

## **Modulation of interferon gamma response through orally administered bovine colostrum in active adolescent boys**

**Mahenderan Appukutty<sup>1,2</sup>, Ammu Kutty Radhakrishnan<sup>3\*</sup>, Kalavathy Ramasamy<sup>4</sup>, Abu Bakar Abdul Majeed<sup>4</sup>, Mohd Ismail Noor<sup>1</sup>, Nik Shanita Safii<sup>1</sup>, Karuthan Chinna<sup>5</sup>, Poh Bee Koon<sup>1</sup>**

<sup>1</sup>Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Malaysia

<sup>2</sup>Faculty of Sports Science and Recreation, Universiti Teknologi MARA, Malaysia

<sup>3</sup>Faculty of Medicine and Health, International Medical University, Malaysia

<sup>4</sup>Faculty of Pharmacy, Universiti Teknologi MARA, Malaysia

<sup>5</sup>Faculty of Computer and Mathematical Sciences, Universiti Teknologi MARA, Malaysia

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### **Abstract**

Bovine colostrum (BC) is a rich source of bioactive components reported to have health promoting effects. The purpose of the present study was to determine the effects of six-week supplementation of BC on the production of gamma-interferon (IFN- $\gamma$ ) by mitogen-stimulated peripheral blood leucocytes (PBL) in active adolescent male. Subjects were recruited from the students who were under-going regular athletics training. The subjects were randomly assigned into control and experimental groups. The control group (n=18) received daily supplementation of skim milk while the experimental group (n=18) received bovine colostrum (20 g/day). Fasting venous blood samples were collected on day-0 and day-42 for measurement of IFN- $\gamma$  produced by concanavalin A (Con A)-stimulated PBL. Interferon- $\gamma$  levels were quantified using ELISA. Changes in IFN- $\gamma$  levels from baseline to endpoints for both groups were compared and presented as mean ( $\pm$  SD). Although baseline levels of cytokine concentrations were similar in both groups, there was a decreasing trend in IFN- $\gamma$  production by the mitogen-stimulated PBL from subjects who received the bovine colostrum supplement compared to controls. The findings of the present study suggest that oral supplementation of bovine colostrum for a period of 6 weeks may modulate *in vitro* IFN- $\gamma$  concentration.

### **Introduction**

Colostrum is a natural product that provides passive immunity against a host of microorganisms in breast-fed newborns [1]. Bovine colostrum (BC) is reported to contain basic nutrients such as carbohydrates, fat, amino acids, vitamins and minerals as well as a mixture of bioac

tive components postulated to boost the immune system [2]. The immunomodulatory components of BC includes

cytokines that promote both cell-mediated (Th1) and humoral (Th2) immunity such as interleukin 1-beta (IL-1 $\beta$ ), interleukin-6 (IL-6), IFN- $\gamma$ , and tumour necrosis factor-alpha (TNF- $\alpha$ ) [3]. Interferon- $\gamma$  coordinates a diverse array of cellular programs through transcriptional regulation of immunologically relevant genes and it is reported that the bovine colostrum contains high levels of IFN- $\gamma$  [3]. It has been suggested that IFN- $\gamma$  orchestrates leucocyte attraction and directs growth, maturation, and differentiation of many cell types [4-6]. In addition, IFN- $\gamma$  has been shown to enhance killing by natural killer (NK) cells [7] as well as regulate B-cell functions such as immunoglobulin (Ig) production and class-switching [4,8].

Bovine colostrum has been well accepted as a functional food supplement over the past decade. Scientific evidence suggests beneficial effects of BC supplements in improving body composition and athletic performance [9]. However, there is a lack of published evidence on the immunomodulatory effects of orally administered BC in active male adolescents. Hence, the present study was designed to assess immune response enhancing effects of BC supplement and specifically to study the enhancement in IFN- $\gamma$  production by mitogen-stimulated PBL in the active adolescent males.

### **Materials and Methods**

#### **Recruitment of subjects**

A total of 40 active adolescent male volunteers aged between 13 to 16 years-old were recruited from the Bukit Jalil Sports School, Kuala Lumpur, Malaysia. The inclu-

sion criteria used include subjects that do not have any known health problems, any medical contraindication or history of any endocrine disorders that requires drug therapy. In addition, subjects must also be actively involved in sports training for a period of more than three-years. Subjects with a history of gastrointestinal tract disease and food allergy (lactose or cow's milk protein intolerance) were excluded. The subjects were asked to refrain from taking other food supplements and to maintain their usual eating habits throughout the study period. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Medical Research and Ethics Committee, Universiti Kebangsaan Malaysia (UKM 1.5.3.5/244/SPP2). Written informed consent was obtained from all subjects and their parents.

### **Training**

All subjects were instructed to maintain their current levels of exercise during the six-week study period. The coach was also informed not to substantially alter their training regime for the 6-week study duration. The training was designed by the school coach, certified by the national coach and the subjects were trained for 2-3 hour/day in the morning and evening. The daily training consisted of a general warm-up and stretching, technical-tactical training, and simulated full or half hockey games and lastly a cool-down phase.

### **Bovine colostrum supplementation**

The volunteers were randomly assigned to control and experimental groups. The subjects in the experimental group ingested a liquid preparation containing 20 g of IgG- rich bovine colostrum powder (IgG plus™ Smart-Naco, Malaysia) while those in the control group were given liquid preparation of 20 g of skim milk. The BC or skim milk given to subjects over the period of six weeks were prepared in the form of a chocolate shake liquid as described elsewhere [10]. Briefly, the BC or skim milk was mixed with local chocolate malt beverage and 100 ml water at temperature not above 50°C as heat *per se* may interfere with the bioactive properties of colostrum [1]. The premixed drinks served to the subjects were identical in appearance. The BC and skim milk powder used in this study are commercially available products approved by the Malaysian Ministry of Health. Twenty-four hour dietary recalls were obtained from all subjects on a random day at the beginning and end of the six-week study. The caloric and macronutrient intake of each subject were ascertained via computerised analyses using Nutrical, a computerised version 1.02 of the Nutrient Composition of Malaysian Foods [11].

### **Sample collection and analysis**

Fasting venous blood samples were collected on day-0 and day-42, i.e. at the start and end of the study for each

subject. The subjects were refrained from any strenuous physical activity a day before the blood collection. Peripheral blood leucocytes were isolated from heparinised blood using the red blood cell (RBC) lysis technique. Briefly, the heparinised blood collected from subjects was aliquoted into 15 ml conical tubes and RBC lysis buffer (G-Dex™ II RBC Lysis Buffer – iNtRON Biotechnology, Seoul, South Korea) was added. The tubes were inverted a few times to ensure mixture was homogenous and incubated at room temperature for 3 minutes to allow the lysis of the RBC to take place. Later, ice-cold phosphate buffer solution (PBS) was added to stop the reaction and the tubes were centrifuged at 4000 rpm for 10 minutes at 4°C. The supernatant was discarded and the cells were washed with cold PBS (Sigma-Aldrich Inc, Missouri, USA). The centrifugation step was repeated and the pellet containing leucocytes was resuspended in 5 ml RPMI-1640 containing 5% (v/v) of fetal bovine serum (FBS), penicillin, streptomycin and L-glutamine (Gibco, Invitrogen Corp, New Zealand). The tubes were kept on ice and cell count was performed using a haemocytometer (Neubauer Dytmatech, Germany). Blood leucocyte cultures were carried out on day 0 and 42. The cultures were incubated for 24, 48 or 72 h at 37°C in a humidified 5% CO<sub>2</sub> atmosphere, in the presence of Con A (Sigma-Aldrich, Inc., St Louis, MO, USA). Culture supernatants were collected and stored at -80°C until assayed for IFN- $\gamma$  production.

### **Immunoassay**

The amount of IFN- $\gamma$  produced in the PBL cultures by Con A on days 0 and 42 were quantified using a human IFN- $\gamma$  ELISA kit as recommended by the manufacturer (eBioscience, San Diego, CA). The amount of IFN- $\gamma$  produced by the Con A-stimulated PBL is expressed as pg/ml. The detection limit of this ELISA kit was 8 pg/ml. The concentration of plasma IFN- $\gamma$  was also determined using the same method.

### **Statistical analysis**

All statistical analyses were performed with SPSS statistical software, version 16.0 (SPSS Inc, Chicago, IL, USA). Changes in the IFN- $\gamma$  levels from baseline (day-0) to end-line (day-42) for both groups were compared and presented as mean values and standard deviations (SD). Data were analysed using two-tailed Student's *t* test at  $P < 0.05$  for comparison of differences between treatments. Data was also presented with effect size of the treatment based on ANOVA test.

## **Results**

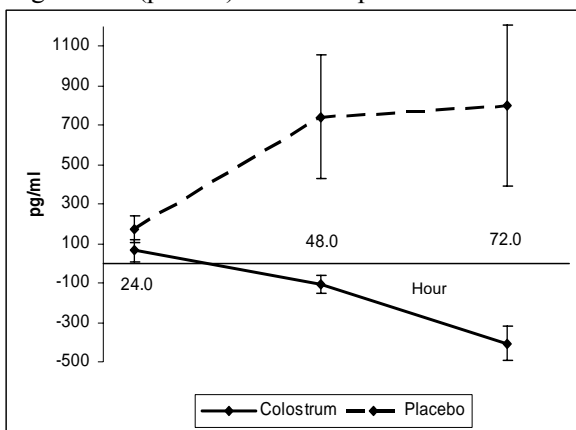
Physical characteristics of subjects in the BC and placebo groups at the initial phase of the study closely matched in age, height and BMI (Table 1). The mean age of the BC

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and placebo groups was 14.3 (SD 1.1) years and 14.4 (SD 1.1) years, respectively. The average reported dietary intake during the measured period was similar in BC ( $2695 \pm 808$  kcal) and placebo ( $2791 \pm 671$  kcal), and there were no differences in macronutrient consumption e.g. carbohydrate ( $50.6 \pm 6.9$  % in BC and  $50.2 \pm 6.4$  % in placebo), protein ( $15 \pm 1.8$  % in BC and  $14.2 \pm 1.9$  % in placebo), and fat ( $34.4 \pm 6.0$  % in BC and  $35.5 \pm 6.7$  % in placebo).

A total of thirty-six subjects were presented for the IFN- $\gamma$  measurements. Table 2a shows the mean value of baseline and endline measurements of IFN- $\gamma$  for the experimental group while Table 2b shows the same for the placebo group. Two subjects did not make it on day-42 for blood withdrawal and another two subjects had readings beyond the detection range of the kit.

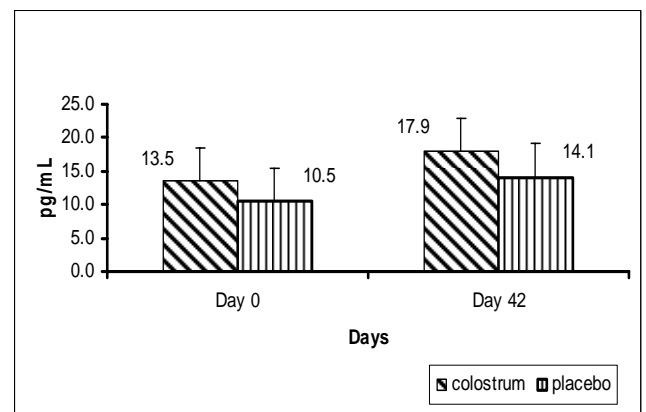
The baseline and endline measurement of IFN- $\gamma$  production by mitogen-stimulated PBL in the control group (n=18) at 24 h were 124.2 pg/ml and 298.8 pg/ml, respectively. The increase of 174.63 pg/ml was found to be significant ( $p < 0.02$ ) with the effect size value of 0.59, which is considered rather high. Following 48 h of culture, the IFN- $\gamma$  increased to 742.41pg/ml, which was also found to be significant ( $p < 0.03$ ) when compared to baseline values.



**Figure 1.** Comparison of mean changes of IFN- $\gamma$  over hours of culture in the placebo and colostrum groups

The effect size value was found to be 0.55 and this was relatively high. The changes in IFN- $\gamma$  levels observed following 72 h of culture was found to be only significant at  $p < 0.10$  level and the effect size value of 0.49 was considered as moderate. When the baseline and endline levels of IFN- $\gamma$  produced by mitogen-stimulated PBL in the colostrum group (n=18) were compared, the differences obtained were not significant and the effect size values obtained were also rather low (Table 2a).

A significant increase in IFN- $\gamma$  production by the mitogen-stimulated PBL was evident following 24 h of culture in the placebo group but not the BC group. After 48 hour of cultures, again a significant increase in IFN- $\gamma$  production was observed in the placebo group where as in the BC group, there was a marginal reduction. Finally, after 72 h, there was a substantial increase in the production of IFN- $\gamma$  in the placebo group, but in contrast there was a reduction of this cytokine in the colostrum-supplemented group. The plots of mean changes over time are illustrated in Figure 1, while the plasma levels of IFN- $\gamma$  are presented in Figure 2 whereby the plasma level of IFN- $\gamma$  increased when compared to baseline data for the BC group.



**Figure 2.** Changes in the plasma IFN- $\gamma$  concentration in the colostrum and placebo groups

**Table 1.** Physical characteristics of subjects

Parameter	Colostrum (n=20)				Placebo (n=20)			
	Baseline		Endline		Baseline		Endline	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Height (m)	1.63	0.06	1.63	0.06	1.63	0.07	1.63	0.07
Weight (kg)	57.4	8.69	57.6	8.91	54.7	6.58	55.1	6.66
BMI (kg/m <sup>2</sup> )	21.4	2.73	21.0	2.74	20.6	1.45	20.1	1.63
Body Fat (%)	16.3	4.67	15.6	4.17	16.7	5.01	14.9	4.42

**Table 2.** Production of IFN- $\gamma$  by Con A-stimulated peripheral blood leucocytes  
a) Colostrum Group (n=18)

Proliferation Time	Baseline	Endline	Difference	SD	p-value	Effect Size
24 hr	190.9	254.8	63.94	230.99	0.29	0.26
48 hr	502.9	398.1	(104.81)	194.77	0.28	0.26
72 hr	1106.8	699.2	(407.58)	373.67	0.06	0.46

b) Placebo Group (n=18)

Proliferation Time	Baseline	Endline	Difference	SD	p-value	Effect Size
24 hr	124.2	298.8	174.63	293.73	0.02	0.59
48 hr	458.1	1200.5	742.41	1343.05	0.03	0.55
72 hr	1393.2	2195.3	802.09	1627.86	0.07	0.49

## Discussion

Bovine colostrum has been shown to possess some potential activity in enhancing specific immune responses in humans [2,12]. Some studies have reported that BC supplementation can protect children and adults against infectious diseases and diarrhoea [13,14]. Several cytokines have been found in BC, including both pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, TNF- $\alpha$ , IFN- $\gamma$  [15], as well as anti-inflammatory components, such as IL-1ra, sIL-1RII and sCD14 [16,17]. Immune interferon or macrophage activating factor, IFN- $\gamma$  is the only Type II interferon, which has antiviral, immunoregulatory and anti-tumour properties [18].

In the present study, we observed a dissimilar trend in the production of IFN- $\gamma$  by the Con A-stimulated peripheral blood leucocytes in BC supplemented individuals when compared to the control group who only received skim milk. In the group supplemented for six-weeks with BC, a downward trend in the production of IFN- $\gamma$  was observed when the Con A-stimulated PBL were cultured for 72 h compared to the initial 24 h. An opposite trend was observed with the Con A-stimulated PBL from individuals who were only supplemented with skim milk for the six-weeks. Alternatively, the production of IFN- $\gamma$  at the systemic level shows a trend towards an increase in the BC group. The specific reasons for this reduction are uncertain and remain speculative. Shing and co-workers [19] have shown that the bovine protein concentrate (BPC) could promote production of IFN- $\gamma$  when peripheral blood mononuclear cells (PBMC) isolated from healthy athletes were co-cultured with 5% BPC and stimulated by lipopolysaccharide (LPS) or phytohaemagglutinins (PHA). While, Biswas and co-workers [19] observed different patterns in IFN- $\gamma$  production when PBMC was stimulated by BC as they found that this could not significantly inhibit production of cytokines [20].

In summary, the present data demonstrated that 6 weeks of oral supplementation with 20 g/day of BC to the trained and healthy young adolescents could modulate *in vitro* IFN- $\gamma$  concentration. However, the specific mechanism responsible for the observed change still remains unclear. Further research is, therefore required to elucidate the immune enhancement mechanism(s) stimulated by BC supplementation.

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**Correspondence:**

Ammu K. Radhakrishnan  
Pathology Division  
Faculty of Medicine & Health  
International Medical University  
No 126, Jalan Jalil Perkasa 19, Bukit Jalil  
57000 Kuala Lumpur,  
Malaysia