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Immunmilk® Feeding Increases Growth and Immune Responses in Broiler Chicks

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Abstract: A study was conducted to measure baseline immunocompetence of Arbor Acre broiler chicks after the birds were raised on hyperimmune bovine colostrum product 'Immunmilk®' for first 7 d of age. Immunological parameters measured were *in vivo* and *in vitro* lymphoproliferation, macrophage functions which included phagocytosis, nitric oxide production and *E. coli* clearance from circulation; and antibody response against SRBC. In addition, body weights (BW) and lymphoid organ weights were also determined. Immunmilk®-treated chicks exhibited significantly higher BW at 7 (18.1% heavier) and 13 d (26% heavier) of age when compared with the chickens in control group. *In vitro* lymphoproliferative response of blood leukocytes to Concanavalin-A was increased ($P = 0.0024$) while *in vivo* lymphoproliferation to Phytohemagglutinin-P was down-regulated ($P \leq 0.01$) in Immunmilk®-treated group. Macrophage phagocytic potential against SRBC was significantly higher and nitric oxide production was reduced in macrophages from Immunmilk® treated chicks. Systemic *E. coli* clearance after i.v. challenge was much more rapid in Immunmilk®-treated chicks. Immunmilk® treatment also increased total and IgM anti-SRBC antibody levels ($P \leq 0.05$) during secondary antibody response. The results of this study showed that Immunmilk® increased growth as well as immune performance of chickens as measured by humoral (antibody) response, mononuclear phagocytic system function as well as lymphoproliferation. However, inflammatory processes such as *in vivo* mitogen-induced T-lymphocyte proliferation and LPS-mediated nitric oxide production by macrophages were down regulated.

Key words: Immunmilk®, colostrum, immune responses, chickens

Introduction

Hyperimmune bovine colostrum is of promise in providing passive immunity against infections in human and livestock populations (Davidson, 1996). Therapy based on colostrum could preclude development of drug resistance as seen in cases of antibiotic therapy (Murray, 1986). Furthermore, the existence of cross neutralizing antibodies in colostrum could provide protection against different antigens that share the same neutralizing sites (Brussow *et al*., 1988; Coulson *et al*., 1986). Previous studies have shown that immunosuppressed individuals that received hyperimmune colostrum were protected against cryptosporidium infection (Saulsbury *et al*., 1980; Losonsky *et al*., 1985) and rotavirus diarrhea (Coulson *et al*., 1986; Tzipori *et al*., 1987; Nord *et al*., 1990; Ungar *et al*., 1990; Rump *et al*., 1992). Immunoglobulins obtained from bovine colostrum after immunizing cows with several *Escherichia coli* (*E. coli*) serotypes, and enterotoxins provided protection against enterotoxigenic *E. coli* infections in human patients (Tacket *et al*., 1988). In a separate study bovine colostral immunoglobulins from non-immunized cows significantly reduced non-specific diarrhea in human population (Stephen *et al*., 1990). Antibodies against *Pasteurella multocida* toxins in colostrum from vaccinated sows can reduce colonization of *Pasteurella multocida* and protect pigs against disease signs of severe progressive atrophic rhinitis (Nielsen *et al*., 1991).

In a study involving chickens, hyperimmune bovine colostrum, that was obtained from cows previously vaccinated against *Eimeria acervulina* antigens, conferred passive immunity in the birds against coccidiosis (Fayer and Jenkins, 1992). Passive immunization through antiserum also protects birds against specific challenges. Chickens that were passively immunized with antibodies against Newcastle Disease virus proteins were resistant against challenge with Texas GB strain of Newcastle disease virus (Umino *et al*., 1987; Reynolds and Maraqa, 2000). Similar study in turkeys has shown protection of birds against clinical signs of turkey coryza after they were treated with antiserum made against whole cells of *Bordetella avium* (Rimler and Robert, 1997).

Immunmilk® is a bovine milk immunoglobulin concentrate, which has been previously used in piglets, calves, broilers and turkeys at various stages of their growth and development. The colostrum product from

first milking is freeze-dried and is water-soluble since it is casein-free. The cattle used as a source of colostrum were vaccinated against *E. coli* and various vaccine-strain viruses. It is, therefore, presumed that this product would have several "natural" or "cross-reactive" and heterologous immunoglobulins. In addition, specific Immunoglobulins against the antigens used to hyperimmunize the cows would pre-dominate the total immunoglobulins present in Immunmilk®.
Therefore, the use of this product could provide passive immunity and protection to a variety of intestinal pathogens present in the recipient. The objective of the present study was to see if the feeding of broiler chicks with this compound starting from the day of hatch would improve growth and baseline immunocompetence of broiler chickens.

Materials and Methods

Chickens and Diets: Day-old Arbor Acre broiler chicks were hatched at the North Carolina State University hatchery and placed in Petersime batteries in temperature and air-controlled poultry housing facilities. A total of 60 chicks were divided into two groups of 30 chicks each and placed in separate pens. Birds were provided starter broiler ration for *ad libitum* consumption. One group of 30 chicks was designated as the control group while the second group of 30 chicks was given Immunmilk® (Phytobiotics GmbH, Rosengasse 9, D-65343 Eltville, Germany) via the drinking water starting from 1-7 d of age. In order to achieve the required dose, our assumption was that in a dilution of 15 g of Immunmilk® in 100 ml of water, one reaches an immunoglobulin concentration of 50 mg per 100 ml since Immunmilk® has an Ig concentration in dry matter of > 30%. Therefore, following dilutions were made to achieve the desired concentration for the number of chicks in this study.

Date	Age (days)	Total Chick Weight (n = 30)	Immunmilk® (g)	Water (ml)
1.8.03	1	1050	15/1000 x 1050 = 15.75	200
1.9.03	2	1350	15/1000 x 1350 = 20.25	300
1.10.03	3	1671	15/1000 x 1675 = 25.06	300
1.11.03	4	2022	15/1000 x 2022 = 30.33	300
1.12.03	5	2588	15/1000 x 2588 = 38.8	300
1.13.03	6	2772	15/1000 x 2772 = 41.58	300
1.14.03	7	3327	15/1000 x 3327 = 49.90	300

Once the required amount of Immunmilk® was calculated and weighed, it was added to a plastic waterer and dissolved in water on a magnetic stirrer. The waterer was then placed inside the pen and the birds allowed to drink untill all of the treated water was consumed. At this point, the *ad libitum* water consumption was resumed until the next day when the Immunmilk® was provided again. In general, the chicks consumed the Immunmilk®-treated water within 3-4 h. After d 7, the Immunmilk® treatment was stopped and both the treated and the control birds were allowed to drink the normal water *ad libitum*.

Body Weights and Relative Lymphoid Organ Weights: The body weights (BW) and the lymphoid organ weights, expressed as a percentage relative to bird's BW, were obtained at 7 and 13 d of age.

Lymphoproliferative response to PHA-P: *In vivo* lymphoblastogenic response was quantitated by using a classic toe-web assay as described by Corrier (1990). The T-cell mitogen, Phytohemagglutinin-P (PHA-P; Sigma-Aldrich, St. Louis, MO 63178), was injected (100 µg/100 µL/bird) intradermally into the toe web of the left foot of 10 birds/group at 8 d of age. The swelling response was measured at 24 and 48 h post injection, by subtracting the pre-injection measurement from the post-injection measurement of the toe web thickness.

Lymphoprolifertaive response to Con-A: *In vitro* lymphoblastogenic response of chicken leukocytes to Concanavalin-A (Con-A; Sigma-Aldrich, St. Louis, MO 63178) was measured as described by Qureshi *et al.* (2000b). Peripheral blood leukocyte (PBL) were incubated with the Con-A (25 μg / 2 x 10^5cells) in 96-well plates. The magnitude of lymphoproliferation was quantitated by a classical MTT assay (Qureshi *et al.*, 2000a). This assay was carried out when the chicks were 7 d of age.

Macrophage Function Assessment

Bacterial Clearance: Mononuclear phagocytic functions were examined at 7 d of age by quantitating the *in vivo* bacterial clearance as described previously (Peterson *et al.*, 1999). An avian strain of *E. coli* was injected i.v. into the brachial vein in 0.2 ml volumes. The blood samples were drawn immediately after bacterial injection and the number of viable bacteria determined by a bacterial plate count method. Three additional blood samples were drawn at 20, 40 and 60 minutes post bacterial injection and the bacterial load quantitated by the plate counts. At 110 minutes post-injection the birds were euthanized and spleens removed aseptically. The splenic homogenate was prepared in sterile PBS (1 ml per spleen) and tested by the plate count method for the viable bacteria.

Phagocytosis, Nitrite Production: Additional macrophage functions, such as phagocytosis of particulate antigens and the metabolite/cytokine production, were assessed at 12 d of age as described previously (Green *et al.*, 1982; Qureshi and Miller, 1991; Dil and Qureshi, 2002). Briefly, abdominal exudate macrophages from both the control and the experimental birds were isolated after Sephadex G-50®

Table 1: Body weights and Lymphoid organ weights[1] of broilers after Immunmilk® supplement in drinking water[2]

Treatment	BW (g) n=30	Thymus (%) n=5	Bursa (%) n=5	BW (g) n=16	Thymus (%) n=8	Bursa (%) n=8	Spleen (%) n = 8
	Day 7			Day 13			
Immunmilk	124.66[a]	0.24	0.20	266.93[a]	0.17	0.26	0.06
Control	105.51[b]	0.21	0.13	211.80[b]	0.17	0.25	0.06
Pooled SEM	2.72	0.006	0.02	6.61	0.01	0.02	0.005

[1]Body weight was taken at 7 and 13 d of age. Thymic lobes from the right side of the neck and bursa of Fabricius were removed and weighed at 7 d of age; spleen was weighed at 13 d of age. Values were computed as percentage of lymphoid organ weight relative to the total body weight from the individual bird. [2] Immunmilk® was included in drinking water of treated broilers for first 7 d of age. ab Means within a column with different superscripts differ significantly at $P \leq 0.05$.

Table 2: Lymphoblastogenic response against PHA-P[1] by broilers on Immunmilk® supplementation[2]

Treatment	n	Toe-web swelling (increase mm) 24h	48h
Immunmilk	8	0.33	0.12[b]
Control	8	0.35	0.25[a]
Pooled SEM		0.05	0.03
P value		0.7972	0.0127

[1]At 8 d of age, PHA-P was injected @ 100mg/bird in the toe web of right foot of 8 chicks per treatment group. Swelling was measured by a constant tension micrometer at 24 and 48 h post injection. The increase in swelling was computed by subtracting the pre-injection value from post-injection value at given time point. [2]Immunmilk® was included in the drinking water of treated broilers for the first 7 d of age. ab Means within a column with different superscripts differ significantly.

(Sigma-Aldrich, St. Louis, MO 63178) elicitation. Macrophages were cultured on glass coverslips and tested for phagocytosis of sheep red blood cells (SRBC). A separate group of macrophages were cultured in 24-well plates and stimulated with *E. coli* lipopolysaccharide (LPS) and the supernatant collected after 24 hours to test for the presence of nitrite.

Antibody Response: Humoral immune response was quantitated by challenging chicks in both groups with SRBC at 7 d of age. Each bird was injected with 1 ml suspension of 5% SRBC made in PBS. Blood samples were drawn at 7 and 14 d post first SRBC injection. At 14 d post-first injection, a booster injection of SRBC was given and blood samples drawn at 7 and 14 d post boost. Total, IgM and IgG anti-SRBC antibody titers were quantitated by using a microplate agglutination assay as previously described (Yamamoto and Glick, 1982; Qureshi and Havenstein, 1994).

Statistical Analysis: Data were analyzed using the General Linear Models procedures of SAS (SAS Institute, 1996). Means were separated for significance by Duncan's multiple range test at significance level of $P < 0.05$ or as indicated.

Results and Discussion

Body Weights and Relative Lymphoid Organ Weights: Body weights and the lymphoid organ weights relative to BW, which were measured at 7 and 13 d of age, are provided in Table 1. At d 7, when the chicks (n = 30) were continuously on Immunmilk® from d 1, the Immunmilk® fed chicks had significantly higher ($P < 0.05$) BW than the chicks in the water-only group. The Immunmilk® treatment was stopped after 7 d of age and the chicks in both treatment groups were allowed to continue to grow on water without any supplementation beyond day 7. At 13 d of age, Immunmilk®-supplemented chicks were 26% heavier than the untreated chicks at 13 d of age ($P = 0.0001$). Furthermore, the Immunmilk® chicks had numerically heavier bursa of Fabricius and thymus weights when calculated as percentage relative to the BW at 7 d of age. However, the statistical differences were lacking, perhaps due to a smaller sample size (n = 5 at d 7, and n = 8 at d 13).
These data indicate that the Immunmilk® treatment given via the drinking water from the day of hatch for a 7 d period improves the development and growth of chicks. In a recent study, calves that were fed unlimited amounts of colostrum for 3 d after birth had higher BW till 1 wk of age, compared with calves that were raised on recommended amounts of colostrum (Hammon *et al.,* 2002). Some of the growth promoting proteins that have been identified in colostrum include insulin-like growth factors 1 and 2 (Francis *et al.*, 1988; Vega *et al.*, 1991), transforming growth factor β (Pakkanen, 1998), platelet derived growth factor like molecule termed colostral growth factor (Shing and Klagsbrun, 1987), and angiogenin (Chang *et al.*, 1997). It is therefore possible that the Immunmilk® improves the growth and integrity of the intestinal tract thereby allowing better nutrient absorption and utilization.

Lymphoproliferative response to PHA-P: *In vivo* lymphoproliferative response of chicks after

Table 3: Lymphoblastogenic response of leukocytes against Concanavalin A[1] after Immunmilk® supplementation in drinking water[2]

Treatment	Stimulation Index[1]	Control	Pooled SEM	P Value
Con-A	0.47a	0.26b	0.04	0.0024

[1]Lymphocytes were isolated by Ficoll gradient at 6 d of age, concentration was adjusted to 0.2 X 10^6 PBL/well, and the cells were then incubated with 25ug/well of Con-A. Lymphoproliferation was quantitated by MTT assay after 24 h of culture and lymphoproliferative index was calculated as: Con-A stimulated - unstimulated/unstimulated. [2]Immunmilk® was included in the drinking water of treated broilers for first 7 d of age. ab Means within a row with different superscripts differ significantly.

Table 4: Bacterial clearance potential[1] of broilers after Immunmilk® supplementation in drinking water[2]

	Time (min)	Immunmilk®	Control	Pooled SEM
		Mean # viable *E. coli* colonies		
Blood	0	883333.33	956666.66	41623.78
	20	42333.33	49533.33	9408.59
	40	25333.33	26800.00	8076.08
	60	12000.00	22800.00	7080.55
Spleen	110	37800.00[a]	81000.00[b]	8548.51

[1]*E. coli* were injected in brachial vein of each chicken (0.2 ml per chick) at 7 d of age. Blood samples were drawn at 0, 20, 40 and 60 minutes post bacterial injection, and the bacterial load was quantitated by plate counts. Spleens were removed at 110 minutes, homogenized and viable bacteria were measured by plate count method. [2] Immunmilk® was included in drinking water of treated broilers for the first 7 d of age. ab Means within a row with different superscripts differ significantly at $P \leq 0.05$.

Immunmilk® treatment is presented in Table 2. No difference was observed in PHA-P mediated swelling response between the chicks with or without Immunmilk® treatment at 24 h post PHA-P injection in toe web. This is the time (i.e., 24 h) when the maximum swelling response, an indication of lymphoproliferation, is expected to take place. By 48 h post injection, the response is expected to decline back to the initial toe-web thickness level. It seems that this reduction was not comparable between the two treatment groups at 48h post injection, *i.e.*, control birds had significantly higher response compared to the treatment birds (P = 0.0127). The exact mechanism as to why Immunmilk® down regulated the observed mitogen-mediated inflammatory as well as possible local T lymphocyte proliferative response is unclear. It is possible that a bovine colostrum factor, colostrum inhibitory factor, which inhibits interleukin-2 gene expression in activated T helper cells (Damaraju *et al.,* 1996), may cause depressed lymphoproliferation of PBL that were challenged with PHA-P. Colostrum also contains TGF β_1, TGF β_2, and glucocorticoids that are shown to suppress immune response (Brabletz *et al.,* 1993; Vacca *et al.,* 1992).

Lymphoprolifertaive response to Con-A: The *in vitro* lymphoproliferative response of peripheral blood leukocytes (PBL) against Con-A is given in Table 3. In this experiment, the mitogen receptors on lymphocytes (presumably T-lymphocytes) present in the PBL come in direct contact with the T-cell mitogen, Con-A, and the lymphocytes undergo cell division. When the cells were allowed to incubate with Con-A, a significantly greater lymphoproliferation in PBL was observed from Immunmilk®-treated birds as compared with the PBL obtained from the control birds (P = 0.0024). This is in contrast to the PHA-P experiment in which the peak swelling response was not different between the two groups. Dissimilar response of T-cells to PHA-P and Con-A could be explained by a previous study (Lassila *et al.,* 1979), which suggests that lymphoproliferative response to PHA-P and Con-A is under different genetic control in White Leghorn chickens. It is possible that broiler strain used in this study shares the genetic control for lymphoproliferation with White Leghorn chickens.

Furthermore, the *in vitro* exposure of lymphocytes to a given mitogen may yield a proliferative response independent of physiological and tissue microenvironmental influences modulating such response *in vivo*. When PBL are stimulated with Con-A, IL-1 is produced by monocytes in PBL fraction, which stimulates proliferation of lymphocytes (Abbas *et al.,* 1991). Although bovine colostrum harbors IL-1 receptor antagonist (Hagiwara *et al.,* 2000), presence of both IL-1 β and IL-1 receptor antagonist in bovine colostrum is considered to counteract the possible inhibitory effect of IL-1 receptor antagonist on proliferation of PBL (Yamanaka *et al.,* 2001).

Macrophage Function Assessment

Bacterial Clearance: The next series of experiments

Table 5: The effect of Immunmilk® on macrophage functions of broilers[1] after Immunmilk® supplementation in drinking water[2]

Treatment	n	Phagocytosis (%)	SRBC/Mϕ(#)	AEC (# x 10^6)
Immunmilk	7	43.54[a]	2.13	2.34
Control	7	27.38[b]	1.89	3.62
Pooled SEM		4.16	0.14	1.32
P Value		0.0125	0.2637	0.4938

[1]Sephadex-elicited abdominal exudate cells were isolated at 2 wk of age. Adherent macrophage-monolayers were established on glass coverslips (3 per chick) and they were fed a 1% suspension of SRBC. After 1 h incubation, coverslips were washed, fixed and stained with Leukostat stain for microscopic evaluation. [2]Immunmilk® was included in drinking water of treated broilers for the first 7 d of age. ab Means within a column with different superscripts differ significantly.

Table 6: Nitrite production by macrophages from broilers on Immunmilk® supplement[1] after Immunmilk® supplementation in drinking water[2]

Treatment	n	Nitrite (µM)
Immunmilk	7	2.33[b]
Control	7	5.73[a]
Pooled SEM		0.11
P Value		0.0001

[1]Sephadex-elicited abdominal macrophages were harvested from 2 wk old broilers and cultured as monolayers in 24-well plates. Cultures were exposed to *E. coli* LPS (1ug/ml) for 24 h, the supernatant was collected and tested for nitrite activity using Greiss reagent. [2]Immunmilk® was included in drinking water of treated broilers for the first 7 d of age.
[ab]Means within a column with different superscripts differ significantly.

dealt with the quantification of mononuclear phagocytic system (MPS) functions, as a measure of the innate immune response. The first experiment was carried out by injecting viable *E. coli* into the blood stream and measuring their clearance from circulation over time. At 110 minutes the birds were euthanized and the splenic bacterial load was quantitated. The chicks in both groups were injected with the same number of bacteria. The data from this experiment are presented in Table 4. It seems that soon after the i.v. injection of *E. coli* (time 0), a higher number of viable bacteria become systemic in the control group chicks than in the chicks on Immunmilk® treatment. It is possible that this difference is due to an immediate trapping of bacteria by the cells of the MPS such as circulating monocytes and fixed tissue macrophages. While the rate of systemic clearance seems to be comparable between the two groups, significant differences were observed between the two groups in terms of viable bacteria in the spleens at 110 minutes post *E. coli* injection. The number of bacterial colony forming units (cfu) in the spleen of the control birds at the termination of the experiment (i.e., 110 minutes) was twice as much (8.47% relative to the time 0 cfu) as in the spleens of the birds on Immunmilk® treatment (4.28% relative to the time 0 cfu). Bovine colostrum has been shown to stimulate non-specific immune response. In an experiment that investigated effect of bovine colostrum on phagocytic activity, peripheral blood leukocytes showed increased phagocytosis of latex particles after they were exposed to bovine colostrum (Sugisawa *et al*., 2001). In a separate study, human colostrum was also shown to contain phagocytosis promoting activity which was much higher in pre-term colostrum compared to in term colostrum (Straussberg *et al.,* 1995).

Phagocytosis, Nitrite Production: The second MPS function experiment was conducted to determine the phagocytic potential of macrophages by using a classical SRBC phagocytosis assay. Sephadex-elicited macrophages from the Immunmilk®-treated and the control group were cultured on glass coverslips and fed a suspension of 1% SRBC. The endpoints included the over all percentage of phagocytic macrophages as well as the number of SRBC internalized per phagocytic macrophage. These data are presented in Table 5. Macrophages from Immunmilk®-treated chicks exhibited significantly higher phagocytic potential as compared with the macrophages from the untreated control chicks (P = 0.0125). No significant differences were observed in the average number of SRBC per phagocytic macrophage or the number of Sephadex-elicited abdominal exudate cells suggesting a comparable *in vivo* chemotactic response of monocytes to an inflammatory signal such as Sephadex. Taken together, the data from the MPS function studies (Table 4 and 5) suggests that Immunmilk® treatment clearly improves MPS function. This would therefore imply that the chickens on Immunmilk® would be more efficient to clear microbial infections, perhaps by increased phagocytic function.
One additional function of macrophages, i.e., the ability to respond to an activation signal such as bacterial lipopolysaccharide (LPS) was examined. The macrophages were cultured in 24-well plates and stimulated with 1 µg LPS. After 24 h, the supernatant fractions were collected and tested for nitrite levels. The

Table 7: Antibody response[1] against sheep red blood cells in Immunmilk® broilers after Immunmilk® supplementation in drinking water[2]

Treatment	Anti SRBC Antibody response			
	Days PPI[3]		Days PSI[4]	
	7	14	7	14
		Mean Total Antibody Log_2 Titers		
Immunmilk	4.62	1.87	9.25a	3.75
Control	4.37	2.25	7.75b	3.50
Pooled SEM	0.61	0.52	0.25	0.56
P Value	0.7764	0.6186	0.0008	0.7582
		Mean IgM Antibody Log_2 Titers		
Immunmilk	4.62	1.87	5.12a	1.50a
Control	4.37	2.12	2.62b	0.25b
Pooled SEM	0.61	0.51	0.55	0.41
P Value	0.7764	0.7367	0.0068	0.0525
		Mean IgG Antibody Log_2 Titers		
Immunmilk	0.00	0.00	4.13	2.25
Control	0.00	0.13	5.13	3.25
Pooled SEM	0.00	0.08	0.59	0.43
P Value		0.3343	0.2550	0.1248

[1]Eight chickens per group were injected with 5% SRBC in 1 ml volume per chick at one wk of age followed by a second injection at 2 wk of age. Antibody response was measured in a microagglutination assay using mercaptoethanol sensitivity as an indicator of IgM or IgG levels. [2]Immunmilk® was included in drinking water of treated broilers for the first 7 d of age. 3,4 PPI = post primary injection, PSI = post secondary injection.
[ab]Means within a column with different superscripts differ significantly.

nitrite levels represent the function of inducible isoform of nitric oxide synthase (iNOS) which is induced in response to LPS stimulation. The iNOS utilizes arginine as a substrate and metabolizes it into effector molecules such as nitric oxide, the levels of which are measured as nitrite in aqueous form in the culture supernatant. As shown in Table 6, the birds on Immunmilk® treatment produced significantly lower levels of nitrite as compared with the control birds group. This observation, coupled with the observation that the birds on Immunmilk® exhibited reduced PHA-mediated swelling response, suggests that Immunmilk® also modulates inflammatory processes. In a previous study, leukocytes from 1 wk old calves were shown to produce less nitric oxide than the leukocytes from older calves (Rajaraman *et al.,* 1998), the authors suggested that suppressive factors in the colostrum, like prostaglandins and transforming growth factor β, are responsible for depression in nitric oxide production.

Antibody Response: The last series of experiments were conducted to determine the humoral immune response of chicks. For this purpose, chicks were injected with SRBC, a T-cell dependent antigen. The immunization regimen included two injections at 14 d apart. This allowed the quantification of both the primary and secondary antibody response.

The data for these experiments are presented in Table 7. The chicks in both groups had comparable total anti-SRBC antibody levels at 7 and 14 d post primary injection (PPI). However, after a booster injection, the Immunmilk®-treated chickens had significantly higher total antibody titers at 7 d post secondary injection (PSI, P = 0.0008) as compared with control chickens. Thereafter, the decline in antibody titers was comparable in both groups.

The second set of antibody level quantification involved the IgM and IgG levels in response to SRBC injection. The chickens in both treatment groups had comparable IgM titers at both 7 and 14 d PPI. However, at 7 d PSI, the chickens in Immunmilk® group had significantly higher IgM titers than the chickens in the control group (P = 0.0068). This increase in IgM titers persisted in Immunmilk® group even at 14 d PSI (P = 0.0525). No significant differences in IgG isotype were observed between the two groups of chickens.

Some of the colostrum factors which could enhance antibody production are soluble CD14 and nucleotides. Soluble CD14 has been shown to induce B cell growth and differentiation in mice (Filipp *et al.,* 2000). Similarly, infant formula fortified with human milk nucleotides cytidine-, uridine-, adenine-, and guanine-monophosphate enhances humoral response against *Haemophilus influenza* infection and diphtheria (Eckhard *et al.*, 2000). The fact that bovine colostrum contains twice as much cytidine- and uridine-

monophosphate as the later stages of mammary secretions (Raezke and Schlimme, 1990) may partly account for promoting impact of colostrum on neonate's humoral immune response.

The present study indicates that Immunmilk® treatment given to chicks soon after hatch improves their growth. This improved growth persisted even with the withdrawal of Immunmilk® after 7 d of treatment. Coupled with an improved growth, several immune function endpoints were also upregulated. These included lymphoproliferation in response to Con-A, a T-cell mitogen, bacterial clearance from circulation, a function of mononuclear phagocytic system function, macrophage phagocytic function, and antibody response against SRBC. Improved macrophage phagocytic function could yield higher microbicidal activities and tumor cell killing (Qureshi *et al.*, 2000a, Dil and Qureshi, 2002). Increased humoral response against SRBC has been correlated with higher antibody titers to vaccination against *E. coli*, Newcastle Disease virus, infectious bronchitis virus, and infectious bursal disease virus (Parmentier *et al.*, 1995). On the other hand, the Immunmilk® treatment seemed to down regulate certain inflammatory response-based endpoints, such as *in vivo* toe web response against PHA-P, and the induction of iNOS activity in macrophages after stimulation with LPS. It must be pointed out that these experiments were only carried out once and therefore must be repeated. Nevertheless, these initial experiments certainly suggest that Immunmilk® can be used as a positive modulator of performance and immune response in chicks at early ages.

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