

## EFFECT OF *CHLORELLA VULGARIS* ALGAE SUPPLEMENTATION ON GROWTH PERFORMANCE OF SUCKLING FRIESIAN CALVES. 18

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### SUMMARY

Twenty newly born male and female Friesian calves with an average live body weight (LBW) of 30.40±0.36 kg were divided into five similar groups (2 male + 2 female in each group) and continued in a feeding trial until weaning to study the effect of supplementing the algae on suckling calves growth performance. Diet of cows and their suckling calves in G1 did not supplemented with algae and served as a control diet. Whereas, cows and their suckling calves were supplemented with algae and their media at levels of 2 ml (G2) and 4 ml (G3) / kg LBW, as low and high levels, respectively. While, cows in G4 and G5 were unsupplemented with algae, but their suckling calves were supplemented with *Chlorella vulgaris* algae and their media at the same above levels. Results showed that groups supplemented with high level of algae (G3 and G5) were recorded significantly (P<0.05) the highest digestibility of all nutrients and feeding values followed by groups supplemented with low level of algae (G2 and G4), while control group (G1) had the lowest values. The DMI were the same for different groups, while the intake of TDN and DCP were significantly (P<0.05) higher for G3 and G5 compared to those of control one (G1), but the low levels of algae rations (G2 and G4) were insignificant higher than those of control ration as well. Mostly, blood serum measurements (total protein, globulin and albumin) in addition of creatinine and liver enzymes activity (AST and ALT) were markedly improved versus to those of control group (G1). Moreover, the concentrations of immunoglobulin fractions (IgA, IgM and IgG) were increased significantly (P<0.05) with chlorella supplementation and increased with increasing the level of chlorella supplement. Weaning weight and average daily gain of suckling calves increased significantly (P<0.05) with chlorella algae supplementation as well as with increasing supplementation level. Chlorella supplementation improved significantly (P<0.05) feed conversion, being decreased the amounts of DM, TDN and DCP required per one kg live weight gain. Also, chlorella supplementation improved economic efficiency, which decreased feed cost per one kg live weight gain and increased the total and net revenue of live weight gain and economic efficiency.

**Keywords:** suckling calves, *Chlorella* algae supplementation, digestibility, growth performance and economic efficiency.

### INTRODUCTION

Microalgae are photosynthetic microorganisms that have the ability to convert sunlight and carbon dioxide (CO<sub>2</sub>) to highly valuable biomass. Algal biomass production has advantages such as use of water that is unsuitable for humans and minimum competition for land with crops. Due to their contents of essential amino acids, fatty acids and other health promoting nutrients, microalgae are attractive feeds or feed supplements for livestock (Janczyk *et al.*, 2006). *Chlorella* is a genus of single-celled green algae belonging to the division Chlorophyta. It is spherical in shape, about 2 to 10 µm in diameter, and is without flagella. *Chlorella* contains the green photosynthetic pigments chlorophyll-a and -b in its chloroplast. Through photosynthesis, it multiplies rapidly, requiring only carbon dioxide, water, sunlight, and a small amount of minerals (Scheffler, 2007).

*Chlorella vulgaris* (*C. vulgaris*) is a genus of unicellular green algae containing high level of protein (50 to 70% of dry matter), lipid, vitamins and minerals (Phang, 1992). *Chlorella vulgaris* is a single-celled freshwater microalgae and contains the highest amount of chlorophyll of any common plant, with protein content of about 600 g/kg dry matter (DM) and 18 amino acids as well as vitamins and minerals (Radhakrishnan *et al.*, 2014). Abedin and Taha (2008) had previously suggested that *C. vulgaris* have a higher antimicrobial activity due to the presence of cyclic peptides, alkaloids, and lipopolysaccharides in vitro. Janczyk *et al.* (2009) also suggested that the inclusion of *C. vulgaris* could benefit the intestinal

lactobacillus diversity in laying hens because of the unknown compounds such as polysaccharides, phenolic substances and aromatic compound (Halama, 1990).

Even when used at small amounts in livestock and aquaculture feeds, algae have been credited with improving immune system (Turner *et al.* 2002), lipid metabolism (Nakagawa, 1997 and Güroy *et al.*, 2011), antiviral and antibacterial action, improved gut function (Michiels *et al.*, 2011), stress resistance (Nath *et al.*, 2012 and Sheikhzadeh *et al.*, 2012) besides providing a source of protein, amino acids, fatty acids, vitamins and minerals, and other biologically active phytochemicals (Pulz and Gross, 2004; Becker, 2004 and Gouveia *et al.*, 2008).

Microalgae are well provided sources of vitamins, fundamental amino acids, minerals, vital fatty acids, and carotenoid pigments for aquatic animals (Takeuchi *et al.*, 2002). Many kinds of microalgae used in feeding trials with fish led to an increase of growth performance (protein accretion), feed utilization, physiological activity, stress response, starvation tolerance, disease resistance, carcass quality (Mustafa and Nakagawa, 1995), carotenoid and protein sources for shrimp (Patnaik *et al.*, 2006; Regunathan and Wesley, 2006). Moreover, it contains some other important dietary components such as unsaturated fatty acids (UFA), glycoproteins and carotenoids (Janczyk *et al.*, 2006). *Chlorella vulgaris* contains a phyto-nutrient called Chlorella Growth Factor (CGF), comprised mainly of nucleic acids and other essential substances, with detoxification and antioxidant properties (Han *et al.*, 2002). Two recent studies (Anele *et al.*, 2016 and Tsiplakou *et al.*, 2016) reported that *C. vulgaris* improved bacterial growth and a shift in the ruminal biohydrogenation pathway by stimulating production of trans C18:1, trans-11 C18:1 fatty acids, monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA).

Chlorella as a feed supplement has been known to have beneficial effects, such as growth, immunomodulation, antioxidant activity and tissue rebuilding (Guzmán *et al.*, 2001).

The objective of this study was to investigate the effect of orally adding of fresh chlorella algae with their media on feed intake, digestibility, blood biochemical and immunoglobulin fractions parameters, body weight gain, feed conversion and economic efficiency of suckling Friesian calves.

## MATERIALS AND METHODS

This study was carried out at El-Karada Animal Production Research Station, Kafr El-Sheikh Governorate, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture and Land Reclamation.

### *Chlorella vulgaris* microalgae:

Lyophilized *C. vulgaris* biomass was cultivated in Cyanobacteria Research Lab., Microbiology Dept., Sakha Agricultural Research Station, Soils, water, and Environment Research Institute, Kafr El-Sheikh, Egypt. Inoculum was prepared as described by El-Sayed *et al.* (2001) using BG-II growth medium (Stainer *et al.*, 1971). Continuous light illumination was provided from daylight lamps (10×40 w). Aeration was achieved using an oil-free air compressor (HIBLOW AIR PUMP, type SPP-100GJ-H, Japan) through a 3-mm polyethylene tube. Room temperature was adjusted to 27±2 °C during the whole incubation period. Incubation was carried out using fully transparent polyethylene bags (75×5 cm<sup>2</sup> and 100 µm thickness) containing 2.5 liters of algal broth. Mass production of *C. vulgaris* was performed within a 1000-litre Zigzag photobioreactor (El-Sayed *et al.*, 2015). For harvesting and cleaning of the obtained biomass, a series of precipitation and washing was performed using tap water and a cooling centrifuge (RUNNE, HIDEBERG, RSV-20, Germany).

### *Animals and experimental groups:*

Twenty newly born male and female Friesian calves with an average live body weight (LBW) of 30.40±0.36 kg were divided into five similar groups (2 male + 2 female in each group) and continued in a feeding trial until weaning. Diet of cows and their suckling calves in G1 did not supplemented with algae and served as a control diet. Whereas, cows and their suckling calves were supplemented with algae and their media at levels of 2 ml (G2) and 4 ml (G3) / kg LBW, as low and high levels, respectively. While, cows in G4 and G5 were unsupplemented with algae, but their suckling calves were supplemented with *Chlorella vulgaris* algae and their media at the same above levels.

**Management procedures:**

Dams of calves in G2 and G3 were orally administrated with chlorella algae every day at the low and high levels of 2 and 4 ml / kg LBW, respectively. Calves were left with dams to suckle their dam's colostrum during the first three days after calving. After that, calves were housed in separate pens at night and free in backyard at day. Calves were fed their allowance during the suckling period (105 days) to cover their nutritional requirements according to NRC (2001) as shown in Table (1). Calves were individually suckled the whole milk in plastic buckets two times daily at 7 a.m. and 4 p.m. Whereas, they were fed in group feeding on calf starter (CS) once time at 8 a.m. and berseem hay (BH) at 11 a.m. Chlorella algae was orally administrated for suckling calves. Fresh water was free available for calves all the day round. Calf starter was consisted (as fed) of 20% soybean meal, 5% linseed cake, 34% ground yellow corn grain, 20% wheat bran, 15% rice bran, 3% molasses, 2% limestone and 1% common salt. Chemical compositions of different feedstuffs are shown in Table (2).

**Table (1): Daily allowances of whole milk, starter, berseem hay for suckling Friesian calves.**

Age	Whole milk (kg)		Calf starter (g)	Berseem hay (g)
	Morning	Evening		
1-3 days			Suckling their dam's colostrum	
4-7 days		2	50	-
Week 2	2.5	2	100	50
Week 3	3	2	150	100
Week 4	3	3	200	150
Week 5	3	2.5	250	200
Week 6	3	2	300	250
Week 7	2.5	2	350	300
Week 8	2.5	2	400	350
Week 9	2	2	450	400
Week 10	3	-	500	450
Week 11	3	-	600	500
Week 12	3	-	700	600
Week 13	2	-	800	700
Week 14	2	-	900	800
Week 15	1	-	1000	900

**Table (2): Chemical composition of calf starter and berseem hay.**

Item	DM %	Composition of DM %					
		OM	CP	CF	EE	NFE	Ash
Calf starter	90.03	89.79	18.40	5.92	2.40	63.07	10.21
Berseem hay	88.83	85.67	14.81	30.11	2.13	38.62	14.33
Basal diet*	26.49	90.13	19.34	11.05	13.46	46.28	9.87

*The composition of whole milk was 3.94% fat, 2.86% protein, 4.32% lactose, 7.88% solids not fat, 11.82% total solids and 0.70% ash.*

*\* Basal diet contained whole milk + calf starter + berseem hay.*

**Live body weight:**

Calves were weighed weekly in the morning before drinking and feeding to the nearest 0.1 kg for each animal during the suckling period and the average daily body weight gain were calculated.

**Digestibility trials:**

Five digestibility trials were conducted at the end week of the suckling period using all calves to determine nutrient digestion coefficients and feeding values of the experimental diets. Acid insoluble ash (AIA) was used as a natural marker as described by Van Keulen and Young (1977). Feces samples were taken from the rectum of each calf twice daily with 12 hours interval for 7 days collection period. Milk samples were analyzed for fat, protein, lactose, solids not fat (SNF), and total solids (TS) by Milko-Scan (model 133B), and ash by difference. While, samples of calf starter, berseem hay and feces were dried in a forced air oven at 65 °C for 48 hours, ground and representative samples were carried out according to the

methods of AOAC (2000). Digestibility coefficient of all nutrients was calculated from the equation given by Schneider and Flatt (1975).

**Blood samples:**

Blood samples were collected from all calves at the end week of suckling period by direct jugular vein puncture into tubes with serum cloth activator (Meus, Piove di Sacco, Italy). Blood samples were allowed to clot at room temperature and then centrifuged at 3000 rpm for 30 minutes to separate serum. The harvested blood serum was dispensed into plastic tubes and stored at  $-20^{\circ}\text{C}$  until analyses. Total protein, albumin, globulin (total protein - albumin), creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically by spectrophotometer (Spectronic 21D, USA) using commercial kits produced by Diagnostic System Laboratories, Inc., USA. The concentrations of immunoglobulin fractions (IgA, IgM and IgG) in blood serum samples were measured using the quantitative ELISA Bovine (IgG), ELISA Quantitation Kit, Bethyl laboratories, UK.

**Feed conversion:**

Feed conversion was expressed as the amount of DM, TDN and DCP required per one kg weight gain.

**Economic efficiency:**

Economic efficiency parameters were calculated according to the prices of year 2018. The prices in Egyptian pound (LE) per ton were 5500 LE for calf starter and 3000 LE for berseem hay. Also, the prices per kg were 5 LE for milk and 70 LE for live weight gain. Whereas the price of chlorella algae with media was 4 LE for one liter.

**Statistical analysis:**

The data of were statistically analyzed using general linear model procedure adapted by IBM SPSS Statistics (2014) for user's guide with one-way ANOVA. Significant differences in the mean values among dietary treatments were analyzed by Duncan's tests within SPSS program set at the level of significance  $P < 0.05$  (Duncan, 1955).

## RESULTS AND DISCUSSION

**Nutrients digestibility and feeding values:**

Results of nutrients digestibility and feeding values for the different experimental rations are presented in Table (3). Rations supplemented with the high level of chlorella algae (G3 and G5) were recorded significantly ( $P < 0.05$ ) the highest values of digestibility for all nutrients and feeding values followed by G2 and G4 those received the low level of chlorella algae, whereas the control one (G1) had significantly the lowest values. Digestibility of all nutrients and feeding values increased by 2.49-2.80% with low level versus 5.49-6.00% with the high level of chlorella algae compared to the control one. The determination of digestibility coefficients is the first step in evaluating the nutritional quality and utilization efficiency of an ingredient in complete diets for animals. These measurements provide an indication of the nutrients or energy fraction of the ingested feedstuffs that are not excreted in the feces, but are used for metabolic processes for animal production (NRC, 2011), thereby positively affecting production rates. It has been suggested that the Chlorella contained growth promoting substance such as S-nucleotide adenosyl peptide complex, which may affect digestibility of the animals (Han *et al.*, 2002). Supplementation of animal feeds with acceptable levels of Chlorella biomass can have a positive influence and probably associated with improvement of nutrients digestibility in pigs (Yan *et al.*, 2012), as well as with higher utilization of minerals (Taranu *et al.*, 2012). Zeinhom (2004) found that fish fed diet containing 15% algae increased significant by the digestibility coefficient of dry matter, crude protein, ether extract and energy. *Chlorella vulgaris* contains  $\beta$ -glucan, which plays a role in scavenging free radicals (Iwamoto, 2004) and thus improves fermentation. Moreover, the *C. vulgaris* microalgae contents of PUFA, carotenoids, phycobiliproteins, polysaccharides and phycotoxins are required for higher microbial growth (Kotrbaček *et al.*, 2015). Tibbetts *et al.* (2016) reported that the dietary effect of algal supplementation on feed digestibility in ruminants is related in part to its lipid content. Two recent studies (Anele *et al.*, 2016 and Tsiplakou *et al.*, 2016) reported that *C. vulgaris* improved bacterial growth and a shift in the ruminal biohydrogenation pathway by stimulating production of trans C18:1, trans-11 C18:1 fatty acids, monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA).

Kholif *et al.* (2017) reported that *Chlorella vulgaris* supplementation to the diet of goats increased apparent diet digestibility compared with a control diet.

**Table (3): Nutrients digestibility and feeding values for the experimental rations.**

Item	Experimental groups					SEM
	G1	G2	G3	G4	G5	
Nutrients digestibility, %						
DM	68.54 <sup>c</sup>	70.46 <sup>b</sup>	72.65 <sup>a</sup>	70.25 <sup>b</sup>	72.31 <sup>a</sup>	0.43
OM	69.76 <sup>c</sup>	71.71 <sup>b</sup>	73.95 <sup>a</sup>	71.50 <sup>b</sup>	73.60 <sup>a</sup>	0.44
CP	68.35 <sup>c</sup>	70.26 <sup>b</sup>	72.45 <sup>a</sup>	70.06 <sup>b</sup>	72.11 <sup>a</sup>	0.42
CF	61.17 <sup>c</sup>	62.88 <sup>b</sup>	64.84 <sup>a</sup>	62.70 <sup>b</sup>	64.53 <sup>a</sup>	0.38
EE	76.93 <sup>c</sup>	79.08 <sup>b</sup>	81.55 <sup>a</sup>	78.85 <sup>b</sup>	81.16 <sup>a</sup>	0.48
NFE	70.28 <sup>c</sup>	72.25 <sup>b</sup>	74.50 <sup>a</sup>	72.04 <sup>b</sup>	74.15 <sup>a</sup>	0.44
Feeding values, %						
TDN	75.80 <sup>c</sup>	77.92 <sup>b</sup>	80.35 <sup>a</sup>	77.70 <sup>b</sup>	79.97 <sup>a</sup>	0.47
DCP	13.22 <sup>c</sup>	13.59 <sup>b</sup>	14.01 <sup>a</sup>	13.55 <sup>b</sup>	13.95 <sup>a</sup>	0.08

*a, b and c: Means in the same row with different superscripts differ significantly (P<0.05).*

**Feed intake:**

Feed intake by suckling calves are presented in Table (4) where the values revealed that the intake of the different ingredients as well as DM intake were typically comparable for the different dietary treatments. These results may be due to that calves in the different groups were fed their recommended requirements according to NRC (2001) as well as they were run in group feeding for calf starter and berseem hay. Whereas, the intake of TDN and DCP were significantly (P<0.05) higher in G3 and G5 which received the high level of chlorella algae (4 ml/kg) compared to those of control one, while G2 and G4 which received the low level of chlorella algae (2 ml/kg) were have the intermediate values without significant differences with the control. In spite of DM intake was the same for different groups, the increase of TDN and DCP intake with chlorella supplementation could be attributed to the increase TDN and DCP contents for groups received chlorella algae (Table 3). These results agreed with those obtained by Glover *et al.* (2012) observed that supplementing diets of lactating cows with marine microalgae at 200 g/day did not affect DMI of fresh forage or silage-based total mixed ration. However, Kholif *et al.* (2017) reported that *Chlorella vulgaris* treatments increased feed intake. Inconsistency between these studies may be due to different diet composition and different algae inclusion doses (Reynolds *et al.*, 2006).

**Table (4): Daily feed intake (kg/head/day) for calves of different experimental diets.**

Item	Experimental groups					SEM
	G1	G2	G3	G4	G5	
Milk	3.69	3.69	3.69	3.69	3.69	
Calf starter	0.45	0.45	0.45	0.45	0.45	
Berseem hay	0.41	0.41	0.41	0.41	0.41	
Chlorella algae (ml)	-	115.32	240.35	116.33	237.85	
DM	1.21	1.21	1.21	1.21	1.21	
TDN	0.92 <sup>b</sup>	0.94 <sup>ab</sup>	0.97 <sup>a</sup>	0.94 <sup>ab</sup>	0.97 <sup>a</sup>	0.01
DCP	0.160 <sup>b</sup>	0.164 <sup>ab</sup>	0.170 <sup>a</sup>	0.164 <sup>ab</sup>	0.169 <sup>a</sup>	0.001

*a and b: Values in the same row with different superscripts differ significantly (P<0.05).*

**Blood serum biochemical:**

Blood serum biochemical of calves in the different groups are presented in Table (5). Almostly, there were significant (P<0.05) differences in the concentrations of total protein, albumin, globulin and creatinine in blood serum among the tested rations and control one. Total protein concentration was significantly (P<0.05) higher in G3 and G5 compared to G1, whereas the values of G2 and G4 were insignificant increased than the control one (G1). However, albumin concentration was significantly (P<0.05) lower in G2 than that of the control one (G1), whereas the concentrations of G3, G4 and G5 were insignificant lower than that of control (G1). At the same time, globulin concentration increased significantly (P<0.05) with *Chlorella* supplementation of all tested rations compared to the control one. Albumin to globulin ratio was

significantly ( $P < 0.05$ ) higher in G1 (1.08) compared to the other groups (0.88-0.93). Albumin to globulin ratio in groups received chlorella improved calves health, which were within the normal range for good health being 0.80 to 0.95. Creatinine concentration decreased significantly ( $P < 0.05$ ) with chlorella supplementation than that of control. Live enzymes activity (AST and ALT) improved markedly ( $P < 0.05$ ) with *Chlorella* supplementation especially in G2 and G3. Increased serum total protein with *C. vulgaris* supplementation may be due to increased DCP intake. Reduced concentrations of AST and ALT are considered as important indicators for liver activity, functionality and hepatotoxicity, suggesting the absence of pathological lesions in the liver. In the present study, feeding *C. vulgaris* microalgae, at both levels, resulted in a significant decrease in AST and ALT concentrations, indicating a probable protective role for *Chlorella vulgaris* against liver dysfunction. Increased serum total protein with *C. vulgaris* supplementation may be due to increased CP and OM intakes and digestibilities. In addition, decreased values of serum urea-N with different *C. vulgaris* treatments indicate the absence of protein catabolism, and normal kidney function (Hosten, 1990). Reduced concentrations of GPT and GOT are important indicators of liver activity, function and hepatotoxicity, suggesting the absence of pathological lesions in the liver. In the present study, feeding *C. vulgaris* microalgae, at both levels, resulted in a significant decrease in GPT and GOT concentrations, indicating a probable protective role for *C. vulgaris* against liver dysfunction. Kholif *et al.* (2017) reported that *Chlorella vulgaris* treatments increased serum total protein concentration but decreased serum AST and ALT concentrations. Also, El-Abd and Hamouda (2017) found that watering chickens with algae caused a reduction in creatinine, AST and ALT.

**Table (5): Blood serum biochemical and immunoglobulin fractions of calves in the different groups.**

Item	Experimental groups					SEM
	G1	G2	G3	G4	G5	
<b>Biochemical</b>						
Total protein, g/dl	7.17 <sup>b</sup>	7.22 <sup>ab</sup>	7.38 <sup>a</sup>	7.20 <sup>ab</sup>	7.35 <sup>a</sup>	0.03
Albumin, g/dl	3.72 <sup>a</sup>	3.37 <sup>b</sup>	3.53 <sup>ab</sup>	3.46 <sup>ab</sup>	3.45 <sup>ab</sup>	0.06
Globulin, g/dl	3.45 <sup>b</sup>	3.85 <sup>a</sup>	3.86 <sup>a</sup>	3.74 <sup>a</sup>	3.90 <sup>a</sup>	0.05
Albumin/globulin ratio	1.08 <sup>a</sup>	0.88 <sup>b</sup>	0.91 <sup>b</sup>	0.93 <sup>b</sup>	0.88 <sup>b</sup>	0.01
Creatinine, mg/dl	1.30 <sup>a</sup>	1.06 <sup>b</sup>	1.10 <sup>b</sup>	1.13 <sup>b</sup>	1.14 <sup>b</sup>	0.04
AST, IU/L	35.33 <sup>a</sup>	31.00 <sup>b</sup>	30.00 <sup>b</sup>	33.33 <sup>ab</sup>	33.00 <sup>ab</sup>	0.83
ALT, IU/L	16.50 <sup>a</sup>	12.83 <sup>b</sup>	13.17 <sup>ab</sup>	14.50 <sup>ab</sup>	14.33 <sup>ab</sup>	0.23
<b>Immunoglobulin fractions</b>						
IgA, mg/dl	28.00 <sup>b</sup>	32.42 <sup>ab</sup>	34.47 <sup>a</sup>	32.67 <sup>ab</sup>	34.50 <sup>a</sup>	0.56
IgM, mg/dl	7.56 <sup>c</sup>	9.83 <sup>b</sup>	10.77 <sup>ab</sup>	10.15 <sup>ab</sup>	11.82 <sup>a</sup>	0.37
IgG, mg/dl	120.92 <sup>b</sup>	138.33 <sup>a</sup>	140.33 <sup>a</sup>	141.83 <sup>a</sup>	140.58 <sup>a</sup>	1.73

*a, b and c: Values in the same row with different superscripts differ significantly ( $P < 0.05$ ).*

#### **Immunoglobulin fractions:**

Results in Table (5) showed significant ( $P < 0.05$ ) increases in the concentrations of immunoglobulin fractions in blood serum with chlorella supplementation. The concentration of IgA was significantly ( $P < 0.05$ ) higher in serum of G3 and G5 (high chlorella level) compared to that of G1, whereas in G2 and G4 (low chlorella level), the IgA values were insignificant increased in relation with that of control (G1). While the concentration of IgM was significant higher for all tested rations in comparison with control one being the highest value was associated with G5. In respect of IgG, its concentration was significant higher with all algae rations than that of control one. Even when used such algae at small amounts in livestock and aquaculture feeds, a favorable affect have been credited with improving immune system (Turner *et al.*, 2002). Guzmán *et al.* (2003) reported that chlorella as a feed supplement has been known to have beneficial effect on immuno-modulation, antioxidant activity and tissue rebuilding. Moreover, Taranu *et al.* (2012) found that natural supplements such as *Chlorella vulgaris* (1%), sodium alginate (0.1%), inulin (1.5%), and a mixture of essential oils (0.04%) had the ability to potentiate the immune function during the initial post weaning period. Dietary supplementation of 1 % chlorella in the form of Chlorella Growth Factor (CGF) and as fresh liquid chlorella increased immune characteristics (number of lymphocytes) and population of Lactobacillus in broiler chickens (Kang *et al.*, 2013).

#### **Live body weight and daily gain:**

Live body weight (LBW) and average daily gain (ADG) of calves fed the dietary treatments during suckling period are presented in Table (6). The initial weight (birth weight) was nearly similar for the different groups. Chlorella supplementation (all tested rations) revealed significant ( $P<0.05$ ) increase in live body weight at 5, 10 and 15 weeks of age in comparison with the unsupplemented one (control). Calves in G3, G4 and G5 showed significantly ( $P<0.05$ ) higher LBW at 5 weeks of age compared to that of control one, whereas the LBW of G2 was insignificant higher than that of control (G1). In the same time, LBW of calves in groups received chlorella algae at 10 and 15 weeks of age was significantly ( $P<0.05$ ) higher than that of control one. Moreover, calves in G3 and G5 (high chlorella level) recorded significantly ( $P<0.05$ ) the highest LBW at weaning (15 week of age) followed by G2 and G4 (low chlorella level), while G1 had the lowest weight.

Concerning average daily gain (Table 6), results showed that ADG during the first 5 weeks of age (1-5) was higher significantly ( $P<0.05$ ) in G3, G4 and G5 compared with G1, while G2 was insignificant higher than that of control (G1). Otherwise, ADG of calves at 10 weeks of age was higher significantly ( $P<0.05$ ) in G2, G3 and G5 compared with G1, while G4 was placed at intermediate with insignificant difference with control or the other tested rations. Moreover, ADG during the third 5 weeks of age (11-15) was significantly ( $P<0.05$ ) higher in G3 compared with G1, G2 and G4, but G5 was insignificant higher than control. Totally, high chlorella level (G3 and G5) revealed significantly ( $P<0.05$ ) the highest ADG over the whole suckling period followed by low chlorella level (G2 and G4), whereas G1 had the lowest ADG. These results are in agreement with those obtained by Guzmán *et al.* (2001) who found that chlorella as a feed supplement has been known to have beneficial effects on growth performance. Additionally, Yan *et al.* (2012) indicated that fermented chlorella supplementation could improve the growth performance of pigs. Recent studies have investigated the potential health benefits of Chlorella to improve the growth and productivity of birds by increasing their intestinal microbial population (Janczyk *et al.*, 2009; Zheng *et al.*, 2012 and Kang *et al.*, 2013).

**Table (6): Live body weight of suckling calves fed the different experimental rations.**

Item	Experimental group					SEM
	G1	G2	G3	G4	G5	
Live body weight (kg)						
Birth	30.30	30.32	30.68	30.43	30.28	0.26
5 week	42.00 <sup>b</sup>	43.53 <sup>ab</sup>	44.78 <sup>a</sup>	45.20 <sup>a</sup>	44.63 <sup>a</sup>	0.39
10 week	60.04 <sup>b</sup>	64.13 <sup>a</sup>	66.14 <sup>a</sup>	64.85 <sup>a</sup>	66.34 <sup>a</sup>	0.64
15 week (weaning)	80.20 <sup>c</sup>	85.00 <sup>b</sup>	89.50 <sup>a</sup>	85.90 <sup>b</sup>	88.65 <sup>a</sup>	0.80
Average daily gain (kg)						
1-5 week	0.33 <sup>b</sup>	0.38 <sup>ab</sup>	0.40 <sup>a</sup>	0.42 <sup>a</sup>	0.41 <sup>a</sup>	0.01
6-10 week	0.52 <sup>b</sup>	0.59 <sup>a</sup>	0.61 <sup>a</sup>	0.56 <sup>ab</sup>	0.62 <sup>a</sup>	0.01
11-15 week	0.58 <sup>b</sup>	0.60 <sup>b</sup>	0.67 <sup>a</sup>	0.60 <sup>b</sup>	0.64 <sup>ab</sup>	0.01
1-15 week	0.48 <sup>c</sup>	0.52 <sup>b</sup>	0.56 <sup>a</sup>	0.53 <sup>b</sup>	0.57 <sup>a</sup>	0.01

*a, b and c: Values in the same row with different superscripts differ significantly ( $P<0.05$ ).*

**Feed conversion:**

Data of feed conversion expressed as the amounts of DM, TDN and DCP required per kg live weight gain are presented in Table (7). Chlorella algae supplementation improved significantly ( $P<0.05$ ) the feed conversion efficiency. All chlorella-algae-rations were recorded significant higher feed conversion efficiency in comparison with control ration, being the lowest amounts of DM, TDN and DCP per one kg live weight gain were recorded with G3 and G5 rations, followed by G2 and G4 rations, while the control ration had the highest amounts of feed/1 kg of gain. These results are in accordance with those obtained by Mustafa and Nakagawa (1995) who reported that algae potentially contributed to an increased in feed utilization by fish. Duck feed supplemented with 0.2 and 0.3 % Chlorella resulted in better nutrient utilization and reduced mortality rates (Redel and Buchta, 2001a,b). Also, Storandt *et al.* (2000) reported a positive effect of Chlorella on feed utilization by piglets, with 1% supplementation being the most effective. Furthermore, Jahn *et al.* (1995) found that feed supplementation of 1% chlorella for 4- to 6-week-old piglets improved feed intake by 11.8% and feed conversion by 13.6%.

**Economic efficiency:**

Results of economic efficiency are shown in Table (7). Average daily feed cost tended to increase with chlorella supplementation. Feed cost per one kg live weight gain decreased significantly ( $P<0.05$ ) with chlorella supplemented rations. Group 1 recorded significantly ( $P<0.05$ ) the highest feed cost/kg gain followed by G2, but G3 had the lowest value. In the other side, total and net revenue and economic efficiency improved significantly ( $P<0.05$ ) with all chlorella supplemented rations. High chlorella level (G3 and G5) recorded significantly ( $P<0.05$ ) the highest total and net revenue and economic efficiency followed by low chlorella level (G2 and G4), while, the control group (G1) had the lowest values. These results are in reflection of the potential improvements in ADG with chlorella supplementation (Table 6). These results in agreement with those obtained by El-Abd and Hamouda (2017) who found that economic efficiency of birds treated with chlorella algae was superior to that of the control group.

**Table (7): Feed conversion and economic efficiency of calves fed the different experimental rations.**

Item	Experimental group					SEM
	G1	G2	G3	G4	G5	
Feed conversion (kg/kg gain)						
DM	2.55 <sup>a</sup>	2.32 <sup>b</sup>	2.16 <sup>c</sup>	2.29 <sup>b</sup>	2.18 <sup>c</sup>	0.03
TDN	1.93 <sup>a</sup>	1.81 <sup>b</sup>	1.74 <sup>c</sup>	1.78 <sup>b</sup>	1.74 <sup>c</sup>	0.02
DCP	0.337 <sup>a</sup>	0.316 <sup>b</sup>	0.303 <sup>c</sup>	0.310 <sup>b</sup>	0.304 <sup>c</sup>	0.00
						3
Economic efficiency						
Feed cost (LE/day)	22.16	22.62	23.12	22.62	23.12	0.08
Feed cost (LE/kg gain)	46.63 <sup>a</sup>	43.44 <sup>b</sup>	41.27 <sup>c</sup>	42.83 <sup>bc</sup>	41.58 <sup>bc</sup>	0.47
Total revenue (LE)	33.27 <sup>c</sup>	36.45 <sup>b</sup>	39.22 <sup>a</sup>	36.98 <sup>b</sup>	38.92 <sup>a</sup>	0.51
Net revenue (LE)	11.11 <sup>c</sup>	13.83 <sup>b</sup>	16.10 <sup>a</sup>	14.36 <sup>b</sup>	15.81 <sup>a</sup>	0.44
Economic efficiency	1.50 <sup>c</sup>	1.61 <sup>b</sup>	1.70 <sup>a</sup>	1.64 <sup>ab</sup>	1.68 <sup>a</sup>	0.02
Economic efficiency*, %	50.13 <sup>c</sup>	61.14 <sup>b</sup>	69.64 <sup>a</sup>	63.48 <sup>b</sup>	68.38 <sup>a</sup>	1.72

*a, b and c: Values in the same row with different superscripts differ significantly ( $P<0.05$ ).*

*\* Economic efficiency, % = net revenue x 100/ feed cost.*

## CONCLUSION

Based on these results it could be concluded that chlorella algae supplementation with their media for diet of suckling calves at the level of 4 ml/kg live body weight potentially improved digestibility, feed intake, blood serum biochemical, immunity, daily gain, feed conversion and economic efficiency. Using milk of cows supplemented with algae in suckling calves did not have any significant effects on their growth performance.

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## تأثير اضافة طحلب كلوريبلا فولجارييس على الأداء الانتاجي للعجول الفريزيان الرضيعة

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استخدم فى هذه الدراسة 20 عجل وعجلة فريزيان حديث الولادة متوسط وزنها  $0,36 \pm 30,40$  كجم قسمت الى 5 مجموعات متماثلة (2 ذكر + 2 أنثى بكل منها) واستمرت التجربة حتى الفطام لدراسة تأثير اضافة الطحالب على أداء النمو فى العجول الرضيعة. لم يضاف الطحلب الى عليقة الأبقار وعجولها الرضيعة فى ج 1 وكانت هى مجموعة المقارنة. بينما أضيف طحلب الكلوريبلا والبيبة النامى عليها للأبقار وعجولها الرضيعة بمعدلات 2 مل (ج2) و 4 مل (ج3) لكل كجم من الوزن الحى للمستويين المنخفض والمرتفع على التوالى. فى حين لم يضاف طحلب الكلوريبلا لأبقار ج4 و ج5، ولكن تم اضافته للعجول الرضيعة بنفس المعدلات السابقة.

توضح النتائج أن المستوى المرتفع من طحلب الكلوريبلا (4 مل/كجم وزن حى) فى ج3، ج5 أظهر معنويا أعلى القيم لمعاملات الهضم والقيم الغذائية تلاهما ج2، ج4 المضاف اليهما المستوى المنخفض من الكلوريبلا (2 مل/كجم وزن حى)، فى حين حققت مجموعة المقارنة (ج1) أقل القيم. أظهر المأكول من مواد العلف والمادة الجافة نفس القيم للمجموعات المختلفة، فى حين ارتفع المأكول من المركبات الكلية المهضومة والبروتين المهضوم معنويا فى ج3، ج5 مقارنة مع ج1، بينما حققت ج2، ج4 زيادة غير معنوية عن ج1.

أدى اضافة طحلب الكلوريبلا الى العلائق الى تحسن ملموس فى معظم قياسات سيرم الدم وأنزيمات الكبد، كما أدى الى حدوث زيادة معنوية تركيز مشتقات المناعة. كذلك اضافة طحلب الكلوريبلا الى علائق الأبقار وعجولها الرضيعة الى تحسن ملموس فى معظم قياسات سيرم الدم وأنزيمات الكبد ومشتقات المناعة.

أدت اضافة طحلب الكلوريبلا الى حدوث زيادة معنوية وزن الفطام ومعدل النمو اليومى فى العجول الرضيعة وكذلك مع زيادة مستوى الاضافة. أيضا أدت اضافة طحلب الكلوريبلا الى تحسن معنوى فى معدل التحويل الغذائى حيث أدت الى انخفاض كميات المادة الجافة، المركبات الكلية المهضومة، البروتين المهضوم اللازمة لانتاج واحد كجم نمو. كذلك أدت اضافة طحلب الكلوريبلا الى تحسن معنوى فى الكفاءة الاقتصادية حيث خفضت من تكلفة التغذية لكل واحد كجم نمو، زيادة العائد الكلى والعائد الصافى من زيادة الوزن.

نستخلص من هذه الدراسة أن اضافة طحلب الكلوريبلا والبيبة النامى عليها للعجول الفريزيان الرضيعة بمعدل 4 مل/كجم وزن حى أدت الى تحسن ملموس فى هضم العناصر، كمية الغذاء المأكول، بعض مقاييس الدم، المناعة، معدل النمو اليومى، معدل التحويل الغذائى والكفاءة الاقتصادية. وأيضا أكدت الدراسة أن اضافة طحلب الكلوريبلا الى علائق الأبقار ليس له تأثير على أداء النمو للعجول الرضيعة.