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## Preliminary investigation of milk production in Angus heifers exposed to different planes of nutrition *in utero*

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### ABSTRACT

Milk yield (MY) and composition were measured on one day in Weeks 7, 9 and 11 in 17 offspring of Angus cows fed for high (1.2 kg/d; HB) or medium (0.5 kg/d; MB) liveweight gain (LWG) for 10 days before insemination and medium (0.5 kg/d; MA) or low (0.01 kg/d; LA) LWG during gestation. Mean MYs in the progeny were; Week 7: 5790 ± 420 g/d, Week 9: 7418 ± 447 g/d, Week 11: 6244 ± 392 g/d. Better feeding before mating had little effect on MY or milk crude protein (CP%) in the progeny but tended to reduce the milkfat% (Week 9; HB (n = 8) 3.50 ± 0.17% versus MB (n = 8) 3.74 ± 0.22%) (P < 0.19), casein (Week 11; HB 2.59 ± 0.03% versus MB 2.68 ± 0.04%) (P = 0.11) and lactose (Week 11; HB 5.18 ± 0.13% versus MB 5.42 ± 0.05%) (P = 0.075). Better feeding of the dam after artificial insemination did not significantly increase MY of the progeny (Week 9; MA (n = 9) 7972 ± 378 g/d versus LA (n = 8) 6794 ± 826 g/d) (P = 0.23), or milkfat%, decreased CP (Week 9; MA 3.22 ± 0.07% versus LA 3.41 ± 0.08%) (P < 0.09) and casein% (at Weeks 7 and 9 identical values of MA 2.52 ± 0.06% versus LA 2.69 ± 0.07%), but increased lactose% after the peak of lactation (Week 11 MA 5.42 ± 0.06% versus LA 5.15 ± 0.14%) (P < 0.08). Feeding before and after conception may affect the nutrition of the grand generation.

**Keywords:** milk yield; milk composition; fetal programming; beef, heifer.

### INTRODUCTION

Epidemiological data from human populations have shown that the mother's health and nutrition has a significant impact on fetal development and lifetime health (Barker *et al.*, 1990). The present study is related to another programme established in 2005 which investigated the effects of ewe size and nutrition during pregnancy on a wide range of fetal, young-animal and mature-animal metabolism, physiology and performance characteristics (Blair *et al.*, 2009; Kenyon *et al.*, 2009; van der Linden *et al.*, 2009). Based on results of Jenkinson (2003) that showed that dam nutrition during gestation can impair fetal mammary gland development, we examined the effects of dam size and dam feeding during pregnancy on milk production of the next generation (G1) ewes. Early results showed that many characteristics were changed in the offspring of the ewes and also in the next generation (Blair *et al.*, 2009).

However, we obtained a surprising and somewhat counter-intuitive result in that G1 ewes born to dams that were fed a restricted diet during pregnancy produced more milk with a higher concentration of protein and lactose, but not fat, during their first lactation (van der Linden *et al.*, 2009). This effect did not persist, however, and in their second lactation the offspring of *ad libitum*-fed ewes produced greater fat yield than did offspring of those allowed a restricted intake, with no difference in protein and lactose (Blair *et al.*, 2009).

In the trial reported here, we examined essentially the same situation in beef cattle. An existing research programme (Hickson, 2009; Hickson *et al.*, 2009) produced G1 heifers, so we examined the effect of the level of nutrition during pregnancy in the G0 dams on the milk production of their G1 progeny. We have obtained some exciting results from our long-term study in sheep, and we would expect that these mechanisms will be consistent across species, meaning that the knowledge should have benefit for other farmed species and potentially humans. This experiment enabled us to examine for the first time, if such trans-generational effects on milk production occur in beef cattle.

The number of heifers available was small, so it was recognised that this preliminary experiment might not show significant treatment differences, should they exist. Nevertheless, an additional objective was to establish a successful milking and sampling protocol and to provide information on milk yield and composition in beef cows in New Zealand, for which there is little published information apart from estimates of calf milk intake by the weight-nurse-weigh method.

### MATERIALS AND METHODS

The experimental procedures applied were approved by the Massey University Animal Ethics Committee (AEC 08/25). All animals used in this experiment were kept on pasture and milking was

carried out in late spring, in October and November, at Massey University's Tuapaka farm, 20 kilometres east of Palmerston North (latitude 40.33° S and longitude 175.73° E).

**Animals and experimental design**

Seventeen two-year-old Angus heifers (G1), were the offspring of heifers in which oestrus had been synchronised using progesterone CIDRs followed by artificial insemination (AI). Their dams were fed at pasture for high (1.2 kg/d; HB, n = 9) or medium (0.5 kg/d; MB; n = 8) live weight gain (LWG) for ten days before insemination and for medium (0.5 kg/d; MA; n = 9) or low (0.01 kg/d; LA; n = 8) LWG after AI, for the first 93 days of gestation (Hickson, 2009; Hickson *et al.*, 2009). Following calving, dams were managed as one group with their calves. Thus, the design was a 2 x 2 Latin square, with either four or five G1 heifers in each group.

G1 heifers were milked on one day during each of Weeks 7, 9 and 11 of lactation. Heifers were placed in a cattle crush and the left hind leg was leg-roped to prevent the milker from being kicked. Milk yield (MY) of the heifers was estimated by the "oxytocin method" (McCance & Alexander, 1959). The dose of oxytocin (50 i.u.) was based upon the label recommendation and was the mean of doses used by Marston *et al.* (1992) (40 i.u.) and Fiss and Wilton (1992) (60 i.u.). Calves were separated from their mothers, which were injected intramuscularly with oxytocin V (Vetpharm Ltd., Hadleigh, Suffolk, UK) and the udder emptied by machine and hand milking. Milking was repeated about 6 hours later, and MY recorded. Twenty mL aliquots of milk were collected from each heifer at the second milking. During the 6-hour period between milkings, the heifers were

returned to grazing in a paddock adjacent to that holding their calves.

Feed intake of the heifers was not estimated but ample pasture of average quality, was available throughout lactation. Intake was assumed to be *ad libitum*, and all heifers were in good condition although condition score was not formally assessed.

Milk samples were preserved with 0.03% bronopol (2-bromo-2nitro-1,3-propanediol (Aldrich Chemical Company Milwaukee, Wisconsin, USA) and refrigerated at 4°C until dispatch to Dairy NZ, Hamilton for analysis of milk fat, crude protein (CP), casein and lactose composition using near infrared spectroscopy (NIRS). The assay was calibrated by comparing NIRS values with those obtained by "wet chemistry" for a range of samples.

**TABLE 1:** Effect of dam nutrition before AI on the mean ± standard error of the mean milk yield and milk composition at Weeks 7, 9 and 11 of lactation, of Angus heifers whose dams were fed either for high liveweight gain (LWG) (1.2 kg/d) before artificial insemination (HB group) or for medium LWG (0.5 kg/d) (MB group).

Group	Week of lactation	Milk yield (g/d)	Milkfat (%)	Milk crude protein (%)	Casein (%)	Lactose (%)
HB (n = 9)	7	5,797 ± 684	3.7 ± 0.1	3.36 ± 0.08	2.60 ± 0.08	4.97 ± 0.03
	9	7,384 ± 675	3.5 ± 0.2	3.26 ± 0.07	2.56 ± 0.02	4.97 ± 0.03
	11	6,256 ± 651	3.2 ± 0.3	3.13 ± 0.03	2.59 ± 0.03 <sup>a</sup>	5.18 ± 0.13
MB (n = 8)	7	5,781 ± 506	3.9 ± 0.2	3.35 ± 0.08	2.59 ± 0.07	5.03 ± 0.02
	9	7,456 ± 621	3.7 ± 0.2	3.36 ± 0.09	2.64 ± 0.08	5.01 ± 0.03
	11	6,231 ± 451	3.6 ± 0.3	3.26 ± 0.08	2.68 ± 0.04 <sup>b</sup>	5.42 ± 0.05
P value		0.99	0.19	0.42	0.47	0.075

P value indicates significance of differences between groups tested by repeated-measures analyses of values obtained at the three sampling times. Differing superscripts indicate trends indicated by univariate analyses for the week indicated: <sup>a</sup> and <sup>b</sup> P = 0.11.

**TABLE 2:** Effect of dam nutrition after AI on the mean ± standard error of the mean milk yield and milk composition at Weeks 7, 9 and 11 of lactation, of Angus heifers whose dams were fed either for medium (0.5 kg/d) (MA group) or low (0.01 kg/d) live weight gain (LA group) during gestation.

Group	Week of lactation	Milk yield (g/d)	Milkfat (%)	Milk crude protein (%)	Casein (%)	Lactose (%)
HB (n = 9)	7	6413±265	3.5±0.2	3.28±0.08	2.52±0.07	4.99±0.03
	9	7972±378	3.6±0.1	3.22±0.07 <sup>a</sup>	2.52±0.06	5.01±0.03
	11	6473±281	3.2±0.3	3.15±0.04	2.61±0.02 <sup>c</sup>	5.42±0.06 <sup>e</sup>
MB (n = 8)	7	5088±797	3.8±0.2	3.43±0.07	2.68±0.07	5.01±0.03
	9	6794±826	3.6±0.3	3.41±0.08 <sup>b</sup>	2.69±0.07	4.97±0.03
	11	5986±793	3.6±0.4	3.25±0.07	2.66±0.05 <sup>d</sup>	5.15±0.14 <sup>f</sup>
P value		0.22	0.42	0.09	0.08	0.12

P value indicates significance of differences between groups tested by repeated-measures analyses of values obtained at the three sampling times. Differing superscripts indicate trends indicated by univariate analyses for the week indicated: <sup>a</sup> and <sup>b</sup> P = 0.07, <sup>c</sup> and <sup>d</sup> P = 0.086, <sup>e</sup> and <sup>f</sup> P = 0.076.

### Statistical analyses

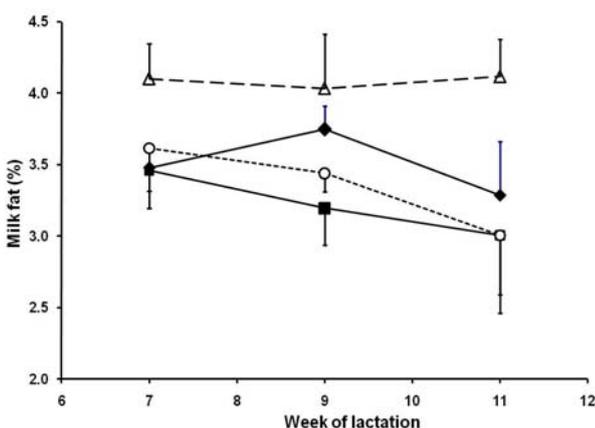
Multivariate, repeated measures, analysis of variance was used to analyse all time-series data. The computer programme was REG (Gilmour, 1990). Data presented are geometric means ( $\pm$  standard error of the mean) since the groups are very nearly balanced for numbers.

## RESULTS

Live weights of heifers did not differ significantly ( $P > 0.7$ ) between treatment groups (mean 453 kg; range 393 to 548 kg) and was not significant in any analyses of MY or composition, so was omitted from the models. Mean MYs of all 17 heifers were; Week 7:  $5,790 \pm 420$  g/d, Week 9:  $7,418 \pm 447$  g/d, Week 11:  $6,244 \pm 392$  g/d, the overall mean being  $5,869 \pm 257$  g/d.

Effects of dam nutrition before AI are shown in Table 1. Dam nutrition treatment before AI had no effect on mean milk yields (MY) of the G1 offspring. Feeding dams for high LWG before AI (HB) resulted in a slight trend ( $P = 0.15$ ) for heifers to produce lower milk fat% compared to those whose dams were fed for med/low LWG (MB). HB had no significant effect on crude protein (CP%) compared to MB. HB resulted in no difference in casein percentage at Week 7 and 9 but tended to be lower ( $P = 0.11$ ) compared to MB at Week 11. Better nutrition treatment before AI decreased lactose percentage in G1 offspring; HB resulted in lower ( $P = 0.075$ ) lactose percentage compared to MB in G1 offspring.

**FIGURE 1:** First-lactation milk fat percentage of heifers born to Angus heifers fed for high (1.2 kg/d) or medium (0.5 kg/d) liveweight gain for ten days before insemination and for medium (0.5 kg/d) or low (0.01 kg/d) liveweight gain during gestation. Groups are HB-LA ( $n = 4$ ;  $\blacksquare$ — $\blacksquare$ ) MB-LA ( $n = 4$ ;  $\triangle$ — $\triangle$ ) MB-MA ( $n = 4$ ;  $\circ$ — $\circ$ ) HB-MA ( $\blacklozenge$ — $\blacklozenge$ ) where HB is high before, MB is medium before, MA is medium after, and LA is low after insemination.



Effects of dam nutrition after AI are shown in Table 2. Heifer offspring born to MA cows had slightly higher MYs compared to those born to LA heifers but the difference was not significant ( $P = 0.22$ ). Milk fat percentage did not differ significantly between MA and LA. MA resulted in a trend ( $P < 0.09$ ) to lower CP% compared to LA. MA resulted in a trend ( $P < 0.08$ ) to lower casein percentage compared to LA. Nutrition after AI had no effect on lactose percentage at Weeks 7 and 9 but MA tended to result in higher ( $P < 0.08$ ) lactose percentage compared to LA at Week 11.

There was a significant ( $P < 0.05$ ) interaction between pre- and post-AI treatments affecting milk fat percentage, MB-LA producing the highest milk fat percentage and HB-LA producing the lowest, with HB-MA and MB-MA intermediate (Figure 1). There were no significant interactions in other analyses so interactions were omitted from the models.

## DISCUSSION

Milk yields peaked in Week nine (Table 1). The average MY ( $5.87 \pm 0.26$  kg/d) was considerably lower than that ( $7.18 \pm 0.81$  kg/d) reported by Fiss and Wilton (1992) for Angus cows in Canada. The difference may reflect the lower age of our cattle, however, Marston *et al.*, (1992) reported an even higher estimate (of  $8.5 \pm 0.3$  kg/d) in heifers of the same age and stage of lactation in an Angus herd conceived by AI. This is comparable to an average peak of 8.2 kg/d reported by Reynolds and Tyrrell (2000) in Hereford  $\times$  Angus primiparous cows that were milked weekly. The very low milk yields for 11 primiparous Angus heifers of  $3.2 \pm 1.2$  kg/d during the first 30 days and  $6.8 \pm 3$  kg/d at the peak on Day 27 reported by Masilo *et al.* (1992), must be seriously questioned since oxytocin was not used. The very high standard errors indicate inconsistent milk let down. The mean milk yield in our study was similar to the mean values 6.2 to 6.7 kg/d on Day 40 reported by Hickson *et al.* (2009) using weigh-nurse-weigh in the dams and "aunties" (G0) of the heifers milked here, and in a previous generation (Hickson *et al.*, 2008). The data are also consistent with previous reports of calves born to 2-year-old Angus heifers grazing at the same farm (Pleasant & Barton, 1987). However, our mean milk yield is somewhat lower than peak values of around 8.2 kg/d obtained by weigh-nurse-weigh in primiparous heifers Grings *et al.* (2008) but is similar to the peak of about 6.1 kg/d reported in primiparous heifers of a composite breed in Queensland, Australia, that received high levels of protein and energy during the first two trimesters (Sullivan *et al.*, 2009).

The average crude protein percentage reported here ( $3.41 \pm 0.03\%$ ) is indistinguishable from that

reported by Marston *et al.* (1992) at 106 days of lactation ( $3.40 \pm 0.03\%$ ), but is a somewhat lower than the values for protein reported by Fiss and Wilton (1992) ( $3.61 \pm 0.17\%$ ), by Masilo *et al.* (1992) ( $3.92 \pm 0.3\%$ ) and by Pareek *et al.* (2007) ( $3.8 \pm 0.2\%$ ). These differences may reflect the lower age of our cattle or a difference in analytical method as none of the above-mentioned authors defined whether they were measuring, crude protein or true protein. Casein percentage in milk of beef cows has not been reported elsewhere.

The average lactose percentage reported here ( $5.03 \pm 0.03\%$ ) is similar to that reported by Fiss and Wilton (1992) ( $4.99 \pm 0.20\%$ ), by Marston *et al.* (1992) at 106 days of lactation ( $4.98 \pm 0.02\%$ ), and in German Charolais (Pareek *et al.*, 2007) ( $5.03 \pm 0.4\%$ ), but considerably different from that reported by Masilo *et al.* (1992) ( $3.96 \pm 0.1\%$ ). However, all of the milk composition data presented by the latter group must be suspect due to the extremely high standard errors. It is expected that milk lactose percentage should be relatively constant within a breed or species because it is the main osmotic component of the milk and if the amount of lactose synthesised alters, the volume of water entering the milk will change correspondingly, thus maintaining a similar lactose concentration (Peaker, 1977).

It is, therefore, most interesting to see that the lactose concentration in the milk of the G1 offspring may have been effected by nutrition of their dams around the time of AI. Better nutrition treatment before AI tended to decrease lactose percentage, whilst better nutrition after AI had the opposite effect. This effect differs from our result in sheep in which first-lactation G1 ewes showed increased milk lactose percentage and milk yield as a result of low feed intake of their dams during gestation (van der Linden *et al.*, 2009) but is similar to the results obtained in the second lactation in the same ewes (S.W. Peterson, Unpublished data) which showed a trend to increased milk lactose percentage ( $P = 0.75$ ) as a result of low feed intake of their dams during gestation. Presumably such changes are epigenetic effects, programming the physiology of the fetus in preparation for a specific environment after birth, at least in the first generation (Blair *et al.*, 2009). The mechanism and reasons for changes in milk lactose concentration are unknown, although higher lactose concentration may be advantageous to brain development in the young mammal since galactose is an important cerebroside (Campbell & Lasley, 1985).

In conclusion, we have established an effective experimental method for estimating milk yield and composition in beef cattle and produced valid data for the composition of their milk.

Furthermore, accepting the limitations of low numbers of animals, it appears that feeding level before AI had no effect on MY of the offspring but

better feeding appeared to have affected milk composition by tending to reduce the percentage of milk fat, casein and lactose. Better feeding of the dam after AI tended to increase MY, decrease crude protein and casein, but increased lactose percentage after the peak of lactation in the G1 offspring. If these results can be repeated, the implications of feeding level of beef cows both before and after conception for the nutrition of the third (grand) generation need further consideration. It also raises questions regarding the management of dairy cows around the time of AI.

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