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Biochemical parameters in the blood of Holstein calves given immunoglobulin Y-supplemented colostrums

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Abstract

Background: In any calf rearing system it is desirable to obtain healthy animals, and reduce morbidity, mortality, and economic losses. Bovine syndesmochorial placentation prevents the direct transfer of bovine immunoglobulins to the fetus, and calves are born hypogammaglobulinemic. These calves therefore require colostrum immediately after birth. Colostrum is rich in immunoglobulins (Ig) and its consumption results in the transfer of passive immunity to calves. The Ig absorption occurs within the first 12 h after birth. Immunoglobulin Y (IgY), derived from chicken egg yolk, has been used in the prevention and control of diseases affecting calves because it is very similar in structure and function to immunoglobulin G (IgG). In the current study, we sought to establish whether administration routes of colostrum supplemented with avian IgY affected passive immunity in calves.

Results: No significant differences were observed with respect to route of administration for colostrum. However, we did observe some differences in certain interactions between the various treatments. Calves fed colostrum containing egg yolk had higher levels of TP, ALB, and IgG, along with increased GGT activity.

Conclusions: Our results suggest that supplementing colostrum with egg yolk has a beneficial effect when given to calves, regardless of administration route.

Keywords: Calves, Colostrum, GGT, IgG, IgY, TP

Background

During calf rearing it is desirable to reduce morbidity and mortality, and to lower costs by avoiding expensive treatments and losses that are a result of late development and delayed production. To achieve these goals, it is necessary to ensure an adequate intake of colostrum to calves during the neonatal period, thereby providing passive immunity [1]. The most important factor in the development of calves is the appropriate and immediate consumption of colostrum post-partum, as it is the first source of nutrients after birth [2]. This should not be delayed for more than 9 h after birth. For the adequate transfer of passive immunity *via* colostrum, different feeding methodologies have been developed that vary in

complexity, accessibility and cost. The transfer of passive immunity is based on different components of colostrum that are absorbed by the gastrointestinal tract of calves [3]. At the end of gestation the mammary gland of the cow produces colostrum, achieving maximum production in the last weeks of pregnancy. Colostrum is an important source of antibodies (Abs) and its absorption is essential in protecting calves against enteric infections, the main cause of death during the first weeks of life [4].

The immunological characteristics of colostrum are high for 4 days after delivery. However, its most potent immunological qualities are lost at 14 h post-partum [5] because immunoglobulins (Ig) concentrations progressively decrease [4]. The number of pregnancies for a cow has a remarkable impact on the volume and quality of produced colostrum. In multiparous cows, colostrum is richer in Abs, thus providing better immunity to calves. Another factor affecting colostrum quality is the handling of the

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dry cow period, where adequate nutrition and rest between drying off and calving must be ensured [6]. Other factors such as udder conformation, teat size, maternal instinct and dystocia have been associated with a failure to transfer passive immunity in calves [7,5].

The function of active Abs in the immune system is to neutralize and opsonize bacteria and other foreign particles invading an organism [8]. The concentration of Igs in cow colostrum ranges 50–150 mg/mL [9] and is composed of immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM). Two subclasses of IgG, IgG1 and IgG2, comprise 80–85% of all colostrum Igs, while IgA comprises 8–10% and IgM 5–12%. These Ig molecules provide immunity against a wide variety of systemic infections and diseases in cattle [10].

Colostrum is the only food source that transfers passive immunity until a calf develops its own active immunity, which takes at least 6 weeks [11]. The absorption of intact Ig molecules occurs for the first 12 h after birth, after which intestinal tract absorption decreases significantly until 72 h after birth, when no Igs are absorbed [4]. Kaske et al. [2] reported the existence of significant changes in Ig absorption that were dependent upon the way colostrum was fed to calves.

Antibodies are employed in various roles in biomedical studies; they are usually obtained from mammals [12]. However, in recent years, chicken IgY has been increasingly used [13] as it can be easily extracted from egg yolks. In addition to aspects related to animal welfare, the levels of Abs produced by chickens are greater than those obtained from various animals, in particular rabbits [14]. From an economic point of view, the use of IgY has a unique advantage. The cost of raising a chicken is no different than that of a rabbit. A significant amount of IgY can be produced from a single hen, between 17-35 g/bird/year. The relatively low cost IgY production allows it to be applied to immunotherapy and immunoprophylaxis of viral and bacterial infections in human and veterinary medicine [12]. Following extraction and purification from egg yolk, the concentration of IgY ranges 100-400 mg/egg yolk, with an average yolk volume of 15 mL [11,15,16]. Variations in the concentrations of IgY are dependent upon chicken strain or breed, and genetics [17-20]. IgY from chicken egg yolk is an important alternative that could help improve the immune system of Holstein calves.

In our study, we sought to establish whether different routes of colostrum administration, and supplementation of colostrum with chicken IgY affected passive immunity during calf rearing.

Methods

Animal study

The study was conducted at the "Las Jarillas" ranch facilities in Aguascalientes City, Aguascalientes, Mexico. The

Animal Care Committee of Universidad Autónoma de Aguascalientes authorized our study in compliance with the Guide for Care and Use of Laboratory Animals [21]. We selected 30 female calves with the following characteristics: not born from dystocia; without signs of congenital or acquired problems; and no colostrum intake. All calves had an average weight of 38.0 ± 3.0 kg, and did not present with signs of diseases. We used randomized blocks with a factorial arrangement ($2 \times 3 \times 6$), resulting in 36 treatments. An esophageal tube or bottle was used to administer colostrum. The amount of egg yolk used to supplement colostrum was 0, 150, and 300 g, corresponding to 0, 1200, and 2400 mg of IgY, respectively. We sampled blood from calves at six intervals (2, 12, 24, 72, 120, and 168 hours).

There were six regimens that we conducted, with each repeated five times. Treatments 1–3 involved colostrum fed by bottle supplemented with 0, 150 and 300 g of egg yolk, respectively. Treatments 4–6 involved colostrum administered *via* an esophageal tube supplemented with 0, 150 and 300 g of egg yolk, respectively.

Calves were weighed and measured immediately after birth and then randomly allocated to one of the six treatment groups. Animals were house in a single hutch with a soil floor that was previously disinfected, dried, and roofed. Buckets for water and food were provided. All calves were fed within the first 2 h after birth with colostrum from their own dam; the amount of colostrum given was 10% of their body weight. We obtained 2610 eggs from a single batch of Hy Line W-36 hens (60 weeks old; average weight, 62.0 ± 3.0 g). The yolks from these eggs were used to obtain IgY with the aid of an IgY Eggs Press Purification Kit (Gallus Immunotech Inc., Canada). Yolks were separated from eggs, and pooled to provide 150 g and 300 g egg yolk preparations, placed in plastic bags and diluted 1:1 with tap water, and then refrigerated until required. Egg yolk preparations were administered at 2, 12, 24, and 72 h post-partum for the respective treatment groups. We obtained blood samples (5 mL) from calves by jugular venipuncture at 2, 12, 24, 72, 120 and 168 h post-partum. Blood samples were centrifuged (3000 rpm, 10 min) and the resulting serum was stored at -20°C until analysis.

Determination of biochemical parameters in blood samples

To determine aspartate transferase (AST), alanine transferase (ALT), and gamma-glutamyl transferase (GGT) activities we used the AST/GOT Spinreact™, ALT/GPT Spinreact and GGT Spinreact kits, respectively, from Spinreact (Girona, Spain). Samples for AST and ALT determination were analyzed by spectrophotometry using an RA-5O Chemistry Analyzer (Bayer) with a wavelength of 340 nm, as described by Murray and Kaplan [22]. Samples for GGT determination were

Table 1 Analysis of variance for certain biochemical parameters in the blood of ne	ewborn calves

Resource	DF	AST	ALT Pr > F	GGT Pr > F	PT Pr > F	ALB Pr > F	IGG Pr > F
		Pr > F					
M	1	0.1994 ns	0.9949 ns	0.3335 ns	0.8802 ns	0.7220 ns	0.3636 ns
D	2	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***
$M \times D$	2	0.4306 ns	0.7771 ns	0.7414 ns	0.8875 ns	0.6599 ns	0.7858 ns
Т	5	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***	<0.0001***
$M \times T$	5	0.9718 ns	0.9758 ns	0.6860 ns	0.9988 ns	0.9994 ns	0.8011 ns
$D \times T$	10	<0.0001***	<0.0001***	<0.0001***	0.0331 ns	0.0031 ns	<0.0001***
$M \times D \times T$	10	1.0000 ns	0.9873 ns	0.8194 ns	0.9995 ns	0.9996 ns	0.5043**

M, method; D, dose, $M \times D$, intersection method and dose; T, time; $M \times T$, intersection of method and time; $D \times T$, intersection of dose and time; $M \times D \times T$, intersection method, dose and time; AST, aspartate transferase; ALT, alanine transferase; GGT, gamma glutamyl transferase; TP, total protein; ALB, albumin; IGG, immunoglobulin G; ns, no significant difference; ***significant difference; ***highly significant difference.

analyzed using the spectrophotometric method described by Genders and Kaplan [23]. Total protein (TP) concentration was determined using a TP Spinreact kit (Spinreat) and the method described by Burthis and Ashwood [24]. The concentration of albumin (ALB) in samples was measured using the spectrophotometric method described by Genders and Kaplan [23] in conjunction with a the ALBUMIN Spinreact kit (Spinreact). We measured the concentrations of IgG in samples using a technique described by Kliks et al. [25].

Statistical analysis

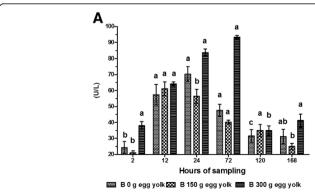
Our data were analyzed through analysis of variance (ANOVA) with a general lineal model (GLM) and Statistical Analysis System (SAS) software [26]. A multiple range of means was compared using Tukey's test. A *P*-value less than 0.05 was considered statistically significant.

Results

The dosage, time and interaction for all variables were highly significant (P < 0.001), as were the interactions between feeding method and dose, and feeding method and time for IgG (Table 1).

The activity of AST in calves given 300 g of egg yolk was highest after 72 h irrespective of administration route (Figure 1A and B). AST activity was lowest when 150 g of egg yolk was administered at 2 h post-partum (bottle fed: 21.10 ± 1.20 U/L; tube fed: 21.00 ± 1.18 U/L). ALT activity was highest (bottle fed: 43.60 ± 1.16 U/L; tube fed: 43.50 ± 1.14 U/L) at 72 h post-partum. ALT activity was lowest in calves given 150 g of egg yolk at 2 h post-partum (bottle fed: 13.75 ± 0.97 U/L; 13.45 ± 1.50 U/L) and in calves that were not given any egg yolk. For both these groups, from 72–168 h after birth, there was a continual decrease in ALT activity (Figure 2A and B).

We did not observe a significant difference in GGT activity between calves receiving colostrum through a tube or a bottle. However, we did observe a significant difference between treatments. GGT activity was highest in calves that received 150 g of egg yolk regardless of administration route (bottle fed: 697.80 ± 13.30 U/L; tube fed: 696.5 ± 13.0 U/L). The GGT activity in animals given 300 g of egg yolk at 12 and 24 h was 637.00 ± 12.61 and 520.6 ± 12.5 U/L, respectively (P < 0.01). GGT activity was lowest at 2 h post-partum for both routes of administration (bottle fed: 18.6 ± 1.2 U/L; tube fed: 18.6 ± 1.18 U/L;



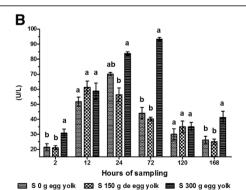


Figure 1 Serum activity of AST in calves. Calves were fed colostrum *via* bottle **(A)** or esophageal tube **(B)**. Blood sampling occurred at 2, 12, 24, 72, 120 and 168 h post-partum. Colostrum was supplemented with tree levels of egg yolk (0, 150 y 300 g) corresponding to 0, 1200 and 2400 mg of lqY respectively. The various letters represent statistically significant difference (*P* < 0.05).

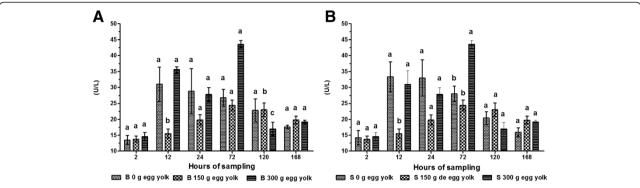


Figure 2 Serum activity of ALT in calves. Calves were fed colostrum *via* bottle **(A)** or esophageal tube **(B)**. Blood sampling occurred at 2, 12, 24, 72, 120 and 168 h post-partum. Colostrum was supplemented with tree levels of egg yolk (0, 150 y 300 g) corresponding to 0, 1200 and 2400 mg of IqY respectively. The various letters represent statistically significant difference (*P* < 0.05).

P < 0.05). The activity of GGT gradually decreased from 24–168 h post-partum (Figure 3A and B).

We observed an increase in TP concentration 12 h after the consumption of colostrum in all treatment groups (Figure 4A and B). This increase was more pronounced in calves that received 300 g of egg yolk by both administration routes (bottle fed: 8.16 ± 0.38 g/dL; tube fed: 8.17 ± 0.39 g/dL). The concentration of TP was lowest in calves at 2 h post-partum (bottle fed: 4.52 ± 0.25 g/dL; tube fed: 4.53 ± 0.26 g/dL). There was a gradual decrease in TP concentration from 72 to 168 h.

The concentration of ALB was highest in calves that received 300 g of egg yolk for both feeding methods (bottle fed: 4.93 ± 0.13 g/dL; tube fed: 4.94 ± 0.12 g/dL) 24 h after birth (P < 0.01; Figure 5A and B). The concentration of ALB was lowest in calves administered colostrum 2 h after birth and where egg yolk was not a supplement (bottle fed: 2.38 ± 0.11 g/dL; tube fed: 2.32 ± 0.08 g/dL; P < 0.05). We also observed a decrease in ALB levels between 72–168 h that was independent of colostrum administration route; this was only seen in treatment groups were egg yolks were excluded from colostrum (P < 0.01).

The highest IgG concentrations were observed 12 h after birth in calves that received 150 or 300 g of egg yolk and were administered colostrum by bottle (14.48 \pm 1.26 mg/mL and 16.36 \pm 1.28 mg/mL, respectively; P < 0.05; Figure 6A and B). Lower IgG concentrations were seen in the control groups fed with a bottle (0.20 \pm 0.07 mg/mL) and in tube-fed calves 2 h after birth (0.029 \pm 0.08 mg/mL). The concentration of IgG in samples did not significantly fluctuate for the duration of the experimental period (168 h). Calves fed colostrum supplemented with 150 g of egg yolk exhibited a gradual increase in IgG concentration from 72 to 168 h (P < 0.05).

Discussion

The measured activities of AST and ALT in blood samples were highest at 72 h and could be considered within the normal reference range. These high levels indicate that the corresponding enzymes were present in the colostrum of newly calved cows. Once AST and ALT, along with other enzymes, are ingested *via* colostrum they are absorbed by the calf intestine before secretion into the bloodstream. The activities of hepatic AST and ALT are

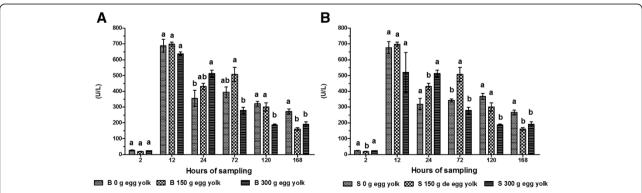


Figure 3 Serum activity of GGT in calves. Calves were fed colostrum *via* bottle **(A)** or esophageal tube **(B)**. Blood sampling occurred at 2, 12, 24, 72, 120 and 168 h post-partum. Colostrum was supplemented with tree levels of egg yolk (0, 150 y 300 g) corresponding to 0, 1200 and 2400 mg of IqY respectively. The various letters represent statistically significant difference (*P* < 0.05).

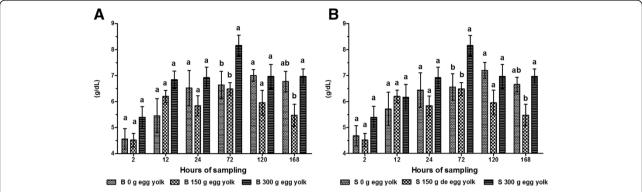


Figure 4 Serum TP concentration in calves. Calves were fed colostrum via bottle **(A)** or esophageal tube **(B)**. Blood sampling occurred at 2, 12, 24, 72, 120 and 168 h post-partum. Colostrum was supplemented with tree levels of egg yolk (0, 150 y 300 g) corresponding to 0, 1200 and 2400 mg of lqY respectively. The various letters represent statistically significant difference (P < 0.05).

increased during pathological processes associated with necrosis. Following this increase in hepatic activity, these enzymes are released into the bloodstream [27]. These two enzymes are used in tests to diagnose liver damage [24,28]. An AST value between 5–40 U/L is considered normal [29], while an ALT value ranging 5–150 U/L is considered normal [30]. In the current study, our results fall within the normal range. We used the activity of these enzymes to verify the increase in GGT activity in calf sera.

The increase in GGT activity at 12–24 h post-partum occurs because the mammary gland is at rest, preparing for the next lactation event, and producing and storing large quantities of colostrum rich in antibodies. GGT is a membrane enzyme found at significantly high concentrations in the cells of alveolar tissue discharged into colostrum. GGT is easily absorbed by newborn ruminants, and serum GGT activity is high in calves that have consumed colostrum [31]. Others have reported 16-fold increases in GGT activity for colostrum-fed calves at 24 h after birth [32]. A correlation between consumption of colostrum

and GGT activity has been shown [33]. Absorption of GGT and Ig in colostrum is limited to the first 24 h after birth. In newborn calves, GGT activity is around 0 U/L but then increases to 1773 U/L at 24 h post-partum following the consumption of colostrum [34]. Our results in the current study support these previous findings, suggesting that serum GGT levels in calves can be used as a qualitative indicator for adequate transfer of passive immunity.

The TP concentration for calves administered colostrum by either route increased by 80.5% at 24 h post-partum. Increases in TP concentration are dependent on the quantity and quality of colostrum provided to calves in the first 2 h after birth. TP is absorbed by the intestine of newborn calves between 12–24 h post-partum. TP concentration can be used diagnostically to reflect the immunity of a calf [3]. A concentration of TP in serum lower than 60 g/L indicates a deficiency in passive immunity as is known as failure transfer of passive immunity (FTPI). Studies have shown that the average TP concentration in calves at birth was 43 g/L, increasing to 74 g/L after 24 h [34]. Other

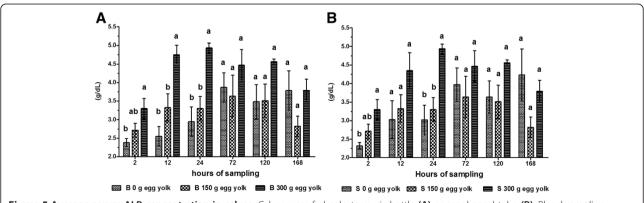


Figure 5 Average serum ALB concentration in calves. Calves were fed colostrum *via* bottle **(A)** or esophageal tube **(B)**. Blood sampling occurred at 2, 12, 24, 72, 120 and 168 h post-partum. Colostrum was supplemented with tree levels of egg yolk (0, 150 y 300 g) corresponding to 0, 1200 and 2400 mg of lgY respectively. The various letters represent statistically significant difference (*P* < 0.05).

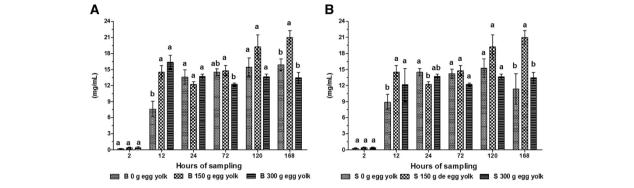


Figure 6 Average serum IgG concentration in calves. Calves were fed colostrum *via* bottle **(A)** or esophageal tube **(B)**. Blood sampling occurred at 2, 12, 24, 72, 120 and 168 h post-partum. Colostrum was supplemented with tree levels of egg yolk (0, 150 y 300 g) corresponding to 0, 1200 and 2400 mg of IgY respectively. The various letters represent statistically significant difference (*P* < 0.05).

studies have reported the serum TP concentration in calves at 72 h post-partum was 60 g/L [35]. Our results are similar to those previously seen in newborn calves (2 h post-partum). However, the concentration of TP at 24 and 72 h post-partum that we determined was 15% higher than those reported by others. Our results show that egg yolks significantly increased TP values and suggest there is a positive correlation between TP and IgG concentrations.

The concentrations of ALB in calves that received the equivalent of 2400 mg of egg yolk in colostrum were 49.3 ± 1.3 and 49.4 ± 1.2 g/L for bottle- and tube-fed animals, respectively, at 24 h post-partum (P < 0.05). In colostrum, similar to what occurs in serum, ALB functions as a transport protein; it is absorbed through the intestine of calves and its levels decrease 3 days after birth [36]. The concentrations of ALB and TP reflect the immunity of calves and can be used diagnostically. The concentrations of ALB we determined in the current study were higher than those previously reported [37,38,39]. Our results suggest that supplementing colostrum with egg yolk influences ALB concentration in the serum of calves.

The concentration of IgG was 16.36 ± 1.28 mg/mL in calves bottle-fed with colostrum containing 300 g of egg yolk at 12 h post-partum. The beneficial effects of Ig in colostrum are generally conferred within the first 2 h of life. These are well absorbed by the intestine before they are discharged into the blood at optimal concentrations. This results in an improved immune status for calves and they become resistant to farm pathogens. It was previously reported that colostrum contains 50-100 g/L IgG [35]. Other researchers have reported a positive correlation between serum IgG and TP concentrations [40,41]. In one study, the plasma IgG concentration in calves 3-8-daysold was less than 10.0 g/L [42]. In another study, plasma IgG levels ranged 3.5–47.0 mg/mL [43]. Our results in the current study indicate higher concentrations of IgG in serum, which conflicts with previous studies. A strong positive correlation between IgG, IgA, IgM and total serum protein concentrations has been demonstrated in newborn calves [44]. In our study, we found a positive correlation between the concentration of either TP or ALB and IgG.

Conclusions

Our findings in this study indicate that egg yolk has a beneficial effect on Holstein calves when administered together with colostrum.

Abbreviations

Abs: Antibodies; ALT: Alanine transferase; ALB: Albumin; ANOVA: Analysis of variance; AST: Aspartate transferase; GLM: General lineal model; FTPI: Failure transfer of passive immunity; GGT: Gamma-glutamyl transferase; Ig: Immunoglobulins; IgA: Immunoglobulin A; IgG: Immunoglobulin G; IgM: Immunoglobulin M; IgY: Immunoglobulin Y; SAS: Statistical analysis system; TP: Total protein.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

TQT research design, experimental design; VLGF conduct research; ROM instrument and technical advice; LEME conduct experiments; AGVF and JLAF statistical and quantitative analysis; ALMN analytical determinations. All authors prepared and approved the final manuscript.

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