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EFFECT OF DIETARY CATION – ANION DIFFERENCES (DCAD) ON RUMEN FERMENTATION, DIGESTIBILITY AND MILK PRODUCTION IN FRIESIAN DAIRY COWS

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SUMMARY

Twenty Friesian cows were used to study the effect of dietary cation-anion differences on digestibility, rumen fermentation and milk production. Animals were divided into four groups. All groups were fed a basal diet consisting of 30 kg berseem, 6 kg concentrate feed mixture (CFM), 6 kg rice straw &75g soya bean meal (SBM) as a carrier for anionic salts/head/day. Anionic salts were used to control DCAD of the ration at the following levels (groups): treatment (1): Control group was fed the basal diet without anionic salts; treatment (2): DCAD was controlled to be 0 mEq/ Kg DM; treatment (3): DCAD was controlled to be negative 150mEq/Kg DM; treatment (4): DCAD was controlled to be negative 150mEq/Kg DM by using Anio - Norel (commercial product).

Animals received treatments three weeks before expected calving date and stopped at delivery day.

Results obtained showed that:

1. Rumen pH was affected by treatment, values were 7.5, 6.9, 6.6 and 7.1 for groups (1, 2,3and 4 respectively).
2. Organic matter digestibility ranged between 59.43% for group (3) and 57.23% for group (4).
3. Crude fiber digestibility was not significantly ($P> 0.05$) affected by treatment.
4. Treated groups showed lower 305 days milk yield when compared with control group ($P< 0.05$).
5. Treatments didn't affect milk components significantly ($P> 0.05$) except for milk fat where it was lower for treatment (4) compared with other treatments.

Keywords: *DCAD, digestibility, rumen fermentation, milk production and dairy cows.*

INTRODUCTION

Manipulation of dietary cation-anion difference (DCAD) is a procedure commonly used in dairy cattle farms to control milk fever. Dietary cation-anion difference (DCAD) can be used to determine the relationship between strong cations (sodium, potassium, calcium and magnesium) and anions (sulphure and chlorine) and thus predict whether a diet will evoke an acidic or alkaline response when fed to a dairy cow (Stewart, 1983 and Oetzel and Barmore, 1993).

DCAD is expressed as mEq/100 g DM or mEq/kg DM. Several equations has been suggested to calculate DCAD:

$(Na^+ + K^+) – (Cl^- + S^-)$ = mEq/100 gm of DM. (Tucker *et al.* (1992)

$(Na^+ + K^+) – (Cl^-)$ = mEq/100 gm of DM… (Mongin, 1981).

The DCAD may also be calculated by using the dietary percent of respective minerals on dry matter basis (DM) with the following equation (Olson, 1991and West, 1993).

DCAD = {(Na%/0.023) + (K% / 0.039)} – {(Cl%/0.0355) + (S%/0.016)}mEq/100g DM.

Several researcher studied the effect of pre-partum anionic supplementation on feed intake, (DeGroot *et al.,* 2010), Hu and Murphy (2004), Hu *et al.* (2007), West *et al.* (1992). They all agreed that DM intake increases as DCAD increases. While Spanghero (2004) reported a decrease in DM intake by animals fed low DCAD diets. Increased DM intake at high DCAD diets might be due to increased rumen PH (Tucker *et al.,* 1988; Sharif *et al.,* 2009; and Sharif *et al.,* 2010) that makes the ruminal environment alkaline, which is pre – requisite for optimum ruminal microbial activity.

DeGroot *et al.* (2010), mentioned that pre-partum diet had no effect on pre-partum dry matter intake while postpartum dry matter intake and milk yield for cows fed anionic diets pre-partum were greater compared with those fed the control diet.

Shahazad *et al.* (2011), used four iso–nitrogenous and iso-caloric diets having different DCAD (-22,-11, +11 and + 22 mEq/100g DM) to study the effect of acidifying diet on DM intake and digestibility in Buffaloes. They concluded that a linear increase in nutrient intake was recorded with increase in the DCAD level. On the other hand Buffaloes fed with high anionic diets had higher nutrients digestibility than those fed with high cation diets. Influence of varying DCAD diets on nutrients digestibility was investigated by Delaquis and Block (1995 a & b) and Tucker *et al.* (1991).They used different positive DCAD values and found no significant effect on ADF and NDF digestibility.

Ganjkhanlou *et al.* (2010) conducted a study to evaluate the effect of three diets with different (DCAD) values (+13, 0 and – 13 mEq/100 g DM) on milk production in Holstein Cows. Their results indicated that production of milk and 3.5% fat corrected milk were increased with decreasing DCAD. On the other hand milk composition was not affected by DCAD.

The main objective of this study was to investigate the effect of pre-parturient DCAD levels on rumen fermentation, digestion and milk production of lactating Frisian cows.

MATERIALS AND METHODS

The field experiment of this study was conducted from April 2014 to August 2015 (treatments started three weeks before expected delivery date and ended on day of parturition, while milk parameters were recorded until the end of lactation season) at El Karada experimental station, Kafr El-Sheikh governorate, which belongs to the Animal Research Institute, Ministry of Agriculture.

Twenty Late pregnant Friesian cows (3 to 4 weeks before calving) were used through the experiment. Animals were divided according to the parity to four groups, five cows each. Animals were housed in four separate open areas provided with water sinks and shades. Animals were tied at feeding time to be fed individually berseem, concentrate feed mixture and rice straw according to NRC (2001). Ingredients of concentrate feed mixture are shown in Table (1) and chemical composition of feedstuffs are shown in Table (2).

All animals were fed on the basal diet and the following additives:

Treatment (1) : Control group received soybean meal without anionic salts (soybean meal was used as a carrier for anionic salts which added to other groups).

Treatment (2): Received (150 gm) anionic salt to achieve DCAD equal zero mEq /Kg DM. composition of anionic salts are shown in Table (3).

Treatment (3): Received (350 gm) Soybean meal + anionic Salt to achieve DCAD equal (-150) mEq /Kg DM.

Treatment (4): Received (350 gm) Anio-Norel compound to achieve DCAD equal (-150) mEq /Kg DM. composition of Anio-Norel is presented in Table (4).

DCAD was calculated according to the following equation:

$$\text{DCAD mEq /Kg DM} = (\text{Na} + \text{K}) - (\text{Cl} + \text{S}).$$

Representative samples were taken from all feed stuffs before, during, and after experimental period. The three samples of each feedstuff were kept frozen and composited till analysis. Rumen samples were collected using stomach tube once after one week of treatments three hours after feeding. Samples were filtered through four layers of cheesecloth's and pH was measures using portable pH meter. Two drops of formaldehyde were added to the sample in ordered to stop microbial growth then samples were kept frozen. Fecal samples were grabbed from the rectum of experimental animals three executive days one week after treatment beginning. Samples were frozen. The three samples of each animal were composited before analysis. Feed and fecal samples were analyzed for proximate analysis according to AOAC (2006) and fiber fractions were determined according to Goering and Van Soest (1970) modified by Van Soest *et al.*, (1991). Digestibility was determined using two internal markers Acid Detergent Lignin (ADL) and Acid Insoluble Ash (AIA).

Milk production was recorded twice daily. Fifty milliliters of milk were obtained weekly for each animal up to the third month of milking then monthly samples were collected up to the end of milking season. Samples were send fresh to be analyzed for fat, protein, total solids and solids nonfat and lactose using Milkoscan device (Foss Electric, Denmark) belongs to International Center for Animal Husbandry in kafr El-Sheihk, Sakha.

All the obtained data were statistically analyzed using the General Linear Model Program of SAS (2000). Statistical models were as follow:

For digestion: $Y_{ijk} = \mu + t_i + m_j + t_i m_j + e_{ijk}$

Were Y_{ijk}, is the dependent valuable; μ is the overall mean. t_i; is the effect of treatment; m_j, is the effect of marker ; t_im_j, is the effect of interaction between treatment and marker; e_{ijk}, is the residual error.

For milk components: $Y_{ijk}= \mu + t_i + w_j + t_iw_j + e_{ijk}$

Were Y_{ijk}, is the dependent valuable; μ; is the overall mean. t_i; is the effect of treatment; w_j, is the effect of week of lactation ; t_iw_j, is the effect of interaction between treatment and week of lactation; e_{ijk}, is the residual error.

For milk production: $Y_{ijkl}= \mu + t_i + m_j + t_im_j + n_k + e_{ijkl}$

Were Y_{ijkl}, is the dependent valuable; μ is the overall mean. t_i; is the effect of treatment; m_j, is the effect of milking time (morning and evening) ; t_im_j, is the effect of interaction between treatment and milking time; n_k is the effect of lactation month e_{ijkl}, is the residual error.

Duncan's new multiple range test (Duncan, 1955) was used to determine the significant differences among treatments.

RESULTS AND DISCUSSIONS

Data in Table (5) showed, a trend of lowered dry matter intake with more anionic diets (i.e. a decrease in DCAD). These findings are similar to those obtained by Oetzel and Barmore(1993) who observed an increasing trend in dry matter intake as DCAD was increased from -109 to +313 mEq/kg DM. In the present study DCAD decreased from +90 for control group to -150 mEq/kg DM in treatments (3&4). However treatment (4) (Anio–Norel group) didn't have this negative effect on dry matter intake. This may be explained by buffering effect of materials included in its composition which kept the rumen PH close to control group. Explanation of the effect of rumen PH on dry matter intake was cleared by Tucker *et al.* (1988); Sharif *et al.* (2009) and Sharif *et al.* (2010). They mentioned that the ruminal environment alkalinity is pre-requisite for optimum ruminal microbial activity. Goff *et al.* (1988) and Shahzad *et al.* (2008a, b) stated that explanation of dry matter intake decreased might be the un-palatability of anionic salts used to reduce the DCAD level. Another possible explanation might be that low DCAD induces slight metabolic acidosis, which reduces dry matter intake (Block, 1994).

Rumen PH values, rumen VFA *mM* and rumen ammonia mg/100 ml are shown in Table (6). Rumen PH tended to be lower with treatments (2) and (3) where anionic salts were added to the diet, compared with control group. However when Anio-Norel was used the PH values increased. To be similar to treatment to ($p > 0.05$) but still significantly less than control and more than treatment (3) ($p < 0.05$).This can be explained by buffer effect of yeast and magnesium oxide included in Anio- Norel mixture. Total volatile fatty acid concentrations were increased with treatments (2) and (3) when compared with control and Anio-Norel groups. Ammonia concentrations were lower with treatments (2) and (3) (14.8mg/100ml) while they were higher for control and Anio- Norel groups (16.4, 16.3 mg/100ml) respectively. In contrast with the present results Apper-Bossard *et al.* (2010) stated that increasing DCAD did not affect mean ruminal pH, neither total VFA concentration nor molar proportion of VFA and rumen ammonia concentration, however levels of DCAD of their studies ranged between 0, 150, or 300 mEq/kg of DM while the present study used DCAD levels of +90, 0, -150 mEq/kg of DM. Moreover they used milking cows rather than dry cows used during the present study. A localized rumen buffering effect could not be excluded and could be linked with a higher amount of HCO3 recycled into the rumen. (Apper-Bossard *et al.,* 2010).

Analysis of variance showed that mean effect (treatments) affected significantly ($p<0.05$) hemi-cellulose, cellulose and organic matter digestibility. While there was no significant effect of treatments on crude protein, fiber, ADF, NDF and dry matter digestibility. Data in Table (7) indicated that treatment (4) (Anio-Norel) decreased significantly ($p<0.05$) the digestibility of cellulose. Hemicellulose digestion was decreased by treatment (2). Organic matter digestibility was higher significantly for treatments (2 and 3) and lower for treatments (1 and 4). Influence of varying DCAD diets (481,327 mEq/kg DM) on nutrients digestibility was investigated by Delaquis and Block (1995a). They reported that DCAD had non-significant effect on ADF and NDF digestibilities in dry cows.

A comparison between apparent digestibility of different nutrients calculated by two different internal markers are presented in Table (8). When Acid insoluble Ash (AIA) and Acid Detergent lignin (ADL) were used to calculate the digestibility of nutrients, values obtained were in general higher significantly ($p<0.05$) forADL when compared with AIA. This is true for all nutrients except for crude protein where differences were not significant. Juvenal Kanani *et al.* (2014) concluded that acid-detergent insoluble ash appear to be appropriate internal markers for predicting fecal output and dry matter digestibility by cattle fed Bermuda grass hay of varying quality. They recommended that such internal markers will facilitate larger studies involving greater numbers of animals and forages to determine the digestibility by applying the marker ratio technique. These studies can then be used to develop more accurate equations to predict energy values of forages based on the relationship of dietary components to digestibility across a wide range of forages

Significant interaction between markers and treatments was only observed with organic matter digestibility (Table 9). Although, in general, values with (ADL) were higher than (AIA), differences were not the same. Differences between organic matter digestibility obtained by the two markers were the highest in control group 33.79 and the lowest in group (2) 29.98. Fluctuations of these differences may be explained by minerals contained in different treatments. In their studies, Juvenal Kanani *et al.* (2014) reported an interaction between marker and diet at significance level of ($P< 0.06$) while the present study find the interaction significant at ($p< 0.05$). The interaction may be explained by different sources of minerals used to manipulate DCAD level.

Total milk yield (Kg/season), milking days and corrected milk yield for 305 days are shown in Table (10) and diagram (1). Data indicated that total milk through the whole season was the highest in group (2) and the lowest in group (3). These may be due to the length of milking days which were 191.2 milk days in group (3) and 341.7 milk days for group (2). When milk production was corrected for 305 days the control group showed the highest values 3421.62 Kg followed by group (2) 3191.91 Kg and Anio- Norel group 3181.29 Kg while group (3) stayed lowest to 612.65Kg.

Results of Table (11) indicated that milk yield was lowered by treatment (3) compared with the other groups which had statistical similar values. These values of milk production may reflect the dry matter intake through the transitional period. In contrast Ganjkhanlou *et al.* (2010) reported that production of milk and 3.5% fat corrected milk were increased with decreasing DCAD. These conflict of results may be explained by variation in length of experimental periods, cows body weight (620 Kg Vs 520 in the present study) and season of lactation. In their study animals were fed experimental diets for 60 ± 5 days comparing with last three weeks before delivery (21 days) in the present study. However Tucker *et al*., (1988) and West *et al.*, (1991) observed increased milk yield in lactating cows as DCAD level increased from -10 to +31 mEq/100g DM. which agree with the finding of the present study.

Morning and evening milk yield through 30 days were presented in Table (12). Morning milk yield was significantly higher than evening.

Monthly milk production decreased with increasing lactation month after parturition (Table 14). The relationship tended to be linear in treatments (1&2) while it was more quadratic in treatment (4) revealing more persistence for Anio-Norel group.

Data in Table (15) present the effect of treatments on milk components throughout the first 16 weeks of lactation. Results indicated that anionic salts didn't affect milk protein, lactose, total solid (TS) and solid not fat (SNF). However milk fat was decreased in treatment (4) when compared with other treatments. Interaction between weeks and treatments were not significant for all milk components. On the other hand protein was the only components affected significantly by weeks after parturition (Table 16). The highest protein content was obtained through the first week and the lowest was through the second week.

CONCLUSION

Anionic salts may be added to the diets through transition period of the dairy cows to decrease the DCAD in order to prevent post-partum clinical or sub clinical milk fever. However, anionic salts may cause a lower rumen PH which may affect some nutrients digestibility and consequently low milk production. Therefore, it is recommended to use a buffering material such as sodium bicarbonate or yeast with anionic salts to ensure alkalinity in the rumen.

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Table (1). Ingredients of the experimental concentrate feed mixture.

Ingredients	%
Cotton seed meal	38
Yellow corn	32
Wheat Bran	24
Molasses	3
Lime stone	2
Sodium Chloride	1

Table (2). Chemical composition of feedstuffs.

Sample	DM%	NDF	ADF	ADL	Hemi	Cellulose	protein	Ash	fiber	0.M	P	Ca
CFM	90	30.1	14.6	3.2	15.5	11.4	12.3	13.8	37.2	86.24	0.2	0.02
Straw	82	70.1	42.3	19	27.8	23.5	2.63	16.8	51.1	83.25	0.1	0.01
Berseem	19	54.4	37.4	5.6	17	31.9	18.6	14.7	39.2	85.26	0.2	0.01

Table (3). Anionic salt composition.

Components	
Magnesium sulfate, g	100
Ammonium sulfate, g	150
Ammonium chloride, g	250
Soybean meal (carrier), g	500
Total, g	1000

Table (4). Anio-Norel composition.

Items	Anio-Norel
Magnesium sulfate, g	100
Ammonium sulfate, g	150
Ammonium chloride, g	250
Molasses flavor, g	0.1
Biomet zinc (15 %), g*	7.5
Actisaf, g **	10
Magnesium oxide , g	50
Soybean meal (carrier), g	449.9
Total, g	1000

**=Chelated Zinc; **=Live Yeast*

Table (5). Feed intake.

Items	Treatments			
	1	2	3	4
DMI, Kg/ head / day	16.02	15.5	15.3	15.95
TDNI , Kg / head / day	12.5	12.2	12.4	12.5
CPI , g / head / day	1.853	1.751	1.770	1.844

Table (6). Rumen Parameters.

Items	Treatments				±SE
	1	2	3	4	
PH	7.5^a	6.9^b	6.6^c	7.1^b	± 0.21
VFA (mM)	62^b	65^a	64^a	61^b	± 2.10
Ammonia(mg/100ml)	16.4^a	14.8^b	14.8^b	16.3^a	± 1.20

a,b: means with different letters differ significantly. (P<0.05).

Table (7). Effect of treatments on nutrients digestibility.

Treatments	CP	Fiber	ADF	NDF	Hemi	Cellu	OM	DM
1	80.51	61.50	70.31	65.66	85.73^a	65.11^a	57.37^b	65.75
2	83.31	65.16	68.74	63.28	82.78^b	59.90^{ab}	58.87^a	66.19
3	84.57	64.49	68.75	62.70	85.48^a	58.55^{ab}	59.43^a	66.10
4	88.12	62.69	69.74	63.06	87.29^a	57.74^b	57.23^b	66.28
S.E	±4.126	±4.20	±0.707	±0.954	±0.791	±1.495	±0.370	±0.594

a,b: means with different letters differ significantly. (P<0.05).

Table (8). Comparison between ADL and AIA as digestibility markers.

Items	CP	Fiber	ADF	NDF	Hemi	Cellu	O.M.	D.M.
ADL	86.34	69.31^a	75.19^a	70.41^a	88.26^a	67.10^a	74.04^a	72.38^a
AIA	81.91	57.60^b	63.59^b	56.94^b	82.38^b	52.56^b	42.41^b	59.78^b
S.E	± 2.917	± 2.972	±0.500	±0.674	±0.559	±1.057	±0.266	±0.420

a,bMeans with different letters differ significantly. (P<0.05).
ADL = Acid detergent lignin; AIA = Acid Insoluble Ash.

Table (9). Effect of interaction between markers and treatments on organic matter digestibility.

Treatments	1		2		3		4		S.E
Marker	A	L	A	L	A	L	A	L	
Mean	40.47^f	74.26^a	43.19^d	74.55^a	44.44^c	74.42^a	41.53^e	72.93^b	±0.532

a,b,c,d,e,f: means with different letters differ significantly. (P<0.05).
A = AIA; L = ADL.

Table (10). Effect of treatments on milk production.

Treatments	Total milk	milk days	Ave. in 305
1	3476.40	308.60	3421.6182
2	3575.95	341.75	3191.9052
3	1810.22	191.20	2612.7467
4	3405.45	327.40	3181.2934

Table (11). Effect of treatment on average monthly milk yield as an average of two milking a day.

Treatments	1	2	3	4
Means.	155.420^a	153.503^a	120.494^b	148.884^a
S.E.	± 4.284	± 4.697	± 5.424	± 4.201

a,b: means with different letters differ significantly. (P<0.05).
Values in the table were calculated according to the following equation:
*(Daily milk /2 * 30) for each animal through 11 months and monthly averages for each group are presented in each cell in the table*

Table (12). Effect of milking time (morning / evening).

Time	Morning	evening	S.E.
means	159.022^{a}	134.559^{b}	± 3.306

a,b: means with different letters differ significantly. (P<0.05).

Table (13). Effect of lactation months on milk yield (average of 2 milking a day).

Month	mean	S.E.
1	193.47^{a}	± 7.572
2	185.32^{ab}	± 7.572
3	179.48^{ab}	± 7.572
4	168.88^{bc}	± 7.572
5	153.31^{cd}	± 7.572
6	148.14^{cde}	± 7.572
7	142.94^{de}	± 7.572
8	128.04^{fe}	± 7.572
9	116.53^{f}	± 7.572
10	110.49^{f}	± 7.801
11	82.16^{g}	± 7.801

a,b,……,g.: means with different letters differ significantly. (P<0.05).

Table (14). Linear & Quadratic relationship of milk production with lactation month.

P values	T1	T2	T3	T4
Significance of linearity	0.0382	0.0005	0.2142	0.0294
Significance of quadratic	0.7387	0.0047	0.2033	0.0002

Table (15). Effect of treatments on milk composition:

Treatment	Fat	Protein	Lactose	T.S.	SNF
1	3.013^{a} ±0.137	2.12±0.052	4.23 ±0.065	10.13 ±0.176	7.12 ±0.105
2	2.913^{a}±0.126	2.22±0.048	4.45 ±0.060	10.29 ±0.163	7.38 ±0.097
3	2.972^{a}±0.161	2.17±0.061	4.32 ±0.077	10.11 ±0.207	7.19 ±0.124
4	2.505^{b}±o.131	2.22±0.050	4.37 ±0.063	9.80 ±0.169	7.29 ±0.101

a,b: means with different letters differ significantly. (P< 0.05).

Table (16). Effect of week on milk protein.

Week	Mean	S.E.
1	2.376^{abc}	± 0.0912
2	1.871^{g}	± 0.0912
3	1.924^{fg}	± 0.0912
4	2.023^{defg}	± 0.0912
5	2.093^{cdefg}	± 0.0912
6	1.987^{efg}	± 0.0912
7	1.979^{efg}	± 0.0912
8	2.177^{bcdef}	± 0.0944
9	2.308^{abcd}	± 0.0944
10	2.308^{abcde}	± 0.0944
11	2.310^{abc}	± 0.1022
12	2.268^{abcde}	± 0.1022
13	2.500^{ab}	± 0.1176
14	2.513^{ab}	± 0.1176
15	2.672^{a}	± 0.1310

a,b……g: means with different letters differ significantly. (P<0.05).

Fig. (1). Effect of treatments on milk production.

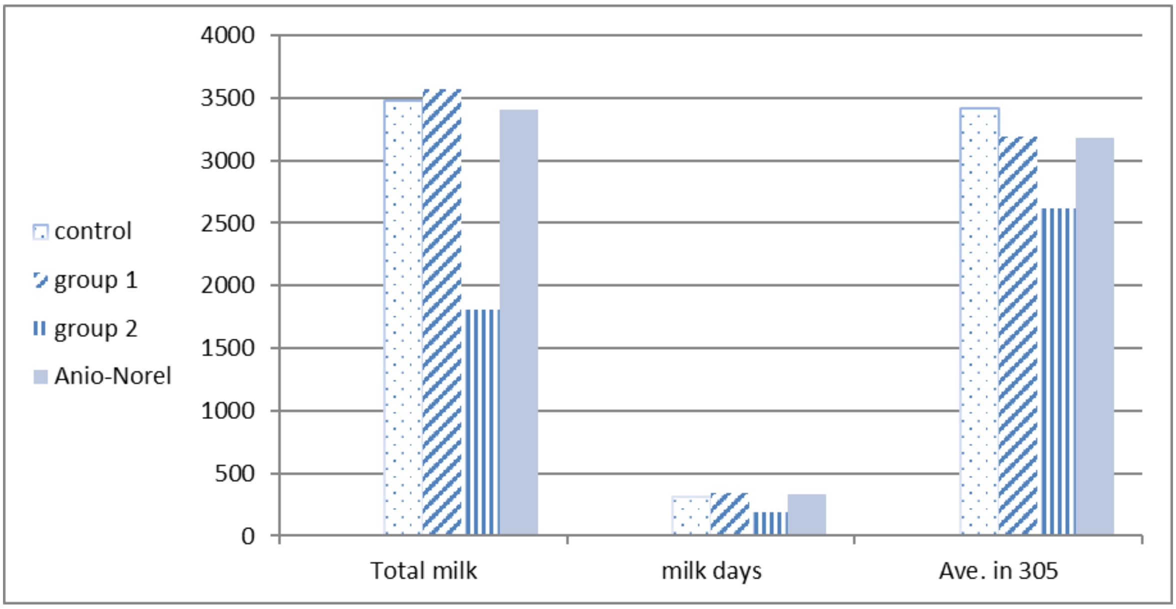
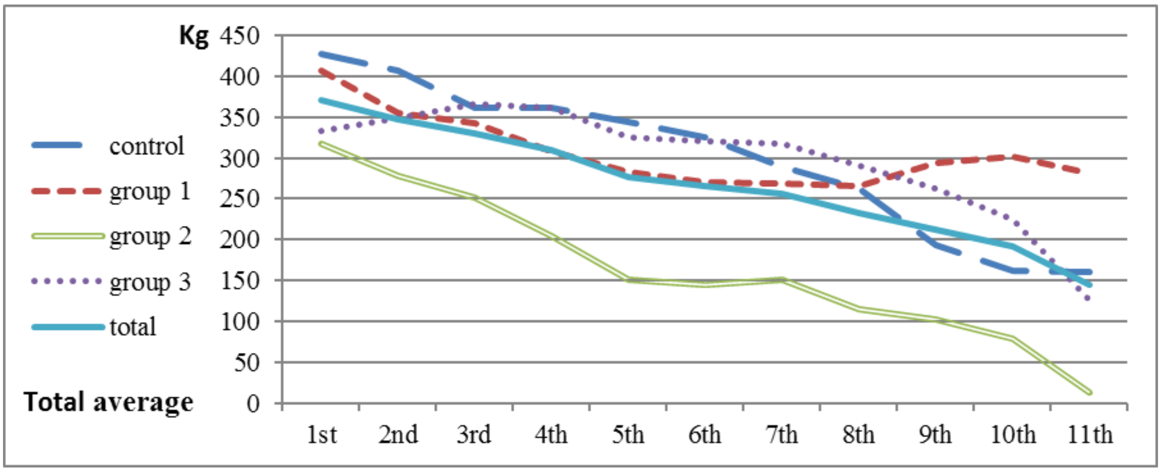


Fig. (2). Effect of lactation month and treatment on milk yield.



تأثير التوازن الانيونى– الكاتيونى للعلائق على تخمرات الكرش والهضم وإنتاج الألبان في أبقار الفريزيان الحلابة.

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تستخدم إضافات الأملاح الأنيونية للعلائق كإجراء وقائي في أخر مراحل الحمل لتجنب الإصابة بحمى اللبن بعد الولادة وهو إجراء أصبح روتينيا في معظم مزارع الألبان.

استخدمت الدراسة عشرين بقرة فريزيان حلابة لدراسة تأثير التوازنبين الكاتيونات والانيونات في العلائق على معدلات الهضم وتخمرات الكرش وإنتاج اللبن.

قسمت الحيوانات إلى أربعة مجموعات تغذت كل المجموعات على عليقة أساسية تتكون من 30 كجم برسيم ، 6 كجم مركزات ، 6 كجم قش أرز، بالإضافة إلى 75جم كسب فول صويا كخامل للأملاح/ رأس / يوم.

وكانت المعاملات كما يلي :

المعاملة الأولى : كانت للمقارنة وتغذت على العليقة الأساسية.

المعاملة الثانية : أضيف إلى العليقة الأساسية 150 جم من مخلوط كسب فول الصويا والأملاح الأنيونية بغرض الوصول إلى فرق بين الكاتيونات والأنيونات يساوي صفر.

المعاملة الثالثة : أضيف إلى العليقة الاساسية 350 جم مخلوط كسب فول الصويا والأملاح الانيونية بغرض الوصول إلى فرق بين الكاتيونات والأنيونات يساوي -150.

المعاملة الرابعة : أضيف إلى العليقة الاساسية 350 جم من مركبالانيونوريل بغرض الوصول إلى فرق بين الكاتيونات والأنيونات يساوي -150.

استمرت المعاملات لمدة ثلاثة أسابيع قبل الموعد المتوقع للولادة وتوقفت يوم الولادة بينما استمر أخذ العينات والقرءات حتى نهاية موسم الحليب وقد كانت النتائج كما يلي :

1- كانت درجة PH الكرش كما يلي : 7.1 , 7.5,6.9,6.6 للمعاملات (1,2,3,4) على الترتيب.
2- تراوحت قيم معاملات الهضم الظاهري للمادة العضوية بين 65.16 في المعاملة (2)و 61.5 في المعاملة (1).
3- لم يتأثر هضم الالياف الخام بالمعاملات.
4- كان إنتاج اللبن المعدل إلى 305 يوم أقل في الحيوانات المعاملة بالمقارنة بالمعاملة الأولى.
5- لم تتأثر مكونات اللبن بالمعاملات إلا في حالة الدهن حيث كان أقل في المعاملة الرابعة عن باقي المعاملات