Harran Üniv Vet Fak Derg, 2022; 11 (2): 201-208 *Research Article*
DOI:10.31196/huvfd.1120644

Investigation of Relationship among Dietary Fatty Acids, Milk Urea Nitrogen and Fertility Problems in Dairy Cattle Farms

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Geliş Tarihi: 24.05.2022 **Kabul Tarihi: 13.10.2022**

Abstract: The aim of this study was demonstrated the relationship between the nutritional variables of ration and the fertility parameters in the postpartum period in dairy cattle farms. All dairy cattle farms used in the present study had fertility problems (calving range ≥14 months and artificial insemination number ≥1.8). Ration and milk samples were taken from selected dairy cattle farms. Fertility records from herd registration systems were examined. In the study, milk urea nitrogen (MUN) levels of the milk samples were different between the farms; the lowest was 7.37 mg/dL, and the highest was 32.92 mg/dL (P<0.001). The artificial insemination number was negatively correlated with the monounsaturated fatty acid (MUFA) concentration of total mix ration (TMR) (r=-0.502; P<0.01). The rations at the beginning of lactation included average 31.09% of w-6 fatty acids, 1.99% of w-3 fatty acids, and 2.95% of w-9 fatty acids. The MUN concentration of milk was negatively correlated with long-chain fatty acids (LCFA) and linoleic acid concentrations of TMR (P<0.05). As a result, it can be said that the easy soluble carbohydrates, crude protein, oleic acid, w-3 and w-6 fatty acids and energy levels that may be related to fertility in dairy cattle should be well adjusted. It was concluded that targeted milk production and fertility could be achieved by feeding as many nutrients as genetic capacity allowed.
Keywords: *Dairy cattle, Energy, Fatty acids, Feed intake, Fertility, Milk urea nitrogen.*

Süt Sığırı Çiftliklerinde Rasyon Yağ Asitleri ile Süt Üre Azotu ve Fertilite Sorunları Arasındaki İlişkinin İncelenmesi

Özet: Bu çalışmanın amacı, süt sığırı işletmelerinde postpartum dönemde rasyonun besinsel değişkenleri ile döl verimi arasındaki ilişkiyi göstermekti. Çalışmada kullanılan süt sığırı işletmelerinin hepsinde infertilite sorunu (buzağılama aralığı ≥14 ay ve suni tohumlama sayısı ≥1.8) vardı. Seçilen süt sığırı işletmelerinde rasyon ve süt örnekleri alındı. Sürü kayıt sistemlerinden fertilite kayıtları incelendi. Çalışmada, süt örneklerinin süt üre nitrojeni (MUN) seviyeleri işletmeler arasında farklı olup, en düşük 7.37 ve en yüksek 32.92 mg/dL idi (P<0.001). Suni tohumlama sayısı, total miks rasyonun (TMR) tekli doymamış yağ asiti (MUFA) konsantrasyonu ile negatif korelasyon gösterdi (r=-0.502; P<0.01). Laktasyon başlangıcında infertilite sorunu rasyonların ortalama yağ asidi konsantrasyonları w-6 yağ asitlerinin %31.09'u, w-3 yağ asitlerinin %1.99'u ve w-9 yağ asitlerinin %2.95'i idi. Sütün MUN konsantrasyonu, uzun zincirli yağ asitleri (LCFA) ve TMR'nin linoleik asit konsantrasyonları ile negatif korelasyon gösterdi (P<0.05). Sonuç olarak, süt sığırlarında fertilite ile ilişkili olabilecek kolay çözünür karbonhidrat, ham protein, oleik asit, w-3 ve w-6 yağ asitleri ile enerji seviyesinin iyi ayarlanması gerektiği sonucuna varılabilir. Hedeflenen süt üretimi ve doğurganlığın, genetik kapasitenin izin verdiği ölçüde besinlerle beslenerek sağlanabileceği sonucuna varıldı.
Anahtar Kelimeler: *Enerji, Fertilite, Süt sığırı, Süt üre azotu, Yağ asitleri, Yem tüketimi.*

Introduction

The ration, such as crude protein (CP), rumen degradable protein (RDP), urea, and fatty acid profile, effects fertility efficiency in dairy cattle. It is desirable to have a certain level of ammonia formed by fermentation in the rumen (Elrod et al., 1993; Otto et al., 2014; Roy et al., 2011). Microorganisms that ferment non-structural carbohydrates (starch and sugar) in the rumen benefit from the protein (microbial protein) formed as a nitrogen source: microorganisms fermenting structural carbohydrates (cellulose, hemicellulose) require only ammonia as the nitrogen source for their metabolism (Russel et al., 1992). In addition to the need for ammonia for microbial growth in the rumen, it has been demonstrated that ammonia is needed for effective fiber digestion (Griswold et al., 1996). The most important factor that causes an increase in blood urea nitrogen (BUN) or milk urea nitrogen (MUN) values are the RDP, which is a ruminal ammonia source (NRC 2001). This excess urea may adversely affect fertility in dairy cattle in the postpartum period (McCormick et al., 1999; Roseler et al., 1993). Notably, high urea nitrogen values in the postpartum period delay the re-

initiation of ovarian activities of dairy cattle in the postpartum period, prolongs the postpartum first insemination period, and increase the interval between conception and calving (Elrod and Butler, 1993; Tamminga et al., 1997). The concentration of MUN in milk is used to determine how much of the CP taken by TMR is not used for microbial protein synthesis by the microorganism but is transferred to the general circulation. The dairy cattle TMR, rich in alpha-linolenic acid, can increase blood progesterone concentration. This hormone is necessary for the healthy continuation of pregnancy in dairy cattle. This hormone stimulates follicular and luteal cells, and progesterone synthesis increases (Lopez et al., 2005). The presented study hypothesizes that the difference in nutritional variables may cause infertility problems in dairy farms under field conditions. The present study aims to investigate, in terms of these nutrition criteria, the reproductive issues such as non-fertility and low pregnancy rates frequently encountered in the postpartum period in existing dairy farms. The data to be obtained will determine what measures the breeders can take against the problems arising from the ration.

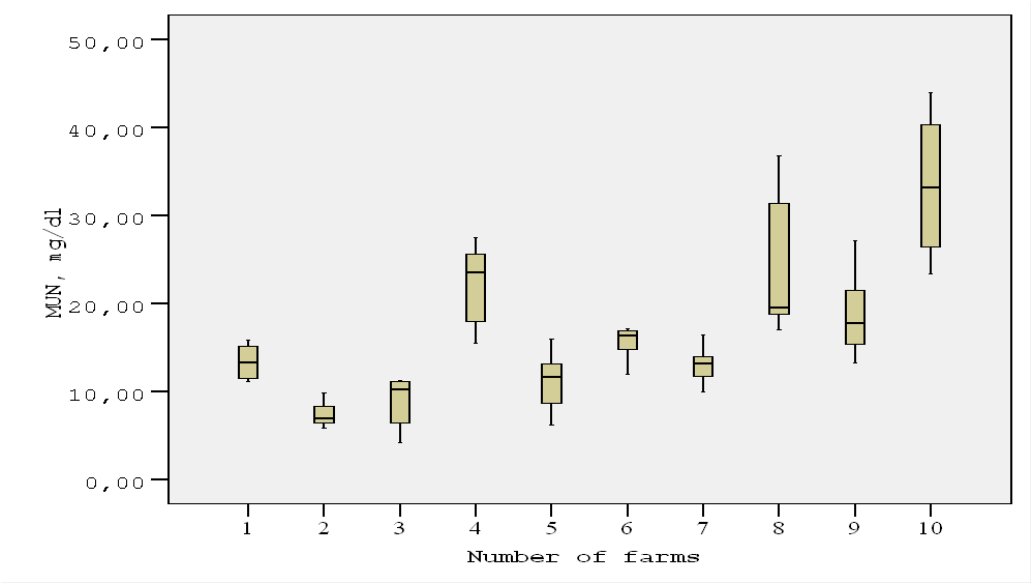

Figure 1. The MUN (milk urea nitrogen) concentrations of milk samples taken from dairy cattle.

Material and Methods

In the study, feed and milk samples were taken in dairy cattle enterprises, so there was no need for ethics committee approval or legal permission since no application was made on live animals.

We investigate the dairy cattle farms (≥25 cows) with infertility problems in the Nevşehir Province of Turkey. The farms' milk production and fertility data were obtained from the Breeding Cattle Breeders Association's e-Breeding Database (Table 1). The total mix ration (forage and concentrate feed) (Table 2) used by these dairy cattle farms were collected and analysed. The feed and milk samples were collected at two-week intervals.

The feedstuffs samples were taken in 2 kg of each ration component and airtight nylon bags (two weeks apart). The dried samples were analysed for crude protein (CP), ash, ether extract (EE), neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) contents (AOAC 1995; Van-Soest et al., 1991). The urea levels of concentrated feeds were determined in the UV-VIS spectrophotometer (SI Analytics, Germany) (R^2 = 0.9995) (Balthrop et al., 2011). All analyses were carried out in triplicate. The non-fibrous carbohydrate (NFC) values of TMR's were calculated according to NRC (2001). The total digestibility nutrient matter (TDN), digestibility energy (DE), metabolizable energy (ME), and net energy lactation (NEL) were calculated according to Donker (1989). Milk samples were taken from individual infertile dairy cattle, approximately 50 mL twice, two weeks apart. The samples were taken from 10 infertile dairy cattle in 10 different dairy farms. The milk samples were analysed for milk urea nitrogen concentration (MUN) using commercial kits in a MUN analyses device (cdR FoodLab Junior MUN, Italy). The EE of TMR's were methylated with the three-stage procedure of Wang et al. (2015). The free methylated fatty acids in n-hexane were detected in a gas chromatograph with a flame ionization detector (GC-FID, Thermo Scientific, USA) (Kara, 2020). The one-way variance analysis was conducted on the parameters tested in different dairy cattle

Table 1. Reproductive efficiency and other parameters of farms having fertility problems.

Number of farms	Breed of cattle	Number of lactation	Average milk yield of farms (L/day)	Number of insemination until conception	Ovarian cyst	Calving range, month	Approximate weight in the first 100 days, kg	Average milk yield, kg/day	DM intake, kg/day	Daily given feedstuffs, kg as DM							
										Commercial concentrate feed	Corn silage	Corn flake	Lucerne hay	Sugar beet pulp	Wheat straw	Oat hay	Meadow hay
1	*Holstein*	Multiparous	22	1.8	+	14.0	625	22	15.85	7.20	-	1.80	3.60	1.45	1.80	-	-
2	*Simmental*	Uniparous	26	1.8	+	14.5	557	26	18.76	5.40	6.16	-	4.50	-	2.70	-	-
3	*Holstein*	Multiparous	20	2.4	+	15.0	680	20	19.70	7.20	5.80	-	4.90	-	1.80	-	-
4	*Simmental*	Multiparous	18	2.5	+	14.5	680	18	16.70	7.20	-	-	3.6	2.32	3.60	-	-
5	*Simmental*	Multiparous	24	2.3	+	15.0	625	24	18.74	7.20	6.14	-	3.60	-	1.80	-	-
6	*Holstein*	Multiparous	24	2.6	+	15.0	680	24	19.45	7.20	4.15	1.80	3.60	-	2.70	-	-
7	*Holstein*	Multiparous	18	1.9	+	16.5	680	18	13.99	5.40	3.39	-	3.40	-	1.80	-	-
8	*Holstein*	Uniparous	26	2.1	+	13.5	557	26	17.33	8.10	-	-	3.60	1.13	2.70	-	1.80
9	*Holstein*	Uniparous	28	2.2	+	14.0	557	28	21.53	9.90	-	1.80	3.60	0.83	1.80	3.60	-
10	*Holstein*	Multiparous	25	2.0	+	14.5	680	25	19.09	7.20	4.69	-	4.50	-	2.70	-	-

Table 2. The chemical analysis values of dairy cattle TMR.

Farm No	CP	EE	Ash	NDF	ADF	HC	HemC	NFC	ADIN*	TDN	ME	NEL	CP**	Urea**
1	12.62^{c}	2.92^{b}	6.79^{c}	38.00^{c}	25.12^{c}	22.86^{ab}	12.88^{c}	39.66^{a}	3.89	61.01^{a}	2.34^{a}	1.39^{ab}	15.85^{ab}	0.13^{a}
2	11.64^{d}	2.79^{b}	8.18^{bc}	55.79^{a}	29.50^{a}	27.33^{a}	26.29^{a}	21.59^{c}	1.65	59.47^{b}	2.27^{b}	1.34^{b}	17.29^{ab}	0.12^{a}
3	11.84^{d}	5.17^{a}	8.95^{b}	46.44^{bc}	26.08^{c}	22.21^{ab}	20.36^{bc}	27.28^{bc}	2.11	60.67^{ab}	2.33^{a}	1.38^{ab}	15.93^{ab}	0.11^{ab}
4	12.15^{c}	3.42^{ab}	8.20^{bc}	49.00^{b}	30.36^{a}	$26.84a^{b}$	18.63^{c}	27.21^{bc}	3.86	59.17^{b}	2.26^{b}	1.34^{b}	15.28^{ab}	0.11^{ab}
5	12.16^{c}	2.83^{b}	9.57^{a}	46.15^{bc}	25.72^{c}	20.37^{b}	20.43^{bc}	30.07^{b}	2.00	60.79^{ab}	2.33^{a}	1.38^{ab}	16.47^{ab}	0.10^{b}
6	11.36^{d}	2.70^{b}	7.72^{c}	48.77^{bc}	23.48^{c}	25.34^{ab}	25.29^{a}	29.43^{bc}	2.47	61.58^{a}	2.37^{a}	1.40^{a}	15.07^{a}	0.11^{ab}
7	13.02^{b}	3.23^{b}	9.93^{a}	47.66^{bc}	25.09^{c}	21.58^{ab}	22.57^{b}	26.15^{bc}	3.50	61.01^{a}	2.34^{a}	1.39^{a}	18.66^{ab}	0.10^{b}
8	13.20^{b}	2.83^{b}	8.95^{b}	42.95^{c}	24.99^{c}	21.94^{ab}	17.96^{c}	32.05^{ab}	1.78	61.05^{a}	2.35^{a}	1.39^{a}	18.43^{ab}	0.10^{b}
9	13.61^{a}	2.98^{b}	8.60^{b}	41.12^{c}	24.42^{c}	21.69^{ab}	16.69^{c}	33.68^{ab}	2.04	61.25^{a}	2.36^{a}	1.40^{a}	19.50^{a}	0.09^{b}
10	12.45^{c}	3.01^{b}	8.25^{bc}	49.45^{bc}	28.68^{b}	27.25^{a}	20.77^{bc}	26.82^{bc}	1.84	59.76^{b}	2.29^{ab}	1.35^{b}	17.66^{ab}	0.11^{ab}
Means	12.40	3.19	8.51	46.53	26.34	23.74	20.19	29.39	2.51	60.57	2.32	1.37	17.04	0.11
Max.	13.66	7.07	10.06	56.65	30.41	28.41	27.19	40.77	4.32	61.60	2.37	1.41	19.80	0.14
Min.	11.26	2.34	6.70	34.55	23.41	12.57	9.56	20.66	1.44	59.15	2.26	1.34	14.44	0.10
SEM	0.12	0.16	0.16	0.95	0.41	0.58	0.80	0.96	0.21	1.43	0.006	0.004	0.35	0.002
P value	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	0.120	<0.001	<0.001	0.004	0.01	0.001

CP: crude protein, EE: diethyl ether extract, NDF: neutral detergent fiber, ADF: acid detergent fiber, HemC: hemicellulose, NFC: non-fibrous carbohydrate, ADIN: acid detergent nitrogen, TDN: total digestible nutrients, ME: metabolic energy (as Mcal/kg DM), NEL: net energy lactation (as Mcal/kg DM). $^{a-c}$: The difference between the average values indicated by different letters in the same column is important.
* The ADIN value is given as % CP in the ADF residue. **: these are as % in concentrate mix feeds, SEM: Standard error of means

Table 3. The compositions of fatty acid (g/100g fat) in dairy cattle TMR's.

Number of farm	w-3	w-6	w-9	w-3/w-6	LA	AA	ALA	EPA	SFA	UFA	MUFA	PUFA	MCFA	LCFA	VLCFA
1	1.17^{d}	31.23^{ab}	5.50	0.037^{cd}	26.15^{a}	44.67^{abc}	0.41^{ab}	0.25^{b}	61.73^{abc}	38.18^{bc}	5.78	32.40^{bcd}	0.06^{b}	97.08^{a}	2.76^{b}
2	1.44^{bcd}	26.26^{b}	5.75	0.055^{bcd}	21.66^{b}	44.94^{ab}	0.29^{b}	0.25^{b}	66.33^{ab}	33.65^{bcd}	5.94	27.71^{cd}	0.07^{b}	94.91^{ab}	4.99^{ab}
3	1.17^{d}	40.25^{a}	2.74	0.029^{d}	26.59^{a}	33.56^{bcde}	0.42^{ab}	0.25^{b}	55.01^{c}	44.99^{a}	3.57	41.42^{a}	0.19^{b}	95.71^{ab}	4.08^{ab}
4	1.26^{cd}	32.55^{ab}	3.34	0.038^{cd}	24.16^{b}	40.66^{abcd}	0.37^{ab}	0.24^{b}	62.62^{abc}	37.40^{bc}	3.58	33.82^{bc}	0.18^{b}	92.72^{ab}	7.11^{ab}
5	2.14^{abcd}	31.71^{ab}	0.29	0.067^{abcd}	24.83^{b}	40.27^{abcd}	0.69^{ab}	0.65^{ab}	65.59^{ab}	34.47^{bcd}	0.61	33.86^{bc}	0.20^{b}	96.08^{a}	3.70^{ab}
6	1.72^{bcd}	28.06^{b}	0.21	0.062^{bcd}	24.51^{b}	47.46^{a}	0.60^{ab}	0.53^{ab}	69.75^{a}	30.25^{d}	0.46	29.79^{bcd}	0.08^{b}	98.12^{a}	1.80^{b}
7	3.19^{a}	32.23^{ab}	1.89	0.099^{a}	22.27^{b}	31.87^{de}	0.88^{a}	0.77^{a}	62.82^{abc}	37.76^{bc}	2.33	35.42^{b}	0.41^{ab}	91.95^{ab}	7.93^{ab}
8	2.38^{ab}	26.34^{b}	3.13	0.091^{ab}	18.07^{b}	28.44^{de}	0.65^{ab}	0.65^{ab}	67.31^{a}	32.12^{cd}	3.39	28.72^{cd}	0.86^{a}	90.19^{ab}	8.15^{ab}
9	2.29^{abc}	31.22^{ab}	2.63	0.073^{abc}	21.66^{b}	32.44^{cde}	0.69^{ab}	0.56^{ab}	63.77^{abc}	36.40^{bcd}	2.88	33.51^{bc}	0.34^{ab}	91.06^{ab}	8.50^{ab}
10	3.13^{a}	31.05^{ab}	4.05	0.102^{a}	19.00^{b}	25.14^{e}	0.66^{ab}	0.57^{ab}	56.70^{bc}	38.63^{b}	4.45	34.18^{bc}	0.94^{a}	83.32^{b}	10.52^{a}
Means	1.99	31.09	2.95	0.066	22.89	36.94	0.56	0.47	63.16	36.38	3.30	33.08	0.33	93.11	5.95
Max.	3.45	42.98	9.44	0.12	32.28	49.22	0.95	0.83	70.59	48.54	9.88	43.73	1.63	99.34	11.65
Min.	0.75	24.90	0.18	0.02	13.42	21.79	0.20	0.06	46.92	29.43	0.38	26.60	0.03	71.16	0.61
SEM	0.79	4.16	2.60	0.02	0.68	1.49	0.04	0.04	1.65	0.79	0.48	0.74	0.35	5.52	3.53
P value	<0.001	<0.001	0.085	<0.001	0.033	<0.001	0.016	0.001	0.001	<0.001	0.109	<0.001	<0.001	0.019	0.008

ALA: α-Linolenic acid (C18:3n3), LA: linoleic acid, AA: Arachidic acid, EPA: cis-5,8, 11,14,17-Eicosapentaenoic Acid (C20:5n3), DHA: cis-4,7,10,13,16,19-Docosahexaenoic Acid (C22:6n3), LCFA: Long chain fatty acids, MCFA: Medium chain fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids, SFA: Saturated fatty acids, UFA: Unsaturated fatty acids, VLCFA: Very long chain fatty acids, SEM: Standard error of means, $^{a-e}$: The difference between the average values indicated by different letters in the same column is important.

Table 4. Comparison of the ration and milk variables analysed with Pearson correlation(*r*).

	ME	NEL	CP	NDF	ADF	HemC	NFC	ADIN	MUN	AIN	MUFA	PUFA	LCFA	LA	ALA
TDN	0.996**	0.990**	0.297	-0.562**	-1.000**	-0.159	0.520**	-0.033	-0.183	0.610**	0.362*	0.021	0.257	0.111	0.419*
ME	1	0.986**	0.336	-0.588**	-0.996**	-0.191	0.538**	-0.027	-0.154	0.599**	-0.362*	0.045	0.224	0.095	0.441*
NEL		1	0.333	-0.581**	-0.990**	-0.187	0.532**	-0.008	-0.209	0.571**	-0.355	0.033	0.272	0.127	0.403*
CP			1	-0.586**	-0.297	-0.547**	0.431*	0.066	0.249*	-0.278	0.078	0.018	-0.384*	-0.378*	0.415*
NDF				1	0.562**	0.906**	-0.953**	-0.187	-0.060	-0.073	0.002	-0.126	-0.055	-0.076	-0.127
ADF					1	0.159	-0.520**	0.033	0.183	-0.611**	0.362*	-0.021	-0.257	-0.111	-0.418*
HemC						1	-0.871**	-0.236	-0.166	0.226	-0.183	-0.140	0.066	-0.034	0.063
NFC							1	0.192	0.067	0.056	-0.012	-0.050	0.143	0.104	0.059
ADIN								1	-0.071	-0.393	0.187	0.136	0.185	0.355	-0.034
MUN									1	-0.065	0.008	0.000	-0.597**	-0.376*	0.189
AIN										1	-0.502**	0.193	0.226	0.174	0.204
MUFA											1	-0.217	-0.327	-0.353	-0.548**
PUFA												1	0.081	0.500**	0.251
LCFA													1	0.781**	0.086
LA														1	0.027

NDF: neutral detergent fiber, ADF: acid detergent fiber, HemS: hemicellulose, NFC: fiber non-carbohydrate, ADIN: acid detergent nitrogen, CP: crude protein, MUN: Milk urea nitrogen, TDN: total digestible nutrients, ME: metabolic energy, NEL: net energy lactation, LCFA: long chain fatty acids, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, LA: linoleic acid, ALA; Alpha linolenic acid, AIN: artificial insemination number. **: P< 0.01, *: P<0.05.

farms. Significance was defined at P values of <0.05. The relationship between the ration and milk variables was determined by the Pearson correlation and SPSS 15.0 package program.

Results

The amount and energy-protein content of rations at the beginning of lactation in dairy cattle farms in the Cappadocia region are given in Table 1. The nutrient matter and energy values of TMR's are given in Table 2. The MUN concentrations of milk samples taken from dairy cattle showed significant differences among farms (P<0.001); the lowest value was 7.37 mg/dL (in number 2 from farms), and the highest value was 32.92 mg/dL (in number 10 from farms) (Figure 1). The ratio of PUFA in the fatty acid profile of the TMR's ranged from 27% to 41%. Linoleic acid levels were between 18.07 and 26.59%. The alpha-linolenic acid level was between 0.29 and 0.88% (Table 3). The ME and NEL values of dairy cattle TMR were positively correlated with ALA and NFC levels of dairy cattle TMR and artificial insemination number and negatively correlated with NDF and ADF values of TMR (P<0.01). The CP value of TMR was positively correlated with MUN concentration (P<0.05). The MUN was negatively correlated with LCFA and linoleic acid of TMR (P<0.05). The artificial insemination number was negatively correlated with the MUFA of TMR (P<0.01) (Table 4).

Discussion

The DM consumption of the dairy cattle in some dairy cattle farms differed according to the amount of milk yield. In some farms, the animals decreased the feed consumption milk yield and the postpartum physiological needs of dairy cattle (NRC 2001). Previous researchers associated the low DM consumption of dairy cattle in early lactation with plasma cholecystokinin increase linearly during postpartum periods (Choi and Palmquist, 1996; Opara et al., 1994). Uterine involution at the beginning of lactation to 30-35 days to continue to reach maximum feed intake by the rumen is another reason (NRC, 2001). The CP levels were slightly lower than the CP requirement calculated by the formula, but these values were lower than the genetic capacity of the herd (NRC, 2001). Likewise, it was found that the ME and NEL values of TMR's were enough according to the current milk yield of the animals, but they were far from the high milk production targeted by the genetic capacity (Alderman et al., 2001). In general, it is understood that dairy cattle in the farms are undernourished in terms of milk yield and have a low milk yield average. For ideal rumen fermentation in dairy cattle diets, 25-33% NDF, 17-21% ADF, and 44-36% NFC in the TMR are required (NRC, 2001). The mean TDN values (60.5%) of dairy cattle TMR were lower than the recommended TDN values (68 or 78%) of TMR's at early lactation for large dairy cattle breeds by NRC (2001). The low energy and TDN of the TMR consumed by dairy cattle may be related to the problem of fertility. Ovarian follicles in dairy cattle contain insulin receptors (Bossaert et al., 2010). Postpartum ovarian activity resumption and average oestrus cycle retention in cows can delay due to insufficient peripheral insulin levels in the immediate postpartum period with low NFC levels of TMR (Van holder et al., 2005). Therefore, the glycogenic TMR's in the postpartum period are recommended to increase peripheral insulin concentrations and for normal ovarian activities of dairy cattle (Gong et al., 2002). The dairy cattle organism directed to gluconeogenesis due to the high energy is needed for milk yield after calving. When plasma glucose levels decrease, the fat mobilizes from the fat stores in the organism and tries to provide the necessary energy until the energy balance shifts to positive (Gong et al., 2002; Adewuyi et al., 2005). In the present study, it is thought that dairy cattle farms experience fertility problems at the beginning of lactation due to low NFC, fat and energy levels, and possible ration digestibility.

The CP contents of dairy cattle TMR's in the investigated farms had a positive correlation with MUN values that were parallel with the results of Elrod et al. (1993). The effect of dietary CP or nitrogenous compounds on milk MUN value can make occur the urea formation in the liver with a breakdown of RDP in the rumen and the absorbed amino acids by the degrading of RUP in the intestine. Under normal conditions, microbial protein is produced by microorganisms in the presence of alpha-keto acids by microbial fermentation from ammonia in the rumen. However, excess ammonia is absorbed from the rumen and mixed with the liver to the general circulation, causing blood and MUN levels to rise (Roy et al. 2011). Consistent with our results, Roy et al. (2011) stated that the high NDF value in dairy cattle TMR's and the increase in rumen pH value could increase $NH4^+$ absorption and blood transfer from the rumen wall and increase the MUN value. The 10-14 mg/dL MUN concentration is considered normal for the MUN value in milk, whereas <10 mg/dL can be association with low CP or RDP in TMR or effective conversion of ruminal ammonia to microbial protein, and >14 mg/dL MUN value may be effective NFC insufficiency in TMR (Aydın and Güler, 2004; Aydın, 2007). In the present study, the milk MUN values in the two farms were

less than 10 mg/dL, although the CP value of TMR's did not lower significantly may be ruminal low NFC concentration. The MUN value was found to be higher than 14 mg/dL in half of the investigated farms. The difference in MUN values despite similar CP consumption in the farms examined suggests that other TMR contents and environmental conditions may also be helpful. Elrod et al. (1993) reported that the protein in the TMR is effective in the uterine environment and that high pH in the ration may be associated with decreased fertility in the uterus. In the present study, it is thought that increasing MUN value may decrease uterine pH and may adversely affect embryo implantation.

Most of the w-3's found in dairy cattle TMR's are obtained from forage. Significant levels of LA, oleic acid and ALA are feedstuffs of sunflower, rapeseed, flaxseed, soybean, corn, safflower, flaxseed, soybean, peanut and canola (Chong et al., 2006). Infertility is considered a grave problem in the dairy industry due to the increased number of artificial inseminations per conception and the irregular shaping of the oestrus (Lopez et al., 2005). A negative correlation between the number of seeding and ration MUFA concentration in the present study seems to be related to oleic acid concentration. Mobilization of fatty acids from adipose tissue during metabolic stress at the onset of lactation will increase the amount of free fatty acids in the blood and follicular fluid and thus affect oocyte quality. In a previous study, the effect of three fatty acids (saturated palmitic and stearic acid and unsaturated oleic acid) on lipid storage and development of the oocyte was investigated and it was found that palmitic and stearic acid had an inhibitory effect on oocyte development, but MUFA oleic acid eliminated this adverse effect and has a positive impact (Aaderma et al., 2011). In the present study, a negative correlation between milk MUN concentration and ration LCFA and linoleic acid levels, and the use of ammonia for microbial protein production by rumen microorganisms may be due to LCFA and linoleic acid reduction.

In conclusion, this study demonstrated that possible problems related to TMR in dairy cattle farms having fertility problems can be listed as follows: It is thought that dairy farms experience fertility problems in the postpartum period due to low energy levels and possible low digestibility (approximately 60% TDN) in TMR's. The low levels of oleic acid and w-3 and w-6 fatty acids are thought to be the cause of fertility problems in dairy cattle. High NDF values in dairy cattle TMR's increase in rumen pH may increase NH_4^+ absorption and increase MUN value in the milk. Not enough ammonia is used in microbial protein production, which may result from low NFC levels in diets, and the urea in general circulation may decrease the pH of the uterus. Due to the high level of NDF and ADF, dairy cattle may enter a negative energy balance.

Çıkar çatışması

Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik izin

Bu çalışma "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik" Madde 8 (k) gereği HADYEK iznine tabi değildir. (Bu durumda "Etik Beyan Formu" veya "Aydınlatılmış Onam Formu" doldurulup tüm yazarlarca imzalanarak sisteme yüklenmelidir.)

Finansal destek

Bu çalışma, Erciyes Üniversitesi Bilimsel Araştırma Projeleri Birimi tarafından TYL-2018-8020 proje numarası ile desteklenmiştir.

Yüksek Lisans Tezi: Bu makale Osman Semih ÇAVDAR'ın *"Kapadokya Yöresindeki Süt Sığırı İşletmelerinde Postpartum Dönemde Besleme ile Döl Verimi Arasındaki İlişkinin Araştırılması"* isimli Yüksek Lisans tez çalışmasından üretilmiştir.

Benzerlik Oranı

Makalenin benzerlik oranının sisteme yüklenen raporda belirtildiği gibi % 13 olduğunu beyan ederiz.

Yazar Katkıları

Fikir/Kavram: OSÇ, KK
Tasarım: KK
Denetleme/Danışmanlık: KK
Veri Toplama ve/veya İşleme: OSÇ, KK
Analiz ve/veya Yorum: OSÇ, KK
Kaynak Taraması: OSÇ, KK
Makalenin Yazımı: OSÇ, KK
Eleştirel İnceleme: KK

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