

Effects of Vitamin and Trace Element Supplementation on Weight Gain and Health of Calves Fed Raw or Pasteurized Waste Milk

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Abstract: The aim of the presented study is to investigate the effects of vitamin-mineral supplement on weight gain and health of dairy calves fed raw or pasteurized milk. The study was conducted on 40 calves from two different herds. Ten calves from herd A, received raw milk and 7.5 g of supplement twice daily (15g/calf/day) for 60 days (AM group); 10 other calves did not receive any supplement and were fed raw milk for 60 days (AC group). Ten calves from herd B (BM group), received 7.5 g of supplement twice daily (15g/calf/day) for 60 days mixed with pasteurized milk (72°C for 15 s). Calves in BC group (n:10) did not receive any supplement and calves in this group were fed with pasteurized milk for 60 days. Body weight of all calves were measured just after birth and on day 60 of the study when calves were weaned. Birth weights (kg±SEM) of groups AM, AC and BM, BC were 34.6±1.59, 34.6±1.68 and 36.4±2.51, 36.8±1.40 respectively. Weaning weights (kg±SEM) of groups AM, AC and BM, BC were 68.2±2.15, 65.9±1.89 and 81.5±3.21, 72.8±2.38 respectively. ADG (g±SEM) of groups AM, AC and BM, BC were calculated as 560±35, 522±25 and 768±30, 600±19 respectively. ADG and weaning weight of AM and AC was similar; however, ADG and weaning weight of BM was significantly higher (P<0.05) than ADG and weaning weight of BC. In conclusion, vitamin and trace element supplementation has beneficial effects on weight gain and health of dairy calves fed pasteurized waste milk.

Keywords: Calf, waste milk, Pasteurization, Vitamin - mineral supplement.

Pastörize veya Çiğ Süt ile Beslenen Buzağılarda Vitamin Mineral Takviyesinin Ağırlık Artışı ve Sağlık Üzerindeki Etkilerinin Araştırılması

Özet: Çalışmanın amacı, vitamin-mineral ikamesinin, çiğ süt veya flaş pastörize süt ile beslenen buzağılarda ağırlık artışı ve genel sağlık durumu üzerindeki etkilerinin araştırılmasıdır. Çalışmada iki farklı işletmeye ait 40 buzağı kullanılmıştır. A işletmesinde, 10 buzağıya günde iki kez çiğ süt ile birlikte 7,5 g. (15g/buzağı/gün) süt ikamesi yapılmış (AM grubu), diğer 10 buzağıya ikame yapılmamış ve 60 gün boyunca çiğ süt ile beslenmişlerdir (AC grubu). B işletmesinde, 10 buzağıya günde iki kez pastörize süt (72°C' de 15sn) ile karıştırılmış şekilde 7,5 g. (15g/buzağı/gün) süt ikamesi yapılmış (BM grubu), diğer 10 buzağıya ikame yapılmamış ve 60 gün boyunca pastörize süt ile beslenmişlerdir (AC grubu). Buzağuların canlı ağırlığı doğumu takiben ve süttten kesildikleri çalışmanın 60. gününde ölçülmüştür. AM, AC ve AM, BC gruplarında doğum ağırlıkları (kg±SEM) sırası ile 34,6±1,59, 34,6±1,68 ve 36,4±2,51, 36,8±1,40 olarak ölçülmüştür. Süttten kesim ağırlıkları (kg±SEM) AM, AC ve BM, BC gruplarında sırası ile 68,2±2,15, 65,9±1,89 ve 81,5±3,21, 72,8±2,38 olarak belirlenmiştir. Günlük Canlı Ağırlık Artışı (GCAA) (gr±SEM), AM, AC ve BM, BC gruplarında sırası ile 560±35, 522±25 ve 768±30, 600±19 olarak hesaplanmıştır. AM ve AC gruplarında GCAA benzer olmasına karşın, BM grubunda GCAA ve süttten kesim ağırlığı BC grubuna göre yüksek (P<0.05) bulunmuştur. Sonuç olarak, vitamin ve iz mineral ikamesinin pastörize atık süt ile beslenen buzağılarda canlı ağırlık artışı ve sağlık üzerine olumlu etkileri olduğu belirlenmiştir.

Anahtar Kelimeler: Buzağı, Atık süt, Pastörizasyon, Vitamin-mineral ikamesi.

Introduction

Un-saleable milk in dairy herds that consists of colostrum milk, milk from cows suffering mastitis, or those that receive drugs; is required to be discarded and is collectively called Waste Milk (WM). Waste milk is used as a cheap alternative to saleable milk for feeding calves in dairy herds. However, bacterial counts in WM is usually higher when compared to saleable milk (Moore et al., 2009). Due to the risk of herd-level, infectious diseases such as tuberculosis (Radostits et al., 2007) and enzootic bovine leucosis (Baumgartener et al., 1976), pasteurization of milk

fed to calves is strictly recommended. Along with that, risk of salmonellosis (Edrington et al., 2012) and mycoplasma pneumonia (Butler et al., 2000) is higher in calves that are fed non-pasteurized waste milk. Feeding pasteurized WM to calves is more cost-effective when compared to milk replacers due to increased daily weight gain, lower morbidity, and lower mortality rates in calves (Godden et al., 2005). The effects of pasteurization, especially on colostrum quality in means of immunoglobulin (Ig) concentrations, have been widely studied (Donahue

et al., 2012; Godden et al., 2003). However, the effects of pasteurization on milk vitamin and trace mineral content and the possible relation of these with calf health is not clearly elucidated. A meta-analysis conducted on the effects of pasteurization on milk vitamins demonstrated that vitamin B12 and E levels in milk decrease after pasteurization (Macdonald et al., 2011). In another study conducted on human milk, pasteurization decreased vitamin C and ascorbic acid levels by 20% and 16%, respectively (Moltó Puigmartí et al., 2011). Similarly in a study conducted in calves fed pasteurized whole milk, calves were found to be vitamin D and E deficient (Blakely et al., 2016). Trace element content of milk could also be influenced with pasteurization. A slight decrease in copper and iron levels occurs after pasteurization (Zurera-Cosano et al., 1994).

As mentioned above, pasteurization is very important for controlling infectious diseases among calves in dairy herds. On the other hand, the pasteurization process has negative effects on vitamin and, to a lesser degree, trace mineral content of milk fed to calves. Thus the aim of the present study is to investigate the effects of a commercial milk supplement containing vitamins A, B1, B2, B6, B12, C, D, E, K and trace elements Cu, Co, Mn, Zn, Fe and Se, on weight gain and basic health status of dairy calves fed either pasteurized WM or raw saleable milk. Milk supplement used in the study had been chosen based on the vitamins it contains as negative effects of pasteurization are mainly reported on vitamin B, C, D and E.

Materials and Methods

The study was conducted in two different herds during the same time period. Herds were selected based on their calf feeding practices; in herd A, calves were fed raw saleable milk while calves in herd B were fed pasteurized waste milk. The distance between the two farms is about 30 kilometers, so climatic conditions between locations were similar during the study period. All calves used in the study were Holstein-Friesian. Serum gamma-glutamyl transpeptidase (GGT) and total protein (TP) levels were routinely measured in every calf 24-36 hours after birth to determine transfer of passive immunity in both herds. Thus we selected only female calves with serum GGT levels higher than 800 IU/L and serum TP levels higher than 6g/dL for the study. Calves from both herds were housed in individual hutches. Calves from herd A were randomly assigned to two groups. Calves in AM group (n=10) received raw non-pasteurized saleable milk and 7.5 g of commercial milk

supplement (MS) (Milkshake®, Mervue Lab., Ireland) containing Vitamins A (600,000 IU/kg), B1 (660 mg/kg), B2 (1520 mg/kg), B6 (160 mg/kg), B12 (1200 mg/kg), C (20,000 mg/kg), D3 (120,000 IU/kg), E (5300 mg/kg), K (66 mg/kg), and trace elements Cu (1000 mg/kg), Co (99 mg/kg), Mn (1300mg/kg), Zn (2600 mg/kg), Fe (2000 mg/kg), and Se (35 mg/kg) (Table1) twice a day for 60 days (15g/calf/day). Calves in AC group (n=10) did not receive any treatment and were fed with raw non-pasteurized saleable milk for 60 days. Calves from herd B were randomly assigned into two groups, Group BM (n=10) received 7.5g of MS twice a day (totally 15g/calf/day) for 60 days mixed with flash pasteurized (72°C for 15 seconds) waste milk and Group BC (n=10) did not receive any treatment and calves in this group were fed with flash pasteurized waste milk for 60 days. Feeding regimes were same for the both herds; all calves received two liters of high quality colostrum (BRIX>21%) in the first 2, 12 and 24 hours after birth. In the second and third day, calves were fed 2.5 liters of second or third milking colostrum twice a day. From days 3 to 10, calves received 2.5 liters of waste or saleable milk, depending on herd, twice a day. From days 10 to 49, daily milk intake of calves was 3 liters twice a day and from day 49 to 60 once a day. All calves were weaned on day 60. Water and calf starter was offered ad libitum to calves from both herds. For ad libitum calf starter intake, calves were offered approximately 500 to 600 g of starter in the first month and 1 kg of starter in the second month of life. The calf starter containing barley grain (10%), sunflower meal (11%), oat bran (25%), molasses (4.5%), corn (32%), soybean (5%), soybean meal (10%) yeast supplement (0.04%) and DCP (2%) was same in both herds. Forages were not offered to both groups till weaning. Body weight of all calves was measured after birth and on day 60 of the study when calves were weaned. Average daily weight gain (ADG) of calves was calculated by dividing the difference between birth and weaning weights by 60. Feces of all calves was monitored daily and animals with enteritis were recorded and treated. Daily body temperature measurement of all calves was standard protocol in both herds. Animals with body temperatures higher than 39.7 °C and harsh sounds detected during lung auscultation were accepted as pneumonic. The number of pneumonia treatments in all groups was also recorded. In case of high body temperature and harsh lung sounds 14 days after the first treatment the animal was again recorded as suffering pneumonia. Treatment protocols for pneumonia and enteritis were the same for herd A and B. Statistical analysis of birth weight, ADG and

weaning weight were performed using Sigma Plot 12 software (Systat Software Inc., USA). A normality test was performed using Shapiro-Wilk test and the data were found to be normally distributed. GGT and TP levels of all four groups were compared by using one-way ANOVA. Birth weight, ADG and weaning weight of groups from the same herd were compared using Student's t-test and $P \leq 0.05$ was considered significant.

Results

All calves survived during 60 days study period. Mean GGT (IU/L \pm SEM) levels of groups AM, AC, BM and BC were 1082 \pm 102, 1106 \pm 72, 1048 \pm 99 and 1065 \pm 74 respectively; and TP (g/dL \pm SEM) levels of groups AM, AC, BM and BC were 6.31 \pm 0.08, 6.33 \pm 0.06, 6.35 \pm 0.05 and 6.41 \pm 0.07 respectively. There was not significant difference between GGT and TP values between study groups. Birth weights

(kg \pm SEM) were 34.6 \pm 1.59 and 34.6 \pm 1.68 for groups AM and AC and 36.4 \pm 2.51 and 36.8 \pm 1.40 for groups BM and BC respectively. Birth weight did not differ between AM-AC and BM-BC groups (Table2). Weaning weights (kg \pm SEM) for groups AM, AC and BM, BC were 68.2 \pm 2.15, 65.9 \pm 1.89 and 81.5 \pm 3.21, 72.8 \pm 2.38, respectively. ADG (g \pm SEM) for groups AM, AC and BM, BC was 560 \pm 35, 522 \pm 25 and 768 \pm 30, 600 \pm 19 respectively. ADG and weaning weight of AM and AC was similar; however, ADG and weaning weight of BM was significantly ($P < 0.05$) higher than ADG of BC. Weaning weights and ADG were similar between AM-AC groups, however weaning weight and ADG was significantly higher ($P < 0.05$) in BM group (weaning weight: 81.5 \pm 3.21 kg; ADG: 786 \pm 30 g) when compared to BC group (weaning weight: 72.8 \pm 2.38 kg ADG: 600 \pm 19 g) (Table-2). Number of enteritis and pneumonia treatments for groups AM, AC, BM and BC were 6, 5, 6, 11 and 3, 3, 5, 7 respectively.

Table 1. Composition of additives per kilogram in the commercial milk supplement used in the study.

Vitamin C (Ascorbic acid) E300	20000 mg
Vitamin E (α tocopherol)	5300 mg
Vitamin A E672	600000 IU
Vitamin D3 E671	120000 IU
Vitamin B1 (Thiamine)	660 mg
Vitamin B2 (Riboflavin)	1520 mg
Vitamin B6 (Pyridoxine Hydrochloride)	160 mg
Vitamin B12 (Cyanocobalamin)	1200 mg
Vitamin K	66 mg
Nicotinic acid	320 mg
Pantothenic acid	1200 mg
Folic acid	32 mg
Biotin	6000 μ g
Copper	1000 mg
Iodine	300 mg
Cobalt	99 mg
Manganese	1300 mg
Zinc	2600 mg
Iron	2000 mg
Selenium	35 mg

Table 2. Weight parameters of calves from the four groups of the study.

	AM	AC	P	BM	BC	P
Mean Birth Weight (kg \pm SEM)	34.6 \pm 1.59	34.6 \pm 1.68	n.s	36.4 \pm 2.51	36.8 \pm 1.40	n.s
Mean Weaning Weight (kg \pm SEM)	68.2 \pm 2.15	65.9 \pm 1.89	n.s	81.5 \pm 3.21	72.8 \pm 2.38	P<0.05
Average Daily Weight Gain (g \pm SEM)	560 \pm 35	522 \pm 25	n.s	768 \pm 30	600 \pm 19	P<0.05

Discussion

The health of newborn calves depends on proper transfer of immunoglobulin (Ig) from dam by colostrum, which is called passive transfer of

immunity (PTI). Serum Ig levels are the gold standard in detecting PTI, but costs, the time consumed, and the need for an experienced

laboratory limit the use of serum immunoglobulin levels. More practical methods for assessing PTI indirectly, such as calves serum TP and GGT 24 to 48 hours after birth are demonstrated to correlate with serum Ig (Parish et al., 1997; Tyler et al., 1996). All 40 calves survived the 60-day study period; this result is probably associated with the selection criteria of calves based on high serum TP and GGT levels.

Lower treatment counts in the BM group fed with supplemented pasteurized WM is in accordance with Krueger et al. (2016), who reported beneficial health effects of vitamin E and D3 supplementation in calves fed with pasteurized whole milk. Lower treatment counts in BM group could be associated with positive effects of vitamin and mineral supplementation on immune status of calves in that group. As, Teixeira et al. (2014) reported greater blood neutrophil function in trace mineral supplemented WM-fed calves. Besides that, Holstein calves supplemented with vitamin E had higher lymphocyte stimulation indices and viral replication of bovine infectious rhinotracheitis virus was inhibited in tissue cultures of vitamin E supplemented calves (Reddy et al., 1986). Similarly, Zinn et al. (1987) reported that vitamin B supplementation lowered morbidity in supplemented calves. Pronounced difference in treatment numbers between groups in herd B could be related to negative effects of pasteurization on milk vitamin and trace minerals, as such difference was not observed in herd A in which calves were fed with non-pasteurized saleable milk.

Birth weights did not differ between groups in the same herd. Similarly, weaning weight and ADG of AM and AC groups did not differ. Birth weights of calves in herd A were about 2 kilograms lower when compared to herd B. This could be related to semen selection criteria of herd A in favor of calving ease. Weaning weight and ADG of BM group were significantly higher when compared to BC group. This finding could be associated with positive effects of supplementation on treatment counts and possibly immunity of calves in BM group. Parallel to our results, Wood et al. (2016) compared the effects of vitamin supplementation in calves fed low or high level pasteurized whole milk and challenged with *E.coli* LPS, and detected higher ADG in vitamin supplemented calves during LPS challenge. On the other hand, Dehghan Banadaky et al. (2015) also compared the effects of vitamin supplementation in *E.coli* lipopolysaccharide (LPS) challenged calves in the first 15 days of life and did not detect any difference between weight gains. The difference between the two studies is the age of the animals, as indicated by Dehghan Banadaky

et al. (2015), fetal depots of vitamin and trace mineral could be sufficient for a short period after birth. A significant difference between ADG in BM and BC group detected in this study could be related to positive effects of vitamin and trace mineral supplementation on health status as the number of treatments between groups was significant. However, although not significant, higher ADG and weaning weight of AM group when compared to AC could indicate that mechanisms other than immune status could influence the effects of vitamin and trace mineral supplementation on weight gain.

Conclusion

In conclusion, the aim of the presented study was to compare the effects of vitamin and trace mineral supplementation in calves fed pasteurized or non-pasteurized milk. Higher treatment count and lower weight gain in calves fed non-supplemented pasteurized WM indicate that vitamin and trace mineral supplementation of pasteurized WM is beneficial in means of health and weight gain in calves. Studies conducted with a higher population in the same herd and evaluating blood vitamin and trace mineral levels would be beneficial for clearly elucidation of the effects of supplementation in calves.

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