EFFECT OF HUMIC ACID AND SELENIUM SUPPLEMENTATION ON IMMUNITY AND PERFORMANCE OF NEWBORN CALVES

H. A. Kanana, Eman A. Elwakeel[*], A. G. Elkomy and S. M.A. Sallam

Department of Animal and Fish Production, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria 21545, Egypt.

[*] ***Corresponding author at the Dept. of Animal and Fish Production, Faculty of Agriculture, Alexandria University, El-Shatby, P.O. Box 21545, Alex. Egypt. emankeel@yahoo.com***

(Received 5/6/2019, accepted 22/7/2019)

SUMMARY

Forty newly born male Holstein calves were distributed randomly into four treatments (n=10) to receive no additive (control), Se (2.3mg/head/d), Humic acid (HA, 15 g/head/d), HA + Se (15 g HA+2.3 mg Se/head/d) to evaluate the effect of treatments on calves performance, serum IgG concentration and some blood parameters. Calves received their respective treatments for four consecutive days after birth as treatments were mixed with colostrum and blood samples were taken at 12, 24, 48 and 72 hrs. after birth and biweekly until 75^{th} day of life. Treatment had no effect on ADG, feed intake or weaning weight. Se and or HA had no effect on IgG concentration at 48 hrs. Se treatment increased ($P<0.05$) serum TP and HA reduced ($P<0.05$) serum TP compared to control and HA+Se. All treatments (Se, HA, HA+Se) increased ($P<0.05$) serum Alb concentration compared to control. Se supplementation increased ($P<0.05$) Glob concentration, however HA+Se reduced ($P<0.05$) Glob. Therefore HA+Se and HA had the higher A/G ratio compared to Se and control. Treatments had no effect on serum glucose (G). Se increased ($P<0.05$) G whereas, HA and HA+Se reduced ($P<0.05$) G significantly compared to control. Treatments with HA and HA+Se decreased ($P<0.05$) serum urea compared to control, whereas HA and increased ($P<0.05$) serum cholesterol but HA+Se group had the least cholesterol concentrations. All treatments reduced ($P<0.05$) serum TG compared to control. Se supplementation increased TP, Glob and G contrary to HA effect. More research is needed to evaluate the effect of Se and HA in animal health and performance.

Keywords*: Humic acid, selenium, calves, growth rates, colostrum and immunoglobulins.*

INTRODUCTION

Calves are born without a functional immune system, absence of immunoglobulin, due to the structure of the bovine placenta that separates the maternal from fetal blood circulations. Therefore, calves depends entirely on maternal immune system by absorption of immunoglobulins from colostrum until development of their own immune system. This would require about four weeks of age (Godden, 2008). The absorption of immunoglobulins from colostrum is greatest within the first 2 hours of life and has stopped completely by the completion of first 24 hours after birth. Calves with adequate passive transfer of immunity had lower morbidity and mortality and ultimately fewer antibiotic treatments compared to animals with failure of passive transfer (Beam *et al.*, 2009).

Humate substances are geological deposits and most abundant in the upper two feet of the earth's crust composed mainly of decaying plant and animal matter through the biological activities of microorganisms (Islam *et al.*, 2005). These compounds apply a protective action on the mucosa of the intestine and have antiphylogistic, adsorptive, antitoxic and antimicrobial properties (Ipek *et al.*, 2007). Griban *et al.* (1991) and Emea (1999) are the first authors who used humic acid (HA) to improve the immune system of calves, as it causes a reduction in stress and play a role in enhancing liver function, as well as stimulation of lymphocyte proliferation.

Selenium (Se) is a trace element that plays a vital role in the health and performance of animals (Spears and Weiss, 2008). It is an essential micronutrient for ruminant animals, affecting both performance and immune functions (Hall *et al.*, 2014). Se deficiency results in white-muscle disease of the newborn and immunosuppression (Koenig and Beauchemin 2009). Adequate Se addition to colostrum increases IgG absorption by newborn calves because calves are always born with Se deficiency. Supplementing Se after birth is an important approach for promoting the development of the immune system (Kamada *et al.*, 2007).

Little information is available about the effect of supplementing HA and Se together or HA alone on the immunity and performance of newborn Holstein calves. Therefore, the objective of the current study was to evaluate the effect of supplementation of HA plus Se to the colostrum given to neonatal Holstein male calves for the first four days of life on their growth performance, serum IgG concentration and some blood biochemical responses.

MATERIALS AND METHODS

This experiment was conducted at the Alexandria Agricultural Company (private dairy farm) located at 75 km Alexandria-Cairo desert road, Egypt. All sample analyses were carried out at the laboratory of Animal Nutrition, Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University, Egypt.

Animals management and experimental design:

In a complete randomized design (CRD), forty Holstein male calves (39.25±0.79 Kg body weight, BW) were enrolled in the experiment from birth till weaning and divided randomly into four groups (10 animals per treatment). All born calves belonged to 2+ parity dams within a 30 days period. Each calf received the respective treatment immediately after birth that lasted for four consecutive days only. As soon as the calf was born, navels were treated with 7% iodine solution. All calves were weighed to record their birth weight and placed in individual hutches measuring 0.8 m × 1.2 m with sandy bedding ground. Fresh colostrum was collected from the first milking of each cow immediately after birth and was given to her own calf either by a nipple bottle *ad libitum.*

Treatments and colostrum management:

Immediately after parturition, animals were divided into four groups with ten calves in each group: first group: received raw colostrum with no supplement (0 HA + 0 Se, Control). Second group: received colostrum supplemented with humic acid (15g HA). Each 100g of Humate substance used in this study (GTX Technologies, Amarillo, Texas, USA) were composed of 90g of humic acid and 10g of minerals. Third group: received colostrum supplemented with 2.3 mg Se) in 1g of Bio Sel plus (Bio Sel plus, manufactured by vetagri consulting Inc. Brampton, Canada. Imported by Bio care Egypt animal health). Fourth group: received colostrum supplemented with 15g HA+ 2.3 mg Se.

The treatments were added to colostrum once daily in the morning feeding for the first four consecutive days of life after birth. All calves were bottle feed twice a day in the morning and the evening with their own mothers colostrum in 12 hours interval. Pooled colostrum was prepared by combining the colostrum from freshly calved cows, stored in a large tank in the calf-housing facility. Two liters of colostrum and transition milk (milk from the second and third milkings) fed to every calf per suckling. The fifth day of life every calf was given the whole milk twice daily (10% of body weight) until weaning. The chemical composition and immunoglobulin concentration of colostrum (Ig) fed to all calves are presented in Table (2).

Feed management and analysis:

In the 10^{th} day of age, calves were offered a total mixed ration (TMR) *ad libitum*, and the daily dry mater intake (DMI) was calculated. Feed intake (DMI) was calculated by subtracting the refused amounts from the offered quantity. Calves were weaned once they had gained specific target weights (100 kg). Age was recorded for all calves. The initial and final body weight was recorded for every calf to calculate body weight gain (BWG) and the average daily gain (ADG). Samples of feed were taken and stored to the subsequent chemical analyses. The feed ingredients of the experimental TMR is presented in Table 1. The total mixed ration (TMR) was subjected to dry mater, organic matter, ash, crude protein and ether extract content analysis according to Association Official of Analytical Chemistry (AOAC, 1995). Organic matter was calculated by ashing at 600°C in muffle oven for 2 hr. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined by a sequential procedure using the methods outlined by Van Soest and Robertson (1991).

Table (1): Ingredients and chemical composition of the total mixed ration fed to the calves.

Ingredient	g/kg
Corn	475
Soya bean meal	400
Clover hay	100
Limestone	13
Trace minerals	3
Mono-calcium phosphate	5
Sodium chloride	3
Nitrotox (antitoxin)	1
Chemical composition	
Item	%
OM	80.88
CP	22.23
EE	13.05
Ash	8.58
NDF	19.85
ADF	6.33
ADL	1.67
Cellulose	4.66
Hemicellulose	13.53

DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NDF: nutrient detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin.

Blood sampling:

Samples (5ml) of blood were collected from the jugular vein before acess to feed at 0, 12, 24, 48 and 72 hours after birth and another set of blood samples were collected starting from day 15 and thereafter every 15 days until the 75^{th} day. Serum was harvested by centrifugation of blood at 3,000 rpm for 20 minutes and then frozen at -20°C before IgG concentration and blood biochemical parameters (total protein, albumin, urea, glucose, triglycerides, cholesterol) analyses. Blood serum Ig concentration of only five calves for each group was determined by the enzyme-linked immunosorbent serologic assay (ELISA) method (Bovine IgG ELISA Kit Cat. No: WBE-005, WKEA MED SUPPLIES CORP). The concentration of IgG (mg/ml) in blood serum samples was calculated from a standard reference curve containing known concentrations of IgG.

Colostrum analyses:

The frozen colostrum was thawed at room temperature then analyzed for fat, protein, lactose, ash, water and solid not fat content using infrared method (EKOMILK-M ultrasonic milk analyzer, EON trading 2000, INC, Bulgaria). Mortality was not detected between all groups throughout the entire experiment.

Table (2): Concentrations of colostrum immunoglobulin (IgG) mg/ml fed to calves and Chemical composition of colostrum (%).

Component	Content
IgG, mg/ml	59.64
Water	76.57 %
Total Solids	23.43%
Solid not fat	16.65%
Fat	6.78%
Protein	12.98%
Lactose	2.2%
Ash	1.47%

Determination of apparent efficiency of absorption (AEA %):

Apparent efficiency of absorption for IgG was determined according to (Quigley *et al.*, 2002) using the following formula:

$$AEA = [\text{serum IgG (g/L)} \times \text{plasma volume (L)/IgG intake (g)}] \times 100.$$

The plasma volume was calculated as follows: plasma volume = 0.089 × [BW at birth (kg)].

Statistical analysis:

Data on feed intake, growth performance, and blood parameters were analyzed as complete randomized design (CRD) with repeated measures over time by using the mixed procedure of statistical analysis system (SAS, 2002). Effects of treatment, time, and the interaction of treatment × time were defined by the F-test of ANOVA. Comparisons among treatments were performed by Tukey's test. Effects were declared significant at $P \leq 0.01$. The model used is: $Y_{ij} = \mu + T_i + D_j + DT_{ij} + E_{ij}$ as Y_{ij} = Observed mean; μ = Overall mean; T_i = Treatment effect (i = 1- 4); D_j = Time effect (j = 1-5); DT_{ij} = Interaction of treatment × time; E_{ij} = Residual error.

RESULTS AND DISCUSSION

Growth performance:

Supplementation of HA and Se alone or combined to colostrum through the first four consecutive days after birth to Holstein calves did not affect any growth performance criterion including final body weight (FBW), body weight gain (BWG), ADG, DMI, weaning age (WA), average daily gain (ADG) and dry matter intake (DMI) (Table 3).

Table (3): Effect of humic acid (HA) and selenium (Se) on growth performance of Holstein male calves.

Parameter	Treatment				*P-value*
	Control	Se	HA	HA +Se	
IBW (kg)	37.8±0.71	40.57±1.32	40.1±0.99	38.89±0.90	0.2717
FBW (kg)	100.18±0.91	101.9±0.69	100.95±2.28	102.57±1.75	0.7478
BWG (kg)	62.38±1.14	61.34±1.04	60.86±2.51	63.68±1.45	0.6374
WA (day)	85.8±2.68	79.9±2.68	88.2±2.68	81.6±2.68	0.633
ADG (kg/d)	0.74±0.03	0.77±0.01	0.70±0.04	0.79±0.03	0.2073
DMI (kg/day)	0.820±0.02	0.838±0.02	0.780±0.03	0.838±0.02	0.2803

Initial body weight (IBW), final body weight (FBW), body weight gain (BWG), weaning age (WA), average daily gain (ADG), dry mater intake (DMI) means ± SE

The results of growth performance were consistent with those reported by Degirmencioglu, (2014) who reported that supplementing HA in three doses 0, 1.0, 3.0 g/kg diet to Saanen goats did not affect BW and DMI. In contrary, others (Huck *et al.*, 1991) reported an increase in body weight gains by HA treatment and reported that the increase in body weight gains in animals may be attributed to the antibacterial and antiviral properties of humic components. In the current study, treatments had no effect in all studied parameters of growth, suggesting that the lack of response to treatment may be attributed to either the short duration of treatment or to lower dose of HA. The inconsistent results observed in different studies mentioned above may be attributable principally to the composition of different humic substances preparation, and supplementation levels, as well as the different animal species and age reported in other studies (Islam *et al.*, 2005).

To our knowledge, several studies have been conducted on the effect of Se on performance. Ghaderzadeh (2016) reported that supplementation of nano-selenium to Moghani male lambs had no significant effect on performance, which is in accordance with other studies that have been done on other species of domestic animals like sheep (Alhidary *et al*,. 2012), lamb (Sushma *et al.,* 2015) and beef calve (Richards *et al.,* 2011). Therefore, the insignificant difference in these studies might be due to feeding the balanced diet with adequate nutritional practice where Se is not deficient.

In contrary, other studies showed positive effect of Se supplementation on lambs (Kumar *et al.,* 2009), beef calves (Hall *et al.,* 2013a,b) and quail (Baylan *et al.,* 2011) that could be attributed to the exposure of these animals to heat stress. As it is well known that Se supplementation during heat stress may ameliorate the adverse effects of thermal stress by improving antioxidant status and thyroid hormone activity and maintain performance parameter compared to control treatments (Alhidary *et al.,* 2012).

Concentration of immunoglobulin (IgG):

Treatment with HA and Se had no effect on serum IgG concentration in newly born calves (Table 4) during the first 48 hrs. Based on the colostrum analyses shown previously in Table 2, overall mean values of colostrum IgG concentration was 59.64±0.44 mg/ml. Overall serum concentrations of IgG were not different among groups of calves at 48 hrs. after birth. However, serum concentrations of IgG were lower (27.35±2.3) immediately after birth (0 hr.) and started to increase and was highest at 48 hrs. (40.3±3.2).

Based on colostrum analyses shown previously in Table 2, overall mean values of colostrum IgG concentration was 59.64±0.44 mg/ml, which is similar to those reported by Kamada *et al.* (2007). When colostrum IgG concentration was higher (100 g/l), the absorption of immunoglobulin G (IgG) was increased by the small intestine of newborn calves (Stott and Fellah., 1983).

Humic substances are considered an important source of macro- and micronutrients, and these materials are able to stimulate oxygen transport (Osterberg and Mortensen 1994). Literature stated that it has growth-related effects as well as health protection capacity by changing some physiology as well as developing immunity in different species of animals (Islam *et al.,* 2005).

Kamada *et al.* (2007) reported that adding 3 mg of Na-selenite/kg to colostrum increased plasma IgG concentrations in Se-deficient dairy calves by 42% at 48 hrs. and by 24% (NS) at 14 d of age. Selenium has been postulated to act directly on intestinal epithelium to activate pinocytosis, which increases IgG absorption by newborn calves (Kamada *et al.,* 2007). It is also possible that supra nutritional concentrations of Se delay turnover of specialized intestinal epithelium, prolonging the pinocytosis process. The period during which the intestine is permeable to proteins, varies; however, it is highest immediately after birth, declines after 6 hrs. ultimately decreases to relatively low levels by 24 hrs. (Quigley and Drewry, 1998).

In the un-suckled calves the concentrations of IgG in the blood are below 0.1 g/l. Quigley *et al.* (2002) reported that inadequate amount of IgG in given colostrum may results in the failure of the passive immune transfer of immune bodies to the calves. In our study, the overall mean of IgG concentration of different treatments were 22.65, 24.58, 24.43 and 25.68 mg/ml for control, Se, HA, and HA+Se, respectively. These levels of serum IgG are closer to common averages indicating that calves included in the current study had normal values of serum IgG concentration and were not suffering from FPT.

Apparent efficiency of IgG absorption (AEA):

Calves supplemented with Se had the highest ($P<0.05$) AEA percentage (64.64%) compared with HA+Se group (45.1±3.81) while HA and HA+Se groups had intermediate AEA (Figure 1). HA and HA+Se decreased AEA by 82.9% and 75.6%, respectively. In contrast, Se group increased AEA by 108.4% as compared with control. AEA percentage depends on plasma volume, which indirectly related to birth weight.

Serum IgG concentration in newly born calves indicates efficiency of the passive immune transfer efficiency (Quigley *et al.*, 2002). Stress conditions (dystocia) during parturition on both the cow and calf may directly impair the ability of calves to acquire adequate passive immune transfer (Hough *et al.*, 1990; Godden *et al.,* 2009). In our study, there were no differences in calf birth weight or calving difficulty. Calves supplemented with Se were higher in AEA% compared to other groups. This further illustrates the ability of Se to directly affect the absorption process through enhancing pinocytosis in the intestinal cells in the first 24 hrs. after birth (Kamada *et al.*, 2007). The percentage of apparent efficiency of absorption is used to measures the efficiency by which IgG can be absorbed. Typically, averages 20 to 35% AEA in first six hours can be reached and it is dependent upon how much was the concentration of IgG in the colostrum (Quigley *et al.*, 2002). More interestingly, a negative relationship was reported between AEA and mass of IgG given to calves. This might suggest the feeding excessive colostrum might impair absorption if IgG particularly with progression of age (Stott *et al.*, 1981; Basser *et al.*, 1985).

Immunoglobulins absorbed from colostrum provide the humoral immunity for the newborn animals until they can produce immunoglobulins on their own in sufficient amounts to provide protection against infectious agents (Meyer and Harvey 2004; Blum 2000). Piccione *et al.* (2009) recorded in calves a decrease of γ-globulins from day 1 until day 15, with a subsequent small increase of values from day 20, which can be related to the development of the lymphoid function. In this study, results showed that A/G ratio decreased gradually by time (1.18±0.96, 0.89±0.83 and 0.65±0.66) for 0, 12 and 24 hrs. respectively, which may due to the increase in serum immunoglobulins concentrations occurred in this period as a result of increasing absorption of IgG from colostrum (Figure 1).

Table (4): Effect of humic acid (HA) and selenium (Se)) on blood serum immunoglobulin (IgG) concentrations (mg/ml) over the first 48 hrs. period after birth.

Time (hour)	Treatment				Overall
	Control	Se	HA	HA +Se	
0	28.7±3.35	32.9±3.35	22.8±3.04	25±7.22	27.35±2.31[b]
12	27.4±1.9	30.2±4.1	31.7±5.0	41.3±8.1	32.65±2.69[ab]
24	38.6±5.2	42.8±3.57	42.8±5.11	33.4±5.76	39.4±2.45[a]
48	43.9±3.97	33.9±8.1	40.4±6.39	43±7.6	40.3±3.2[a]

[a, b] *Means with different letters in the same column differ (P<0.05)*

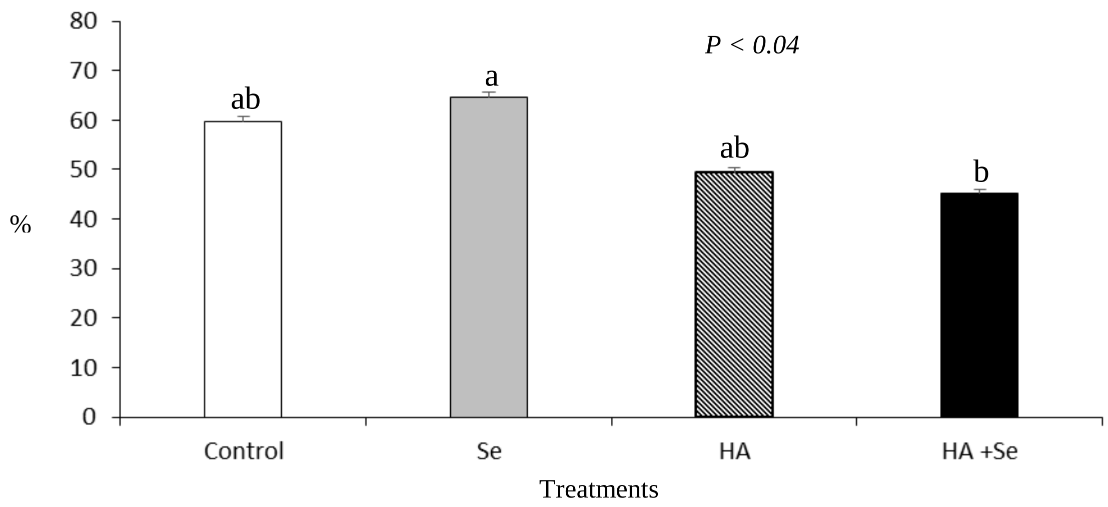

Figure (1): Apparent efficiency of absorption (AEA, %) of colostrum by control, Se, HA and HA+SE supplemented calves during the first 24 hours.

Blood metabolites:

Effects of selenium (Se) and humic acid (HA) on blood serum TP, Alb, Glob, A/G ratio, glucose, urea, cholesterol and triglycerides at 0, 12, 24, 48, and 72 hrs. after birth of Holstein male calves are shown in Table 5. Effects of Selenium (Se) and humic acid (HA) on blood serum TP, Alb, Glob, A/G ratio, glucose, urea, cholesterol and triglycerides at 15, 30, 45, 60, and 75 days after birth of Holstein male calves are shown in Table (6).

Serum total protein, albumin and albumin/globulin (A/G) ratio over first 72 hrs. after birth and from days 15 to 75:

There were no treatment × time interactions during the first 72 hrs. after birth on all determined blood parameters. Concentration of serum total protein increased gradually with time, where it reached the highest level at 24 and 48 hrs. in all groups. Overall serum total protein concentration (5.56±0.16) in Se group was significantly ($P<0.01$) higher than control (4.82±0.15), HA (4.38±0.13) and HA+Se (4.66±0.11) (g/dl) groups.

Time has no significant effect on concentration of serum Alb among treatment groups. Overall serum albumin concentrations in HA+Se group (2.62±0.03) was significantly ($P<0.01$) higher than HA, Se, and control groups 2.1±0.06, 1.95±0.03 and 1.76±0.07 (g/dl), respectively.

Concentration of serum globulin increased ($P<0.01$) gradually by time, where it reached the highest level at 48 hours in all groups. There was significant differences ($P\leq0.01$) between treatments on overall serum Glob concentrations. Supplementations of humic acid either alone (HA) (2.15±0.1 g/dl) or combined with selenium (HA+Se) (2.1±0.09 g/dl) decreased serum globulin concentrations compared with (Se) group (3.48±0.13 g/dl) and control (2.1±0.09 g/dl). Further, selenium supplementation alone (Se group) had the highest levels of serum globulin (3.48±0.13 g/dl) compared to control and other treatments.

Time had significant ($P<0.001$) effect on A/G ratio over the first 72 hrs. where it decreased gradually by time. Treatment significantly ($P<0.001$) affected A/G ratio in all groups. Control and Se groups had the lowest values compered to HA which was lower than HA+Se.

There were no treatment × time interactions ($P=0.75$) during the first 75 days after birth on serum total protein. Overall concentrations of serum TP were increased ($P<0.01$) with advancement of age starting from day 60 after birth (5.42±0.11 g/dl) and reached its highest level at day 75 of age (5.64±0.13 g/dl) in all groups. The effect of treatments on blood serum TP was significant ($P<0.01$). Overall concentrations of TP were greater in the Se group (5.42±0.13 g/dl) than HA (4.69±0.11 g/dl) and HA+Se (5.1±0.11 g/dl) groups but not the control (5.1±0.11 g/dl).

There were no treatment × time interactions ($P=0.24$) during the first 75 days after birth on serum albumin. Overall concentrations of serum albumin (g/dl) were increased ($P<0.01$) with advancement of age starting from day 30 after birth (2.47±0.09) and reached its highest level (2.68±0.8) at day 75. The effect of treatments on serum Alb was significant ($P<0.01$). Overall concentrations of serum Alb (g/dl) were greater in the Se and HA groups than control, whilst HA+Se group had the highest serum Alb concentrations.

There were no treatment × time interactions ($P=0.45$) during the first 75 days after birth on serum globulin. The highest levels of serum Glob were recorded on days 60 and 75 after birth (2.75±0.12 and 2.97±0.15 g/dl) respectively. On the other hand, treatments have significant effect ($P<0.01$) on serum globulin. Overall concentrations of serum globulin were significantly greater ($P<0.01$) in Se group (3.16±0.17 g/dl) than HA (2.28±0.09) and HA+Se (1.93±0.08 g/dl) but not with control (3.14±0.1g/dl).

There were a significant ($P<0.05$) treatment × time interactions during the first 75 days after birth on A/G ratio. The highest levels of serum A/G ratio were recorded on days 30-75 after birth in HA+Se group compared to other groups (Fig 2).

Results showed that the concentration of total protein (TP) of the two of HA and HA+Se groups were significantly lower compared to the Se and control groups (Table 5). However, Se group was superior compared to all groups over the first 72 hrs. and this effect remained to the end of the experiment (day 75). These results were in agreement with those of Rzasa *et al.* (2014) who found that serum TP was lowered with the addition of HA in two doses of 5% and 10% of HA given to New Zealand rabbits. Our results showed that the concentration of serum albumin was higher in HA+Se group compared with control (Table 5).

In a study by Soliman *et al.* (2012), ewes that injected with vitamin E + Se in period of late gestation and suckling times had significantly increased total protein and globulin without a change in plasma albumin compared to control ewes. These data are in accordance with those of El-Shahat and Abd El-Monem (2011) reporting that Baladi ewes given 50 mg vitamin E + 0.3 mg of Se per kg diet, 15 days before breeding and continued during pregnancy upto lambing increased serum TP and globulin. However, ewes given only Se had lower albumin concentrations. These data highlights the lack of effect of Se when given alone on serum albumin to sheep (Hamam and Abou-Zeina, 2007). They reported that Vit. E + Se-supplemented animals had more total serum globulin, in the form of γ-globulins, than the control or Se ewes. However, in buffalo calves, Shinde *et al.* (2009) found no difference in serum TP and globulin after supplementation with Se and Vit. E. This was coinciding with results reported by Trckova *et al.* (2017). This was expected to some extent based on acquired immunity (positive transfer of immunity) of calves throughout their lifetime.

Serum urea over first 72 hrs. after birth and days15 to 75:

No effect of time was found on serum urea concentrations throughout the 72 hrs. period. However, there was a significant (P<0.01) effect of treatment on overall serum urea concentrations. Either HA alone or combined with Se (HA+Se) decreased urea (10.14±0.6 and 11.96±0.8 mg/dl, respectively) compared to control (23.59±1.7 mg/dl) and Se (36.21±1.5 g/dl) groups. Serum urea concentration in Se group was significantly (P<0.01) higher than control and all other groups.

Time has no effect on serum urea concentrations throughout the 75 days. Se group had the highest urea 38.93±1.66 g/dl compared to other treatments while humic acid supplementation decreased urea either alone (HA) or with Se. (HA+Se) 12.6±1.03 and 12.76±0.87 g/dl, respectively. The results of this particular study obtained that supplementation with HA in the colostrum of neonatal dairy calves decreased the level of serum urea concentration in the two groups of humic acid (HA and HA+Se) compared to control while there was an increase in the Se group compared to control.

The results of this particular study revealed that supplementation with HA in the colostrum of neonatal dairy calves decreased the level of serum urea concentration in the two groups of humic acid (HA and HA+Se) compared to control while there was an increase in the Se group compared to control. These results of our study are in agreement with those reported by Trckova *et al.* (2017) who mentioned that there was an increase of serum urea in the Se group compared to control.

Serum glucose over first 72 hrs. after birth and from days 15 to 75:

Concentration of serum glucose increased (P<0.01) by time, where it reached the highest level at 48 and 72 hrs. In addition, treatment effected (P<0.05) on overall serum glucose concentrations. Supplementations of HA+Se increased (P<0.05) serum glucose concentrations compared to HA or Se alone but not the control group. There was no significant differences between Se and HA groups in blood serum glucose.

Overall concentrations of serum glucose increased with advancement of age Table (6). The highest levels of serum glucose were recorded on day 75 (82.7±5.4 mg/dl) of age while the least one was obtained on day 30 (33.51± 3.3mg/dl). It was evident that treatments have significant effect (P<0.05) on serum glucose concentration. Significant treatments × time interaction was found. The general trend of serum glucose concentrations (mg/dl) in all treatments included the control increased with the advancement of age.

Glucose is associated with various metabolic processes, and its concentration in blood is specifically regulated by complex mechanisms (Braun and Sweazea, 2008). Our results showed a significant increase in blood serum glucose for the group of HA+Se (61.71 mg/dl) at the first 72 hours. While on the long term over the day 75, the HA group was the highest concentration (63.39 ±5.4 mg/dl) among all groups. These results were in agreement with Šamudovská and Demeterová (2010) who reported that with the supplementation of natural HA and sodium humate (HNa) to the diets of chickens, the HNa group showed an increase in blood serum glucose after 35 days of the study compared to control. Mista (2007) demonstrated that humic additive caused an increase in the involvement percentage of propionic acid to the overall poll of VFA created by the microflora of the intestine *in vitro*, and this fatty acid is involved in gluconeogenesis. Alhidary *et al.* (2012) revealed no significant effect of Se injection to Australian Merino sheep on blood serum glucose. Soliman *et al.* (2012) reported that glucose concentrations were not altered in ewes or in their lambs after injection with vit E+Se compared to control. Also, Shinde *et al.* (2009) indicated that supplementation of Se alone to buffalo calves or vit E + Se did not affect serum glucose concentrations. In contrast, Singh *et al.* (2002) lower glucose concentration were observed in buffalo calves fed wheat straw + Se. It should be also understood that glucose in ruminants, is constantly synthesized from VFA, the main source of energy in the liver as the change in circulating glucose levels is limited (Udum *et al.*, 2008).

Serum cholesterol and triglycerides over first 72 hrs. after birth and from days15 to 75:

The concentration of serum cholesterol increased gradually by time (P<0.01), where it reached its highest level at 72 hrs in all groups. Results showed a treatment effect (P<0.01) on overall serum cholesterol concentrations. HA increased significantly serum cholesterol concentration compared with all other groups while control and Se groups had the lowest serum cholesterol concentrations.

Overall concentration of blood serum TG increased gradually by time (P<0.01), where it reached its highest level at 48 and 72 hrs (72.41±6.3 and 72.53±6.4 (g/dl), respectively. A treatment effect (P<0.01) was detected on blood serum TG. Supplementations of Se alone and HA alone or combined (HA+Se) decreased serum TG compared with control. However, HA supplementation decreased significantly serum TG concentration (39.29±2.8 mg/dl) compared with Se group (54.24±3.1 mg/dl).

Colostrum supplementation with HA alone or in combination with Selenium increased blood serum cholesterol throughout the first 75 days (99.89±1.6 and 70.95±1.9 g/dl) for HA and HA+Se groups, respectively compared with control (47.72±3.2 g/dl) and Se (47.07±2.1 g/dl) that showed no differences from the control. A treatment × time interaction (P<0.01) was detected on blood serum cholesterol. Humic acid plus Se (HA+SE) group had the lowest blood serum cholesterol throughout most of time points compared to other treatment groups.

A time effect (P<0.01) was recorded on serum triglycerides (TG). The highest serum TG concentration was recorded on day 60 (65.98±5.4 g/dl). A treatment effect (P<0.01) was recorded on TG. HA+Se, Se and HA groups had lower overall serum TG (50.04±3.0, 48.83±2.7 and 38.14±2.3 g/dl, respectively) compared with control (90.56±6.0 g/dl). A treatment × time interaction (P≤0.01) was detected on blood serum TG. Humic acid (HA) group had the lowest blood serum TG concentration 38.14±2.3 g/dl throughout all time points compared to other treatment groups including the control group.

Table (5): Effect of humic acid (HA) and selenium (Se) on some overall blood metabolites over the first 72 hours after birth.

Time	Treatment				*P-value*		
	Control	Se	HA	HA +Se	Trt.	Time	Trt. × Time
TP	4.82±0.15[b]	5.56±0.16[a]	4.38±0.13[c]	4.66±0.1[bc]	0.01	0.01	0.80
Alb.	1.76±0.07[d]	1.95±0.03[c]	2.1±0.1[b]	2.62±0.03[a]	0.01	0.07	0.99
Glob.	3.13±0.16[b]	3.48±0.1[a]	2.28±0.1[c]	2.04±0.1[c]	0.01	0.01	0.42
A/G	0.59±0.61[c]	0.57±0.3[c]	0.96±0.7[b]	1.34±0.5[a]	0.01	0.01	0.98
Glucose	52.82±4.5[ab]	50.4±2.8[b]	49.65±3.3[b]	59.71±3.1[a]	0.04	0.01	0.44
Urea	23.59±1.7[b]	36.21±1.5[a]	10.14±0.6[c]	11.96±0.8[c]	0.01	0.12	0.90
Cholesterol	47.72±3.2[c]	47.07±2.1[c]	99.89±1.6[a]	70.95±1.9[b]	0.01	0.01	0.65
triglyceride	93.72±6.8[a]	54.24±3.1[b]	39.29±2.8[c]	44.66±3.4[bc]	0.01	0.01	0.80

[a, b,c] *Means with different letters in the same row differ (P<0.05)*

Table (6): Effect of humic acid (HA) and selenium (Se) on blood serum biochemical parameters during days 15 to 75.

Time	Treatment				*P-value*		
	Control	Se	HA	HA +Se	Trt	Time	Trt × Time
TP	5.23±0.11[ab]	5.42±0.13[a]	4.69±0.11[c]	5.1±0.11[b]	0.01	0.01	0.74
Alb.	2.1±0.04[c]	2.41±0.04[b]	2.41±0.05[b]	3.17±0.05[a]	0.01	0.01	0.24
Glob.	3.14±0.1[a]	3.16±0.17[a]	2.28±0.09[b]	1.93±0.08[c]	0.01	0.02	0.45
A/G	0.67±0.31[c]	0.77±0.31[c]	1.07±0.48[b]	1.68±0.66[a]	0,01	0.05	0.01
Glucose	55.23±5.1[ab]	56.28±3.6[ab]	63.39±5.4[a]	51.21±3.4[b]	0.07	0.01	0.01
Urea	26.79±2.21[b]	38.93±1.66[a]	12.6±1.03[c]	12.76±0.87[c]	0.01	0.90	0.25
Cholesterol	79.53±5.0[c]	69.9±2.1[d]	140.58±3.0[a]	110.71±2.6[b]	0.01	0.01	0.01
triglyceride	99.76±6.0[a]	48.83±2.7[b]	38.14±2.3[c]	50.04±3.0[b]	0.01	0.05	0.01

[a, b,c] *Means with different letters in the same row differ (P<0.05)*

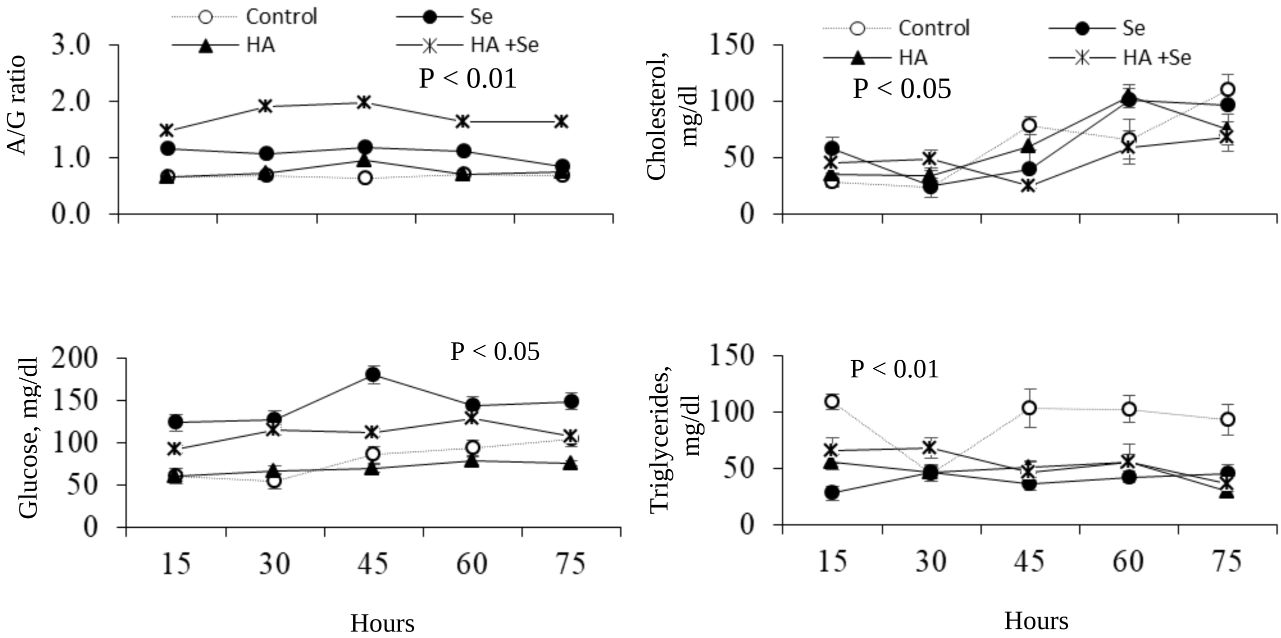

Figure (2): Treatment × time interaction of serum concentrations of albumin/globulin (A/G) ratio, glucose, cholesterol and triglycerides over 75 days after birth in Holstein calves receiving control, Se, HA and HA+Se supplementation with colostrum.

Concentrations of cholesterol (Cho) increased gradually by time as highest serum cholesterol concentrations recorded were at days 45, 60 and 75. Humic acid (HA) treatments either alone or combined with Se had the highest serum cholesterol concentration. Serum triglycerides (TG) followed a different trend where all treatments had lower TG concentrations compared to controls. However, it is difficult to compare the effect of HA across studies due to the different sources and preparations of HA used, because animals reared in various regions of the world are exposed to different climates and environmental conditions (Islam *et al.*, 2005). This effect lasted for the end of the experiment except for Se group, which became significantly lower than control. In spite of this increase of Cholesterol concentrations were within the normal values, which indicate that there is no effect on liver dysfunction. But in the case of TG we observed that supplementation with HA and Se decreased serum TG during the first 75 days compared with control. This effect remained for the end of the experiment as well.

CONCLUSION

In conclusion, addition of HA did not produce the promising results it was anticipated to have as promoting supplement to newly born calves' in colostrum compared with some other previous data. The difference may be due to verities of HA sources, doses, duration of treatments, animal species or the environmental conditions. We recommend different doses of both HA and Se must be considered for longer durations to assess either beneficial or harmful effect on the animal performance and immunity.

ACKNOWLEDGEMENTS

Thanks to the Department of Animal and Fish Production, Faculty of Agriculture, Alexandria University for providing financial support for the research and use of research facilities.

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تأثير إضافة حمض الهيوميك والسيلنيوم على مناعة وأداء العجول حديثة الولادة

حسن علي قنانه ، إيمان علي الوكيل ، أحمد جمال الدين الكومي و صبحي محمد عبد الله سلام

قسم الإنتاج الحيواني والسمكي - كلية الزراعة- جامعة الإسكندرية- الإسكندرية- جمهورية مصر العربية.

أجريت هذه الدراسة على أربعين عجل هولشتين ذكر حديث الولادة (39,25 ± 0,79كجم) قسمت لاربع مجاميع: الكونترول : (بدون إضافات) , المجموعة الثانية : تم إضافة 1 جم سيلنيوم , المجموعة الثالثة: تم إضافة 15 جم من حمض الهيوميك , المجموعة الرابعة : تم إضافة 15 جم من حمض الهيوميك + 1جم سيلنيوم إلى السرسوب يومياً لمدة أربعة بعد الولادة لدراسة تأثير ذلك على تركيز الأجسام المناعية (الإمينوجلوبيولين) في سيرم الدم وأداء النمو من حيث معدل الزيادة الكلية في الوزن وعمر الفطام ومعدل الزيادة اليومية في الوزن والمادة الجافة المأكولة وبعض القياسات الكيميائية لمكونات الدم. تم أخذ عينات الدم عند الولادة مباشرة قبل رضاعة السرسوب ثم عند 12 , 24, 48 و 72 ساعة ثم روعي أخذ عينات الدم بعد ذلك مرة كل أسبوعين حتى نهاية التجربة.

أظهرت النتائج انه لم يكن هناك تأثير معنوي للمعاملات على مقاييس النمو التي تم دراستها. إضافة كل من حمض الهيوميك والسيلنيوم معاً أو كل على حدة لم يؤثر معنوياً على تركيز الأجسام المناعية (الأمينوجلوبيولين) في سيرم دم العجول بينما أدى إستخدام السيلينيوم إلى تحسين كفاءة امتصاص الأجسام المناعية في القناة الهضمية. إزداد تركيز البروتين الكلي والجلوبيولين في سيرم دم العجول تدريجياً مع الوقت حيث وصل لإقصي معدلاته عند 24 و48 ساعة وكان الأعلي معنوياً في مجموعة السيلنيوم مقارنة بباقي المجموعات. تركيز الألبيومين كان أعلى معنويا في الهيومك + السلينيوم من باقي المجموعات. أدت إضافة الهيومك والسيلنيوم معاً إلى إرتفاع معنوي في مستوى الجلوكوز في الدم عن باقي المجاميع وذلك في المرحله الاولي من التجربه. ولكن في المرحلة الثانية من عمر 15 – 75 يوم كانت مجموعة الهيوميك أعلى معنويا من الهيوميك + السيلينيوم ولكن ليس بالنسبة للكونترول أو السيلنيوم. في المرحلة الأولى والثانية من التجربة أدت إضافة الهيوميك، الهيوميك + السيلينيوم إلى إنخفاض معنوي في مستوى يوريا سيرم الدم مقارنة بالكونترول. إضافة حمض الهيوميك أدى إلى إرتفاع معنوي في تركيز الكوليسترول بين كل المجموعات بينما حققت مجموعة الكونترول والسيلنيوم أقل قيمة بين باقي المجموعات ولم يكن هناك تفاعل بين الوقت والمعاملة . إضافة السيلنيوم و حمض الهيوميك معاً أو كل على حدة أدى إلى إنخفاض في قيمة الدهون الثلاثية مقارنة بالكونترول.

نستخلص من هذه الدراسة أن إستخدام حمض الهيوميك مع أو بدون السيلنيوم لم يحسن النمو أو تركيز الأجسام المناعية في الدم بينما أدى إستخدام السيلينيوم إلى تحسين كفاءة امتصاص الأجسام المناعية في القناة الهضمية.