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Associations between milk fat, protein and fat-to-protein ratio with some reproductive indices in dairy cows

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ABSTRACT

Postpartum induced lipolysis by negative energy balance (NEB) causing an increase in milk fat, a decrease in milk protein, and as a consequence an increase in milk fat to protein ratio (FPR). The aim of this study was to evaluate the relationship between milk FPR and first service conception risk (FSCR), days to first service (DFS) and calving to conception interval at first service (CCIFS). Therefore, milk and reproduction data of 1,375 primiparous and multiparous Holstein dairy cows from 10 commercial dairy farms located on sub-tropical region were collected on days 30 and 60 of days in milk (DIM) and near the first service. The Pearson correlation test of milk compositions revealed only a significant correlation between milk protein at day 30 DIM and DFS, but in Logistic regression analysis it did not have a constant effect on reproductive indices. On the other hand, the effect of previous dry-off duration and AI season on FSCR were significant. Based on the result of the present study, it is concluded that milk compositions such as fat, protein and FPR had no correlation with the result of the first AI.

Keywords: Dairy cows, Fertility indices, Milk fat to protein ratio

Over the past decades, with increasing the capacity of dairy cattle to produce higher milk per cow (Patton *et al*. 2007), problems such as decreasing in fertility has been manifested (De Marchini *et al.* 2014). One of the most important aspects in this regard is NEB (Ricardo and Chebel 2007). This period lasts for 10–12 weeks after parturition (Butler 2003) and its consequences including altered ovarian function (Waldmann *et al.* 2006), decreased estrus expression and increased days open (Wathes *et al.* 2007) and increased calving to first ovulation interval (Butler 2003).

In NEB due to postpartum lipolysis, the percentage of milk fat is increased and of milk protein is decreased (De Marchini *et al.* 2014), so increasedFPR is a fixed outcome. Use of FPR in the first milk test is recommended as a diagnostic tool in individual dairy cows (Friggens *et al.* 2007).

Thus, the present study was aimed to investigate the relationship between milk fat, protein and FPR of 30 and 60 DIM, and also near the first AI with fertility indices such as first service conception risk (FSCR), days to first service (DFS), and calving to conception interval at first service (CCIFS) in Holstein dairy cows.

MATERIALS AND METHODS

Within one year period, 1,375 lactation records from primiparous and multiparous Holstein dairy cows (parity ≤5) were collected from 10 commercial dairy herds in sub-tropical regions of Iran. Record data such as milk yield in previous lactation period, previous dry-off duration, BCS on a 5-scale system (where 1 = very thin to 5 = extremely fat), seasons of AI, DIM, milk yield on days 30, 60 postpartum and at the time of AI, calving season, AI technician and sperm type were considered as confounding variables and their effect on reproductive indices were evaluated.

Milk fat and protein percentages were measured at 30±7, 60±7 DIM and near the time of the 1st AI postpartum with ±4-day interval and considered as independent variables. Thereafter, FPR were calculated for given periods and classified as follows: less than 1.09, 1.1–1.34 and above the 1.34 (Podpecan *et al.* 2010).

In all herds, cows were fed twice daily a TMR consisting of barley, alfalfa hay, corn silage, concentrate and it is designed to contain 18% to 19% crude protein and 4.8% to 5.1% crude fat, in order to meet or exceed the minimum nutritional requirements for high producing dairy cows (NRC 2001). All dairy cows were housed on confined free-stall system and had *ad lib.* access to feed and water. All dairy cows milked three times daily.

Reproductive activity was year-round and the voluntary waiting period (VWP) was considered approximately 60 days postpartum in all herds. The most common fertility program was Double-Ovsynch (Souza *et al.* 2008) for

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primiparous and Presynch-Ovsynch (Moreira *et al*. 2001) for multiparous dairy cows. Pregnancy diagnosis was performed by ultrasonography at day 35±3 post-AI.

Statitical analysis: The One-Sample Kolmogorov-Smirnov test was used to evaluate the distribution of quantitative data with respect to normalization. The Mann-Whitney U (the only two groups) and the Kruskal-Wallis (multiple groups) analysis were used for the comparison of quantitative data that were not in normal distribution. For comparison of absolute and relative distributions of qualitative data, we used Chi-Square and Fisher's exact tests. Cox regression was used for measuring the different variable effects on CCIFS and the determination of Hazard ratio. Multiple logistic regression model was used to evaluate the constant effect of various variables on FSCR and determining the odds ratio. Statistical associations were evaluated with the following tests: Pearson analysis for scale, Spearman rank correlation for ordinal and Kendall test for nominal variables. The statistical associations between milk composition variables and the fertility indices such as DFS, CCIFS and FSCR were evaluated.

RESULTS AND DISCUSSION

The amount of milk fat, protein and FPR at different days postpartum in both pregnant and non-pregnant cows are illustrated in Table 1. Mann-Whitney U test showed only 0.1% increase in milk protein percentage in non-pregnant compared to pregnant cows at day 30 DIM (P=0.036).

Inconsistent to the result of our study, other researchers found that milk fat differences between the first 2 months of lactation were the best indicator for NEB. But, in their study milk protein and FPR were not changed by NEB and concluded that milk protein percentage and FPR are not useful indicators for determining energy condition (De Vries *et al.* 2000). On the other hand, in another study it is shown that FPR is a useful indicator for predicting energy condition and has an inverse relationship with it (Toni *et al.* 2011).

The Pearson correlation test revealed a significant correlation between protein at day 30 DIM and DFS ($r = -0.079$, $P<0.01$). There were no significant correlations between stated reproductive indices and milk parameters at day 60 DIM and the time of first AI.

High milk protein in the first month of lactation is related to short duration from calving to appearance of luteal tissue, decreased DFS, increased CR in the first AI and decreased number of services per conception (Morton *et al.* 2016). It is likely the relationship between milk protein and reproductive performance are mediated by milk yield in an inverse association. Also, they showed that milk protein from parturition till day 30 DIM in contrast to day 30–60 DIM has no significant association with reproductive performance, which is not in agreement with our finding. So, it seems that the association between milk protein and reproductive performance is probably due to other factors except NEB in early lactation. In addition, it is proposed that milk fat, protein, and FPR in the first 2 weeks of lactation can be used for predicting subclinical ketosis, but its sensitivity and specificity are moderate (Lima *et al.* 2012). So, because of its limitation, milk compositions should be used along with other criteria.

Previous milk yield (P=0.844) and days in milk (P=0.349), and current milk yield on days 30 (P=0.230) and 60 (P=0.1) after parturition and at the time of AI (P=0.433) were analyzed in this study, but did not reveal any significant difference between pregnant and non-pregnant cows following the first AI.

The association between high milk yield and poor reproductive performance is controversial. Negative or no relationship is found in one research (Luci 2001). Moreover, it is reported that milk yield in the first month of lactation or milk yield at the time of AI and/or its peak are not related remarkably to reproductive performance, which corresponds with our result (Patton *et al.* 2007). On the other hand, it is found that the association between milk yield and calving to first ovulation is important only at those cows that earlyovulated (Butler 2003). Findings of different experiments and individually cow differences suggest that factors other than milk yield probably have critical role in determining calving to first ovulation index.

Both calving and AI seasons were analyzed in our experiment and the correlation of the AI season with the result of first AI was significant (P = 0.00). The Chi-Square test showed that CR in the first AI in spring, summer, fall, and winter were 65.8, 49.9, 14.6 and 26.1%, respectively

Table 1. Fat, Protein and FPR at different days postpartum in pregnant and non-pregnant cows

Results of first AI		Fat 30	Protein30[†]	FPR 30	Fat 60	Protein 60	FPR 60	Fat AI	Protein AI	FPR* AI
Pregnant (N=517)	Mean	2.85	2.76	1.03	2.95	2.74	1.07	2.83	2.66	1.06
	SE**	0.05	0.04	0.015	0.05	0.04	0.02	0.05	0.04	0.02
	Median	3.01[a]	3.00[a]	1.02[a]	3.12[a]	2.92[a]	1.05[a]	3.0[a]	2.90[a]	1.03[a]
Non pregnant (N=858)	Mean	2.79	2.71	1.02	2.96	2.74	1.08	2.73	2.60	1.05
	SE	0.04	0.03	0.01	0.04	0.03	0.02	0.04	0.03	0.01
	Median	3.00[a]	3.1[b††]	1.00[a]	3.09[a]	2.92[a]	1.05[a]	3.00[a]	2.90[a]	1.02[a]
Total (N=1,375)	Mean	2.81	2.73	1.02	2.95	2.74	1.07	2.77	2.62	1.05
	SE	0.03	0.03	0.01	0.03	0.02	0.01	0.03	0.03	0.01
	Median	3.00	3.00	1.01	3.10	2.92	1.05	3.00	2.90	1.02

[†]The Mann-Whitney U test showed P=0.036; [††]Different superscripts as lower-case letters indicate a statistical significance between groups in the relevant column; *, FPR (Fat to Protein ratio); **SE, Standard error of mean.

(P=0.00). Also, the Logistic regression model showed the same results.

Consistent with our result, an experiment in England showed that calving season has not any effect on the risk of pregnancy till 100 DIM, but affects the reproduction result till 150 DIM (Cook and Green 2016). Explaining this finding is difficult because data show that milk yield and its composition are different in a variety of seasons in England (Madouasse *et al.* 2010) and another critical influencing factor on milk fat is feed regime (Bauman and Griinari 2003).

The Mann-Whitney U test revealed the median of previous dry-off duration in pregnant cows following the first AI (64.1±2.4 d, MED: 58) was significantly less than non-pregnant cows (68.7±1.7 d, MED: 63) (P=0.01).

Historically it is believed a dry period as long as 6–8 weeks for dairy cattle is beneficial to increase milk production in the next lactation. But nowadays, this issue is controversial, since decreased dry-off duration causes milk production shift from the important period postpartum to the weeks before it. This shift may enhance the EB of animal, its health and fertility. Recently, a meta-analysis reviewed the current knowledge regarding dry-off duration in dairy cattle and its correlation with milk production, EB, fertility and health (van Knegsel *et al.* 2013). In agreement with our results, they found both decreasing and eliminating the dry period reduces milk production in the next lactation, increases milk protein percentage and reduces the risk of ketosis postpartum, but had no effect on milk fat percentage. Moreover, decreasing the dry-off duration do not change the odds ratio for fertility indices, metritis and mastitisin the next lactation. Economically in dairy herds, the financial losses for each average cow due to clinical metritis and clinical mastitis are $37 and $15, respectively (Yildiz 2018). On the other hand, short or no dry period compared to conventional one improved EB of cattle postpartum (Rastani *et al.* 2005).

The Mann-Whitney U test showed decrement of BCS from parturition till the 2nd months of DIM has a statistically significant difference between pregnant and non-pregnant cows following the first AI (P=0.041) and the other BCS records or decrements were not statistically significant among them (P>0.05). Cows losing or gaining BCS from parturition till the 2nd months of lactation were categorized into 4 groups; A (–1 to 0), B (0 to 0.49), C (0.5 to 0.99) and D (>1), and Chi-Square and Log Linear tests revealed that pregnant cows in group A, B, C, and D were 2.1%, 55.9%, 39.1%, and 2.9%, and non-pregnant cows were 1.6%, 51%, 43.8%, and 3.5%, respectively (P>0.05).

In cows with low BCS at parturition or cows losing their BCS severely postpartum, there will be consequences in their reproduction such as less probability of ovulation, decreased CR in the first AI postpartum, increased number of services per conception and increased CCIFS (Berry *et al.* 2007, Sharma *et al.* 2018). The more BCS loss in cattle, the more negative effect on CR. Corresponding to the result of this study, it is demonstrated that both severe BCS loss postpartum and low BCS at the time of AI cause a remarkable increase in CCIFS (López-Gatius 2003). In another study, cows losing their BCS severely after parturition require more AIs for pregnancy and have long CCIFS, although calving to first AI interval is not influenced (Gillund *et al*. 2001).

An interesting experiment was carried out regarding the effect of different housing systems on reproductive indices and BCS in sheep (Kochewad *et al*. 2018). They found higher CR in ewes maintained in intensive system compared to semi-intensive or extensive ewe systems. Moreover, BCS was significantly higher during pregnancy in intensive ewes. The Cox survival model showed among the various confounding variables, only season of the first AI had a constant effect on CCIFS (Hazard ratio=0.47, P<0.037), but not BCS, milk fat, protein, and their ratio. The Multiple logistic regression model showed among the various studied confounding variables, just season of the first AI had a constant effect on the FSCR (P=0.00, odds ratio= 2.83).

The Kendall test revealed that AI technician and sperm type as confounding variables did not have any correlation with discussed reproductive indices.

In conclusion, between BCS and milk composition indices (fat, protein, and FPR) as two important indicators of net energy balance, only milk protein at day 30 DIM had correlation with reproductive indices and it was 0.1% more in pregnant compared to non-pregnant cows; in spite of significant correlations of AI seasons and previous dry-off duration with FSCR, none of the milk fat, protein or FPR had constant correlation with it.

REFERENCES

Bauman D E and Grinari J M. 2003. Nutritional regulation of milk fat synthesis. *Annual Reviewof Nutrition* **23**: 203–27.

Berry D P, Roche J R and Coffy M P. 2007. Body Condition Score and Fertility—More Than Just a Feeling. *Proceedings of Fertility in Dairy Cows –Bridging the gaps*. Liverpool Hope University, Liverpool, UK 107–118.

Butler W R. 2003. Energy balance relationships with follicular development, ovulation, and fertility in postpartum dairy cows. *Livestock Production Science* **83**(2–3): 211–18.

Cook J G and Green M J. 2016. Use of early lactation milk recording data to predict the calving to conception interval in dairy herds. *Journal of Dairy Science* **99**(6): 4699–4706.

De Marchi M, Toffanin V, Cassandro M and Pensa M. 2014. Invited review: Mid-infrared spectroscopy as phenotyping tool for milk traits. *Journal of Dairy Science* **97**(3): 1171–86.

De Vries M J and Veerkamp R F. 2000. Energy balance of dairy cattle in relation to milk production variables and fertility. *Journal of Dairy Science* **83**(1): 62–69.

Friggens N C, Ridder C and Lovendahl P. 2007. On the use of milk composition measures to predict the energy balance of dairy cows. *Journal of Dairy Science* **90**(12): 5453–67.

Gillund P, Reksen O, Grohn Y T and Karlberg K. 2001. Body condition score related to ketosis and reproductive performance in Norwegian dairy cows. *Journal of Dairy Science* **84**(6): 1390–96.

Kochewad S A, Traghunandan T, Rao K, Reddy K K, Kumari N, Ramana D, Kumar S, Meena L R and Singh S P. 2018.

Reproductive performance and BCS of Deccani sheep during various physiologic stages in different farming systems. *The Indian Journal of Animal Sciences* **88**(12): 1379–82.

Lima F S, Risco C A, Pereira R V K, Galvao K N and Santos J E P. 2012. Assessment of daily milk fat and protein composition and the milk fat-protein ratio early postpartum as a predictor for sub- clinical ketosis in dairy cows. Conference: 2012 ADSA-ASAS-CSAS Joint Annual Meeting 07/2012.

López-Gatius F. 2003. Is fertility declining in dairy cattle? A retrospective study in northeastern Spain. *Theriogenology* **60**(1): 89–99.

Luci M C. 2001. Reproductive loss in high-producing dairy cattle: Where will it end? *Journal of Dairy Science* **84**(6): 1277–93.

Madouasse A, Huxley J N, Browne W J, Bradley A J, Dryden I L and Green M J. 2010. Use of individual cow milk recording data at the start of lactation to predict the calving to conception interval. *Journal of Dairy Science* **93**(10): 4677–90.

Moreira F, Orlandi C, Risco C A, Mattos R, Lopes F and Thatcher W W. 2001. Effects of presynchronization and bovine somatotropin on pregnancy rates to a timed artificial insemination protocol in lactating dairy cows. *Journal of Dairy Science* **84**(7): 1646–59.

Morton J K, Auldist M J, Douglas M L and Macmillan K L. 2016. Associations between milk protein concentration, milk yield, and reproductive performance in dairy cows. *Journal of Dairy Science* **99**(12): 10033–43.

NRC. 2001. *Nutrient Requirements of Dairy Cattle*. 7th rev. ed. The National Academies Press, Washington, DC.

Patton J, Kenny D A, Mc Namara S, Mee J F, O'mara F P, Diskin M G and Murphy J J. 2007. Relationships among milk production, energy balance, plasma analytes, and reproduction in Holstein-Friesian cows. *Journal of Dairy Science* **90**(2): 649–58.

Podpecan O, Mrkun J and Zrimsek P. 2010. The evaluation of fat to protein ratio in milk as an indicator of calving to conception interval in dairy cows using various biostatistical methods. *Acta Veterinaria* (*Beograd*) **60**(5–6): 541–50.

Rastani R R, Grummer R R, Bertics, S J, Gumen A, Wiltbank M C, Mashek D G and Schwab M C. 2005. Reducing dry period length to simplify feeding transition cows: Milk production, energy balance, and metabolic profiles. *Journal of Dairy Science* **88**(3): 1004–14.

Ricardo C and Chebel Z. 2007. Mastitis effects on reproduction. *NMC Regional Meeting Proceedings.* pp.43–55.

Sharma A, Singh M, Sharma A and Kumar P. 2018. Effect of BCS and parity on uterine involution, ovarian rebound and various fertility parameters in postpartum dairy cows. *The Indian Journal of Animal Sciences* **88**(5): 526–29.

Souza A H, Ayres H, Ferreira R M and Wiltbank M C. 2008. A new presynchronization system (Double-Ovsynch) increases fertility at first postpartum timed A I in lactating dairy cows. *Theriogenology* **70**(2): 208–15.

Toni F, Vincentti L, Grigoletto L, Ricci A and Schukken Y H. 2011. Early lactation ratio of fat and protein percentage in milk is associated with health, milk production, and survival. *Journal of Dairy Science* **94**(4): 1772–83.

Van Knegsel A T M, van der Drift S G A, C¡ermáková J A C and Kemp B. 2013. Effects of shortening the dry period of dairy cows on milk production, energy balance, health, and fertility: A systematic review. *The Veterinary Journal* **198**(3): 707–13.

Yildiz A S. 2018. Effects of some diseases observed at postpartum period of cows in dairy farms: Economic perspective. *The Indian Journal of Animal Sciences* **88**(6): 645–50.

Waldmann A, Jevgeni Kurykin U, Tanel K, Madis A, Mihkel J, Lembit M and Peeter P. 2006. The effects of ovarian function on estrus synchronization with PGF in dairy cows. *Theriogenology* **66**(5): 1364–74.

Wathes D C, Fenwick M, Cheng Z, Bourne N, Llewellyn S, Morris D G, Kenny D, Murphu J and Fitzpatrick R. 2007. Influence of negative energy balance on cyclicity and fertility in the high producing dairy cow. *Theriogenology* **68**(1): 232–41.