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The Effect of Aditional Lyzozyme to Milk on Growth Performances of Holstein Calves

[1]Serap Goncu, [1]Murat Gorgulu and [2]Gokhan Gokce
[1]Department of Animal Science, [2]Research and Practice Farm,
Faculty of Agriculture, Cukurova University, Adana, Turkey

Abstract: This study was planned to investigate the effect of aditional lyzozyme to milk on growth performances of Holstein calves. About 15 female and 18 male Holstein calves were used in this study. Between day 4 and 60, calves were fed with whole milk from a pail twice a day such that each calf received 228 L whole milk (4 L day^{-1}) over the whole experiment. All calves were fed *ad libitum* with calf starter along with alfalfa hay. Calves was fed with milk which is additional (10 mg L^{-1}) lysozyme during milk fed period. During 0-35 days period of the experiment, lysozyme supplementation decreased daily gain and increased feed to gain ratio ($p<0.05$) and male calve had higher daily gain, feed intake and better feed to gain ratio in the same period ($p<0.01$). The effects of lysozyme and gender on daily gain and feed to gain ratio have disappeared during 35-60 days period. Lysozyme decreased feed intake ($p<0.05$) during 35-60 days of experiment. Similarly overall feed intake, daily gain and feed to gain ratio were not affected by lysozyme ($p>0.05$). Male calve had higher daily gain and weaning weight and lower feed to gain ratio than female calve throughout the study ($p<0.05$). Lysozyme x gender interaction affected feed intake in all experimental period and male receiving control diet had higher feed intake than female but lysozyme supplemented male calves had lower feed intake than female ones ($p<0.05$). The results revealed that lysozyme may deteriorate of adaptation of the microflora of the gastrointestinal system of 5 weeks old calves and be tended to decrease diarrhea cases during preweaning period.

Key words: Lysozyme, milk, feeding, holstein calf, performances, Turkey

INTRODUCTION

Lysozyme, also known as muramidase is a type of glycanhydrolase which hydrolyzes the b-1,4-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in the peptidoglycan of bacterial cell walls (Humphrey *et al*., 2002). Lysozyme is a antimicrobial protein found in the tears, saliva and milk of all mammals (Maga *et al*., 2006a; Ito *et al*., 1993). Bovine milk contains very little lysozyme compared with human milk, 13 μg/100 mL instead of 30 mg 100/mL (Chandan *et al*., 1964) which showed variation during the lactation. Lysozyme concentration of colostrum fluctuated within the range of 0.15-0.65 μg mL^{-1} with an average of 0.30 μg mL^{-1}. Gueorguiev *et al*. (1996) reported that the lysozyme activity in the colostrum of cows with high and low milk yield changes from 0.401±0.09 and 0.327±0.07 μg mL^{-1} at parturition to 0.096±0.03 and 0.073±0.01 μg mL^{-1} on the 3rd post partum day, respectively (Paulik *et al*., 1985). The benefits of lysozyme present in milk to improve immunity and prevent infection (Paulik *et al*., 1985; Gueorguiev *et al*., 1996; Maga *et al*., 2006a). It increases the levels of beneficial intestinal microflora and strengthens disease resistance in new born animals. These effects are believed to occur through the lysis of certain potentially damaging gram positive bacteria and a few gram-negative bacteria in the gastrointestinal tract (Maga *et al*., 2006a). Standard human lysozyme was found to be effective at significantly slowing the growth of the milk cold-spoilage organism *Pseudomonas fragi* of a clinical isolate of the mastitis-causing organism *Staphylococcus aureus* and of a nonpathogenic strain of *E. coli* (Maga *et al*., 1998). Researcher reported that the milk from transgenic mice secreting human lysozyme in their milk at an average concentration of 0.38 mg mL^{-1} was found to be bacteriostatic against the cold-spoilage organisms. Recently, several investigators (Irwin and Wilson, 1989; Irwin *et al*., 1989; Kato *et al*., 1998) have elucidated at a significant extent the genetic structure of the gene (or genes), coding for lysozyme in the various animal species and its importance in systemic defense against pathogens. Maga *et al*. (2006a) reported that earlier developed a line of transgenic dairy goats which express hLZ at 67% (270 mg L^{-1}) of the level found in human milk. Researchers suggested that if lysozyme-rich milk from

Corresponding Author: Serap Goncu, Department of Animal Science, Faculty of Agriculture, Cukurova University, Adana, Turkey

transgenic animals is applied to dairy cattle, the potential for even more widespread benefit could be realize (Maga *et al*., 2006a, b; Brundige *et al*., 2008, 2010). Yang *et al*. (2011) have produced 17 healthy cloned cattle expressing recombinant human lysozyme using somatic cell nuclear transfer. The expression level of the recombinant lysozyme was up to 25.96 mg L^{-1}. The researchers then fed the lysozyme-rich milk to young goats and pigs to see if it affected the bacteria population in animal guts. They reported that lysozyme had different effects in monogastric and ruminants. High lysozyme milk increased total coliform bacteria in small intestine of kids but decreased it in small intestine of piglet (Brundige *et al*., 2010). However, there is limited study in preruminant with lysozyme. The study was therefore, planned to examine the effect of additional lysozyme to cow milk on calves growth performances.

MATERIALS AND METHODS

About 15 female and 18 male Holstein calves were used in this study and allocated into four experimental groups with similar birth weights. All calves were kept together with their mothers for the first 3 days after calving and then were housed in calf hutches. All hutches had soil floors with a straw bedded which is commonly used in intensive dairy farm for calf comfort. Calves were fed colostrums as soon as possible after birth during the 3 days period. After 3 days between day 4 and 60 days, calves were fed with whole milk from a pail twice a day, 228 L whole milk (2 L each) over the whole experiment. Water was provided in the bucket used for the milk feeding. All calves were fed *ad libitum* with calf starter along with alfalfa hay. Live weight, live weight gain and feed intake were determined weekly. The chemical composition of calf starter and alfalfa hay is given in Table 1.

Calves were fed with milk which is enriched with 10 mg L^{-1} chicken type lysozyme and EDTA during milk fed period. The experiment was carried out in a completely randomized design with 2 (Gender) ×2 (Lysozyme) factorial arrangements. The data obtained were analyzed by GLM procedure of SPSS with Duncan's Multiple Range test.

Table 1: The chemical composition of calf starter and alfalfa hay

Chemical composition (as fed basis)	Starter	Alfalfa
Dry matter	93.77	93.24
Crude protein	16.70	11.60
Crude fiber	9.65	34.52
Ether extract	4.14	0.80
Ash	7.85	7.63

RESULTS AND DISCUSSION

During 0-35 days period of the experiment, lysozyme supplementation decreased daily gain and increased feed to gain ratio ($p<0.05$, Table 2) and male calve had higher daily gain, feed intake and better feed to gain ratio in the same period ($p<0.01$, Table 2).

The effects of lysozyme and gender on daily gain and feed to gain ratio have disappeared during 35-60 days period. Lysozyme decreased feed intake ($p<0.05$, Table 2) during 35-60 days of the experiment. Similarly overall feed intake, daily gain and feed to gain ratio were not affected by lysozyme ($p>0.05$, Table 2). Male calve had higher daily gain and weaning weight and lower feed to gain ratio than female calve throughout the study ($p<0.05$, Table 2). Lysozyme x gender interaction affected feed intake in all experimental period and male calves receiving control diet had higher feed intake than female but lysozyme supplemented male calves had lower feed intake than females ($p<0.05$). A total of 18 calves in groups of lysozyme only had 11% (2/18) diarrhea case while 33% of the control calves (5/15) suffered diarrhea.

It is well known that male calve may grow better than females however, both gender preruminant stage may had similar growth rate as reported in the some studies (Sahani *et al*., 1998; Bilgic and Alic, 2004; Kocak *et al*., 2007). Lysozyme is one of the main non specific immunological component present in body fluids (tear, gastric juice, milk, etc.) contribute by defensing against infection by patogenic organism, the stimulation of beneficial gut microflora and maturation of the intestinal tract (Lonnerdal, 2003), depress superoxide generation of neutrophyls and enhance lymphocyte proliferation (Dominguez-Bello *et al*., 2004). Lysozyme is a bacteriolytic enzyme posses mureinolic activity which hydrolyzes the β-1, 4 glycosydic bond of the peptidoglycan of cell wall of certain bacteria. The lysozyme supplemented calves had lower daily gain as a reflection of worsening feed conversion especially in the 1st 5 weeks of the experiment. Later stage of the study (35-60 days), feed intake decreased markedly with lysozyme supplementation. These could be explained by a delay to establish balanced intestinal flora with high level of lysozyme usage for supplementation. Generally monogastric animal responded better to lysozyme supplementation due to lower levels of coliform bacteria in the small intestine including fewer *Escherichia coli* (Brundige *et al*., 2010). Similarly, Humphrey *et al*. (2002) reported that chick receiving transgenic rice expressing lactoferrin and lysozyme may have a potential to be used instead of antibiotics. However, there is marked differences in

Table 2: Response of milk fed calves to supplemental lysozyme

	Control		Lysozyme (L)		Significancy		
Gender (G)	Female	Male	Female	Male	L	G	L*G
No. of calve	7	8	8	10	-	-	-
Birth weight (kg)	36.26±1.650[a]	37.12±1.5200[a]	35.44±1.520[a]	37.37±1.360[a]	0.853	0.362	0.726
From birth to 35 days old							
Daily gain (g day^{-1})	142.57±32.23[c]	397.50±30.150[a]	124.25±30.15[c]	268.40±26.96[b]	0.020	0.000	0.070
Total feed intake (g day^{-1})	251.86±9.340[bc]	313.63±8.7400[a]	286.50±8.740[b]	272.60±7.820[b]	0.720	0.010	0.000
Feed to gain	2.06±0.330[b]	0.80±0.3100[a]	3.04±0.310[c]	1.24±0.270[ab]	0.030	0.000	0.420
35 days body weight	41.24±1.600[b]	51.03±1.5000[a]	39.79±1.500[b]	46.77±1.330[a]	0.060	0.000	0.350
From 36 days old to weaning (60 days old)							
Daily gain (g d^{-1})	422.71±74.26[a]	511.13±69.460[a]	471.38±69.46[a]	516.20±62.13[a]	0.700	0.340	0.750
Total feed intake (g day^{-1})	669.43±22.49[ab]	731.13±21.030[a]	675.00±21.03[ab]	640.20±18.81[b]	0.050	0.530	0.030
Feed to gain	1.65±0.290[a]	1.61±0.2700[a]	1.77±0.270[a]	1.58±0.240[a]	0.870	0.680	0.790
From birth to weaning							
Daily gain (g day^{-1})	282.65±35.74[c]	454.206±33.44[a]	297.77±33.44[bc]	392.29±29.91[ab]	0.490	0.000	0.260
Total feed intake (g day^{-1})	460.64±13.09[b]	522.38±12.240[a]	480.75±12.24[b]	456.40±10.95[b]	0.070	0.140	0.000
Feed to gain	1.69±0.170[bc]	1.18±0.1600[a]	1.81±0.160[c]	1.26±0.140[ab]	0.520	0.000	0.920
Sutten kesim Aoyrlyoy	56.04±2.310[b]	68.91±2.1600[a]	56.29±2.160[b]	64.83±1.930[a]	0.380	0.000	0.320
Diarrhoea case	3	2	2	0	-	-	-

gastro intestinal system in monogastric and ruminants. Establishing and maintaining normal intestinal microorganisms is a basic issue for milk fed-young ruminant rather than increase productive performance (i.e., gain and efficiency). Normally lysozyme is present in herbivore including bovine, ovine and caprine species. It is produced immediately after birth (Guilloteau *et al*., 2009) and its production decrease throughout the preruminant stage. High level of lysozyme supplementation may therefore have negative effect on early establishment of intestinal flora due to its strong antimicrobial properties. Maga *et al*. (2006c) evaluated milk from transgenic goats expressing human lysozyme in mammary gland with piglet and kids. Pig consuming milk from transgenic goats had fewer numbers of coliforms and *E. coli* in their intestine than did those receiving milk from non-transgenic animals. They also reported that kids receiving transgenic milk had high number of coliforms, *E. coli* and lower diarrhea incidence than those receiving non-transgenic milk.

When the results were evaluated for weaning stage there is a decrease in diarrhea incidence with lysozyme supplementation and there is no improvement in daily gain. As mentioned earlier however, the main purpose for the preweaning period for rearing in young ruminants is to reduce digestive problem, morbidity and mortality.

CONCLUSION

It could be therefore concluded that lysozyme may be considered to reduce diarrhea incidence during preweaning period but more research are warranted to reach better and conclusive comments in preweaning period of young ruminants.

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