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The Effect of *Aloe vera* Gel on Performance and Serum Electerophoresis Pattern in New born Calves

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ABSTRACT

In this study the effect of Aloe vera leaf gel on absorption of colostral immunoglobulin from intestinal mucosa in new born calves was investigated at 48 h of age(time I) and 72 h of age (time II). Weight gain was assessed until weaning weekly. Thirteen Holestein calves with average 45 kg were divided into two groups, randomly. Daily, 15 ml Aloe vera gel was given to treatment group with colostrum and milk. Blood specimen was collected from jugular vein at both times (I,II). Total protein γ-, globulin, and weight gain were assayed. The result of acetate cellulose electerophoresis showed the level of γ- globulin in treatment and control groups were 1/33 g/dl and 1/25 g/dl at time I that was not significant but correlation between serum protein and gamaglobulin levels was significant in treatment group (r=0/5832, P=0/05) against control (r=0/0141) at time I. Increasing immunoglobulin absorption and also decreased FPT in treatment group (35 %) in comparison with control (44%) could be the cause of significant correlation. At time II, the level of gamaglobalins decreased briefly in treatment group, conversly. The level of serum IgG by sodium sulfite precipitation test revealed a boot 20 % calves had less than 500 mg/dl IgG in serum in treatment group in contrast to control at time II. The more important correlation in treatment group(r=0/7646 P=0/01) than control (r= 0/5404 P=0/05) represented increasing serum non imunoglobulin proteins in treatment group at time II. On the other hand, differences between r^2=0/5846 in treatment group and r^2=0/2925 in control may be represented modified protein metabolism in consequence of Aloe vera effect on regulation of protein metabolism in treatment group at time II. predicted serum protein concentration at 1 g IgG /dl serum in treatment and control groups at time II and treatment group at time I were approximately 5.8 g/dl. Therefore cutoff value was not affected by use of Aloe vera gel in three groups. This means Aloe vera does not interfere with diagnosis FPT by measurement of total protein concentration in new born calves. Also, the consumption of Aloe vera gel have not significant effect on gain weight until weaning. In conclusion Aloe veragel has favorable effect on the level of gamaglobulin and decreasing of FPT at time I but it has no effect on gain weight in new born calves.

Keywords: *Aloe vera* , colostrum, Immunoglobulin, , gain weight.
Abbreviation key: FPT: Faiuler of Passive Immunity.

INTRODUCTION

Transfer of immunoglobulins in Some of Species neonates for instance human, Guinea pig and rabbit occur by means placenta [1]. The placental structure of ruminant prevents the transmission of immunoglobulins from dam to the fetus in utreo [1]. Also rising of glucocorticoids level in blood at birth attenuates cellular immunity. Therefore the young calves are agamaglobulinemic at birth [2, 1]. So the best and the most rapid mechanism for obtaining passive immunity in calves is the transfer of immunoglobulin from colostrum across the small intestine [3]. Colostrum is the early milk produced by female mammals and in the case of bovin is the first four days of milk post parturition [4]. It contains essential component for a new born for the first few days of life. The most important component of colostrum are immunoglobulins that transfer passive immunity to newborn Calves [5]. Colostrum approximately contains IgG% 85-90, IgM %7 , IgA %5 [5]. New born calves rely on antibodies present in

colostrum for protection against common environmental pathogens [6]. These immunoglobulin molecules may constitute 50 to 75% of The colosteral proteins. The IgGimmunogloblins can be divided into two subclasses IgG1 and IgG2. IgG1 selectively transported in unaltered form by udder from the circulation to the lacteal secretions. Hence IgG1 is the principal immunoglobulin for passive immunization in calves. In contrast to IgG1 little IgG2 enter the lacteal secretions [7]. Absorbed immunoglobulins and the acquired immunity remain for 14to 67 days [7].Neonates are immunocompetent at the birth [6] but endogenous antibody production does not reach to the protective level until 1 month of age [8] and maximum level until at least 2-3 month of age [6, 9]. Immunoglobulins appear in the Serum of a new born calf within one to three hours after the feeding of colostrum and reach a maximum at 6 to 24h [7]. Ideally, Colostrum should be consumed by the calves in the first four hour Postpartum [10]. Transfer of passive immunity is generally considered to be adequate if serum concentration IgG would be more than 1g/dl in calves that have been fed by colostrum when sampled between 24 and 48 hour of age [6]. Failure of passive transfer (FPT) alone is not considered as a disease however, it predisposes neonates to infection and septicemia if it is not immediately identified and Corrected [3]. The ingested immunoglobulins in colostrum nonselectively absorb across the small intestine by pinocytic mechanism of fatal vacuolated enterocyte [12] and released into the lymphatics by exocytosis. Then, enter circulatory system through the thoracic duct [13]. This process refers to as open gut [12]. The ability of colosteral immunoglobulin by enterocytic cells begin to decline at 8-12 h of age and would be completed by 24 h of age [14]. The cessation of macromolecular absorption by enterocytesic cells refers to as closure. The exact mechanism of this process does not elucidate but it Probably due to the replacement of fatal vacuolated enterocyte by adult type enterocyte [15]. This replacement occurs during 5-7 day after birth [14].The fatal enterocyte cells are capable of immunolgoblin uptake but adult enterocyte cells are incapable of that. Thereby this replacement contributes to intestinal barrier closure [14, 16]. Therefore, the base on above description, the factors which are capable to increase permeability of enterocyte and finally absorption ofγ-globulins increase from colostrum across the small intestine could be very important. In this study we have assessed the effect of consumption *Aloe vera* gel on the level of γ- globulins absorption from colostrum across the small intestine and acquired passive immunity in new born calves at 48 and 72h of ages (time I,II).

The direct and indirect effects of *Aloe vera* gel on immune system has been investigated by other researchers but there is no any research on the effect of *Aloe vera* gel on Passive immunity in calves. *Aloe vera* is one of the medicinal Plants belonging to liliaceae family and grow in hot dry climate [17]. *Aloe vera* leaf can be divided into two major parts namely the outer green rid including the vascular bundle and inner colorless parenchyma containing *Aloe vera* gel [17]. More than 75 ingredient have been identified in it [17]. *Aloe vera* extract possesses many biological activities such as antidiabetes [18], anti-inflammation [19], anti-cancer [20], anti-microbial, anti-fumgal[21] anti-viral [22] and anti-oxidant acti-vities23] Other activities includes macrophage activation [24, 25], wound healing [19], biological membrane permeation [26]. Therefore in this study the effect of Aloe veva gel on the permeability of biological membrane specially entrocytic cell has been considered. Before time it was shown that *Aloe vera* gel increased significantly the transport of the macromolecular peptide drug, insulin , across the caco- 2-cell mono layer [26]. Besides, *Aloe vera* gel is used as alternative medicine antibiotics in treating mastitis [27] and for treatment poultry infectious disease[28].

MATERIALS AND METHODS

In this study thirteen Holestein calves with average 45Kg were divided into two groups, treatment and control, randomly. Treatment group were fed with 15 ml *Aloe vera* gel that was Produced by Barij Essence pharmaceutical co, with colostrum and milk from birth to eight week of age on daily basis. Blood specimen were taken from jugular vein before Feeding with colostrum at 48 and 72 h of age. Then it was centrifuged at 3000 rpm for 10 minutes and separated sera stored until analysis at- 20^0C.Serum Protein concentrations assayed by referactometric method [29] (Refractomer model erma, Made in Japan). Serum concentrations of albumin, α- globulin, β- globulin and γ-globulin were assayed by acetate cellulose electerophoresis (Electerophores apparatus Model Helena, made in America). IgG concentration was assessed by sodium sulfate precipitation test [30]. In this method, the utilization of 3 concentration of sodium sulfate 14g/ 100, 16g/100, 18g/100 allowed scoring of serum immunoglobulin concentration as less than 500 mg /dl, 500-1500 mg/dl, and over than 1500 mg/dl IgG in serum respectively. The assessment of the performance of calves was done by weekly weighting before feeding until eight weeks of age.

The statistical analysis of data for each parameters were based on the average value obtained. All result expressed as Means ± Standard Error of the Means ± (SEM), P values $\leq$0.05 and $\leq$0.01 were considered statistically significant. Data were analyzed by using SPSS 16 Software. Processing of regression models was done by exele software.

RESULTS

Table 1. Mean value (± SEM) of total serum protein concentrations (g/dl) and mean value of the concentrations of each fraction of serum protein electrophoresis. Alb, α- globulin, β- globulin, γ- globulin and the ratio of albumin to globulin (A:G) in treatment and control groups at 48 (time I) and 72 h (time II) of ages.

Parameters	Mean value		SEM	P	Mean value		SEM	P
	Treatment group Time I	Control group Time I			Treatment group Time II	Control group Time II		
Albumin (g/dl)	3.1	3.12	0.17	ns	3.1	3.07	0.187	ns
α-globulin (g/dl)	0.927	1.106	0.34	ns	0.81	0.83	0.127	ns
β–globulin (g/dl)	1.3	1.05	0.166	ns	1.28	1.3	0.558	ns
γ-globulin (g/dl)	1.33	1.25	0.213	ns	1.44	1.47	0.221	ns
Total protein (g/dl)	6.65	6.54	0.31	ns	6.54	6.64	0.217	ns
A:G ratio	0.98	1.01	0.136	ns	1.01	0.92	0.136	ns

ns= not significant; A:G ratio= Albumin to globulin ratio; Alb = Albumin

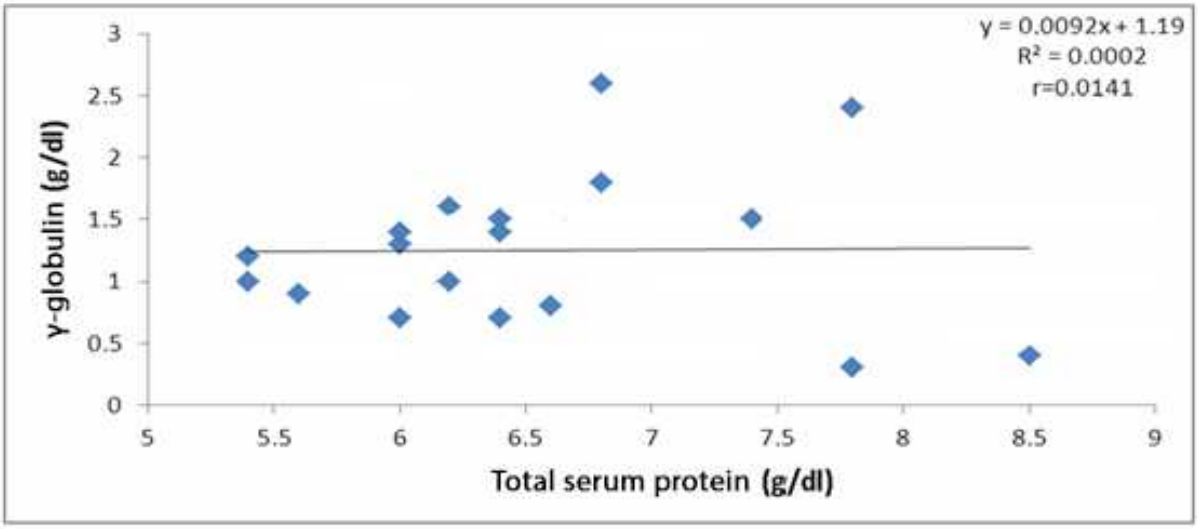

Figure 1.Relationship between total serum protein concentration and γ- globulin concentration in newborn calves in control group at 48 h of age (time I)

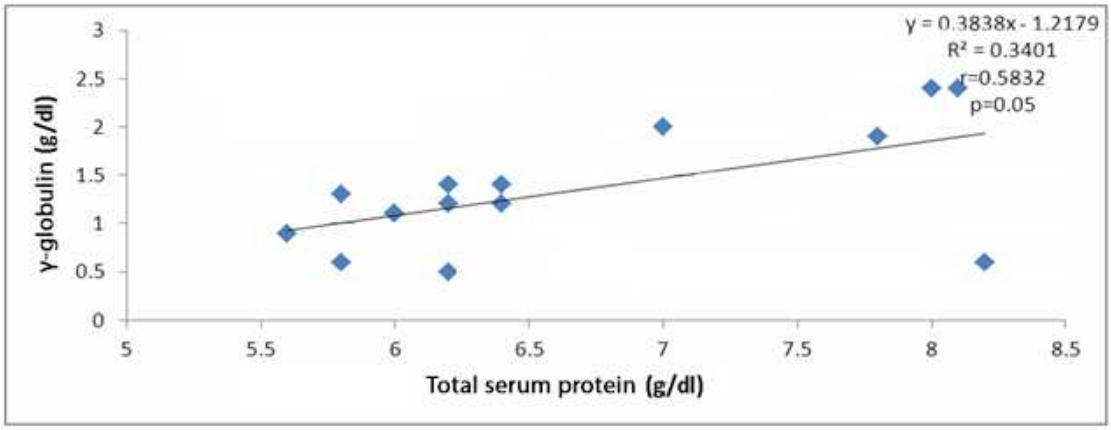

Figure 2. Relationship between total serum Protein concentration and γ- globulin concentration in new born calves in treatment group at 48 h of age (time II)

Table 1shows that the mean value concentrations of total serum protein, β- globulin and γ- globulin in treatment group is more than, control group but the mean value of Alb concentration and the ratio of albumin to globulin (A: G) in treatment group is less than control group at time I. The mean value of total serum Protein concentration and The mean value of concentrations of α and β globulines in treatment group is less than control group but the

concentration of Alb and the ratio of albumin to globulin is more than control group at time II. The observed differences between above parameters are not statistically significant.

Regression line and regression equation in Figure 1do not show any relationship (r= 0/0141) between total serum protein and γ- globulin concentration in control group at time I but regression line and regression equation in figure 2 show relatively close relationship (r= 0/5832 P≤0.05) between two parameters in treatment group at time I.

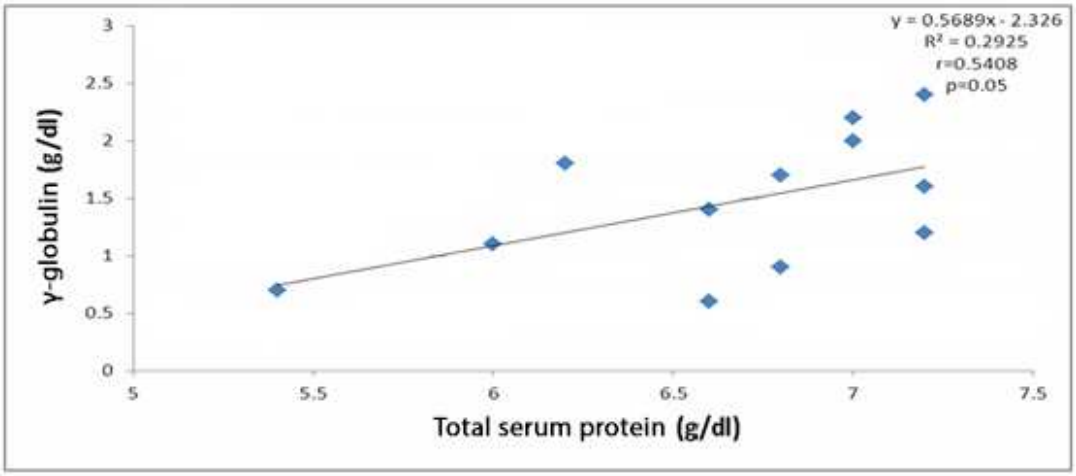

Figure 3. Relationship between serum total protein concentration and γ- globulin concentration in newborn calves in control group at 72 h of age (time II).

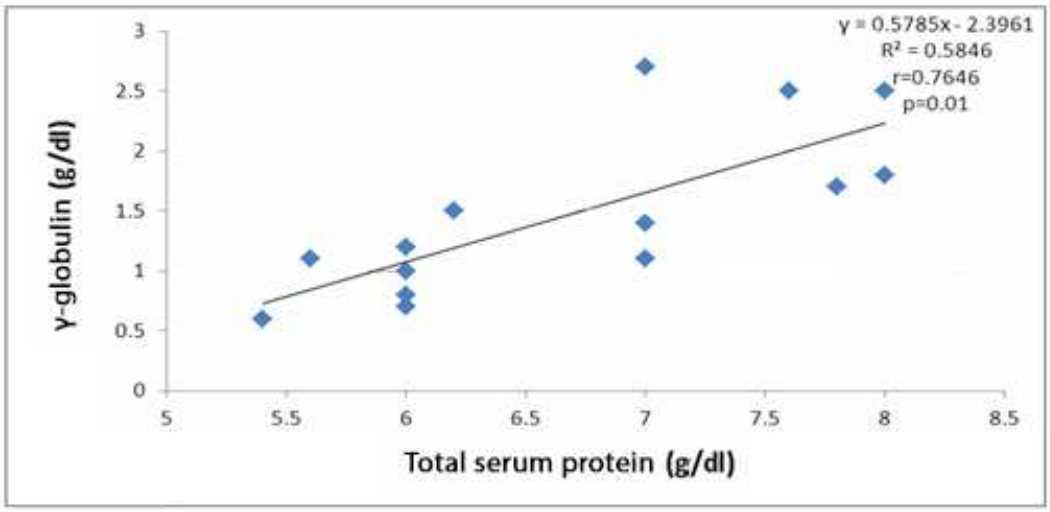

Figure 4. Relationship between total serum protein concentration and γ- globulin concentration in newborn calves in treatment group at 72 h of age (time II)

Regressions line and regression equation in Figure 3 show relatively close relationship (r= 0/5408 P≤0/05) in control group at time II, whereas figure 4 show close relationship (r= 0/7646 P≤0/01) in treatment group at time II.

Table 2. The frequency of IgG concentrations and Chi-Square test for greater than 500mg/dl IgG and less than 500 mg/dl IgG in serum in control and treatment group at 72 h of age (time II)

		Less than 500 mg/dl IgG	greater than 500 mg/dl IgG
	Treatment group (time II)	19 %	81 %
Frequencies	Control group (time II)	1 %	99 %
Chi- Squre test	---------	1.5211**	1.99

*** = Significant at p<0/01*

Table 2 shows that the Population frequency for the amount of greater than 500 mg/dl IgG were 99% and 81% in control and treatment groups at time II respectively. On the basis of Chi-Squre test this difference was not statistically significant, whereas population frequency for the amount of less than 500 mg/dl IgG were 1% and18% in control and treatment group at time II respectively. On the basis of Chi-Squre test this difference was statistically significant (P≤0/01).

Table 3. The mean value of body weight of calves ± SEM during eight weeks from birth and the percentage of gain weight ± SEM at eight week age in related to birth weight of calves in control and treatment groups

Time(week)		1	2	3	4	5	6	7	8	gain weight after eight week (%)
Weight (kg)	Treatment group	45.8	45.23	46.09	49.9	48.32	50.14	56.72	59.55	24.1 %
	Control group	45.23	45.32	45.32	49.18	48.41	50.59	56.05	58.12	23.04 %
SEM	--------	0.898	0.921	0.959	1.082	1.305	1.084	1.074	1.086	0.979
P	--------	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns = not significant

Table 3 does not show significant difference weekly in gain weight in Treatment and control groups during eight week from birth. Also the percentage of gaining weight at eight week of age relating to the birth weight were 24/1% and 23/04 % in treatment and control groups respectively. This difference also does not statistically significant.

DISCUSSION

In this study our results revealed separately four protein bands by acetate cellulose electerophoresis includes Alb, α-globulin, β- globulin and γ- globulin. This findings were in agreement with the studies of Kaneko [31]. The mean value of total serum protein and mean value of each fraction of protein electrophoresis that were shown in table 1 for control group at 48h of age (time I) were in accordance to reference value for Holestein cow at 1-14 days of age [32]. The mean value of γ-globulins were respectively 1/25 g/dl and 1/74 g/dl for control groups that were fed with colostrum at 48 and 72 h of ages. These results showed slight increase in the absorption of γ-globulins from colostrum in control group at 72 h of age compared with control group at 48 h of age (time I). Although this result is not statistically Significant but findings of Piccione and Stelania [33] also demonstrated Slight absorption of γ-globulines increase during the third day after birth for Limousine cow. The obtaining result in our studies seems to be rational, since the exchange of fatal vacuolated enterocyte with adult enterocyte accomplished within 5-7 day after birth and gut closure completely happens [14] of course There are another factors That influences the Level of γ- globalins absorption from gut in newborn calves. One of which is the level of trypsin inhibitor in colostrum that protects immunoglobulins from proteolysis, so that, the highest amount of its exists in the first milking and then decreases [34].Furthermore, another factor is Probably the occurrence of enzyme modification with advancing age in cow stomach [14]. The mean value of total serum protein in treatment and control groups at 48 h of age (time I) were 6.65 g/dl and 6.56 g/dl and mean value of Albumin to globulin ratio were 0.98 and 1.01 respectively. This results explain that the slight rise in serum protein concentration is due to increasing of globulins concentration in serum. Although this result was not statistically significant, the assessment of relationship between serum protein concentration and γ- globulin concentration was revealed better consequences. Linear regression and Linear equation showed week association between two parameters in control group at 48 h of age (r= 0/0141) (Figure 1) but significant linear association between two parameters was shown in treatment group at 48 h of age (r=0/05832, P≤ 0.01)(Figure 2).Also less proportion of FPT was seen in treatment group (35%) compared with control group (44%) at 48 h of age.

Therefore decreasing of FPT in treatment group compared with control group under similar management could demonstrate that Aloe vere gel increases permeability of entrocytic cells and finally increases of γ– globulins from colostrum and decreases FPTin Treatment group at 48h of age (time I). Approvingly the previous studies showed *Aloe vera* gel decreased the transepithelial electrical resistance of intestinal cell monolayer [26]. *Aloe vera* gel was also able to significantly increase the transport of macromolecular peptide drug, insulin, across the caco-2 cell monolayer [26].

Of course for obtaining more significant results in the following studies we suggest the increase of the amount of *Aloe vera* gel and the increase number of calves in the experiment. Our results showed the mean value of γ–globulins concenteration in control and treatment group at 72 h of age (time II) were 1/47 g/dl and 1/44 g/dl respectively. This result showed slight decrease of γ–globulins in treatment group compared with control. Although this result was not statistically significant, in another approving experiment, measuring of IgG concentration by sodium bisulfit precipitation test revealed serum IgG value was less than 500 mg/dl for 20% of calves (p≤0/01) (table 2) in treatment group compared with control group at 72h of age (time II). There can be several reasons for the observing results. The one of which is simultaneously decreasing trypsin inhibitor in colosterum on the third day after birth [34] and the existence carboxypeptidase in *Aloe vera* gel [35] that help destroy proteins such as γ–globulins. The other reason is the exchanging of neonatal vacuolated enterocyte with adult entrocyte on third day with advancing age from birth [14] and Probably ineffective of *Aloe vera* gel on adult enterocyte. The last reason may be due to normal consumption of IgG in response to environmental pathogens regarding to the activating macrophages by *Aloe vera* gel [24].

In this study relationship between total serum protein and γ– globulins concenterations was assessed in treatment and control groups at 72 h of age (time II). Linear regression and linear equation showed more significant association in Treatment group (r= 0/7646, p≤0.01) (Figure 4) Compared with control group (r= 0/5408, p≤0.05) (Figure 3). The observed differences in r values means at equal concentration of γ– globulins there was more amount of protein in treatment group compared with control group. This finding could due to more consumption of IgG in treatment group than control group or due to the effect of *Aloe vera* gel on modifying protein metabolism in treatment group at 72 h of age (time II) in that to some extent of amino acids entrance to gluconeogenesis Pathway or protein biosynthesis in new born calves.

In accordance with present reports FPT occurred when The concentration of IgG in blood serum is less than 1g/dl in calves. In This study prediction of serum protein concentration at 1 g/dl IgG in treatment groups at 48 and 72 h of age (time I , II) and Control group at 72 h of age (time II) were 5.8, 5.86, 5.8 g/dl respectively. Therefore the use of *Aloe vera* gel with colostrum in new born calves have not effect on cutoff point value. Thus it does not interfere with assessment of FPT by the measurement of total serum protein concentration in new born calve. In this study the effect of feeding calves with *Aloe vera* gel together with colostrum or milk on calves performance and gaining weight during eight week from birth were assessed. Calves performance were assessed by weekly weighting of calves. On the basis Table 3 the percent of gaining weight at eight week of age relating to the birth weight were 24/1% and 23/01% in treatment and control groups respectively that was not statistically significant. However in spite of existence of biologically active components in *Aloe vera* gel that were shown proliferative cell effect for example, veracylglucan C, βsitoestrol, different molecular weight glycoproteins and lectins [35] and also, overall effect of *Aloe vera* gel on health, we expected better growth.

In conclusion, the *Aloe vera* gel have beneficial effect on γ– globulins absorption and decreasing of FPT in new born calves at 48 h of age (time I) but does not have any effect on the performance and the growth of calves during weaning. In the following studies for obtaining more important result we suggest increasing the amount of *Aloe vera* gel consumption and the number of calves in each experiments. In addition, another suggestion is inactivating *Aloe vera* carboxypeptidase in a way that it can not effect on activity of other component in *Aloe vera* gel.

REFERENCES

[1] G.H. Arthur, In: G.H. Arthur, D.E. Nokes, H. Peasron (ED.), The development of conceptus. Pregnancy and parturition in veterinary reproduction and obstetrics, 7thedn, (WB. Saunders, Philadelphia, **1996**), 51-109.
[2] W.J. Penhale, G. Christie, A.D. McEwan, E.W. Fisher, I.E. Selman, *Brit. Vet. J*., **1970**, 126, 30-37.
[3] T.C. Mcguire, N.E. Pfiffer, J.M. Weikel, R.C. Bartsch, *J. Am. Vet. Med. Assoc*., **1976**. 169, 713-718.
[4] P.K. Gopal, H.S. Gill, *J. Nutr*., **2000**. 84, 68-74.
[5] T.J. Newby, C.R. Stokes, F.J. Bourne, *Vet. Immunol. Immunop*., **1982**, 3, 67-94.
[6] D.M. Weaver, J.W. Tyler, Van Metere, D.E. Hostetler, G.M. Barrington, *J.Vet. Intern. Med*., **2000**, 14, 569-577.
[7] J.E. Butler, *Vet. Immunol. Immunop*., **1983**, 4, 43-52.
[8] G.M. Barrington, *Food. Anim. Practice*., **2001**, 17, 463-476.
[9] A.J. Husband, M.R. Brandon, A.K. Lascalles, *AUS. J. Exp. Biol. Med. Sci*., **1972**, 50, 491-498.
[10] P. Michanek, M. Ventrop, B. Westrom, *Res. Vet. Sci*., **1989**, 43, 375-379.
[11] O.M. Rudostits, D.C. Gaycc Blood, K.W. Hincheliff; A Textbook of the Disease of Cattle, Sheep, Pigs, Goats, and horses, WB Saunders, London, **1999**, 9thedn.
[12] C.W. Broughton, J.G. Lecce, *J. Nutr*., **1983**, 4, 43-52.
[13] T.E. Staley, C.D. Corles, L.J. Bush, *Anat. Rec. J*., **1979**, 172, 559-579.
[14] P. Guilloteau, R. Zabilski, J.W. Blum, *J. Physiol. Pharmacol*., **2009**, 60(3), 37-46.
[15] G.H. Stott, D.B. Marx, B.E. Menefee, *J. Dairy, Sci*., **1979**, 62, 1632-1638.
[16] J.F. Trahair, P.M. Rohinson, *J. Anat*., **1989**, 166, 103-110.
[17] G.H. Hammana, *Mol*., **2008**, 13, 1599-1616.
[18] S. Rajasekaran, K. Ravi, K. Sivagnana, S. Subramanian, *Clin. Exp. Pharmacol. P*., **2006**, 23, 232-237.
[19] T. Reynolds, A.C. Dweck, *J. Ethnopharmacol*., **1999**, 68, 30-37.
[20] V. Stenkamp, M.J. Stewart, *Pharma. Biol*., **2007**, 45, 411-420.
[21] O.C. Agarry, M.T. Olaleye, C.O. Betlo-Michael, *Afr. J. Bio*., **2005**, 4, 1412-1414.
[22] D.S. Alevs, S.L. Perez-Fon, A. Estepa, V. Nicol, *Biochem. Pharmacol*., **2004**, 63, 18-21.
[23] L. Lenfmead, R.J. Makins, D.S. Rampton, *Aliment. Pharm. Ther*., **2004**, 19, 521-527.
[24] N. Pugh, S.A. Ross, M.A. Elsahly, D.S. Pasco, *J. Agr, Food. Chem*., **2001**, 49, 1030-1034.
[25] L. Zhang, I.R. Tizard, *Immunopharmacology*., **1996**, 35, 119-128.
[26] W. Chen, Z. Lu, A. Viljoen, J. Hammana, *Planta. Med*., **2009**, 75(6), 587-595.
[27] J. Duval, *Ecol. Agr. Pro*., **1997**, 110, 320-388.
[28] M. Mwale, E. Bhelhe, M. Chiamonyo, T.E. Halimani, *Int. J. Appl. Res. Vet. M*., **2005**, 3(2), 162-170.

[29] D.B. Hand, *J. Biol. Chem*., **1935**, 108(3), 703-707.
[30] N.E. Pfeiffer, T.C. Mcguire, R.B. Bendel, *Am. J. Vet. Res*., **1977**, 38, 693-698.
[31] J.J. Kaneko, In: J.J. Kaneko, J.W. Harvey, M.L. Bruss (Ed), Serum proteins and the dysproteinemias. Clinical Biochemistry of Domestics Animals (5thedn, CA, Academic Press, San Diego), **1997**, 117-137.
[32] J.H. Lumsden, K. Mullen, R. Rowe, *Can. J. Comparat. Med*., **1982**, 44, 24-31.
[33] G. Piccione, C. Stefania, G. Claudia, V. Irene, *Acta. Vet*., **2009**, 59(11), 413-422.
[34] J.D. Quigely, R. Martin, H. Dowln, *J. Dairy. Sci*., **1995**, 78, 1573-1577.
[35] S. Choi, M. Chung, *Sem. Int. Med*., **2003**, 1(1), 53-62.