Milk Production with a Particular Reference to Milk Essential Fatty Acids of Lactating Cows under Grazing and Indoor Feeding Conditions

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ABSTRACT

An experiment was conducted to study the milk quality of dairy cattle under conditions of group grazing and loose housing on high quality forage intake. Eight crossbred cows (93% Holstein and 7% *Bos indicus*) all in their second to fourth lactation and at 100–120 d in the mid-late period of lactation were divided equally into two groups of four cows each under a pair comparison design. The first group was allowed free grazing on pasture consisting mainly of Guinea grass using a rotational grazing procedure. The second group was kept in a shaded cow shed and fed cut-and-carried pasture from an equal-sized area of the same pasture grazed by the first group. Both groups were supplemented with *Leucaena leucocephala* at 3%, based on the intake requirement for body weight maintenance and milk production for the 45 d experiment. The daily milk yield was recorded throughout the experiment and weekly milk sampling was evaluated for composition. A composite sample of morning and evening milk was analyzed for the percentages of milk fat, protein and lactose, solid not fat (SNF) and essential milk fatty acids—linoleic acid (C18:2*n-6*; Omega 6), linolenic acid (C18:3*n-3*; Omega 3) and conjugated linoleic acid (CLA) contents (grams per 100 g of fat) were also analyzed. The results showed that the milk yields, the percentages of milk fat, protein and SNF, and the contents of linolenic acid (C18:3 *n-3*) and CLA were not significantly different between treatments. However, the lactose percentage and the content of linoleic acid (C18:2 *n*-6) were significantly different ($P < 0.01$ and $P < 0.05$, respectively). The outdoor grazing system significantly increased the higher milk content of linoleic acid.

Keywords: Guinea grass, *Leucaena leucocephala*, linoleic acid (C18:2*n-6*; Omega 6), linolenic acid (C18:3*n-3*; Omega 3), conjugated linoleic acid (CLA)**,** grazing and indoor feeding.

INTRODUCTION

Most dairy farmers in Thailand adopt a cut-and-carry system of feeding their livestock as they believe that shaded, indoor conditions provide a better environment for cows and result in higher milk yields (Tudsri and Sawasdipanit, 1998). However, milk production and composition were reported to be similar under both grazing and indoor feeding with cut-and-carried fresh grass (Hongyantarachai *et al.*,1989; Prasanpanich *et al.*, 2002). These data suggest that dairy cows will

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produce satisfactory milk yield and composition when not housed (as is common in Thailand) and instead are grazed outdoors. Outdoor grazing should result in not only a reduction in farm costs (Lekchom *et al.*, 1989) but also improve milk quality in terms of essential milk fatty acids (Tanaka, 2005).

It is generally known that milk fat synthesis results from high roughage feeding containing fatty acids which are the important precursors in dairy products (Jiang *et al.*, 1996). Conjugated linoleic acid (CLA), a mixture of positional and geometric fatty acid isomers of linoleic acid (C18:2 *n-6*) with conjugated unsaturated double bonds, is produced in milk products of ruminant animals by animal diet manipulation, especially grazing pasture (Dhiman *et al.*, 1999). Consumer's demand is now for food products of superior health quality, with milk products from pasture being high in quality and safe (Hellgren, 2010). Hopefully, consumers will have renewed interest in modifications related to the milk fatty acid composition from the richest natural feed sources which are believed to have several important human physiological functions, including immune-modulating with anti-carcinogenic and anti-artheriosclerosis agents, growth promotion and lean body mass promotion (Pastuschenko *et al.*, 2000; Whigham *et al.*, 2000).

The current experiment lasting 45 d was conducted to examine milk production between two groups of Friesian-crossbred dairy cows in mid lactation which were either grazed outdoors on a Guinea grass pasture or fed the same pasture (cut-and-carry) while housed indoors in an open-sided barn. Both groups had daily access to an abundant supply of fresh pasture supplemented with *Leucaena leucocephala*, the aims being to investigate potential milk yield and milk quality in terms of the essential milk fatty acid content as milk CLA under the two feeding systems.

METERIALS AND METHODS

Animals and pasture management

The study was conducted at the Organic Dairy Research and Development Unit on approximately 0.8 ha of Guinea grass pasture (*Panicum maximum* cv. Purple guinea) established under the Dairy Farming Promotion Organization of Thailand, Muaklek, Saraburi, Thailand (latitude 14°50´, longitude 101°10´E, altitude 220 m above sea level). The experiment compared two groups of feeding systems using a pair comparison design between grazing and loose housing groups:

Group1—four cows strip-grazed on purple Guinea grass pasture for 24 h daily, apart from twice daily milking (in the same milking barn as Group 2), and rotated around the 15 sub-paddocks allocated to them; Group 2—four cows fed indoors under loose housing with pasture cut-and-carried from an equal area of the same sward. The barn was 7 m long × 4 m wide × 2 m high with a tiled roof and its long axis was oriented east-west.

The cows were all crossbred animals (93% Holstein and 7% *Bos indicus*), all in their second to fourth lactation and at 100–120 d of lactation when the experiment began. The animals were balanced for these factors across the two groups, and account was also taken of their average live weight of 450 kg and previous milk production. All cows also had individual access *ad libitum* to fresh *Leucaena leucocephala* supplementation at milking times. A pre-experimental period of 1 wk was allowed on the experimental area and indoor feeding for the animals to adapt to their changed management conditions and for preliminary milk production data to be collected.

The experimental pasture was divided into 15 paddocks all approximately 0.052 ha. Each paddock generally provided 3 d of grazing and was subdivided into strips by an electric fence, providing 25% of the area cut-and-carried to the four cows indoors and the remaining 75% of the area was grazed during the whole day by the four cows outdoors. The daily subdivision ensured that each cow was allowed a minimum of 35 kg of fresh pasture of similar quality each day. Natural shade and water were provided to the outdoor animals. Drinking water was provided in a trough, which was moved to the end of each new forage strip.

The forage available in each paddock before grazing was estimated by cutting three random quadrats (each 1 × 1 m) with hand shears to a height of 8 cm and a pasture sample was collected for the analyses of the dry matter (DM) content, Kjeldahl nitrogen (Tecator system 1002), neutral and acid detergent fiber (NDF and ADF; Goering and van Soest, 1970). After grazing, each strip was mechanically slashed to a height of 8 cm. Sprinkler irrigation was applied when necessary to ensure optimal soil moisture conditions for pasture growth.

The mean pasture intake of the indoor cows was estimated as the difference between the daily herbage offered and the residual uneaten herbage while that of the grazed cows was based on Chapas (1966) using the pasture yield in the cage at the end of each grazing period (two cages per paddock, each 1 × 1 × 1.5 m). At the same time, post grazing samples were also taken from an equal area outside the cages. Then, pasture intake was calculated using Equation 1:

$$\frac{(a-b)\,(\log_e c - \log_e b)}{\log_e a - \log_e b} \qquad (1)$$

where a is the pre-grazing pasture yield, b is the post grazing pasture yield and c is the pasture yield in the cage at the end of each grazing period all measured in kilograms of dry matter.

The daily individual *Leucaena leucocephala* intake of the indoor and outdoor cows was determined from the difference between the feed offered and the feed left during evening milking time. It was also analyzed for the DM content, Kjeldahl nitrogen (Tecator system 1002), fat content (Association of Official Analytical Chemists, 1980), and NDF and ADF. Separated parts of grass leaf and stem and whole *Leucaena leucocephala* were also analyzed for the fatty acid precursor of CLA (Christie, 1982; Hara and Radin, 1978) by gas chromatography (Model 6890 Series II; with Autoinjector; Shimadzu Corp.; Kyoto, Japan).

All cows were milked twice daily at 06.30 and 17.30 hours. The milk yield was recorded for each cow at each milking and a composite sample of morning and afternoon milk for each cow was analyzed at weekly intervals for fat, protein, lactose and solids-non-fat (SNF) using a Milkoscan Tester (Roche; Copenhagen, Denmark). A yield of 4% fat correctedmilk (FCM) was calculated by the formula of Walker *et al*. (2001) shown in Equation 2:

$$\text{FCM} = \text{Milk yield} \times [0.4 + 0.015 \times \text{Fat content}] \qquad (2)$$

where FCM and milk yield are measured in kilograms per cow per day and the fat content is measured in grams per kilogram.

Milk samples were also stored at -20°C until analysis for their fatty acid profile (Hara and Radin, 1978; Christie, 1982). The live-weight change was recorded before the experiment began and at fortnightly intervals thereafter.

Measurement of climatic conditions

On-farm meteorological data on the maximum and minimum temperature, dry- and wet-bulb temperatures (DB and WB; by dry- and wet-bulb thermometer, respectively), rainfall and relative humidity (RH; by the conversion of DB and WB temperatures) were recorded daily in a Stephenson screen located at the farm station. The temperature-humidity index (THI) was then calculated using Equation 3 for lactating dairy cows (McDowell, 1972):

$$\text{THI} = 0.72\,(\text{DB} + \text{WB}) + 40.6 \qquad (3)$$

Statistical Analysis

Statistical analysis of the milk yield and composition was carried out using a t-test with animals as the experimental unit (SAS, 2000).

Experimental period

The experimental period was commenced on 17 July and terminated on 31 August, 2011. A pre-experimental period of 1 wk was allowed for the animals to adapt to procedures.

RESULTS AND DISCUSSION

Nutritive values of the experimental feeds

The nutrient composition of the experimental feeds is shown in Table 1. The crude protein content of 9.77% in 45-day-old Guinea grass exceeded the normal requirement of a 7% ruminal protein level for cellulolytic bacterial activity (Hennessy, 1980) and was also at the critical dietary crude protein level below which voluntary intake would be depressed (Milford and Minson, 1967). Such a high protein level was possibly due to the high leaf stem ratio at the young age of 45 d (Reling *et al.*, 2001). The supplement (*Leucaena leucocephala*) in this experiment had a protein content of 24.63% which was higher than the content from meal concentrate which ranged from 16 to 21% for lactating cows (National Research Council, 2001).The benefits of using a protein-rich tree legume as a supplement to replace meal concentrate include improving the energy and protein intake, increasing feed efficiency and the availability of minerals and vitamins and encouraging rumen function (Poppi and Norton, 1995). However, high contents of NDF and ADF in *Leucaena leucocephala* would be a limiting factor when fed with pasture. The total NDF intake from both feeds would depress voluntary intake. There is substantial evidence that NDF alone is inadequate as its filling effect varies with particle size, particle fragility, the rate and extent of NDF digestion, retention time in the rumen and total chewing activity (Kennedy, 1984; Teimouri *et al.*, 2004).

The two important fatty acids—linoleic acid (C18:2 *n-6*) and linolenic acid (C18:3 *n-3*) in Table 2 are in different portions in the leaf (18.58 and 16.22 g per 100 g of fat, respectively) and stem (43.10 and 10.01 g per 100 g of fat, respectively) of Guinea grass. As linoleic acid (C18:2 *n-6*) and linolenic acid (C18:3 *n-3*) are C:18 substrates for rumen bio-hydrogenation (Elgersma, 2005), the feeding of fresh grass from either cutting or grazing provides more linoleic and α- linolenic acid (C18:3 *n-3*) as precursors of CLA which appears to be a simple and effective means of enhancing the CLA content in ruminant products (Tanaka, 2005).

Climatic conditions

Mean maximum and minimum temperatures, ambient temperature, RH, rainfall and THI are presented in Table 3. However, the average ambient temperature of 24.6 °C was close to the acknowledged upper critical temperature of

Table 1 Chemical composition (% dry matter) of the experimental roughages provided to the animals.

Items	Guinea Grass	*Leucaena leucocephala*
DM	22.77	32.21
CP	9.77	24.63
Fat	1.79	1.22
NDF	72.95	35.42
ADF	43.50	25.64

DM = Dry matter; CP = Crude protein content; NDF = Neutral detergent fiber; ADF = Acid detergent fiber.

26 °C for Friesian cattle (Johnson *et al.*, 1961). The high RH of 92.61% could have affected the THI values by exceeding the critical value of 72 for Holstein cows. Whichever THI value is most appropriate, it is apparent from Table 3 that the grazed cows in the study were subjected to similar levels of high heat stress and thus production would be likely to fall (Johnson, 1987).

Feed Intake

The estimated daily pasture DM intakes were 4.37 and 8.0 kg per cow for the grazed and housed groups, respectively (Table 4) which only provided an estimate of the mean intake value for both groups and thus did not allow statistical analyses to be performed. Housed cows consumed more forage than the grazed group; it is probable that the grazed cows were markedly affected by heat stress during the day (Hongyantarachai *et al.*, 1989; Prasanpanich *et al.*, 2002) resulting in compensation by *Leucaena leucocephala* intake which appeared to have been greater than for the housed group (Table 4). Cows in Groups 1 and 2 averaged 608.75 and 565.28 kg live weight at the start of the experiment and gained 2.5 and 2.0 kg, respectively, during the study. The initial weight, final weight and body weight changes of both groups were not affected and better quality roughage has been reported to support milking performance (Humphrey, 1991) and also to produce a greater gain in live weight during the mid-late period of lactation (Chilliard, 1989).

Milk yield and composition

Under the conditions of this study, there was no significant difference in the milk yield and composition (Table 5; except for the lactose content) of cows grazed outdoors and cows fed indoors which supported the findings of Hongyantarachai *et al.* (1989) and Prasanpanich *et al.* (2002). However, as a result of the lower feed intake which occurred in the grazed cows under heat stress, cows grazed outdoors might have a greater opportunity to select the more nutritious parts of plants (Stobbs, 1975).

The milk yield was correlated with higher milk protein and lactose percentages (McDonald *et al.*, 1988). The lactose content of both groups

Table 2 Linoleic acid (C18:2 *n-6*) and linolenic acid (C18:3 *n-3*) concentrations (grams per 100 g of fat) in *Leucaena leucocephala* and grass components of leaf and stem.

Item	Guinea grass		Leucaena leucocephala
	Leaf	Stem	
Linoleic acid (C18:2 n-6)	18.58	16.22	19.4
Linolenic acid (C18:3 n-3)	43.10	10.01	42.9

Table 3 Average on-farm meteorological data throughout the experiment measured in a Stephenson screen.

Item	Mean
Rainfall (mm)	6.77
Maximum temperature (°C)	31.52
Minimum temperature (°C)	17.62
Average temperature (°C)	24.60
Relative humidity (%)	92.61
Temperature humidity index	78.65

was highly significant different ($P < 0.01$) because of the increase in the total dry matter intake for the indoor cows. In particular, a greater roughage intake of 8 kg DM in indoor cows could produce higher blood glucose (Church, 1979; McDonald *et al.*, 1988). Subsequently, a high correlation between blood glucose uptake for mammary lactose synthesis and milk yield was found in cows consuming high fiber diets as roughage (Kittivachra *et al.*, 2007). Milk fat contents of 3.66 and 3.72% from the grazed and indoor cows, respectively, were greater than the value of 3.35% reported by National Bureau of Agricultural Commodity and Food Standards (2010) which are of benefit to milk pricing.

The fatty acid profile (in particular CLA) is considered to be a substantial part of the richest dietary sources and the CLA is believed to have several important physiological

Table 4 Live weight and feed intake of housed and grazed animals.

Item	Grazing group	Loose housing
Live weight (kg)		
Initial weight	608.75±54.23	565.28±55.15
Final weight	611.25±54.43	567.25±54.02
Body weight change	2.50± 2.50	2.00± 2.41
Dry matter (DM) intake (kg DM per day)		
Guinea grass	4.37	8.00
Leucaena leucocephala	0.67	0.42
Total dry matter intake	5.04	8.42

Table 5 Milk yield, composition and milk fatty acid content of housed and grazed animals (mean ± SE).

Item	Grazing group	Loose housing
Actual milk yield (kg.d^{-1})	10.15±0.64	10.30±2.50
4 % FCM (kg.d^{-1})	9.58±0.31	10.51±0.48
Milk composition (%)		
Fat	3.66±0.11	3.72±0.15
Protein	3.22±0.05	3.29±0.04
Lactose	4.27±0.05**	4.50±0.03**
Solid not fat	8.39±0.10	8.32±0.10
Fatty acid profile (g per 100 g of fat)		
Conjugated linoleic acid	2.10±0.20	1.84±0.07
Linoleic acid (C18:2 *n-6*)	2.16±0.50*	1.77±0.12*
Linolenic acid (C18:3 *n-3*)	0.40±0.03	0.35±0.05

FCM = Fat corrected milk.

* = Mean values within the same row with different superscripts are significantly different at $P < 0.05$.

** = Mean values within the same row with different superscripts are highly significantly different at $P < 0.01$.

functions, including immune-modulation, anti-carcinogenic and anti-artheriosclerosis properties, growth promotion and lean body mass promotion (Pastuschenko *et al.*, 2000; Whigham *et al.*, 2000; Tanaka, 2005). It was found that the amounts of linolenic acid (C18:3 *n-3*) and CLA in both groups were not significantly ($P < 0.05$) different but the amount of linoleic acid (C18:2 *n-6*) in both groups was significantly ($P < 0.05$) different. It is well documented that CLA is a mixture of positional and geometric fatty acid isomers of linoleic acid (C18:2 *n-6*) with conjugated unsaturated double bonds and is clearly present in milk products of ruminant animals by animal diet manipulation, especially from either fresh-cut or grazing pasture (Dhiman *et al.*, 1999) with a correct forage-to-concentrate ratio suitable in relation to increased milk CLA content.

However, the CLA contents from both groups were not significantly ($P < 0.05$) different, with the content from grazed cows appearing to be higher than in housed animals (2.1 ± 0.2 and 1.84 ± 0.07 g per100 g of fat, respectively). Cows grazing outdoors had greater opportunity for selection of leafy material and their intake may well have been different from the likely, more stemmy diet of the cows fed indoor (Stobbs, 1975). It is apparent in Table 2 that Guinea grass provides animals with higher contents of linoleic acid (C18:2 *n-6*) and linolenic acid (C18:3 *n-3*) in the leaves than in the stem. The cows grazing on pasture had a higher content of milk and linoleic acid (C18:2 *n-6*) than the housed cows (2.16 ± 0.5 and 1.77 ± 0.12 g per 100 g of fat, respectively). Clearly, the grazed animals could select and ingest the upper layers of the pasture containing leafy plant parts with higher lipid concentrations and proportions of fatty acid in the herbage (Elgersma *et al.*, 2012). Subsequently, linoleic acid (C18:2 *n-6*) known as a major dietary unsaturated fatty acid in forage crops (Jiang *et al.*, 1996) was clearly present in the milk products of ruminant animals.

CONCLUSION

There was no significant difference in the yield and composition (except for the lactose content) of cows grazed outdoors and cows fed indoors. However, an improvement in the milk CLA content was achieved by selective grazing with a higher content of linoleic acid (C18:2 *n-6*) in the leaf fraction resulting in higher levels of milk linoleic acid (C18:2 *n-6*; Omega 6) and linolenic acid (C18:3*n-3*; Omega 3). The different feeding patterns in lactating cows (either grazing or indoor feeding with fresh-cut pasture) should be considered with regard to optimizing milk quality and to promote healthy dairy products.

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