Changes of milk fat, crude protein, true protein, NPN and protein:fat ratio in Holstein cows fed a high concentrate diet from early to late lactation

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Summary

Percentages and yields of fat, crude protein (CP), true protein (TP) and non-protein nitrogen (NPN) were assessed in milk of four groups of Holstein cows, at early, mid and late lactation (days in milk 20-70, 71-110, 111-150 and 151-210), on a diet rich in concentrates during a hot summer. Four milk samples were taken from individual cows at 10-day intervals. Milk fat percentages were close to breed average and increased toward the end of lactation with a tendency ($P = 0.055$) for significant difference between the early and late lactation. Percentages of CP ($P = 0.14$) and TP ($P = 0.478$), however, were low similarly among all groups and did not follow the normal pattern of the breed. Percentage of NPN was significantly higher in groups III and IV ($P = 0.005$). Yields of fat ($P = 0.035$), CP ($P = 0.002$) and TP ($P = 0.001$), but not NPN ($P = 0.589$), decreased toward the end of lactation. There was a negative correlation ($r = -0.354$, $P<0.001$) between the percentages of NPN and TP. However, no correlation was observed between the percentages of NPN and CP. Despite a very strong correlation between yields of CP and TP ($r = 0.983$, $P<0.001$), the correlation between the yield of CP and that of NPN was much weaker ($r = 0.473$, $P<0.001$). It is concluded that percents and yields of milk fat, close to breed average are attainable at any stage of lactation on high concentrate diets in hot weather. However, regarding milk protein, cows may not perform well. TP may be more affected than CP.

Key words: Milk, Fat, protein, NPN, Lactation cycle

Introduction

Fat and protein are the two major milk components affecting milk price, usually concerned by dairy producers and dairy industries. Higher fat and protein are recovered in milk by feeding high forage diets during late lactation and/or improving the energy status of cows during early lactation. Moving of cows into positive energy balance during late lactation also leads to higher percentages of fat and protein in milk (Bachman, 1992; Hutjens, 2002). However, practices for feeding high forage diets and/or improving energy status of cows may not be applicable under some circumstances. High producing cows may remain on low fiber, high concentrate diets from early to late lactation. This is partly due to their high production and partly due to grouping facilities and the necessity for reestablishing appropriate body condition scores in cows before moving them to a lower group. In addition, exposure of cows to hot weather (more than 27ºC) depresses energy intake by reducing dry matter intake (DMI) (McDowell *et al.*, 1976) and reduces consumption of forages (Huber, 1996). The aim of present study was to determine if the depression in milk fat and protein remains inevitable on high concentrate diets during hot weather even after the energy balance of the cow improves from negative to positive through a lactation cycle. Changes of fat, crude protein (CP), true protein (TP) and non-protein nitrogen (NPN) in milk of high producing cows were assessed under field conditions.

Materials and Methods

The study was carried out on 32 adult (second lactation or later) Holstein Friesian cows in a commercial dairy farm, located in Marvdasht area, Fars province, Iran. The cows were at various stages of lactation cycle from early to late lactation and were divided into 4 groups according to their days in milk (DIM) and their theoretical energy balance (Table 1). No adjustment was made in routine management practices of the farm. The cows were grouped in the high string according to their daily production (35 kg or more) and/or their body condition score (less than 2.75 using a 5-point scale with quarter point divisions) regardless of their stage of lactation. Thus, all cows received a same isocaloric and isonitrogenous diet regardless of their physiological status. The diet contained 11.4 kg (43.25%) forage and 15 kg (56.75%) concentrate on dry matter basis (Table 2) and was allocated in 9 meals per day. Forages were either mixed partially with concentrates or were fed separately. The cows were milked three times a day at 0500, 1300 and 2100. The study was completed during July and August when maximum ambient temperature approached to 40ºC or more during days and the relative humidity was around 32%. The temperature-humidity index (THI), calculated by the following equation, can be used for confirming heat stress: THI = td-(0.55-0.55 RH)(td-58); where td is dry bulb temperature (Fahrenheit) and RH is relative humidity expressed in decimal form. THI estimates higher than 72 represent heat stress (Armstrong, 1994). In the present study maximum daily THI was around 86 for several hrs during days. Averages of rectal temperature and respiration rate of cows at 1100 were 39.2 ± 0.2ºC and 80 ± 3 min^{-1}, respectively. The cows were housed in open sheds with shades on resting areas and bunks but not on the holding area and waterers. The cows were cooled under showers (but not fans) at least two times per day at 1100 and 1500 in the holding area. No other cooling practice was performed.

Milk samples were collected four times, at 10-day intervals. Beginning at 1300, milk production was recorded at each milking time and double samples of composite milk of individual cows (2 × 20 ml) were collected for further analyses. Samples were refrigerated until they were transferred to the laboratory early in the next morning. When in the laboratory, samples were first analysed for their fat percentages using Gerber's method (Horwitz, 1975) and the remainder was stored at -20ºC. Within less than 2 months, the samples were thawed at room temperature to be analysed for CP and NPN percentages as Kjeldhal N × 6.38 (Horwitz, 1975). For measuring NPN, 5 ml of 12% trichloroacetic acid was added to 5 ml of milk in order to precipitate TP of milk. Then, the mixture was centrifuged at 3000 × g for 20 min and NPN was measured in the supernatant. Percentage of TP and weights of CP, TP and NPN were calculated in 24 hrs milk from the values obtained as above.

Data were compared statistically among groups with one-way ANOVA and Duncan's multiple range test. Correlations of various factors measured in milk were determined using two–tailed Pearson correlation test. The SPSS statistical software (version 10) was used to perform statistical analysis.

Results

Milk volume decreased significantly (P = 0.002) as could be expected due to normal decline in production from early to late lactation (Fig. 1). Milk fat percentage tended to increase through groups as DIM progressed, with a tendency (P = 0.055) for significant difference between groups I and IV (Fig. 2a). Percentages of CP (P = 0.14) and TP (P = 0.478), however, were almost similar among all groups (Fig. 2a). Percentage of NPN was significantly higher in groups III and IV (p = 0.005) (Fig. 3a).

Yield of milk fat was the highest in group II and the lowest in group IV (Fig. 2b). It was significantly lower in groups III and IV (p = 0.035). Yields of CP and TP also had a decreasing pattern and being lower in groups III and IV. This was much more significant than that of milk fat yield (P = 0.002 for CP and P = 0.001 for TP) (Fig. 2b). Yield of milk NPN, on the other hand, was relatively constant showing no

Table 1: Groups of cows according to their days in milk and theoretical energy balance

Groups	n	DIM	Lactation stage	Energy balance
Group I	8	20-70	Early lactation	Negative
Group II	8	71-110	Mid lactation	Balance/negative
Group III	8	111-150	Mid lactation	Balance
Group IV	8	151-210	Late lactation	Positive

DIM = days in milk

Table 2: Ingredients and chemical composition of the diet on dry matter basis

	Feed name	Weight (kg)		Feed name	Weight (kg)
Forages:	Alfalfa, hay	4.50	Concentrates:	Beet pulp, with molasses	2.70
	Alfalfa, fresh	3.75		Barley, ground	5.00
	Corn silage	3.12		Wheat bran	1.80
				Cottonseed meal	2.31
				Cottonseed, with lint	2.43
				Mineral/vitamin supplements	0.475
				Sodium bicarbonate	0.200
Total		11.37 (43.25%)			14.92 (56.75%)
Crude protein (kg)		4.35 (16.54%)			
NEL (mcal)*		42.35 (1.61 mcal/kg)			
NDF (kg)		9.7 (36.89%)			
NSC (kg)*		9.28 (35.30%)			
EE (kg)		1.11 (4.22%)			
Calcium (kg)		0.237 (0.9%)			
Phosphorus (kg)		0.144 (0.55%)			

NEL = net energy for lactation; NDF = neutral detergent fiber; NSC = non structural carbohydrates; EE = ether extract; *= calculated

significant difference among groups (P = 0.589) (Fig. 3b).

Correlation coefficients between milk values are depicted in Table 3. As it is obvious, there is no significant correlation between the percentages of CP and NPN (r = 0.035, P = 0.719). There is a negative correlation between the percentage of TP and that of NPN (r = -0.354, P<0.001). Despite a very strong correlation between weights of CP and TP (r = 0.983, P<0.001), the correlation between the weight of CP and that of NPN was found to be much weaker (r = 0.473, P<0.001).

Discussion

The average milk fat percentage for Holsteins is reported to be 3.66 (Hutjens, 2002). In cows with less than 50 DIM, low fat concentrations can reflect energy shortage. From 50 to 150 DIM, fat percentage will be at its lowest point and milk fat concentrations between 3.0 and 3.3% for high producing Holsteins are not a concern. From day 150 towards the end of lactation, milk fat should be close to the average for the breed. Milk protein test patterns should follow those of milk fat (Hutjens, 2002). A normally fed group of Holstein cows can be expected to have a milk protein percentage of 2.7 to 3.0% by week 5 to 6 of lactation which then increases to as high as 3.6 to 3.8% by the end of the lactation. Examining the relationship between DIM and milk protein percentage is valuable in determining whether the milk protein percentages in a particular herd are relatively high, low, or normal for the different stages of lactation (Robinson, 2000).

The results of the present study indicate: (1) fat concentrations close to breed average, increasing toward the end of lactation, (2) low and almost constant protein concentrations (both CP and TP) and (3) increasing percentages of milk NPN. Provision of substantial amounts of digestible fiber from beet pulp, wheat bran and whole cottonseed could result in fat

Table 3: Correlation coefficients between milk parameters*

	Weight	Fat (%)	CP[1] (%)	TP[2] (%)	NPN[3] (%)	Fat yield **	CP yield	TP yield
Fat (%)	-0.221 (0.017)							
CP (%)	-0.436 (0.000)	0.187 (0.045)						
TP (%)	-0.371 (0.000)	0.226 (0.000)	0.911 (0.000)					
NPN (%)	-0.039 (0.684)	-0.095 (0.324)	0.035 (0.719)	-0.354 (0.000)				
Fat yield	0.734 (0.000)	0.475 (0.000)	-0.262 (0.004)	-0.182 (0.050)	-0.095 (0.322)			
CP yield	0.940 (0.000)	-0.180 (0.054)	-0.111 (0.235)	-0.073 (0.439)	-0.033 0.731	0.714 (0.000)		
TP yield	0.914 (0.000)	-0.146 (0.117)	-0.079 (0.400)	0.028 (0.768)	-0.201 (0.035)	0.710 (0.000)	0.983 (0.000)	
NPN yield	0.497 (0.000)	-0.228 (0.014)	-0.211 (0.023)	-0.520 (0.000)	0.803 (0.000)	0.307 (0.001)	0.473 (0.000)	0.308 (0.001)

*Figures in parentheses indicate p-values; **Weights are in kilogram; [1]Crude protein; [2]True protein; [3]Non-protein nitrogen

concentrations close to breed average despite high proportion of concentrates in diet (57% DM). The percentage of NDF in the ration (36.9%) was higher than the recommended values (28-30%) (Radostits, 2001). There are several explanations for long lasting low protein concentrations:

1- Forage:concentrate ratio: Robinson and McQueen (1997) reported lower protein yield and percent with diets containing 42% forage and 58% concentrate compared with diets containing 55% forage and 45% concentrate in mid lactation cows. They concluded that this might be the result of suppressed rumen bacterial growth associated with the acidotic rumen fermentation conditions that are typical of situations where rations contain high levels of concentrate.

2- Heat stress: Heat stress may decrease milk protein percent up to 0.2 to 0.3 units (Robinson, 2000) by suppressing DMI (McDowell *et al.*, 1976), increasing maintenance requirements (Huber, 1996) and predisposing cows to ruminal acidosis (Mishra *et al*., 1970). The primary nutritional factor that affects milk protein percentage and yield is energy intake, which is dependent upon DMI and energy density of diet (Bachman, 1992). Perhaps the most detrimental effect of heat stress is reduction in feed intake (McDowell *et al.*, 1976), resulting in lower energy intake. Body heat production, rectal temperature and respiration rate are greater for high forage compared with moderate or high concentrate diets. Greater heat increment has been associated with higher acetate production in the rumen of cows fed high forage diets (Tyrell *et al.*, 1979). Often cows voluntarily limit their forage consumption during hot weather, even to the extent of drastically shifting acetate to propionate ratios and lowering butterfat content of milk (Huber, 1996). High quality forages produce less heat in fermentation (Huber, 1996). In the present study, all forages were of high quality to increase their utilization. Nevertheless, as forages were not totally mixed with concentrates, the cows potentially had the opportunity to select among the components of their feed. An average of 1.2 kg DM day^{-1} (mainly corn silage) was not consumed. Thus, it is

Fig. 1: Daily milk production in different groups (Mean ± SEM)

Fig. 2: Percentages and weights of milk fat, crude protein, and true protein in different groups (Mean ± SEM)

Fig. 3: Percentage and weight of NPN in milk of different groups (Mean ± SEM)

Concluded that while dramatic shortage in fiber intake could be prevented by concentrate feeds such as beet pulp, shortage of energy intake could remain as a potential problem. Maintenance requirements of lactating dairy cows increase by about 10 to 30% if ambient temperatures are raised from 20 to 30 and 40ºC, respectively for 6 hr d^{-1} (McDowell *et al*., 1976). In the present study no adjustment was performed to increase energy and protein density of the diet. Thus, it is concluded that the cows were subjected to energy and protein intakes lower than their actual needs.

Changes of acid-base balance of cows during heat stress predispose them to ruminal acidosis. Cows pant and exhale carbon dioxide during hot weather (Dale and Brody, 1954) and the total amount of buffering capacity within their body fluids may decrease. In a study that tested the effect of ambient temperature on rumen environment (Mishra *et al*., 1970), lactating Holstein cows were fed high roughage or high concentrate diets at either ambient temperatures of 16ºC (cool) or 29.4ºC (hot) with relative humidities at 50% and 85%, respectively. Ruminal pH was lower at the higher temperature and on the higher concentrate diet ($P \leq 0.01$). Ruminal changes appear to be responses to ambient, not ruminal temperatures (Gengler *et al*., 1970). According to a conclusion made by Robinson and McQueen (1997), acidotic conditions in the rumen may cause lower protein concentration in milk. It appears from the results of the present study that the detrimental effects of conditions described above are mainly reflected on milk protein percentage rather than milk fat.

3- Pectin (sugar beet pulp) and fat (whole cottonseed) as energy sources in the diet: Pectin is fermented slowly and fats are non-fermentative sources of energy and both may have some adverse effects on synthesis of microbial protein in rumen. This may lessen milk protein. Although beet pulp is valuable in maintaining normal milk fat concentrations with high concentrate diets (Ensminger *et al*., 1990), it may cause reduction in milk protein because of its high content of pectin. Pectin may yield less microbial protein than starch (Hall and Herejk, 2001). Rodriguez *et al*., (1997) showed that dietary addition of fats is associated with reductions in milk protein percentage. Milk protein yield is generally unchanged or slightly increased. Supple-mental fat is predisposed to have a greater impact on production of milk fat and lactose, the latter of which is the major regulator of milk volume, than on milk protein production.

Despite milk fat percentages close to breed averages at various DIMs, examining the milk protein:fat ratio (using percentage values) revealed subnormal values among all groups, ranging from a maximum of 0.83 in group I to a minimum of 0.77 in group IV. Normal milk protein:fat ratio for Holsteins is 0.85 to 0.88 (Mahanna, 1999). Lower values could mean protein intake problems from too little intake of total protein or insufficient rumen undegraded protein. These observations together with increasing percentage of milk NPN may be the result of utilization of feed and body proteins to fulfill increased maintenance requirements for energy and protein. Compounds of NPN fraction in milk are similar to those found in cow urine and could be the end products of N metabolism in the cow (Alston-Mills, 1995). As cows move into positive energy balance by consuming more energy and protein than they are using, they produce milk of normal protein content due to an overall increase in energy and protein intake. The CP content of the diet in the present study appeared to be insufficient for early lactation cows (16.54% vs 18% DM, Radostits, 2001). Unexpected low milk protein even during late lactation could mean extreme utilization of body proteins during early and probably mid lactation, extension of increased protein catabolism into late lactation and the priority for replenishment of this protein instead of incorporation of amino acids into milk protein.

Correlation coefficients of milk com-ponents (Table 3) show that the percentage and the yield of TP rather than those of NPN mostly affect the CP content of milk. Thus, the less the protein available, the less the protein secreted into milk. Ruminal acidosis (not documented in this study) may be another cause of low protein and increasing NPN concentrations in milk. In a study on the effect of nutritionally induced acidosis on milk composition (Gentile *et al*., 1986), casein and the ratio of casein to protein tended to decrease and the ratio of NPN to protein tended to increase. Genetics may also be the basis for lower milk protein. However, according to some complementary experiments performed during cool periods (autumn), milk protein concentration of high producing cows of the same herd was above 3%.

It is concluded that the percents and yields of milk fat close to the breed average are attainable at any stage of lactation on high concentrate diets in hot weather. However, regarding milk protein, cows may not perform well due to climatic and dietary factors. TP may be more affected than CP. If the bulk milk protein content is affected severely, some approaches may be necessary to alleviate the problem. Provision of some sources of rumen undegradable protein and maintaining synchronization in utilization of energy and synthesis of microbial protein may alleviate the problem. Evaluating the ration with modern ration evaluator softwares is recommended. However, any approach in manipulating the diet should be evaluated economically and the effects of heat stress should also be kept in mind.

Acknowledgements

This work was financially supported by the office of vice-chancellor for research at University of Shiraz. Contribution of Mr. P. Asghari and Mr. A. Elaahi is highly appreciated.

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