individual
282 cow milk yield was recorded (Dairymaster, Causeway, Co.Kerry, Ireland) at each milking.
283 Milk fat, protein and lactose concentrations were determined with the Milkoscan 203 (DK-
284 3400; Foss Electric, Hillerød, Denmark) from one successive evening (Tuesday) and morning
285 (Wednesday) milk sample for each cow during each measurement period. Daily milk solids
286 yield (kg/d) was calculated as the sum of fat (kg) plus protein (kg) for each cow.
287
288 ***Digestibility calculations.*** Apparent digestibility was calculated using Equation 1:
289
290 $$Digestibility = \frac{(x - y)}{x}$$
291 **Equation 1**
292 where *x* and *y* are equal to the intake in herbage and the output in feces of the relevant
293 component, respectively. This equation was used to calculate the apparent digestibility for
294 DM, OM, N, NDF and ADF.
295
296 ***Abundance of selected rumen microbes***
297 A third component study was undertaken in late September 2010 to examine the relative
298 abundance of specific rumen microbial populations potentially involved in cellulose
299 digestion. For this study a further 15 of each HF, JE and JE×HF cows that were part of the
300 genotype × stocking rate study mentioned above and described briefly by Thackaberry et al.
301 (2011) were sampled. These cows were grazing pasture and not the same cows used in the in
302 vivo digestibility study. The mean (SD) parity of JE, JE×HF and HF cows was 2.9 (1.55), 2.9

(1.58) and 2.7 (1.50), respectively. Samples of rumen fluid from cows were collected after morning milking using the transesophageal sampling device (FLORA rumen scoop, Guelph, ON, Canada) described by Geishauser et al. (2012). The scoop was inserted and allowed to settle in the rumen, after 1 min the scoop was opened for 1 min to collect fluid then closed and removed. This procedure was designed to avoid contamination with saliva.

A 20 ml aliquot of the collected rumen fluid was transferred using a pipette and sterilized tip into a separate labeled sterilized container, immediately frozen in liquid N and stored at -80°C until processing. Total microbial DNA was extracted from rumen fluid samples by adaptation of the repeated bead beating and column purification (RBB + C) method (Yu and Morrison, 2004), which provides efficient recovery of PCR-quality microbial DNA (Carberry et al., 2012). The integrity of microbial DNA and successful removal of RNA were verified by agarose gel electrophoresis. The concentration and quality of DNA was determined at A_{260} nm and A_{280} nm with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Quantitative real time PCR (**qPCR**) assays were performed to measure the relative abundance of a number of rumen microbial populations potentially involved in cellulose digestion; *viz* protozoa, anaerobic fungi and celluloytic and fibrolytic bacteria, *Fibrobacter succinogenes* and *Ruminococcus flavefaciens,* as described by Carberry et al. (2012). Genus/species-specific primer sets used in this study to amplify genus/species specific partial 16S rRNA/18S rRNA gene regions are presented in Table 1. All primer sets were commercially synthesized (Sigma-Aldrich Ireland Ltd. Dublin, Ireland) and end point PCR was conducted to validate the specificity of the primers against target species. Aliquots of 10 μL PCR products were analyzed by electrophoresis on a 2% agarose gel (w/v) to verify the presence and size of the

amplicons. Negative controls without template DNA were included in parallel. Amplicons corresponding to specific microbial groups were subjected to sequence analysis to verify their primer specific identity (Macrogen, Seoul, Korea).

Relative qPCR assays were performed on an ABI 7500 Fast Real-Time PCR system using Fast Power SYBR Master Mix (Applied Biosystems, Warrington, UK) as described by Carberry et al. (2012). Optimization of assay conditions were performed for both primer and template DNA concentrations. To reduce PCR inhibition, total microbial DNA was diluted to 1 ng/ul. A primer concentration of 10 μM was found to be optimal for each assay. Real time PCR amplification efficiencies (***e***) were estimated for the primer sets from a linear regression of the threshold cycle (**Ct**) for each dilution versus the log dilution using the formula: $e = x^{-1/slope}$, where x = fold dilution (Pfaffl, 2001). Efficiencies of the primers sets are presented in Table 1. These efficiencies ranged from 197% to 201%, close to the optimum value of 200% which is representative of the doubling effect of the target sequence during the qPCR cycle. Adhering to the MIQE guidelines (Bustin et al., 2009), qPCR data was processed using the software package GenEx 5.2.1.3 (MultiD Analyses AB, Gothenburg, Sweden) as previously described (O'Loughlin et al., 2011). Changes in microbial communities due to genotype were expressed relative to total bacteria. Specifically, abundance of microbial populations were expressed as a proportion of total estimated rumen bacterial 16S rDNA as described previously (Chen et al., 2008; Guo et al., 2008) according to the equation: relative quantification = $2^{-(Ct\ target-Ct\ total\ bacteria)}$, where Ct represents threshold cycle (Carberry et al., 2012).

Statistical analysis

All data were statistically analyzed using SAS (2002). In the first component study heart, lungs, liver and kidneys data were analyzed (n = 83) and pancreas, reticulo-rumen, omasum, abomasum, intestines and total GIT data were also analyzed (n = 77). Some data were excluded from analysis as the scales malfunctioned on one of the days of slaughter. All data (n = 95) were available for statistical analysis of metabolic BW ($BW^{0.75}$). Organ mass was expressed as g/kg BW and was analyzed using PROC GLM. Genotype, day of slaughter, parity and all interactions were included in the model. In the second component study herbage composition data during the in vivo digestibility trials were analyzed using PROC GLM. Time period was included as the fixed effect in the model. Time period refers to the weeks of the 4 digestibility trials (week beginning: August 2, August 16, August 30 and September 13). Milk yield, fat and protein concentration and milk solids yield data were analyzed using PROC GLM. Genotype, herbage allowance, time period and all interactions among genotype, herbage allowance and time period were included as fixed effects in the model. Intake and digestibility data were analyzed using PROC MIXED with individual cow as the random variable. Genotype, time period, herbage allowance and all interactions were included as fixed effects in the model. In the third component study PROC MIXED was used to determine the effect of genotype on the relative abundance of rumen microbial populations with the individual cow as the random variable and genotype as the fixed effect in the model. For all data the Tukey-Kramer multiple range test was used for mean separation ($P< 0.05$).

RESULTS

Abundance of selected rumen microbes

Table 1 shows the relative abundance of rumen microbial populations in grazing HF, JE and JE×HF cows. There were no significant differences observed in the relative abundance of bacteria, protozoa, general anaerobic fungi and *F. succinogens* populations among genotypes.

There was a decrease in the relative abundance of *R. flavenfaciens* in the rumen microflora of JE compared with HF and JE×HF cows ($P < 0.001$).

Post-slaughter anatomical data

There was a genotype effect on BW and the unadjusted anatomical data (Table 2). The HF were heavier than both the JE and JE×HF ($P < 0.001$). The HF had a heavier heart, lungs, liver, kidneys, reticulo-rumen, omasum, abomasum, intestines and total GIT compared with the JE ($P < 0.01$). The HF had heavier lungs, kidneys, intestines and total GIT compared with the JE×HF ($P < 0.05$). There was no difference in abomasal weight or in lungs and pancreas weights between the JE and JE×HF ($P > 0.05$). There was a genotype effect on anatomical data normalized to BW (Table 3) except for liver, kidneys and intestines ($P > 0.05$). On a per unit BW basis, the HF had a lighter heart, lungs, pancreas, reticulo-rumen, omasum, and total GIT than the JE ($P < 0.05$). There was no difference in lungs or omasum weights between the HF and JE×HF ($P > 0.05$). The JE×HF had a proportionally heavier heart, pancreas, reticulo-rumen and total GIT compared with the HF ($P < 0.001$). There was no difference between the JE and JE×HF in kidney, liver, lungs, reticulo-rumen, omasum, intestines and total GIT ($P > 0.05$).

In vivo digestibility trials

Herbage composition and herbage measurements. The average (SD) pre-cutting sward height of the harvested herbage offered during each of the 4 time periods was 14.9 (0.80), 9.2 (0.53), 15.4 (1.08) and 11.1 (1.50) cm. The average (SD) post-cutting sward heights were 5.2 (0.62), 4.3 (0.44), 4.5 (0.29) and 4.4 (0.95) cm respectively. The average (SD) regrowth interval for the swards was 23 (2.1), 20 (0.9), 23 (0.0) and 17 (4.6) d

401 respectively. The chemical composition of the herbage offered to the cows during the 4 time
402 periods is shown in Table 4.
403
404 ***Milk production.*** With the exception of milk solids yield, there was a genotype effect
405 on all milk parameters recorded ($P < 0.01$; Table 5). The HF cows had the highest milk yield,
406 JE had the lowest and JE×HF were intermediate ($P < 0.001$). Milk fat and protein
407 concentration was highest for the JE, lowest for the HF and JE×HF were intermediate ($P <$
408 0.001). Similarly, kg milk solids/100 kg BW was highest for the JE, lowest for the HF and
409 JE×HF were intermediate ($P < 0.001$).
410 There was an effect of herbage allowance on milk production. Milk yield and milk
411 solids yield was higher for the cows offered the high herbage allowance (16.2 kg ± 0.31 and
412 1.6 kg ± 0.03, respectively) than the cows offered the low herbage allowance (14.2 kg ± 0.31
413 and 1.4 ± 0.03, respectively; $P < 0.01$). There was no effect of herbage allowance on milk fat
414 and protein concentration.
415
416 ***Herbage intake and milk solids per kg DMI.*** Genotype had a significant effect on all grass
417 intake parameters investigated ($P < 0.05$; Table 6). The JE×HF and HF consistently had a
418 higher intake of DM, OM, N, NDF and ADF than the JE ($P < 0.05$; Table 6). Intake (DM/100
419 kg BW) tended to be different among treatments and was numerically highest for JE and
420 lowest for HF. The JE cows had a higher yield of milk solids/kg DMI than the HF and JE×HF
421 cows (Table 6; $P < 0.01$). Although numerically in favor of the JE×HF, there was no
422 significant difference between the HF and JE×HF regarding milk solids/kg DMI ($P > 0.05$).
423

There was an effect of herbage allowance on herbage intake. Dry matter intake was higher for the cows offered the high herbage allowance (16.3 kg ± 0.13) than the cows offered low herbage allowance (14.8 kg ± 0.13; $P < 0.001$).

Herbage digestibility. For all digestibility parameters investigated JE cows had a higher digestibility than HF cows ($P < 0.05$; Table 7). The JE×HF cows were intermediate to the HF and JE cows for all parameters except for DM digestibility. The DM digestibility of HF and JE×HF cows was similar ($P > 0.05$). Jersey cows were able to digest 2.2% more DM than both HF and JE×HF cows.

There was no effect of herbage allowance on any of the digestibility parameters investigated ($P > 0.05$).

DISCUSSION

This study investigated GIT weight, apparent total tract digestibility and rumen microbial population composition to determine if differences existed among dairy cow genotypes. Previous research has proven that JE and JE×HF are highly efficient milk producers (Prendiville et al., 2011a; Vance et al., 2012). Total tract digestibility, GIT size and rumen microbial populations are factors that may contribute to production efficiency (Richardson and Herd, 2004).

Dry matter intake is affected by several factors. Reviews by Allison (1985) and Allen (1996) suggest that one factor limiting DMI is GIT capacity and in particular the capacity of the reticulo-rumen. Rumen capacity was not measured in the present study but the reticulo-rumen weight was measured and it has been shown that there is a positive correlation between rumen capacity and rumen weight (Purser and Moir, 1966). In the present study, differences in

reticulo-rumen weight as a proportion of BW are reflected in differences in GIT weight as a proportion of BW among genotypes. The proportionally heavier GIT found in the JE in the present study, and also by Nagel and Piatkowski (1988), suggests that JE have a proportionally greater DMI capacity than HF. In the present study there was indeed a tendency for an effect of genotype on DMI capacity, measured as DMI/BW. This is in contrast to a study by Smith and Baldwin (1974), who found no significant difference between JE and HF regarding proportional GIT weight. The study of Smith and Baldwin (1974) is, however, 40 years old and the cows in that study, compared to modern dairy cow genetics, are likely to be considerably different. In the present study, JE×HF also had a proportionally (relative to BW) greater reticulo-rumen and total GIT weight than HF which helps explain previous reports of a greater DMI capacity for the JE×HF compared to the HF (Prendiville et al., 2010; Xue et al., 2011; Vance et al., 2012) and supports the tendency for an effect of genotype on DMI capacity measured in the present study. It should however be noted that the omasum and intestinal tissues were weighed containing digesta and this may affect the differences observed. Herbage DMI is one of the most important factors influencing milk production in grazing dairy cows (Dillon, 2006). Kolver and Muller (1998) attributed the lower milk production of herbage-fed cows compared to TMR-fed cows to the lower DMI of the herbage-fed cows compared to cows offered TMR. The proportionally heavier GIT, and particularly the greater reticulo-rumen size, of the JE and JE×HF compared to the HF explains their greater intake capacity and is one biological difference which likely contributes to the previously reported production efficiency difference among these genotypes (Prendiville et al., 2009).

Increasing herbage allowance resulted in an increase in DMI and milk solids yield. This agrees with previous research (McEvoy et al., 2010). The topic of herbage allowance was not the main focus of the present study and will therefore not be discussed further.

Daily milk yield was higher for HF compared with both JE and JE×HF and milk fat and protein concentration exhibited the opposite ranking order, agreeing with previous research (Heins et al., 2008; Prendiville et al., 2009; Olson et al., 2010). Milk solids/BW were lower for the HF than for either the JE or JE×HF. These differences have been discussed by the afore-mentioned authors in detail and are presented in the present study for the purpose of confirmation only. These results will therefore not be discussed here.

Digestibility is expected to decrease with increasing DMI due to a faster rate of passage (Thornton and Minson, 1972; Tyrrell and Moe, 1975). Equally a lower DMI is associated with a slower rate of passage and is expected to increase digestibility (Tyrrell and Moe, 1975). The present study and previous studies have shown that JE have a greater DMI as a proportion of BW (Goddard and Grainger, 2004; Prendiville et al., 2009) and accordingly Ingvartsen and Weisbjerg (1993) and Aikman et al. (2008) showed that JE had a faster rate of passage than HF, thus it would be expected that JE would have a lower digestive efficiency than HF. The present study found however that JE were more efficient for all digestive parameters measured, which may be partly attributed to the relatively larger GIT of the JE. Increased relative GIT size indicates a relatively larger area available for absorption of nutrients, allowing for greater nutrient absorption and thus increased digestibility (Van Soest, 1994). Additionally, research has shown a simultaneous increase in digestibility and intake on high quality herbage-only diets (Baumont et al., 2007). The differences in digestibility among genotypes are in contrast with previous studies that found no difference in DM digestibility

among dairy breeds offered corn silage (Blake et al., 1986) or a TMR diet (Ingvartsen and Weisbjerg, 1993). Diet type (e.g. high vs. low forage) can however greatly affect passage rates and diet digestibility (Colucci et al., 1982). Therefore passage rates among dairy cow genotypes should be investigated further with cows offered a high quality herbage diet to determine if differences among genotypes exist.

The higher digestibility exhibited by the JE cows may also be explained by their greater number and frequency of grazing and ruminating mastications compared with HF (Prendiville et al., 2010). Mastication plays a part in digestion by physically disrupting the food and breaking it into smaller particles to facilitate microbial attack (McAllister et al., 1994). This would result in smaller particles entering the JE rumen which are potentially digested more rapidly (Fritz et al., 2009).

The increased fiber digestibility observed in the JE further confirms that they are well suited to grazing systems as herbage typically contains more fiber and less energy than concentrate feeds (Hendy et al., 1995; O'Mara, 2000; Coleman et al., 2010). Aikman et al. (2008) found that there were differences between HF and JE regarding NDF digestibility of a TMR diet, consistent with the present study. Unlike the present study however Aikman et al. (2008) observed no differences among genotypes regarding ADF digestibility. The ADF digestibility values in the present study are higher than the values reported by Aikman et al. (2008), but the diet in that study was a TMR, compared to herbage in the present study.

Although in the present study, JE had a greater N digestibility than HF this was likely of no benefit to the JE as N was not limiting in the high CP grass diet being offered. Generally, in herbage-based diets, energy intake is the factor most limiting to animal performance (O'Mara,

2000) and therefore the increased OM digestibility is of greater significance, although of course the increased N digestibility is a contributor to this. Blake et al. (1986) found no difference between JE and HF regarding N digestibility during the first trimester of lactation, although JE had a higher N digestibility than HF in the second trimester of lactation. There was no difference in N digestibility between HF and JE×HF which was also found by Xue et al. (2011).

Increasing the digestibility of a feed means that more of the feed is utilized and less is excreted as waste product. This will result in increased energy available to the animal. The present study shows that there is a difference among breeds in total tract digestibility. The JE had a higher total tract digestibility indicating an increase in energy available for milk solids production (Coulon and Rémond, 1991). This increase in total tract digestibility likely contributes to the difference in milk production efficiency measured among these dairy cow genotypes (Prendiville et al., 2010). The JE were able to digest 2.2% more of the grass than the HF, which is the equivalent of an increase in the energy content of grass from 1.01 UFL/kg DM to 1.05 UFL/kg DM. One UFL (unite fourragère lait) of energy is defined as the net energy content of 1 kg of standard barley for milk production, which is 1,700 kcal. This increases the energy available for milk production for JE by 0.56 UFL per day. This corresponds to the JE being able to produce an extra 0.90 kg of milk per day (39.6 g protein/kg, 76.9 g fat/kg).

Rumen microorganisms, particularly cellulolytic bacteria, protozoa and fungi, are critically important for the fermentation of feed (Van Soest, 1994; Gordon and Phillips, 1998) into short-chain fatty acids, including propionate, acetate, butyrate, lactate and succinate (Hungate, 1966). These serve as major carbon and energy sources for the ruminant. It is widely accepted

that diet has a role to play in shaping the microbial communities of the rumen (de Menezes et al., 2011; Carberry et al., 2012; Boots et al., 2013). Typically animals on forage-based diets will have more fibrolytic bacteria and less starch-digesting amylolytic bacteria than animals on a starch-based diet (Van Soest, 1994; Beever and Mould, 2000). All 3 genotypes had similar abundance of protozoa, anaerobic fungi and *F. succinogens*, however, JE cows had a reduced abundance of *R. flavefaciens* compared to HF and JE×HF. This cellulolytic bacterial species is associated with fiber digestion in the rumen (Baldwin and Allison, 1983; Van Soest, 1994). Despite this JE cows had a higher NDF and ADF digestibility than HF cows. Differences in NDF and ADF digestibility may be due to differences in microbial populations that were not evaluated in the present study. A more comprehensive approach, such as sequencing of rumen metagenomic DNA, may uncover differences responsible for the observed differences in fiber digestibility and may also provide evidence for other biological differences among the dairy cow genotypes.

CONCLUSION

Earlier studies demonstrated that modern JE genetics are well suited to herbage-based systems because of their ability to achieve high herbage intakes and efficiently convert herbage to milk solids. Deductions from this study are that the JE genotype has greater digestibility and a different rumen microbial population than the HF. Jersey and JE×HF cows had a proportionally greater GIT weight than HF. These differences are likely to contribute to the production efficiency differences among genotypes previously reported.

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737 digesta and fecal samples. Biotechniques 36:808-812.

738 **Table 1.** The effect of dairy cow genotype (n = 15 per genotype) on the relative abundance of ruminal microbial populations[1]

		Primers(5'- 3')			Genotype[2]				
Target Taxon	SSU rRNA[3]	Forward	Reverse	*e*[4]	HF	JE	JE×HF	*SEM*	*P* - value
Bacteria[5]	16S	CCTACGGGAGGCAGCAG	ATTACCGCGGCTGCTGG	200					
Protozoa	18S	GCTTTCGWTGGTAGTGTATT	CTTGCCCTCYAATCGTWCT	201	2.80	1.35	2.33	1.980	ns
General anaerobic fungi	18S	GAGGAAGTAAAAGTCGTAACAAGGTTTC	CAAATTCACAAAGGGTAGGATGATT	199	1.28	0.96	1.33	0.440	ns
Ruminococcus flavefaciens	16S	CGAACGGAGATAATTTGAGTTTACTTAGG	CGGTCTCTGTATGTTATGAGGTATTACC	202	1.64[a]	0.71[b]	1.44[a]	0.420	< 0.01
Fibrobacter succinogenes	16S	GTTCGGAATTACTGGGCGTAAA	CGCCTGCCCCTGAACTATC	197	1.03	1.09	1.22	0.310	ns

739 [1]Microbes measured as a proportion of total estimated rumen bacterial 16S rDNA, relative quantification = $2^{-(\text{Ct target-Ct total bacteria})} \times 100$

740 [2]HF = Holstein Friesian; JE = Jersey; JE×HF = Jersey × Holstein Friesian

741 [3]Small Sub Unit ribosomal RNA gene targeted

742 [4]Amplification Efficiency %

743 [5]Primers used for qPCR normalization

744 [a-b]Means within a row without a common superscript differ ($P < 0.05$).

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Table 2. The effect of dairy cow genotype on BW and mass of body components from cows slaughtered in February 2010, December 2010 and November 2011

	Genotype[1]				
Variable	HF	JE	JE×HF	SEM	*P* – value
BW (kg)	557[a]	406[c]	486[b]	7.9	< 0.001
Heart (kg)	2.3[a]	2.0[b]	2.2[a]	0.05	< 0.001
Lungs (kg)	5.4[a]	4.4[b]	4.8[b]	0.16	< 0.001
Pancreas (kg)	0.8	0.8	0.8	0.02	ns
Liver (kg)	7.7[a]	6.2[b]	7.4[a]	0.17	< 0.001
Kidney (kg)	1.5[a]	1.1[c]	1.4[b]	0.03	< 0.001
Reticulo-rumen (kg)	13.5[a]	11.7[b]	13.8[a]	0.38	< 0.001
Omasum (kg)	16.1[a]	13.8[b]	15.5[a]	0.44	< 0.001
Abomasum (kg)	4.0[a]	3.3[b]	3.6[ab]	0.20	< 0.05
Intestines (kg)	37.3[a]	28.7[b]	33.0[c]	0.91	< 0.001
Total GIT[2] (kg)	71.2[a]	57.2[c]	66.2[b]	1.55	< 0.001

[1]HF = Holstein Friesian (n = 33); JE = Jersey (n = 35); JE×HF = Jersey × Holstein Friesian (n = 27)

[2]Total GIT (gastrointestinal tract) = sum of reticulo-rumen, omasum, abomasum, intestines

[a-c]Means within a row without a common superscript differ ($P < 0.05$).

Table 3. The effect of dairy cow genotype on mass of body components expressed on a per unit BW basis from cows slaughtered in February 2010, December 2010 and November 2011

	Genotype[1]				
Variable	HF	JE	JE×HF	SEM	*P* - value
			g/kg of BW		
Heart	4.1[c]	5.0[a]	4.6[b]	0.10	< 0.001
Lungs	9.7[b]	11.0[a]	10.1[ab]	0.36	< 0.05
Pancreas	1.5[c]	1.8[a]	1.7[b]	0.04	< 0.001
Liver	13.8	13.3	13.3	0.30	ns
Kidney	2.6	2.7	2.8	0.07	ns
Reticulo-rumen	24.3[b]	29.3[a]	28.3[a]	0.79	< 0.001
Omasum	29.2[b]	33.9[a]	31.8[ab]	0.86	< 0.001
Abomasum	7.2	8.2	7.5	0.38	0.09
Intestines	67.1	70.1	68.3	1.67	ns
Total GIT[2]	128.8[b]	142.5[a]	136.8[a]	2.87	< 0.001

[1]HF = Holstein Friesian (n = 33); JE = Jersey (n = 35); JE×HF = Jersey × Holstein Friesian (n = 27)

[2]Total GIT (gastrointestinal tract) = sum of reticulo-rumen, omasum, abomasum, intestines

[a-c]Means within a row without a common superscript differ ($P < 0.05$).

Table 4. The chemical composition of grass offered to Holstein-Friesian, Jersey and Jersey × Holstein Friesian lactating dairy cows during in vivo digestibility studies conducted on 4 occasions in 2010 (week beginning: August 8, August 22, September 5, September 19)

Variable	August 8	August 22	September 5	September 19	SEM	*P* - Value
DM %	18[a]	17[ab]	16[b]	15[b]	6.2	< 0.05
OM (g/kg of DM)	926[a]	907[b]	927[a]	924[a]	13.1	< 0.001
CP (g/kg of DM)	186[b]	237[a]	191[ab]	240[a]	32.7	< 0.05
NDF (g/kg of DM)	481[a]	419[b]	416[b]	457[ab]	33.7	< 0.05
ADF (g/kg of DM)	309[a]	254[b]	256[b]	299[a]	20.9	< 0.001

[a-b]Means within a row without a common superscript differ ($P < 0.05$).

Table 5. The effect of dairy cow genotype (n = 16 per genotype) on milk yield and composition during in vivo digestibility studies conducted on 4 occasions in 2010 (week beginning: August 8, August 22, September 5, September 19)

	Genotype[1]				
Variable	HF	JE	JE×HF	SEM	*P* - value
Milk yield (kg/d)	16.93[a]	12.81[c]	15.33[b]	0.449	< 0.001
Milk fat concentration (g/kg)	56.7[c]	76.9[a]	64.7[b]	2.11	< 0.001
Milk protein concentration (g/kg)	34.3[c]	39.6[a]	36.6[b]	0.62	< 0.001
Milk solids yield (kg/d)	1.55	1.49	1.53	0.047	ns
BW (kg)	576[a]	434[c]	501[b]	8.65	< 0.001
Milk solids (kg/100 kg BW)	0.27[c]	0.35[a]	0.31[b]	0.011	< 0.001

[1]HF = Holstein-Friesian; JE = Jersey; JE×HF = Jersey × Holstein-Friesian

[a-c]Means within a row without a common superscript differ ($P < 0.05$).

Table 6. The effect of dairy cow genotype (n = 16 per genotype) on grass intake during in vivo digestibility studies conducted on 4 occasions in 2010 (week beginning: August 8, August 22, September 5, September 19)

	Genotype[1]				
Variable	HF	JE	JE×HF	SEM	*P* - value
DM Intake (kg/d)	16.71[a]	13.93[b]	15.96[a]	0.165	< 0.001
DM Intake (kg/100 kg BW)	2.98	3.22	3.09	0.054	0.08
OM Intake (kg/d)	14.58[a]	12.42[b]	14.24[a]	0.276	< 0.001
N Intake (kg/d)	0.57[a]	0.47[b]	0.54[a]	0.009	< 0.001
NDF Intake (kg/d)	7.77[a]	6.46[b]	7.49[a]	0.111	< 0.001
ADF Intake (kg/d)	4.29[a]	3.58[b]	4.15[a]	0.061	< 0.001
Milk solids (kg/kg DMI)	0.093[b]	0.108[a]	0.096[b]	0.003	< 0.01

[1]HF = Holstein-Friesian; JE = Jersey; JE×HF = Jersey × Holstein-Friesian

[a-b]Means within a row without a common superscript differ ($P < 0.05$).

Table 7. The effect of dairy cow genotype (n = 16 per genotype) on apparent total tract digestibility of grass during in vivo digestibility studies conducted on 4 occasions in 2010 (week beginning: August 8, August 22, September 5, September 19)

	Genotype[1]				
Variable	HF	JE	JE×HF	SEM	*P* - value
DM Digestibility (%)	78.8[a]	80.6[b]	79.0[a]	0.44	< 0.01
OM Digestibility (%)	79.5[a]	81.7[b]	80.6[ab]	0.40	< 0.01
N Digestibility (%)	79.8[a]	82.4[b]	81.0[ab]	0.53	< 0.01
NDF Digestibility (%)	78.6[a]	81.0[b]	79.6[ab]	0.52	< 0.05
ADF Digestibility (%)	70.5[a]	74.4[b]	72.2[ab]	1.05	< 0.01

[1]HF = Holstein-Friesian; JE = Jersey; JE×HF = Jersey × Holstein-Friesian