Short Paper

The effects of oral administration of ascorbic acid on serum γ-globulin concentrations of neonatal calves

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Summary

The effect of ascorbic acid (vitamin C) on the immune system is well-known. Ascorbic acid stimulates either humoral or cell-mediated immunity in many species. This experiment was conducted to evaluate the effect of oral administration of ascorbic acid on serum γ-globulin concentration of colostrum-fed newborn calves. During a cold winter season, 20 dairy Holstein calves from a group of 40 calves were supplemented with ascorbic acid from birth to 3 weeks of age (treatment). The other 20 calves did not receive any ascorbic acid supplementation (control). Ascorbic acid was administered per os and treatment was as follows: 3 g/day for the first week, 2 g/day for the second week, and 1 g/day for the third week. All calves were housed in elevated metal pens during the winter and at low temperature from birth to 60 days of age. Serum samples were collected immediately after birth, before taking colostrum and on the 2nd 14th and 28th days of age via jugular vein. There was no significant difference in serum γ-globulin concentrations for the two groups before taking colostrum and at 2 and 14 days of age. The serum γ-globulin concentration of the supplemented group at 28 days of age was significantly higher than that of calves not receiving ascorbic acid. The results of the present study showed that oral administration of ascorbic acid increases γ-globulin concentration of neonatal calf serum.

Key words: Ascorbic acid, Calf, Gamma-globulin

Introduction

A significant relationship between low serum immune globulins and a high incidence of disease (Boyd, 1972) and even mortality (McEwan et al., 1970; Irvin, 1974) has been observed in calves.

In commercial herds from 20% to 40% of dairy calves may be hypogammaglobulinemic (Selman et al., 1970; Brignolle and Scott, 1980). Even 10% of colostrum-fed dairy calves have low or negligible serum immune globulin concentrations (Selman et al., 1970). This hypogammaglobulinemia in colostrum-fed calves is usually attributed to a- inadequate colostrum ingestion, b- late feeding of colostrums, or c- the early loss of absorption capability in calves (Scott et al., 1979). The effect of supplementation of ascorbic acid (vitamin C) on the immune system of human and several animal species has been previously reported (Cummins and Brunner, 1989, 1991). Dietary supplemented ascorbic acid has stimulated either humoral (including nonspecific immunity) or cell-mediated immunity in catfish (IL and Lovell, 1985), rainbow trout (Verlac and Gabaudan 1990), mice (Mafison and Manwaring, 1937), guinea pigs, human (Prinz et al., 1977) and chickens (Franchini et al., 1994). The mechanism responsible for the effect of this vitamin on plasma antibody
concentration and immune response has not still been explained. However, it appears to be attributed to the effect of stress and associated endocrine changes (Cummins and Brunner, 1991).

There is a high concentration of ascorbic acid in cells of hypophysis, adrenal gland and leukocytes. During stress and increased glucocorticosteroid synthesis, the zona fasiculata of the adrenal cortex is enlarged and the concentration of ascorbic acid is lowered (Kolb, 1992). Both parameters are negatively correlated. In the first few weeks of life, the biosynthesis of ascorbic acid in calves, other young animals and chicks is not fully developed. The high ascorbic acid content in colostrum of the first milking after parturition, declines rapidly and is relatively low in the milk (Wegger and Mustgaard, 1982; Kolb, 1992). Dairy calves do not synthesize endogenous ascorbic acid in first few weeks of life and thus rely only on a relatively low concentration of ascorbic acid in milk (Wegger and Mustgaard, 1982; Kolb, 1992).

The effect of supplementation with ascorbic acid on enhanced resistance to infection (enteric infections, pneumonia, etc.) in young calves of age has been reported (Hemingway, 1991; Seifi et al., 1995).

Calves and piglets which are deficient in colostrum immunoglobulins are highly susceptible to septicemia. Colostrum provides protection against colisepticemia and many other infection diseases (Radostitis et al., 2000). Diarrhea in colostrum-fed calves was prevented by ascorbate at doses of 3, 2 and 1 g day during weeks 1 to 3 of age (Seifi et al., 1995).

This experiment was conducted to determine the effect of dietary supplementation of ascorbic acid on serum γ-globulin concentrations of colostrum-fed calves that were housed in elevated metal pens during a cold winter.

**Materials and Methods**

In this experimental study, 40 newborn Holstein calves were divided into two equal groups. One group received ascorbic acid as the free acid (250 mg tablets, Loghman Pharmaceutical Co., Tehran, Iran), in their daily feed up to 3 weeks of age (treatment group). The other group did not receive ascorbic acid (control group). All calves received colostrum immediately after birth by bucket nearby their dams. All Holstein calves received two liters of fresh colostrum during the hours after birth beside their dams, were removed from cows during three hrs after birth and housed in elevated metal pens (1.5 × 1.01 m). All the calves received four liters fresh colostrum during the first two days of life. The calves were housed in these pens up to 60 days of age. In the treatment group, supplementation of ascorbic acid was as follows: 3 g/day (12 tablets) for the first week, 2 g day (8 tablets) for the second week and 1 g/day (4 tablets) for the third week after birth. The daily supplementary ascorbic acid was dissolved in whole raw milk and fed in two equal doses. All calves were fed approximately 4–5 liters whole milk daily and offered a calf starter ad libitum after the first week.

Blood samples were collected from the jugular vein using vacuum tubes (Pars Khavar, Ghazvin, Iran) immediately after birth, before ingestion of colostrum and on 2, 14 and 28 days of age. Sera were removed and stored at -20°C until analysis.

Estimation of γ-globulin was carried out by cellulose acetate electrophoresis. Total protein was estimated by the Biurea method using biochemical autoanalyser (Eppendorf, Germany). Then γ-globulin concentration was measured on the basis of the samples’ total protein (g/dl) with automated densitometry (LRE Pherotrosis, Germany) (Peters et al., 1982; Tietz, 1990).

Data obtained were processed by SPSS software (Nie et al., 1986). Mean values were compared by one-way analysis of variance (ANOVA) followed by Fischer’s LSD as the post hoc test with the significance level set at <0.05.

**Results**

Serum immunoglobulin concentrations for the treatment group were significantly greater than for the control group at 28 days
of age (P<0.05). Mean serum γ-globulin concentrations of 2.782 and 1.717 g/dl were found for calves receiving ascorbic acid on days 2 and 28 of age, respectively. In the control group, however the corresponding values were, 2.884 and 1.395 g/dl (Fig. 1).

Treatment had no effect on γ-globulin concentrations on days two and 14 of age (P>0.05). Serum total protein was not affected by ascorbic acid administration (P>0.05) (Fig. 2).

There was no significant difference in albumin to globulin ratio between the treatment and control groups. Colostrum feeding tended to increase serum γ-globulin concentrations on day two of age, but γ-globulin concentrations progressively decreased on days 14 and 28 of age. Calves supplemented with ascorbic acid over 21 days of age had a slower rate of reduction in immunoglobulin concentrations(Fig. 1).

**Discussion**

The results of the present study are different from the previous studies in which variable responses to dietary ascorbic acid supplementation were reported (Cummins and Bruner, 1989, 1991).

Calves deprived of colostrum and housed in metal pen with strict confinement (and high humidity as subjectively observed in this facility) during cold winter months, responded to oral supplementation of ascorbic acid with increased plasma IgG concentrations (Blair and Cummins, 1984). In another experiment performed in the same laboratory, colostrum deprived calves, which were housed in hutchs during warmer weather, did not respond to dietary ascorbic acid with increased plasma immunoglobulin concentrations (Cummins and Brunner, 1989). They concluded that immunostimulatory effects of ascorbic acid may be more evident in animals with low plasma immunoglobulin (IgG) concentrations that in those subjected to stressful conditions (Cummins and Brunner, 1989).

It has been reported that ascorbic acid supplementation could not increase plasma immunoglobulin concentrations of colostrum-fed calves (Blair and Cummins, 1984; Cummins and Brunner, 1989). Actually, in one study, colostrum-fed calves that received ascorbic acid had slightly lower plasma IgG concentration on 14 and 28 days of age than those received no ascorbic acid (Cummins and Brunner, 1989).

In the present study, colostrum-fed calves that received ascorbic acid had higher serum concentrations of γ-globulin on 14 and 28 days of age, than calves, which not received ascorbic acid. The difference between the two groups on 28 days of age was statistically significant (P<0.05).

In calves with low post-colostral immunoglobulin levels, the concentration progressively increases after birth. There is

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**Fig. 1:** Serum γ-globulin concentrations (Mean ± SE, n = 20) in calves with and without ascorbic acid supplementation

**Fig. 2:** Mean serum total protein concentrations in the treatment and control calves
also a progressive decrease in serum immunoglobulin of calves with high post-
colostral levels (Boyd, 1972). In calves with low initial level of passive acquired
immunoglobulin, the overall rise is due to the formation of autogenous globulin,
whereas in calves with high initial level, destruction of initially high level of
passively acquired immunoglobulins masks any formation of autogenous antibody. The
estimated half-life of passive acquired immunoglobulin is 21.5 days (McEwan et al.,
1970). It has been shown that the ability to produce immunoglobulin or antibody in response to certain forms of
antigenic stimulation is present at birth and the detectable immunoglobulin during the
second week of life is probably a measure of immune response to antigenic stimuli started
immediately after birth (Franchini et al., 1994).

In the present study, a progressive decrease in γ-globulin concentrations in the
two groups of treatment and control was observed.

Reduction in the serum γ-globulin level in control calves, during the study was faster
than for the calves receiving ascorbic acid. It was probably because of the higher
production of autogenous antibody in the treatment group (Fig. 1). Halving the
concentrations of passively-acquired γ-globulin in the control group occurred by
approximately 21 days of age. In the treatment group, it had not quite halved even at 28 days of age. This is probably due to an
increase in autogenous synthesis of γ-globulin. Oral administration of ascorbic acid to colostrum-fed neonatal calves
significantly increases the concentration of γ-globulin on day 28 of age that might
protect them from infection.

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References

1- Blair, L and Cummins, KA (1984). Effect of
dietary ascorbic acid on blood
immunoglobulin concentration in dairy
2- Boyd, JW (1972). The relationship between
serum immune globulin deficiency and
disease in calves: a farm survey. Vet. Rec.,
90: 645-649.
3- Brignolle, TJ and Scott, GH (1980). Effect of
sucking followed by bottle-feeding colostrum
on immunoglobulin absorption and calf
4- Cummins, KA and Brunner, CJ (1989).
Dietary ascorbic acid and immune response in
of calf housing on plasma ascorbate and
endocrine and immune function. J. Dairy Sci.,
72: 1582-1588.
6- Franchini, A; Bertuzzi, S and Tosarelli, C
(1994). Chronobiological influence of
vitamin C on chicken immune functions.
Arch. Geflügelk. 58: 165.
7- Hemingway, DC (1991). Vitamin C in the
8- IL, Y and Lovell, RT (1985). Elevated levels of
ascorbic acid increase immune responses in
colostrum-deprived calves. Vet. Rec., 94:
105.
10- Kolb, VE (1992). Neur erkenntnisse zur
betung der askorbin saure fur haustier und
zu ihrer anvendung in der veterinarmedizin.
Tierarztl. Umschau. 47: 163.
11- Mafison, RR and Manwaring, W (1937).
Ascorbic acid stimulation of specific antibody
402.
12- McEwan, AD; Fisher, EW and Selman, IE
(1970). Observation on the immune globulin
levels of neonatal calves and their
relationship to disease. J. Comp. Pathol., 80:
259.
13- Nie, NH; Handalhull, C; Jenkins, JG;
Steinbrenner, H and Bent, DH (1986). SPSS:
Statistical package for the social science.
14- Peters, TJ; Biamonet, GT and Doumas, BT
(1982). Protein in serum, urine and CSF,
selected method of clinical chemistry. 3rd.
Edn., Vol. 9, AACC (American Association


18- Selman, IE; McEwan, AD and Fisher, EW (1970). Serum immune globulin concentrations of calves left with their dams for the first two days of life. J. Comp. Pathol., 80: 419.


