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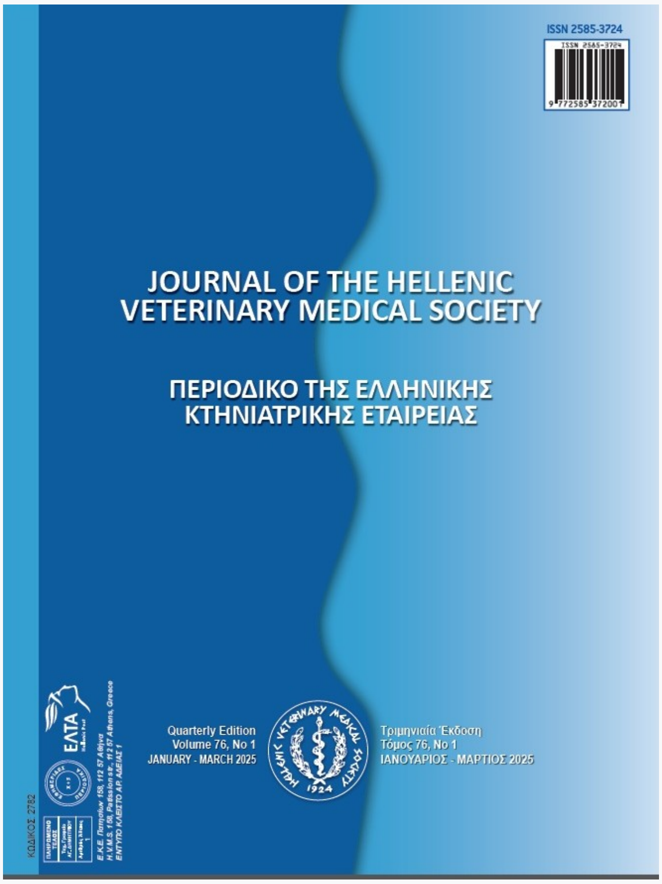

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Milk Yielding-Associated Hypokalemia and Treatment with Potassium Chloride in Holstein Dairy Cows

A.A. Nikvand*, M. Nouri, M.R. Jalali, M. Makki, A.K. Mogahi

Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

ABSTRACT: Potassium (K) is a major electrolyte in intracellular fluids. Milk as an intracellular fluid contains large amounts of K, and high-milk yielding cows always obligatory excreted significant amounts of K in milk, so they may be exposed to hypokalemia. Hence, this study aimed to investigate the serum K changes related to different milk production levels and to evaluate the treatment of the possible hypokalemic dairy cows with gelatin capsules of potassium chloride (KCl). 135 and 170 lactating dairy cows were studied from two farms (Isfahan and Behbahan, respectively) with different milk production levels. Blood samples were taken and serum K values were determined. In Behbahan farm, 12 out of 23 high-producing hypokalemic cows were randomly enrolled to treatment with KCl. Each cow received two capsules (each containing 100 gr) at a 12-h interval and blood samples were taken at five-time points after administration. The results revealed that 31.6% of very high-producing (VHP), 11.4% of high-producing (HP), and 12.5% of medium-producing (MP) cows in Isfahan and 28.7% of HP, 11.1% of MP, and 8% of low-producing (LP) cows in Behbahan farm were hypokalemic. A significant K rising was observed only at 1/2 and 1 day after KCl feeding ($p < 0.05$), but it was below the physiologic level. It is speculated that, HP dairy cows may be at the highest risk for hypokalemia compare to the MP and LP ones, without underlying diseases and these hypokalemic cases require more and longer K doses than the examined dose.

***Keywords**:* Dairy cow; Gelatin capsule; high-milk yielding; hypokalemia; potassium

Corresponding Author:
Ali Abbas Nikvand, Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
E-mail address: a.nikvand@scu.ac.ir

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INTRODUCTION

Potassium (K) is the main intracellular cation in most tissues of the body. It is also involved in most essential biological reactions such as intracellular osmotic pressure, neurotransmission, intracellular enzymatic reactions, normal renal function, and skeletal, smooth, and cardiac muscle function (Harrison et al., 2011). K homeostasis is affected by two variables: 1) the external balance of K, which is the difference between the getting from the diet and excreted from urine, feces, and milk, and 2) the internal balance, which depends on K distribution between extra and intracellular fluids (Sattler and Fecteau, 2014).

K should be provided through diet daily, because of its small reserves in the animal's body. As a major electrolyte in intracellular fluids, it has a concentration of about 150 to 155 mEq/L, whereas its concentration is low in the extracellular fluids (NRC, 2001). Because milk is an intracellular fluid, it contains great amounts of K (38 mEq/L) (Harrison et al., 2011). Approximately 13% of the K absorbed through the diet is secreted into the milk in dairy cows (Constable et al., 2017). Also, it has been addressed that about 1.4 gr/L of K is always obligatory secreted in milk (Grunberg et al., 2006), so it may be problematic to sustain K homeostasis in the high-yielding dairy cows. Different amounts of K are secreted in milk at each stage of lactation, so that in the early stage 42 mEq/L, middle stage 40 mEq/L, and in the late stage of milk production, 27 mEq/L (Constable et al., 2017), hence, it seems that high-yielding dairy cows to be highly predisposed to hypokalemia.

Nennich and associates determined that the early lactation dairy cows are in a negative K balance, due to greater fecal excretion and greater secretion of K into the milk in cows that are at less than 75-day of lactation (Nennich et al., 2006). The high rate of hypokalemia in early lactating sick dairy cows is probably due to a combination of reduced appetite, concomitant alkalemia following abomasal disorders, aldosterone secretion in response to hypovolemia, and K losses in milk (Grunberg et al., 2006). For example, a remarkable prevalence rate of hypokalemia (53%) at the cutoff point <3.9 mEq/L has been determined in dairy cows with left displacement of abomasum (Constable et al., 2013). Sead et al (2020) has also recorded a high rate of hypokalemia (58.1%) with concomitant hypocalcemia in dairy cows during transition period.

Various studies have reported different levels of low serum K as the cut-off point for defining hypokalemia in cattle. For example, serum K values less than 3.9 mEql/L (Constable et al., 2013) and < 3.5 mEq/L (Wittek et al., 2019) have been identified as cut-off points for hypokalemia in cattle.

Oral administration of K has been accepted as the method of choice for treating dairy cows with hypokalemia (Constable et al., 2014). Hypokalemia a secondary sequel to abomasal displacement, mastitis, as well as diseases that cause inappetence, was treated in dairy cows (Constable et al., 2013). A conventional proposed method for treating dairy cattle with mild to moderate hypokalemia involves a total dose of 60 to 120 gr of KCl/day (Constable et al., 2003). Hitherto, KCl has not been used to treat probable cases of hypokalemia subsequent to high milk yielding in apparently healthy dairy cows.

Treatment with high doses of KCL may be associated with adverse effects. It has been reported a decrease in plasma magnesium (Mg) value in cows under feeding high (4.6 percent) compared with the low (1.6 percent of dry matter (DM)) dietary K content (Fisher et al., 1994). Increasing dietary K has also been shown to reduce the Mg absorption from the ruminal epithelium (Constable et al., 2017).

To the best of the author's knowledge, few relevant published papers have been conducted to determine hypokalemia in the context of high milk production and preventive measures in probable milk yielding associated hypokalemia in Holstein dairy cows (Greenlee et al., 2009; Constable et al., 2014). So, it seems to be an interesting and important topic for research. Accordingly, the present study was performed with the following objectives: 1) To evaluate serum K concentrations in the high, medium, and low milk-producing Holstein dairy cows in the early lactation period. 2) To identify the responsiveness of the hypokalemic dairy cows to the suggested dose of KCl. 3) To evaluate serum chloride (Cl) and Mg changes in response to treatment with KCl.

MATERIALS AND METHODS

The present cross-sectional study was performed on 305 Holstein dairy cows in June and August 2020 in the two industrial farms in the Isfahan and Khuzestan provinces, center and southwest of Iran, respectively.

Animal selection based on milk production level

In the Isfahan farm with 4000 Holstein dairy cows, 135 apparently healthy cows with no history of recent anorexia or hyporexia, at the different levels of milk production were randomly selected and based on the milk production values were divided into three groups: VHP (with daily milk production of more than 60 liters) (n= 76), HP (with daily milk production between 30 to 60 liters) (n= 35), and MP cows (with daily milk production between 25 to 30 liters) (n= 24). In the Khuzestan province, from a farm with 400 Holstein dairy cows in the Behbahan city, 150 dairy and 20 dry cows (DC) were randomly assigned to evaluation and based on the milk production values were divided into four groups: high-producing cows (with daily milk production between 30 to 60 liters) (n= 80), medium-producing cows (with daily milk production between 25 to 30 liters) (n= 45), low-producing cows (with daily milk production of lesser than 25 liters) (n= 25), and dry cows (n=20). The recruited lactating cows on both farms were in the early and middle stages of lactation with a parity of 2 to 5.

Primary blood sampling

Immediately after the morning milking, a blood sample (5 ml) was obtained from the coccygeal vein into a free anticoagulant tube and sent to the laboratory. Blood samples were centrifuged at 2500 g for 10 min at room temperature. Sera were separated into micro-tubes and kept at -20 °C before analysis. The effect of hemolysis on the measured K value is well documented (Azman et al., 2019). So, immediately after serum separation, the hemolyzed samples were detected by visual inspection and comparing those with hemolytic chart and blood sampling was repeated, due to the availability of the animals. Serum K values were measured and levels less than 3.8 mEq/L were considered hypokalemia (Constable et al., 2013).

Dietary DCAD and K contents

A balanced TMR was fed to meet the requirements of lactating dairy cows based on the National Research Council recommendations (NRC, 2001). Water availability was ad libitum during the study. According to the report of farms nutritional managers, all presented analyzes have been performed on rations of lactating dairy cows. The amount of dietary Cation-Anion difference (DCAD) and K values were reported to be + 36.8 mEq/100 g and 1.42 percent of DM in Isfahan, and + 22 mEq/100 g and 1.35 percent of DM in Behbahan farm, respectively.

The recommended K requirement for lactating dairy cows has been reported in the range of 1.3% to 1.4 % on a dry-weight basis (Constable et al., 2017), so primary dietary K deficiency was ruled out in both studied herds. KCl is not able to unwilling changes in the DCAD of the diet, because as K increases DCAD, Cl will reduce its value (Harrison et al., 2011).

The diet chemical compositions and feed ingredients of the two studied farms are presented in table 1.

Table 1: The rations of the lactation cows in the two studied farms

Isfahan farm	Behbahan farm	
26.2	25.6	DMI (Kg)/day
	Feed ingredients (% DM)	
12.1	14.95	Alfalfa hay
29	27.19	Corn silage
3	3.7	Wheat straw
0	0.86	Corn gluten meal, dried
0	4.4	Wheat bran
1.4	0	Cotton meal
0.3	0	Urea
5.6	17.71	Ground barley
29.7	7.8	Corn grain, cracked, dried
5.9	10.62	Canola meal
0	9.62	Soybean meal
8.4	0	Poultry meat powder*
1.4	0	Fatty acid powder
0	0.5	Bentonite
0.9	1	Calcium carbonate
0.4	0.25	Salt (white)
0.22	0.15	Magnesium oxide
1.2	0.75	Sodium bicarbonate
0.5	0.5	Minerals (low Ca and P)
	Chemical composition	
37	26.6	FNDF (%DM)
29.9	34.8	NDF (%DM)
1.7	1.5	NEL (Mcal/kg)
16.5	16.76	CP (%DM)
28	10.25	MP (%DM)
-	11.5	RDP (%DM)
41	32.5	RUP (% CP)
0.91	0.87	Ca (%DM)
-	0.44	P (%DM)
-	1.97	Ca:P
44.1	50.24	Forage ratio (DM)

CP: crude protein; DM: dry matter; DMI: dry matter intake; MP: metabolizable protein; NEL: net energy for lactation; NDF: neutral detergent fiber; FNDF: forage NDF; RDP: rumen degradable protein; RUP: rumen undegradable protein.
* The poultry meat powder which is consisted of processed by-products from slaughtered chickens is legally used to feed dairy cows in Iran.

Treatment with gelatin capsule of KCl

Out of 80 high-yielding dairy cows (mean daily milk production of 40 liters) in Behbahan dairy farm, 23 were diagnosed with hypokalemia (K < 3.8 mEq/L). The mean milk production of hypokalemic cows was 42 liters/d. Twelve out of 23 hypokalemic cows were randomly enrolled for treatment with gelatin encapsulated KCl (Zofa Parnian Co. Iran). One day after primary blood sampling, just after morning milking, each hypokalemic cow received two capsules (each capsule containing 100 g of elemental KCl) at a 12-h interval by a metal balling gun. It means that each cow received 200 g of KCL. Although the logical approach to the treatment of healthy cows with subclinical hypokalemia would be to supplement the diet with K but to ensure that the prescribed doses of KCl be accurately obtained, the authors treated the individual subclinical hypokalemic cows with gelatin encapsulated KCl with a balling gun.

Secondary blood sampling after KCl administration

Blood samples were taken at time points of 0 (just before first KCL treatment), 1/2, 1, 3, and 7 days after first treatment, and isolated sera were analyzed to measure K, Cl, and Mg values. All studied hypokalemic cows in Behbahan farm had the same diet and were in the same environmental and managerial conditions.

Serum electrolytes analysis

Serum concentrations of K were determined using the flame spectrophotometric technique (Flame Photometery, Corning 410-C, England). Serum concentrations of Mg and Cl were measured by xylidyl blue and mercuric thiocyanate colorimetric endpoint methods (respectively) using commercially available kits as recommended by the manufacturer (Pars Azmon, Iran) using Autoanalyzer (BR-1500, Italy).

Data analysis

Due to the differences in the milk production levels and the ingredients and chemical compositions of diets, the data of two studied farms were analyzed separately.

All the data had a normal distribution in the Shapiro-Wilk test and were represented as means ± standard deviation. One-way analysis of variance and complementary Tukey tests were used to compare the mean serum K values between the groups of very high, high, medium, and low-producing lactating cows. A repeated measure ANOVA was further employed to analyze the overall trend of data changes over the five-time points of 0, 1/2, 1, 3, and 7 days after KCl administration in the hypokalemic cows. The SPSS, version 21, was employed for statistical analyses (IBM SPSS statistics for windows, IMB Inc., version 21.0, NY, USA). The significance level was set to $p < 0.05$.

RESULTS

Comparison of serum K values in the Holstein lactating cows with the various milk yielding levels in Isfahan farm

The mean serum K values and the daily milk production of the three groups of VHP, HP, and MP lactating cows in Isfahan farm are shown in Table 2. Based on the one-way ANOVA test, serum K value in the VHP group had significant differences with the HP ($p = 0.02$) and MP ($p = 0.01$) groups. Serum K value in the HP group was also significantly higher than in the MP group ($p = 0.03$). Considering the cut-off point of less than 3.8 mEq/L of serum K to confirm hypokalemia, 24 out of 76 (31.6 %) VHP, 4 out of 35 (11.4 %) HP, and 3 out of 24 (12.5 %) MP cows were found to have hypokalemia. No relevant Clinical signs were observed in the hypokalemic cases.

Table 2: Serum K values (mEq/L) and daily milk production (liter) in three groups of VHP, HP, and MP Holstein cows in Isfahan farm

Daily milk production			Serum K			Groups	
Min	Max	Mean ± SD	Min	Max	Mean ± SD		
60	81	65.3 ± 3.4	2.3	4.6	3.94 ± 0.4^{c}	Total (n= 76)	VHP
62	81	66 ± 3	2.3	3.7	3.4 ± 0.4	Hypokalemic (n= 24)	
33	45	35.7 ± 6.7	2.9	4.8	4.16 ± 0.3^{b}	Total (n= 35)	HP
39	45	42 ± 3	2.9	3.8	3.5 ± 0.3	Hypokalemic (n= 4)	
23	29	27.3 ± 2	3.6	5.6	4.41 ± 0.5^{a}	Total (n= 24)	MP
28	29	28.7 ± 0.6	3.6	3.7	3.6 ± 0.05	Hypokalemic (n= 3)	

Different superscript letters in the same column denote significant differences between the groups (c-b: $p < 0.02$; c-a: $p < 0.01$; b-a: $p < 0.03$). VHP: Very high-producing; HP: High-producing; MP: Medium-producing.

Comparison of serum K values in the Holstein lactating cows with the various milk yielding levels in Behbahan farm

The mean serum K values and the daily milk production of the three groups of the HP, MP, and LP lactating cows in Behbahan farm are shown in Table 3. Based on the one-way ANOVA test, serum K value in the HP group was significantly lower than the MP and LP groups ($p = 0.047$). Twenty-three out of 80 (28.7 %) HP, 5 out of 45 (11.1 %) MP, 2 out of 25 (8 %) LP, and 4 out of 20 (20 %) DC were found to have hypokalemia.

The mean serum K values of the HP, MP, LP, and DC hypokalemic cows in Behbahan farm are presented in table 3. No relevant Clinical signs were observed in the hypokalemic cases.

Serum K, Cl, and Mg changes following KCl administration in the hypokalemic cows

The mean serum K, Cl, and Mg values in the treated hypokalemic cows at the time points of 0, 1/2, 1, 3, and 7 days after KCl administration are shown in Table 4. Based on the repeated measures ANOVA, the administration of KCl caused a significant rising trend in serum K value at the time points of 1/2 and 1 day ($p < 0.05$), but failed to bring it to the physiological serum level (Figure 1).

The overall trend of changes showed a significant increase in serum Cl values at the time points of 1/2 and 1 day in treated hypokalemic cows ($p < 0.01$), (Figure 2).

No significant change was seen in serum Mg value over the studied time points in the treated hypokalemic cows ($p > 0.05$).

Table 3: Serum K values (mEq/L) and daily milk production (liter) in three groups of the HP, MP, and LP, as well as dry Holstein cows in Behbahan farm

Daily milk production			Serum K			Groups	
Min	Max	Mean ± SD	Min	Max	Mean ± SD		
34	54	40.1 ± 6	1.8	4.9	3.6 ± 1^{bc}	Total (n= 76)	HP
36	54	42.7 ± 6	1.8	3.8	2.7 ± 0.7	Hypokalemic (n=23)	
25	29	26.7 ± 1.6	3	4.3	4.1 ± 0.35^{a}	Total (n= 45)	MP
25	28	26 ± 2	3	3.7	3.4 ± 0.3	Hypokalemic (n= 5)	
10	24	16.4 ± 4	3.7	4.4	3.9 ± 0.24^{ab}	Total (n= 25)	LP
12	20	16 ± 5.6	3.6	3.7	3.6 ± 0.07	Hypokalemic (n= 2)	
-	-	-	2.7	4.3	3.8 ± 0.5^{b}	Total (n= 20)	DC
-	-	-	2.7	3.8	3 ± 0.5	Hypokalemic (n= 4)	

Different superscript letters in the same column denote significant differences between the groups ($p < 0.05$). HP: High-producing; MP: Medium-producing; LP: Low-producing; DC: Dry cows.

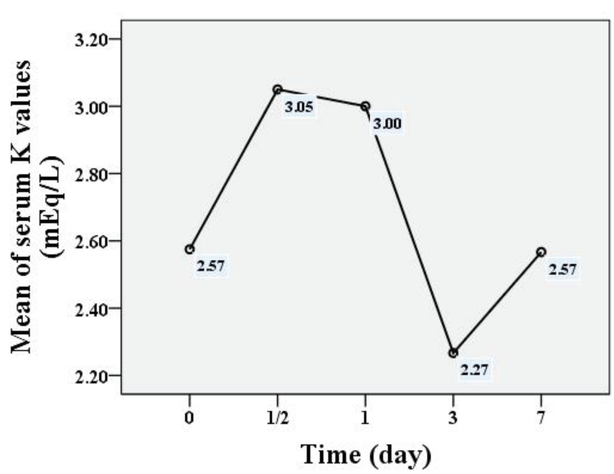

Figure 1: Serum K variations of the hypokalemic dairy cows following KCl treatment at the studied time points.

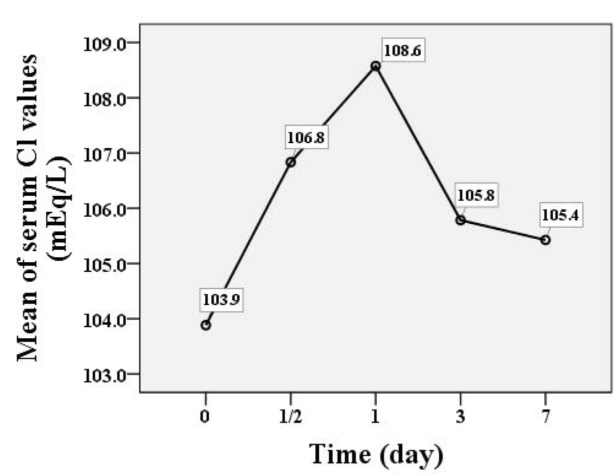

Figure 2: Serum Cl variations of the hypokalemic dairy cows following KCl treatment at the studied time points.

Table 4: Mean ± SD of serum K, Cl, and Mg values in the KCl treated hypokalemic cows at the studied time points (day)

7	3	1	1/2	0	
2.6 ± 0.8^{b}	2.3 ± 0.6^{b}	3 ± 0.7^{a}	3.1 ± 0.5^{a}	2.57 ± 0.5^{b}	K (mEq/L)
105.4 ± 2.1^{b}	105.8 ± 1.8^{b}	108.6 ± 3.3^{a}	106.8 ± 2^{a}	103.9 ± 2^{b}	CL (mEq/L)
2.4 ± 0.2^{a}	2.3 ± 0.1^{a}	2.3 ± 0.2^{a}	2.4 ± 0.1^{a}	2.3 ± 0.2^{a}	Mg (mg/dL)

Different superscript letters in the same row denote significant differences among times ($p < 0.05$)

DISCUSSION

Effects of milk yielding levels on serum K values

The most important findings of this study were that serum K decreased in high-producing cows and administration of gelatin capsule of KCl increased serum K values but could not sustain it in the reference range for a long time. The reference range of serum K in cattle is reported to be 3.9 to 5.8 mEq/L (Constable et al., 2013). Different serum K values have been reported as the cut-off point for defining hypokalemia in cattle. For example, a cut-off point of < 3.9 mEq/L (Constable et al., 2013) and < 3.5 mEq/L (Wittek et al., 2019). The Values below 3.8 to 2.3 have also been considered mild to moderate hypokalemia (Peek et al., 2002). Accordingly, in the present study, the dairy cows with serum K values less than 3.8 mEq/L were considered hypokalemic. According to the results of Isfahan farm, 31.6 % of very high, 11.4 % of high, and 12.5 % of medium-producing cows were found to have hypokalemia. Similar results were also obtained on Behbahan farm. As a whole, it is understood that the higher the milk production, the higher the rate of hypokalemia. In consistent with our findings, Harrison et al. reported that cows under 75-day of lactation tended to excrete more K than cows over 75-day, while the K intake of both groups was similar (Harrison et al., 2011). They also suggested that cows are in a negative K balance in the early lactation period, which supports our results.

It has been found that when high-yielding dairy cows excrete large amounts of K in milk, urinary K excretion decreases, so that serum K is maintained at its lowest physiological level (Berg et al., 2017). The mentioned mechanism can explain the lower and narrower normal range of serum K in the very high-producing cows (3.9 ± 0.4 mEq/L) in Isfahan farm. The marginal and low serum K values in the high-producing cows become important when they become anorexic for any reason; this can predispose the mild hypokalemic cows to severe hypokalemia.

Although the present study was conducted in the warm months of June and August, the studied farms, as routine work, had cooling equipment such as fans and sprinklers to overcome the thermal stress. The role of heat stress has been accepted in lowering blood K through sweating and decreased appetite in cattle (Beede et al., 1983; Schneider et al., 1986). Beside, some recent studies presented controversial data. For example, Srikandakumar and Johnson (2004) reported that heat stress significantly increased plasma K in Holstein cows. Joo et al. (2021) also reported that thermal stress conditions could not induce a significant decrease in serum K value in Holstein dairy cows. However, in the present study, at the same ambient temperature, high-yielding cows had a significant lower serum K compared to medium and low-yielding cows, which could support the probable role of high milk production in induction of hypokalemia in both studied farms.

Serum K, Cl, and Mg changes in response to the gelatin capsule of KCl

In the present study, a significant increase was notable in serum K value at time points of 1/2 and 1 day following treatment with gelatin capsule of KCl, although failed to reach the normal value. In line with the present study, Constable and colleagues reported an increase in serum K value up to 3 mEq/L at 12 and 24-h after administering a gelatin capsule (200 g) of KCl in dairy cows with average milk production of 32 liters/d (Constable et al., 2014). Bolus and solution forms of KCl in hypokalemic Simental cows with average milk production of 9.4 liters/d could increase blood K value up to 4.2 mEq/L at 24-h after administration (Wittek et al., 2019). In our study, the gelatin encapsulated KCl administration increased serum K from 2.5 up to 3.1 mEq/L at 12 and 24-h after treatment in hypokalemic cows (with the mean milk production of 42 liters/d). It seems that the higher levels of milk production in the present and the Constable et al. (2014) studies compare to the study of Wittek et al., could prevent the increase of serum K to the normal range after KCl administration.

In a previous study, treatment of 10 hypokalemic cows with gelatin capsule of KCl resulted in an increase in serum K concentration in all cows but 4 of them only returned to normal K value (Sielman et al., 1997). Sattler and Fecteau (2014) have also suggested continuing the treatment of hypokalemic patient cows with a dose of 60 to 100 g/100 kg b.w. /d for 3 to 5 days. On the other hand, according to Peek et al. study, it should not be used more than twice a day and more than half a pound (Ib) of KCl at each time, because it causes diarrhea in cows (Peek et al., 2000). However, in the present study, following the treatment of hypokalemic cows with two doses of 100 gr of KCl, like the study of Constable et al. and Sielman et al., serum K values did not reach the physiological range. In general, on the one hand, the high milk production and on the other hand, the forced excretion of about 1.4 g/L of K into milk may explain the lack of increase in serum K value to the reference range in the present study (Constable et al., 2014). A lack of proper response to KCl administration may be due to the more need for K for HP hypokalemic dairy cows. Therefore, it is suggested that the longer periods of treatment with KCl with daily monitoring of serum K should be studied in hypokalemic dairy cows.

The authors also determined the effect of oral KCl on serum Cl values. Serum Cl values were in the normal range at day zero in the high-producing hypokalemic cows. In other words, hypokalemia was not associated with hypochloremia in the high-producing lactating cows. In this regard, it has been reported that the amount of milk Cl secretion at the early-lactation period was not more than the amount of it at the late-lactation period (Gaucheron, 2005). Prado et al. (2019) did not observe a significant difference between the serum chlorine ion values in the high and low-producing cows (Prado et al., 2019). Administration of KCl was associated with the significant increases in the serum Cl compared with the K in the presently studied cows. According to the Gaucheron et al. (2005) study, healthy cows secrete 1.2 to 1.7 g/L of K into the milk while excreting 0.7 to 1.2 g/L of Cl. It seems that the lower milk Cl secretion compared to K has led to significant increases in serum Cl following the administration of KCl.

Constable et al. (2014) reported a decrease in plasma Mg concentration following treatment with a similar dose rate of KCl to our study (0.4 g/kg b.w.) in experimentally induced hypokalemic cows. They have also advised supplying Mg to inappetant lactating dairy cattle being treated with oral KCl. It is speculated that a 50 % decline in feed intake rearing to injection of isoflupredone acetate and furosemide in the Constable et al study could induce concomitant hypomagnesemia in the treated cows with KCl. It should be noted that in the present study neither hypokalemic cows were hypomagnesemic nor did KCl treatment cause hypomagnesemia. It seems that differences in the study designs and objectives could explain these discrepancies. So, further relevant studies are recommended in this regard.

CONCLUSIONS

It is speculated that very high-yielding dairy cows may be at the highest risk for hypokalemia compare to the high, medium, and low milk-producing ones. High milk yielding may predispose dairy cows to hypokalemia without underlying diseases or disorders. The marginal and low serum K values become important in the high-producing cows when they become anorexic for any reason; this can predispose the mild hypokalemic cows to severe hypokalemia. It is recommended that longer periods of treatment with KCl with daily monitoring of serum K be considered to survey in cows with high-producing associated hypokalemia.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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