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RESEARCH ARTICLE

The Immunoglobulin G Concentration in Colostrum and Blood Serum from Sarabi Calves in Iran

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Abstract: The objective of the present study was to measure immunoglobulin G (IgG) levels in colostrum and blood serum of Sarabi calves as a genetic source of Iran. IgG levels determined in the first colostrum after calving by a SRID kit and also in the blood serum by ELISA and SRID. Twenty colostrum samples were taken from cows' immediately after parturition and stored at -20 °C until analysed. Venous blood samples for determination of plasma IgG concentration were obtained from 20 calves immediately before colostrum administered and after 24 h, 48 h and 8 days of birth. The maximum, minimum and mean (± SE) levels of IgG in the colostrum samples were 17000, 2000 and 8825±1206 mg/dl, respectively. The IgG levels (Mean± SE) in blood serum of calves were 451±17, 493 ±16, 508±26, and 503±24 mg/dl before colostrum intake, 24 h, 48 h, and 8 days after birth, respectively. The maximum concentrations of IgG in the blood serum (by ELISA) were 634, 631, 697 and 696 mg/dl in different time-points, respectively. IgG concentration in 4 calves that measured by SRID were 180, 1138, 1017, and 897 mg/dl in different time, respectively. The results showed that plasma IgG in calves was lower than 10 g/l, indicating existence of failure of passive transfer (FPT) in all the studied animals. Therefore, the Sarabi calves are prone to present clinical signs of FPT syndrome.

Keywords: Colostrum, ELISA, IgG, Sarabi, SRID.

INTRODUCTION

Inadequate transmission of colostral immunoglobulin to the calf, known as failure of passive transfer (FPT), after birth has been associated with increased risk of neonatal mortality [1 - 4]. Factors including timing of colostrum ingestion, the method and volume of colostrum, the immunoglobulin concentrations of the colostrum ingested, and the age of the dam have been implicated in affecting the optimization of absorption. The colostrum pooling, the breed and presence of the dam may influence passive transfer [4, 5]. IgG have different values in different breeds. The type of placenta in ruminants (syndesmochorial) cause infants not taking IgG during pregnancy thus neonatal immunity depends on colostrum ingestion after birth. The FPT in calves may lead to pneumonia and diarrhea syndrome, loosing body weight, and high percentage of mortality during growing. The lowest concentrations of IgG in the colostrum required to prevent FPT is 1000 mg/dl. Therefore, taking colostrum as soon as possible after birth (less than 6-12 hours), could protect calves from FPT syndrome [5, 6]. Colostrum samples were collected from the first milk secretion of multiparous cows. In a study, ELISA and agar gel immunodiffusion (AGID) tests were used for measuring IgG concentration in the blood of calves and it was estimated the agreement between results of 2 mentioned methods was 94% [7, 8]. The ELISA method was also used for measuring the IgG concentrations of adulteration of bovine milk [9]. It is assumed that the incidence of diarrhea and application of antimicrobial treatments will significantly

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reduce but weight gain is higher in the group of calves received the supplemental IgG [6]. The aim of the present study is to measure immunoglobulin G (IgG) levels in colostrum and blood serum of Sarabi calves as a genetic source of Iran.

MATERIALS AND METHODS

The research was carried out in Sarab, a city in the East Azerbaijan of Iran 700 Km to Tehran, the capital of Iran.

IgG concentrations were measured in the first milking colostrum after calving by a SRID kit (Kents Lab, USA). Twenty samples with 200 ml volumes were taken from cows in different ages from first milking and from 4 quarter in a plastic container and stored at -20°C until assayed. Collecting of colostrum was done in four seasons of the year. The age of dam, season of calving, single or twining, gender, weight at parturition, normal calving or dystocia, and duration of dry period were recorded. The characteristic of calves such as sex, time of birth (morning, evening, and night), season of year, type of calving (normal or dystocia), age of the mother at parturition and single or twin calves also were recorded. Calves were bottle-fed with colostrumas soon as possible after birth and then 2 times after 4 h. All of calves were fed 3 L of colostrum until 24 h after birth. Twenty blood samples were taken by a Venoject (EDTA) from Jugular vein after birth (before colostrum intake), 24 h, 48 h, and 8 days after birth. The blood sera were separated by a centrifuge (3000 rpm for 10 min) as soon as possible and were stored at -20°C until analyzed. Wilson *et al.* revealed that the activity of serum gamma-glutamyl transferase (GGT) should be used and restricted to assess passive transfer status in beef calves beneath 8 days. We did not determine the activity of serum GGT as findings by Wilson study are not in agreement with some other papers. Since Wilson and some other researchers have been focused on days being selected less than 10 so that we did so. IgG levels were determined in blood samples by ELISA (Koma, South Korea) and SRID (Triple J Farms, Redmond, WA) kits. All characteristics related to the cows and calving were recorded. Statistical analyses of the data were performed by SPSS (version 16) using one and two way ANOVA, or student *t*-test. The results were statistically analyzed by ANOVA test under probability ($P<0.05$) using Minitab program.

Single Radial Immunodiffusion Assay (SRID)

The SRID Kit was used for detection of bovine immunoglobulin G. The sera were separated from the blood and preserved at -20^{o}C until further experiment. Briefly, 5 µl bovine IgG standards and serum or colostrum samples were loaded in the wells and incubated in a humid chamber at room temperature (RT) overnight. The diameter of the precipitation ring was measured with a millimeter ruler and compared with the standard values (1-2). All samples were run in duplicate.

ELISA

ELISA microplates were coated with 100 µl of Affinity purified Sheep anti-Bovine IgG antibody (1:1,000 dilution in 50 mM Carbonate-Bicarbonate Buffer, pH 9.6) overnight at 4ºC. Then, the plate blocked with 200 µl blocker solution (4% non-fat dry milk in PBS, pH 7.4) and incubated at RT for 1 h. The plate was washed with PBST (0.1% Tween-20 in PBS, pH 7.4) three times. The plate was incubated with serial concentrations of Bovine Reference Serum as standard solution (20,000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.8 ng/ml) and diluted samples at RT for 1 h. The wells were incubated with HRP conjugated Sheep anti-Bovine IgG (1:10,000 dilution in PBS, pH 7.4) and incubated for 1 h at RT. 50 µL Substrate solution was added after washing well. The color intensity was measured at 450 nm by a microplate-reader (Stat Fax, St. Louis, MO, USA). All samples were run in duplicate.

RESULTS

The minimum, maximum, and mean (±SE) concentrations of IgG in the colostrum were 2000, 17000, 8825±1206 mg/dl, respectively (Table **1**). The age of dam, season of calving, single or twining, gender, weight at birth, normal calving or dystocia of dam, and duration of dry period did not affect the IgG levels in colostrum of Sarabi cows ($P>0.05$). Sixteen blood samples (10 males and 6 females) were collected and analyzed by ELISA. Body weight at birth was 26±0.88 kg (Mean± SE) and IgG levels of the blood were 451±17, 493 ±16, 508±26, and 503±24 mg/dl before colostrum intake, 24 h, 48 h, and 8 days after birth, respectively (Table **2**). The maximum and minimum levels of blood IgG (by ELISA) was 634, 631, 697, 696 mg/dl and 352, 402, 332, 359 mg/dl in different time respectively (Table **3**). Gender of calves (10 males and 6 females), time of birth (morning 3, evening 4 and night 9), season of year (spring 8, summer 6, fall 1 and winter 1), type of calving (normal 9 and dystocia 7), age of the mother at calving (heifer 13, second 1, third 1, sixth calving 1) and single or twin calves did not influence serum levels of IgG ($P>0.05$). IgG levels in 4 (2 females and 2 males) calves that measured by SRID were 180, 1138, 1017, and 897 mg/dl in different time

respectively. In both methods blood levels of IgG in blood samples in different time were not significantly different (P>0.05) with each other but showed significant differences in the colostral levels of IgG (P<0.05). Altogether, the results demonstrated that blood levels of IgG were 1/10 (one tenth) of colostral levels of IgG in both methods (P<0.05).

Table 1. The maximum, minimum and Mean± SE levels of IgG (mg/dl) in 20 colostrum samples.

Maximum	Minimum	Mean± SE
17000	2000	8825±1206

Table 2. The blood levels of IgG in the calves measured by ELISA [Mean± SE (mg/dl)] and SRID (mg/dl).

ELISA [Mean± SE (mg/dl)] in 16 calves				SRID (mg/dl) in 4 calves			
Before intake	24 h	48 h	8 days of age	Before intake	24 h	48 h	8 days of age
451±17	493 ±16	508±26	503±24	180	1138	1017	897

Table 3. the maximum and minimum levels of IgG (mg/dl) were measured by ELISAin blood of calves at different times.

	Before colostrum intake	24 h	48 h	8 days after birth
Maximum	634	631	697	696
Minimum	352	402	332	359

DISCUSSION

IgG have different values in different breeds. The type of placenta in ruminants (syndesmochorial) causes infants not taking IgG during pregnancy. Thus, neonatal immunity depends on colostrum ingested after birth [8, 9]. The minimum level of IgG in the colostrum to cease FPT is 1000 mg/dl. The calves' colostrum intake after birth (less than 6-12 hours) could protect against FPT syndrome. SRID is preferred to ELISA for measurement of IgG levels in the colostrum because of turbidity appeared in colostrum [1]. SRID was also applied to determine the colostral levels of IgG in some other animals such as goats described by Arguello *et al*. [10]. In an experiment performed on Norwegian dairy cows and SRID was applied to measure colostral levels of IgG, 57.8% of the samples contained less than 50 g of IgG/L of colostrum. In other study, calf age up to 4 hours neither had significant effect on the calf's ability to ingest colostrum nor on serum level of IgG after 48 h [11 - 14]. Calf sex, calf birth weight, and season of calving were not significant predictors for detection of serum IgG in precolostral samples [15]. The findings of the present study were in agreement with results of research done by Chigerwe *et al*. In another study performed on the sheep in Iran, the serum levels of IgG was not affected by sex, litter type, number of ewe parturition and birth weight [16] that were parallel with our findings on calves. The study of Cheryl L. *et al*. (2009) showed that the twining, dystocia and calves born from a heifer will intake IgG lower than 16 g/L of colostrum which is in contrary with our findings. The research demonstrated that the blood levels of IgG are the same by ELISA and SRID . Furthermore, serum levels of IgG in our study are similar to results of Lee SH and his colleagues. Since the Sarabi calves did not intake pasteurized or freezed colostrum, or colostrum replacer and always feeding by fresh colostrum so we could not compare our finding from this viewpoint (IgG and total protein in blood) with other research. Some studies suggested that the colostrum replacer product failed to routinely provide adequate IgG concentration compared with the fresh colostrum and claves fed fresh colostrum had significantly higher serum total protein and IgG concentrations [14, 15, 17]. Both volume and method of colostrum feeding are important in terms of serum level of IgG, especially if small volume is utilized (*i.e*. 1.5 L). In the other hand, if a large volume of colostrum is fed (3 L), the method of feeding (bottle or esophageal tube) will not affect IgG absorption and passive transfer indices [18, 19]. In present study all calves received colostrum only *via* bottle as explained in the material and methods. Wilson *et al*. demonstrated that the activity of serum GGT should be used and restricted to assess passive transmission status in beef calves beneath 8 days of age [20]. We did not measure the amount of GGT activity and the findings obtained from experiment of Wilson were not in parallel with those of other papers. A new research presented that the adding the selenium to colostrum increases the amount of IgG absorbed by newborn calves. It seems that this might directly activate physiological pinocytosis of intestinal epithelial cells because of being reaction occurred rapidly [21]. However, supplementing colostrum with other nutrients did not influence passive immunity and IgG levels [22, 23]. Bender *et al*. believe that measurement of IgG merely is not sufficient and could not represent the absorption patterns so plasma protein content or activity of serum GGT should be determined. Values of hormones (*e.g*. insulin, glucagon, insulin-like growth factor-I, including its binding proteins, and cortisol), essential and non-essential fatty acids and fat-soluble vitamins (*e.g*. β-carotene, retinol, and α-tocopherol) are dependent

on time and volume of colostrum fed [2, 24 - 26]. This evidence may be adapted also to quality of maternal colostrum. Other studies are recommended to be done and GGT activity or total protein values must be measured to complete the results of present study.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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