Evaluation of Possible Stress Factors Affecting Physiological Level of Gamma Interferon During First Six Months of Life in Healthy Calves

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Abstract | Background and Aims: To determine the possible stress factors affecting normal physiological level of gamma interferon in calves. Methods: Forty-two healthy calves with age range (1-6 months) were included. Five milliliters of venous blood were collected and stored at -20°C for evaluation of gamma interferon (INFγ). Complete investigation about the age, gender, source of feed, source of water, health status of breeding environment and season was recorded. Results: The mean INFγ concentration was (126.2714 ± 14.43685 pg/ml). Significant difference was reported between age groups according to INFγ level (p value = 0.000). INFγ level positively correlated with calves age group (1-2) month (p value = 0.043); female calves (p value = 0.014), while significant negative correlation was reported between the male calves and INFγ level (p value = 0.032). INFγ level positively correlated with drinking of tap water (p value = 0.003) and milk feeding (p value = 0.050). INFγ level positively inversely correlated with grass feeding (p value = 0.050); breeding of calves in poor health status of environment (p value = 0.0050). Although significant fluctuation in INFγ level was obvious throughout the year, no significant correlation between season and mean INFγ level. Conclusions: Normal physiological level of INFγ in calves positively increased during 1-2 months after birth, positively correlated with gender mainly females; drinking of tap water; milk feeding. Normal physiological level of INFγ in calves inversely correlated with males; grass feeding; breeding of calves in poor health status of environment. Normal INFγ level in calves not affected by season.

Keywords | Physiological level of INFγ, Calves, Stress factors

INTRODUCTION

Systems physiology is a scientific discipline combining theoretical, computational, and experimental studies to increase our understanding of the physiology of living creatures (Kitano, 2010). Understanding calves biology requires a systems physiology approach to dissect molecular and cellular mechanisms that regulate phenotypes, development and disease of calves. Homeostasis is essential for cattle to achieve and sustain health and, indirectly, food productions and requires hormones, powerful substances secreted by various organs in the body responsible for stimulating a cell-specific response.

Stress is defined as a condition in an animal that results from the action of one or more stressors of either external or internal origin. A variety of environmental factors can cause strain on animals and evoke stress responses. Stressors can be separated into three main types: psychological stress, physical stress, and a combination of both (Kim et al., 2011). A psychological stressor does not physically affect the body directly, but can be perceived as stressful or dangerous. For example, when animals are transported, handled, and mixed and/or put in isolation, they view...
these situations as psychological stress (Kim et al., 2011). Physical stress, including extreme temperatures or food shortage, directly induces stress responses in the body. Some stressors such as noise, pain, restraint, and weaning combine both psychological and physical stress (Kim et al., 2011).

Adjusting to stress induces a wide range of behavioral and physiological responses including endocrine changes in the hypothalamus-pituitary-adrenal (HPA) axis thus releasing corticosteroids and aldosterone (Bova et al., 2014). The overall effects on the animal are multifaceted, so different physiological outputs must be studied in order to understand the global effects of stimuli on animals and how these influence animal health, production, and quality food products.

The cytokine family consists mainly of smaller water-soluble proteins and glycoproteins with a mass of between 8 and 30 kDa. Interferons contain oligosaccharide chains (glycans) covalently attached to their polypeptide backbones (Mendelsohn et al., 2014). The carbohydrate is attached to the protein in a cotranslational or posttranslational modification (Schultz et al., 2004). Interferons are classified as a type of cytokine that produced by leukocytes, T lymphocytes, and fibroblasts and used in organisms as signaling compounds (Kasper and Reder, 2014). These chemical signals are similar to hormones and neurotransmitters and are used to allow one cell to communicate with another (Chousterman et al., 2017).

The cytokines are particularly important in both innate and adaptive immune responses (McCance and Huether, 2018). Due to their central role in the immune system, cytokines are involved in a variety of immunological, inflammatory, and infectious diseases. However, not all their functions are limited to the immune system, as they are also involved in several developmental processes during embryogenesis (Coico and Sunshine, 2015).

The interferons are the most important group of cytokines which present in different types such as alpha, beta, gamma, tau, omega, and so forth (Ealy and Wooldridge, 2017). Furthermore, interferons can be placed in classes (I, II, III) according to the receptor they bind. For example, the fibroblast or beta interferon (IFN-β) and the leukocyte or alpha family of interferons (IFN-α) are placed together as two major subtypes in type I IFNs. The only known interferon of type II is IFN-γ, which is produced exclusively by lymphocytes (Delves et al., 2017). Stress influences humoral and cellular immune systems that are regulated by the nervous and endocrine systems via cytokines, hormones, neurotransmitters, and receptors, which are in constant communication to maintain homeostasis and orchestrate coordinated responses to imbalances and pathologies (Del Rey and Besedovsky, 2019). The level of IFN-γ appear to be seriously affected and may be dawn regulated in case of chronic stress under experimental conditions and subsequently all cytotoxic mechanisms that facilitate in the continuous protection of the body (Mazal and Ramy, 2018).

Current study aims to determine the possible effect of age, gender, the season, type of drinking water, type of feed, health status of breeding environment as a stress factors on the normal physiological level of gamma interferon in calves.

**MATERIALS AND METHODS**

**STUDY AREA AND STUDY POPULATION**
This study was conducted on 42 calves, age range (1-6) months calves, living in the Baqubah city -Diyala province 33°45'34.71"N; 44°36'23.97"E, Northeast. Full investigation about age, gender, source of drinking water, source of feed, health status of breeding environment and season were reported (Figure 1).

![Figure 1: Location map of Baqubah city -Diyala province](https://www.google.iq/maps/place/Baqubah)

**BLOOD SAMPLES COLLECTION AND PROCESSING**
Five ml of venous blood were collected from jugular vein. First disinfecting the area with 70% ethanol and using a disposable syringe with a 23-gauge needle after applying a tourniquet. The blood was placed in a plain tube and left to stand for one hour at room temperature for clot formation. The tube was centrifuged for 10 minutes at room temperature at 2000X g for serum collection (Al-Ezzy and Abood, 2016). The serum was then aspirated by using a Pasteur pipette and dispensed into sterile glass tubes (1 ml each) and stored at -20°C until used. The repetitive freezing and thawing of serum sample should be avoided (Vaught, 2006).

**DETECTION OF GAMMA INTERFERON IN SERUM OF CALVES**
This immunoassay allows for the in vitro quantitative

**Test Principle**
The microtiter plate provided in the kit has been pre-coated with an antibody specific to gamma interferon. Standards or samples are then added to the appropriate microtiter plate wells with a biotin–conjugated polyclonal antibody preparation specific for gamma interferon and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain gamma interferon, biotin–conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of γ interferon in the samples is then determined by comparing the O.D. of the samples to the standard curve. The minimum detectable bovine INFγ is typically less than 1.0 pg/ml. While the detection range 6.25 pg/ml-200 pg/ml.

**Statistical Analysis**
Demography and cross tabulation were calculated by statistical analysis using SPSS for windows TM version 17.0. Chi square was used to verify possible association physiological concentration of INFγ and exposure with different factors. Significant of correlations (Pearson, spearman) include also 0.01 (two-tail). Statistical analysis was performed using SPSS for windows TM version 17.0, and Microsoft Excel for windows 2010 (AL-Ezzy, 2015; Rosner, 2015).

**Results**
As shown in Table (1), the minimum physiological concentration of INFγ in apparently healthy calves was (32.70 pg/ml), maximum physiological concentration was 307.60 pg/ml, while the mean level was (126.2714± 14.43685 pg/ml). As shown in Table (2), the minimum age of calves was 1 month, and the maximum was 6 months. Significant difference was reported between age groups according to physiological level of INFγ (p value =0.000). Significant positive correlation was reported between the age group (1-2) month and physiological level of INFγ (p value =0.043).

As shown in Table (3), males represent (71.4%) with mean physiological level of INFγ (106.8800 ±24.93423 pg/ml), while the rest (28.6%) was female with mean physiological level of INFγ (174.6250 ±64.92596 pg/ml). Significant positive correlation was reported between the females and physiological level of INFγ (p value =0.014), while significant negative correlation was reported between the males and physiological level of INFγ (p value =0.032). As shown in Table (4), calves drinking tap water represent (7.1%), with mean physiological level of INFγ (276.70±0.000 pg/ml), those drinking river water (57.1%) with mean physiological level of INFγ (106.7625±29.25018 pg/ml) while those drinking river water mixed with tap water (35.7%) with mean physiological level of INFγ (127.4000±47.98049 pg/ml). Significant positive correlation was reported between the drinking of tap water and physiological level of INFγ (p value =0.003). As shown in Table (5), calves feed on milk represent (14.3%), with mean physiological level of INFγ (124.1000±1.100000 pg/ml), those feed on grass (85.7%) with mean physiological level of INFγ (126.6333±30.10350 pg/ml). Significant positive correlation was reported between the milk feeding and physiological level of INFγ (p value =0.050). Inverse correlation was reported between the grass feeding and physiological level of INFγ (p value =0.050). As shown in Table (6), breeding of calves in poor health status of environment represent (42.86%), with mean physiological level of INFγ (99.4167±41.14292 pg/ml). Breeding of calves in middle health status of environment represent (14.28%) with mean physiological level of INFγ (117.6000 ±7.600000 pg/ml). Breeding of calves in good health status of environment represent (42.86%), with mean physiological level of INFγ (156.0167 ±44.32858 pg/ml). Strong inverse correlation was reported between breeding of calves in poor health status of environment and physiological level of INFγ (p value =0.005). As shown in Table (7), although significant fluctuation in physiological level of INFγ was obvious throughout the year, current study reported no significant correlation between season and mean physiological level of INFγ.

**Discussion**
During the early stage of development, the neonatal calf has a heightened susceptibility to a variety of infectious diseases. Peripheral blood mononuclear cells (PBMC) from 1-wk-old calves fed colostrum and milk are functionally hyporesponsive when compared to PBMC from adult cattle. The capacity of PBMC from young calves to produce interferon (IFN)-γ, a pivotal cytokine in cell-mediated immunity, differ substantially from the capacities of PBMC from adult cattle (Nonnecke et al., 2003). In addition, age-related differences in leukocyte populations from newborn calves are characterized by a higher proportion of γδ-T cells likely contribute to the increased susceptibility of the neonatal calf to infectious disease (Foote et al., 2007). Nutritional insufficiency impacts immune function and infectious disease susceptibility. Conceivably, improved nutrition would promote immune competency at an earlier age (Foote et al., 2004).
In current study, significant difference was reported between age groups according to physiological level of INFγ (p value = 0.000). Significant positive correlation was reported between the age group (1-2) month and physiological level of INFγ (p value = 0.043). These results come in line with (Foote et al., 2007), stated that physiological level of INFγ positively correlated with age.

In current study, significant positive correlation was reported between the females and physiological level of INFγ (p value = 0.014), while significant negative correlation was reported between the males and physiological level of INFγ (p value = 0.032). These results come in accordance with that reported by (Hughes et al., 2014), stated that gender plays a role in an animal’s ability to fight stress factors. The main reason for such differences is steroid hormones.
specifically estrogens, androgens and progestins, that have immunomodulatory effects. Estrogen play vital role in enhancing of immune response against stress, conversely, androgens tend to suppress certain aspects of the immune response (Hughes et al., 2014). On the other hand (Hughes et al., 2014), support current results and stated that estradiol and progesterone also influence the antigen presenting cells functions of dendritic cells, and estradiol may influence dendritic cells to stimulate Th-2 responses, while simultaneously causing a decrease in production of the Th-1 cytokines, TNF-α and INFγ. Other evidence support current results come from (Schuurs and Verheul 1990), stated that testosterone suppresses immune cell differentiation and macrophage activation in mice and rats, whereas production of IL-2 and INFγ in peripheral T-cells increased following castration of male rats, improving their ability to overcome viral and bacterial infection (Hughes et al., 2014).

Current study revealed significant positive correlation between the drinking of tap water and physiological level of INFγ (p value =0.003). This result may attributed to the presence of water pollutant such as heavy metal even in small quantities which leads to a disproportion of natural cytokines balances, and thus may result in a negative effect on immune system. Similar conclusion was reported by (Radbin et al., 2014), who found that drinking water contaminated with leads and cupper causing actual disturbance in immune cell synthesis of proteins including INFγ.

Current study revealed that (85.71%) of calves were feed grass throughout the period of study, (6 months) on the
other hand, they weaned from milk weather dam milk or artificial milk replacers. During this period current study determine an inverse correlation between grass feeding and serum INFγ concentration which come in line with that reported by (Hickey et al., 2003; Cray et al., 2009; Kim et al., 2011; Campistol et al., 2016). This result appear to be logical and reflect the physiological stress that the calves exposed to throughout the time to enforce them for changing their nutritional behaviors (Carroll and Forsberg, 2007). This stress leads to fluctuation of serum INFγ concentration when compared with those based on milk feeding, possibly due to direct effect of weaning stress on hypothalamus–pituitary axis pathway and resulted in the release of glucocorticoids in the stressed calves and hence affecting the normal function of immune system (Sivakumar et al., 2010). This result come in accordance with (Hulbert and Moisá, 2016), reported that the weaning and other stresses affecting the hypothalamus pituitary axis which in turn affecting on the levels of glucocorticoids and subsequently on the cytokines level, accordingly this include INFγ concentration. The hypothalamus pituitary axis pathway controls the release of glucocorticoids from the adrenal cortex. Both glucocorticoids and pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α are potentially direct stimulators of the acute phase response (Kurash et al., 2004). Therefore, weaning as a stressor induced two major stress pathways and stimulated the production of hepatic acute phase proteins. However, there is some contradiction related to the production of pro-inflammatory cytokines in the stress response as glucocorticoids also suppress the synthesis and release of cytokines (Kim et al., 2011). Both physical and psychological stressors have been shown to suppress blastogenesis of T- and B lymphocytes, natural killer cell activity, and IFN-γ production (Kim et al., 2011).

On the other hand current study reported positive correlations between milk feeding and INFγ concentration. This come in line with that reported by (Chase et al., 2008), stated that one indicator of a developing immune system in neonatal calves is the shift from a reliance on T helper (Th) 2 cells to Th 1 cells. As the IFN-γ is produced by Th 1 cells, earlier production of IFN-γ can signal earlier maturation of the immune system. Milk feeding during early stage of development after birth enhance the maturation of immune system and subsequently increase the production of IFN-γ to fight the pathogens at this critical period of life (M’Rabet et al., 2008).

Current study revealed strong inverse correlation was reported between breeding of calves in poor health status of environment and physiological level of INFγ (p value =0.0050). The fluctuation of INFγ may attributed to air contaminant of barns including pathogens which leads to stimulation of Th1 to produce large quantity of INFγ which come in line with that reported by (Sun et al., 2011). Current study revealed that although significant fluctuation in physiological level of INFγ was obvious throughout the year, current study reported no significant correlation between season and mean physiological level of INFγ which may attributed to small sample size under investigation which come in agreement with (Bova et al., 2014), stated that the environmental factors of climate, nutrition, and management are considered major stressors on animal health and production. Those external factors or stimuli are transduced by different receptors and may result in epigenetic changes in the absence of any changes in gene sequence in cattle. On the other hand (Sapolsky, 2005, Gupta et al., 2007) proved that stress due to overheating or cooling for calves environment activates the adrenal-cortical axis, increases cortisol and catecholamine production and, in the long term, can affect the cardiovascular function, fertility, immunosuppression and neurologic dysfunction. Continued exposure to heat stress has several known physiological effects such as an increase in plasma progesterone which in turn leads to decrease in IFN-γ (Hughes et al., 2014). Others reported that cows stalled in refrigerated barns had serum cortisol concentrations lower than those of cattle housed outside and thus have direct effect on hypothalamus–pituitary axis pathway and resulted in the release of glucocorticoids in the stressed calves and hence affecting the normal function of immune system (Bova et al., 2014). This result come in accordance with (Hulbert and Moisá, 2016), reported that stresses affecting the hypothalamus pituitary axis which in turn affecting on the levels of glucocorticoids and subsequently on the cytokines level accordingly this include INFγ concentration.

In conclusions, normal physiological level of INFγ in calves positively increased during 1–2 months after birth. INFγ positively correlated with females gender; drinking of tap water and milk feeding. Normal physiological level of INFγ in calves inversely correlated with males gender; grass feeding and breeding of calves in poor health status for environment. Normal physiological level of INFγ in calves not affected by season.

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CONFLICT OF INTEREST
The authors declare no conflict of interest

AUTHORS CONTRIBUTION
All the authors have equal contribution in the develop-


**REFERENCES**


- Del Rey A, H Besedovsky (2019). “The immune system as a sensor able to affect other homeostatic systems.” Immunopsychiatry: A Clinician’s Introduction to the Immune Basis of Mental Disorders. 83.


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