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EFFICACY OF USING PROBIOTICS CONTAINING *Bacillus subtilis* AND *Bacillus licheniformis* SPORES ON PERFORMANCE AND HEALTH OF HOLSTEIN SUCKLING CALVES

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ABSTRACT: The objective of this study was to evaluate the effect of *Bacillus subtillis* (*BS*) and *Bacillus licheniformis* (*BL*) spores supplementation on performance, general health and some serum constituents in Holstein suckling calves. A total number of 36 newborn pure-bred Holstein calves (5 days old, with an average body weight of 44.27±0.37 and 42.87±0.55 Kg for males and females, respectively) were randomly divided in to three homogeneous groups (12 calves per group, 6 of each sex). For 30 consecutive days, all groups were fed whole milk three times daily. Once a day, 0, 10 and 20 g of the tested probiotics powder dissolved in the whole milk to represent G1 (control), G2 and G3, respectively. Each gram of this powder contained 2×10^9 cfu of *BS* + 2×10^9 cfu of *BL*. Offering the starter mixture to calves began on the 8^{th} day of life. The same parameters were investigated for another 30 days after the end of the probiotics treatment. The obtained results revealed that the overall TDMI value did not affected the first 30 days, but it significantly (P<0.001) increased in post-treatment period in G2 than those in G1 and G3. During the two experiment phases, the ADG of G2 calves was better (P<0.001) than those in the other tested groups. At the end of the trial, the G2 calves were 8.68 Kg heavier than the control calves. Consequently, the weaning age was earlier in G2 than in other tested groups. The incidence of diarrhea and its duration declined in G2 calves compared with G1and G3 calves. The highest percentage of pneumonia incidence was observed with the calves in G1. The concentrations of serum glucose, total protein, creatinine and activities of ALT and AST did not vary (P≥0.05) among all tested groups. On the other hand, albumin level were decreased (P=0.02) in G2 and G3, while globulin level was elevated (P=0.02) in G2 compared with those in G1, but these levels stayed within the normal range. Additionally, the triglycerides and cholesterol mean values were significantly (P=0.001) lower in G2 and G3 than in G1. However, serum immunoglobulin G concentrations were significantly (P=0.001) increased in G2 and G3 after 15 days of the probiotics addition compared to that in G1. The economic evaluation showed better return with the low dose of probiotics (G1) than the other groups. Conclusively, supplementing whole milk with *BS* plus *BL* spores had beneficial effects on the performance and health of the suckling Holstein calves.

Key words: Probiotics, *Bacillus Subtillis, Bacillus licheniformis*, Holstein calves, performance, health.

INTRODUCTION

The world is becoming densely populated day by day. There is desperate need for some efficient yet solution to supply this population's increasing demand for animal protein source. Probiotics have a vital role in solving this food production problem and replacing the harmful antibiotic use in farm industries. **FAO/WHO (2002)** addressed probiotics as live active microbes that offer health values for the host animal when appropriately supplemented.

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Currently, farmers provide probiotic feed supplements to poultry, ruminants and fishes. Probiotics are mostly gram-positive bacteria but there also gram-negative bacteria, yeast and fungi (**Arora and Kaur, 2020; Park *et al.*, 2016; Afsharmanesh and Sadaghi, 2014; Zhang *et al.*, 2020; Zhang and Kim, 2014; Bai *et al.*, 2013**).

Probiotics exert their effectiveness through diverse mechanisms. Probiotics inhibit and control pathogens along with improving the functioning and production capacity of animals (**Maas *et al.*, 2021; Van Zyl *et al.*, 2020; Layus, 2020; Chen *et al.*, 2020; Sharma *et al.*, 2018; Mookiah *et al.*, 2014**).

Probiotics can enhance milk production (**Ma *et al.*, 2020**), digestibility (**Boyd *et al.*, 2011**), immune system (**Signorini *et al.*, 2012**), improve dietary intake in newborn calves (**Muya *et al.*, 2015**) and promote the viability and balance of rumen microorganisms (**Chen *et al.*, 2020**).

Considering the limited and scarce research on using probiotics in newborn animals, more insight into their efficacy and mode of action is needed. The objective of this study was to evaluate the effect of probiotics containing 2×10^9 cfu of *Bacillus subtillis* (*BS*) and 2×10^9 cfu of *Bacillus Licheniformis* (*BL*) on growth performance and health status of Holstein suckling calves.

MATERIAL AND METHODS

The trial was conducted at the Global dairy farm (29°24'26"N30°52'00"E), Sinnuris, Al-Fayoum, Egypt, during the year 2020 (March to May).

Animals and Management

A growth experiment was performed on thirty-six neonatal pure-bred Holstein calves weighing 44.27±0.37 and 42.87±0.55 Kg for males and females, respectively. The trial started from the 5th day of age and continued till reaching the weaning weight (115 Kg). The animals were randomly divided into three similar groups (12 calves per group, 6 of each sex). Each calf was individually placed in a suckling box (2×1×1 meters) on a sandy bed under shading. All calves were healthy and kept under the same managerial procedures.

Experimental Design and Treatments

The calves received the whole milk supplemented with 0, 10 and 20 g of the probiotics as a single daily dose to represent G1 (control), G2 and G3, respectively. These probiotics are a formula in a powder form prepared especially for this research in Agrivit Company for manufacturing feed additives, Cairo, Egypt. Each gram of this powder contained $2\times10^{\mathbf{9}}$ cfu of *BS* and $2\times10^{\mathbf{9}}$ cfu of *BL*.

Before the evening meal (from 1 to 3 p.m.), the probiotics powder was dissolved well in the suckling bucket and offered. The treatment lasted for 30 days after the colostrum period. Animals always have free access to clean water.

Feeding Regime

Within an hour of birth, all calves received the first meal (4 liters of colostrum, from their dams) by a stomach tube. Thereafter, calves were fed the transition milk till the end of the 4th day by using suckling buckets provided with nipples. Then, the treatment started and all groups were fed the whole milk three times daily in the same technique. Calves obtained standard quantities milk of according to the suckling routine followed on the farm. The offered quantities of whole milk from 1st week of life up to 12 weeks increased gradually with the age (5 – 12 Kg / day). This routine is named the accelerated growth program for milk-fed calves and uses the modifications of **NRC (2001)** equations illustrated by Cornell-Illinois (**Van Amburgh and Drackley, 2005**).

Calves were fed starter mixture (SM) *ad libitum* in buckets from the beginning of the 2nd week of life up to the end of the experimental period. The SM consisted of 90% a mash concentrate feed mixture plus10% wheat straw. The formulation and proximate chemical analysis of the SM are displayed in Table 1.

Performance Parameters

Live body weight of calves was estimated by measuring the chest circumference using the standardized weight tape at birth, 15, 30, 45, 60 and the weaning day. The average daily gain (ADG) was calculated by dividing the live weight gain (g) by the growth period length (day).

Table 1. Formulation and proximate chemical analysis of the starter mixture and milk (on DM basis)

Items	
Ingredients of the starter:	Kg / Ton
Yellow corn	430
Soya bean meal (46%)	350
Wheat bran	100
Salt	10
Limestone	5
Minerals mixture	3
Vitamins mixture	1.5
Anti-toxin	0.5
Wheat straw	100
Nutrients content:	(%)
Starter mixture:	
Dry matter	90.20
Organic matter	92.37
Crude protein	21.1
Ether extract	4.35
Crude fiber	16.17
Nitrogen free extract	50.75
Ash	7.63
Acid insoluble ash	2.44
GE Mcal/Kg DM	4.87
DE Mcal/Kg DM	3.59
ME Mcal/Kg DM	3.19
Whole Milk:	
Dry matter	12.80
Crude protein	3.35
Fat	3.89
Lactose	4.81
Ash	0.75
GE Mcal/Kg	0.739
DE Mcal/Kg	0.584
ME Mcal/Kg	0.560

Gross energy (GE), digestible energy (DE), and metabolizable energy (ME) of the starter mixture and the whole milk (Mcal/Kg) were calculated from as described below:
GE of the starter mixture = (CP %× 0.057) + (EE% × 0.094) + (Total carbohydrate% × 0.0415)
DE of the starter mixture = GE × 0.82
ME of the starter mixture = (DE × 1.01) - 0.45 + (0.0046 × Fat % - 3)
GE of Milk = (CP% × 0.057) + (Fat% × 0.092) + (Lactose% × 0.0395)
DE of Milk = GE × 0.97
ME of Milk = DE × 0.96.

The dry feed intake was recorded daily from the 8th day of life till the weaning by subtracting amounts of the residual from the offered. Feed conversion ratio (FCR) was estimated by dividing the total dry matter intake of both milk and the SM (Kg) by the live weight gain (Kg).

Protein and energy utilization parameters were estimated as follows:

Protein content of gain (PCG) = 30 × 6.25 × ADG (Kg), according to **NRC (2001)**.

Efficiency of crude protein utilization (ECPU %) = (PCG/CPI) ×100, where, CPI is the crude protein intake

Energy conversion ratio (ECR) = ADG gain/ MEI, where MEI is the metabolizable energy intake.

Disease Incidence

Observations of diarrhea incidence (DI), diarrhea duration (DD) and pneumonia incidence (PI) were detailed as daily notes. Feces were scored according to **Renaud *et al.* (2020)**. Where: 0, 1, 2, and 3defined as normal, soft, fluid, and aqueous types of feces, respectively, and the score of ≥2 indicated the presence of diarrhea. The diarrhea duration (scores 2 or 3) is the total number of disease days. However, the percentage of diarrhea incidence is the number of calves in diarrhea divided by the total number of calves in each group.

Blood Sampling

The first blood sample were collected before first meal of colostrum and the other blood sample were collected after the meal of the morning by 3 hours on day 30th and 45th from the treatment start. Samples were collected via the jugular vein by using a sterile syringe and then transferred to sterile tubes. The collected samples were centrifuged at 1006 g for 10 minutes. Sera were preserved at -18°C until tested. Commercial kits (DiaSys, Diagnostic Systems GmbH, Germany) were used to assess liver functions, kidney functions, metabolites and immune response parameters.

Analytical Procedures

Proximate chemical analysis

Nutrient contents of the milk and SM samples were determined according to **AOAC (1990)** while, Nitrogen free extract in the SM was obtained by the difference.

Blood biochemical and immunological parameters

Levels of serum total protein and albumin were determined by the methods of **Bakker and Mücke (2007)**. While, serum globulin concentration was calculated as follow Globulin (g/dL) =Total protein (g/dL) **-** Albumin (g/dL). Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the methods of **Thomas (1998)**. Levels of serum glucose and creatinine were estimated by methods described by **Young** *et al.*, (2000).Serum triglycerides and cholesterol levels were measured according to **Rifai *et al.* (1999).** Immunoglobulins IgG was quantitatively determined by Sandwich Enzyme Linked Immunosorbent Assay (ELISA) according to **Engvall and Perlmanm (1971**).

Economic Evaluation

The economic evaluation was gauged in line with the prevailing market prices. The Relative profit (RP) was computed as follows:

RP = (profit of each tested diet/profit of the control diet) × 100

Statistical Analysis

Data handling and statistical analysis was carried out at the Dept. of animal production, Faculty of Agriculture, Zagazig University. Analysis was done using SPSS/PCT, (Statistical Package for Social Sciences version 22.0) (IBM Corp., Armonk, NY, USA) software. Results were reported in means ± SEM (Standard Error of Mean). The value of $P<0.05$ was used to indicate statistical significance. The statistical method was ANOVA test (one way analysis of variance) to test the differences in control and probiotics groups. The Duncan multiple range tests are also used (**Duncan, 1955**).

RESULTS AND DISCUSSION

The current work was achieved to investigate the impacts of two doses of the probiotics (*BS* and *BL*) on the growth performance, health and economical feed efficiency of Holstein suckling Holstein calves.

Effect of Probiotics Supplements on Growth Performance

The effect of probiotics supplementation on averages of total dry matter intake (TDMI), live body weight (LBW), daily gain (ADG) and feed conversion ratio (FCR) all over the environmental period are introduced in Table 2.

The probiotics addition had no significant impact on the TDMI values except G2 which significantly ($P<0.05$) improved in the post treatment period compared with the other groups (G1 and G3). However the averages of LBW, ADG and FCR were positively ($P<0.05$) influenced by the lower dose (10 g/day) of probiotics (Table 2). In contrast the higher dose (20 g/day) of probiotics addition (G3) negatively affected the average of LBW, ADG and FCR during the treatment period and significantly ($P<0.05$) improved in the post treatment period, and showed no significant differences between G3 and G1 (83.16 vs. 82.24, respectively) at the end of the trial.

The probiotics as additive in G2 enhanced the overall means of LBW, ADG and FCR by 5.16, 18.14 and 15.05%, respectively, in the treatment period and 10.55, 13.27 and 9.45% in the post treatment period, respectively, compared with the control group (Table 2). At the end of the trial the supplemented calves with lower dose were 8.68Kg heavier than the control and 7.76 Kg than the calves received the higher dose.

The performance improvement of calves fed diet supplemented with 10 g probiotics (G2) positively reflected on the weaning age (Fig. 1) than the control and G3 (78.33 vs. 84.92 and 86.08 days, respectively).

The calf sex did not significantly affect the averages of TDMI and LBW. Conversely, males had superior ($P<0.05$) values of ADG and FCR compared with females (Table 2). The interaction effect between the probiotics and sex did not significantly affect the tested growth parameters through the experimental period.

The results are in agreement with **Smock *et al.* (2020)** who noted an improvement in ABG and LBW during the initial 56-day feed-lot receiving phase when *B. subtillis* PB6 was supplemented to high stressed feed steers. Also, **Mousa and Marwan (2019)** found that buffalo calves at 15th day old, showed insignificantly greater body weight when supplemented group fed *Bacillus spp*. than the unsupplemented group. However the supplemented group showed significantly increase in body weight at 30th day than the control one. Similar results were recorded by **Khalifa *et al.* (2016)** in lambs and **Kochewad *et al.* (2009)** in growing kids. The researchers discussed the improving DBG of animals fed probiotics may be a result to favorable growth of useful bacteria which colonized in intestine more quickly the pathogenic bacteria.

Also, **Liao *et al.* (2010) and Kowalski *et al.* (2009)** showed that bacterial probiotics supplementation numerically improved DM intake and feed conversion ratio in suckling and post-partum period compared with the control. The significant increase may reflect higher ruminal fermentation in the treated groups than in control. However, **Aikman *et al.* (2011)** observed no difference in DM intake between the control and treated cows fed two TMR’s differing in level of concentrate and supplemented with direct-fed-microbial (DFM).

On the other hand, **Mostafa *et al.* (2014)** found insignificant effect of dietary supplementation of two probiotics on live body weight of cow during pre-partum, calving and post-partum.

The result of feed efficiency, calculated as consumption of metabolizable energy intake (MEI) and crude protein intake (CPI) (Table 3) showed significant ($P<0.05$) improved the efficiency of crude protein utilization (ECPU) and energy conversion ratio (ECR) with the lower dose(G2) compared with control (G1) and the higher dose (G3) which reflected on the better final live weight of calves. These results are in agreement with **Kowalski *et al.* (2009)** who found that the supplement calves with probiotics were significantly heavier as a result of improving the feed efficiency of consumed ME and CP.

Effect of Probiotics Supplements on Blood Constituents

Table 4 presents the results of serum biochemical parameters in Holstein calves that suckled milk supplemented with spores of *Bs* and *Bl*.

Table 2. Effects of probiotics, calves sex and their interaction on growth performance (Mean ± SE) during the treatment period and after treatment period

Items	Initial weight Kg	During treatment period				After treatment period			
		DMI Kg/day	30^{th} day weight Kg	ADG g/day	FCR	DMI Kg/day	60^{th} day weight Kg	ADG g/day	FCR
Groups									
G_1	43.08±0.66	1.03±0.01	58.24^{b} ±0.64	498.33^{b}±12.66	2.06^{b}±0.01	1.72^{b}±0.01	82.24^{b} ±0.60	866.67^{c}±16.92	2.01^{a} ±0.04
G_2	43.67±0.31	1.04±0.01	61.25^{a} ±0.30	588.75^{a}±11.80	1.75^{c}±0.04	1.78^{a}±0.01	90.92^{a} ±0.36	981.67^{a}±11.13	1.82^{b} ±0.02
G_3	43.54±0.87	1.03±0.01	55.39^{c} ±0.70	396.67^{c}±18.01	2.68^{a}±0.13	1.72^{b}±0.01	83.16^{b}±0.22	930.56^{b}±21.89	1.89^{b}±0.05
***P* value**	1.00	0.43	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Sex									
Males	44.27±0.37	1.03±0.01	58.60±0.79	474.91^{b}±22.64	2.27^{a}±0.12	1.74±0.01	86.60±0.94	918.52±18.52	1.93±0.04
Females	42.87±0.55	1.03±0.01	57.96±0.68	514.26^{a}±20.87	2.06^{b}±0.10	1.74±0.01	85.57±0.80	934.07±17.14	1.89±0.04
***P* value**	0.07	0.53	0.73	0.02	0.02	0.94	0.82	0.44	0.40
Groups × Sex									
G_1× Males	45.25±0.85	1.04 ±0.01	59.75±1.11	485.83±20.38	2.15±0.09	1.73±0.01	85.00±1.08	844.44±28.11	2.07±0.06
G_2× Males	43.67±0.56	1.03±0.01	60.84±0.40	572.22±18.09	1.79±0.06	1.78±0.01	90.50±0.56	988.89±7.03	1.81±0.02
G_3× Males	44.20±0.49	1.03±0.01	55.00±0.71	366.67±14.91	2.88±0.16	1.72±0.02	83.20±0.37	922.22±23.83	1.90±0.06
G_1× Females	42.11±0.68	1.01±0.01	57.56±0.71	510.83±15.06	1.98±0.06	1.72±0.01	83.89±0.73	888.89±16.48	1.94±0.04
G_2× Females	43.67±0.77	1.04±0.01	61.67±0.42	605.28±13.27	1.72±0.04	1.78±0.01	91.34±0.42	974.44±21.79	1.83±0.04
G_3× Females	43.13±1.39	1.03±0.01	55.63±1.08	426.67±29.08	2.48±0.18	1.72±0.02	83.13±0.30	938.89±38.88	1.89±0.09
***P* value**	0.26	0.05	0.16	0.64	0.32	0.78	0.32	0.50	0.36

G1(control): calves suckled whole milk without additives, G2: calves suckled whole milk supplemented with 10 g of probiotics ($2×10^9$ CFU of *Bs* + $2×10^9$ CFU of *Bl*), G3: calves suckled whole milk supplemented with 20 g of the same probiotics. a, b and c: Means in the same column with different letters are significantly (P<0.05) differ.

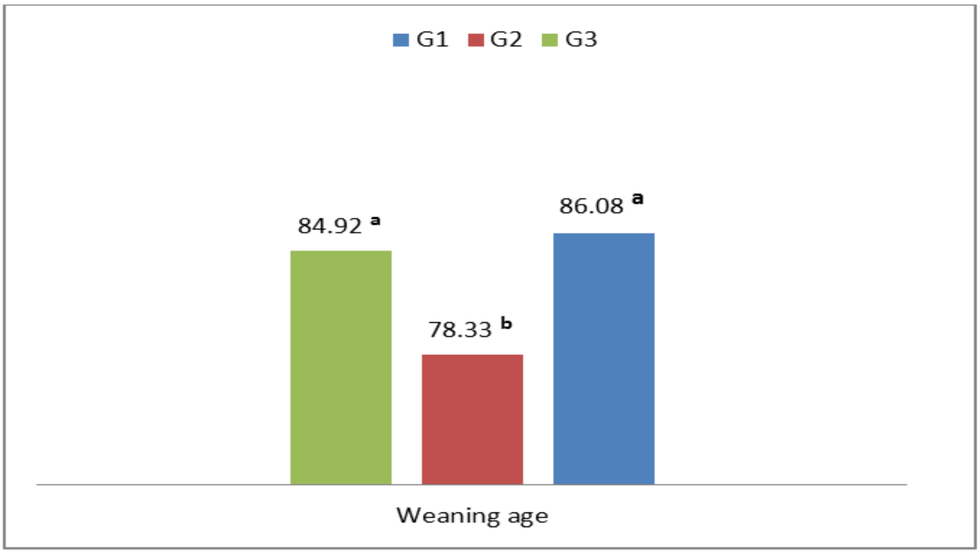


Fig. 1. Effects of probiotics supplementation on the weaning age in the suckling Holstein calves. a and b: Means with different letters are significantly (P<0.05) differ

Table 3. Effects of probiotics, calves sex and their interaction on protein and energy utilization (Mean ± SE) during treatment and a month post-treatment

Items	During of treatment					
	CPI g/dad	**MEI Mcal/day**	**ADG g/day**	**PCG g**	**ECPU %**	**ECR g/Mcal**
Groups						
G_1	262.10 ±1.12	4.78±0.02	498.33 ^{b}±12.66	93.44^{b}±2.37	35.87^{b}±0.86	105.15^{b}±2.50
G_2	264.79±0.94	4.83±0.02	588.75 ^{a}±11.80	110.39^{a}±2.21	41.72^{a}±0.76	122.34^{a}±2.24
G_3	263.73±0.75	4.81±0.01	396.67 ^{c}±18.01	74.38^{c}±3.38	28.33^{c}±1.37	83.08^{c}±4.04
***P* value**	0.11	0.11	<0.001	<0.001	<0.001	<0.001
Sex						
Males	263.70±0.71	4.81±0.01	474.91^{b}±22.64	89.05 ^{b}±4.24	33.87^{b}± 1.62	99.31^{b}± 4.77
Females	263.38±0.89	4.80±0.02	514.26^{a}±20.87	96.42^{a}± 3.91	36.75 ^{a}±1.43	107.74^{a}± 4.18
***P* value**	0.75	0.75	0.02	0.02	0.02	0.02
Groups × Sex						
G_1× Males	264.35±1.38	4.82±0.02	485.83±20.38	91.09±3.82	34.74±1.27	101.92±3.72
G_2× Males	263.47±1.57	4.81±0.03	572.22±18.09	107.29±3.39	40.89±1.19	119.90±3.48
G_3× Males	263.29±0.79	4.80±0.01	366.67±14.91	68.75±2.79	25.98±1.18	76.11±3.49
G_1× Females	259.86±1.27	4.74±0.02	510.83±15.06	95.78±2.82	37.01±1.07	108.39±3.07
G_2× Females	266.10±0.84	4.85±0.01	605.28±13.27	113.49±2.49	42.55±0.92	124.78±2.76
G_3× Females	264.17±1.33	4.82±0.02	426.67±29.08	80.00±3.38	30.68±2.16	90.05±6.35
***P* value**	0.05	0.05	0.64	0.64	0.50	0.50
	Post-treatment					
	CPI g/d	**MEI Mcal/day**	**ADG g/day**	**PCG g**	**ECPU %**	**ECR g/Mcal**
Groups						
G_1	425.34^{b}±0.89	8.03^{b}±0.01	866.67^{c}±16.92	162.50^{c}±3.17	38.26^{b}±0.74	108.10^{b}±2.10
G_2	437.47^{a}±0.92	8.24^{a}±0.02	981.67^{a}±11.13	184.06^{a}±2.09	42.07^{a}±0.47	119.19^{a}±1.35
G_3	425.38^{b}±2.25	8.03^{b}±0.04	930.56^{b}±21.89	174.48^{b}±4.10	40.78^{a}±1.03	115.18^{a}±2.89
***P* value**	<0.001	<0.001	<0.001	<0.001	0.01	0.01
Sex						
Males	429.47±1.77	8.10±0.03	918.52±18.52	172.22±3.47	39.97±0.74	113.04±2.11
Females	429.32±1.89	8.10±0.03	934.07±17.14	175.14±3.21	40.76±0.72	115.28±2.05
***P* value**	0.94	0.94	0.44	0.45	0.39	0.39
Groups × Sex						
G_1× Males	426.30±1.51	8.05±0.03	844.44±28.11	158.33±5.27	37.11±1.08	104.88±3.07
G_2× Males	436.85±1.60	8.23±0.03	988.89±7.03	185.42±1.32	42.34±0.43	119.93±1.20
G_3× Males	425.25±3.27	8.03±0.06	922.22±23.83	172.92±4.46	40.47±1.20	114.30±3.36
G_1× Females	424.37±0.92	8.02±0.02	888.89±16.48	166.67±3.09	39.41±0.86	111.32±2.43
G_2× Females	438.08±0.99	8.25±0.02	974.44±21.79	182.71±4.09	41.80±0.88	118.45±2.52
G_3× Females	425.51±3.39	8.04±0.06	938.89±38.88	176.04±7.29	41.09±1.78	116.06±5.02
***P* value**	0.76	0.76	0.50	0.50	0.46	0.46

G1(control): calves suckled whole milk without additives, G2: calves suckled whole milk supplemented with 10 g of probiotics (2×10^9 CFU/g of *Bs* + 2×10^9 CFU/g of *Bl*), G3: calves suckled whole milk supplemented with 20 g of the same probiotics. CPI: crude protein content intake, MEI: metabolizable energy intake, ADG: average daily gain, PCG: protein content of gain = 30×6.25×ADG (Kg) according to **NRC (2001)**, ECPU %: efficiency of crude protein utilization= (PCG/ CPI) ×100, ECR: energy conversion ratio= ADG gain/MEI. a, b and c: Means in the same column with different letters are significantly (P<0.05) differ.

Table 4. Effects of probiotics supplementation on the serum biochemical parameters (Mean ± SE) in the sulking Holstein calves

Items		Groups			P value
		G_1	G_2	G_3	
Glucose	**mg/dL**				
Day 1		117.32 ± 5.52	116.66 ± 6.20	119.32 ± 6.04	0.46
Day 30		94.00 ± 2.21	92.00 ± 4.57	93.50 ± 5.28	0.06
Total protein	**g/dL**				
Day 1		4.72 ± 0.05	4.62 ± 0.05	4.73 ± 0.08	0.45
Day 30		6.69 ± 0.21	6.89 ± 0.05	6.50 ± 0.09	0.21
Albumin	**g/dL**				
Day 1		2.67 ± 0.04	2.58 ± 0.05	2.68 ± 0.08	0.46
Day 30		3.39^{a} ± 0.17	2.92^{b} ± 0.04	3.11^{b} ± 0.02	0.02
Globulin	**g/dL**				
Day 1		2.05 ± 0.01	2.04 ± 0.01	2.05 ± 0.01	0.72
Day 30		3.30^{b} ± 0.15	3.97^{a} ± 0.01	3.39^{b} ± 0.08	0.01
ALT	**IU/L**				
Day 1		7.93 ± 0.35	8.00 ± 0.57	8.13 ± 0.29	0.95
Day 30		9.46 ± 0.29	7.94 ± 0.02	8.00 ± 0.57	0.05
AST	**IU/L**				
Day 1		53.33 ± 1.45	52.66 ± 1.45	53.00 ± 0.57	0.93
Day 30		48.86 ± 0.94	51.96 ± 2.26	49.00 ± 0.57	0.05
Triglycerides	**mg/dL**				
Day 1		39.00 ± 0.57	39.10 ± 0.37	38.90 ± 0.55	0.96
Day 30		59.66^{a} ± 0.88	37.00^{c} ± 0.57	48.00^{b} ± 0.57	0.001
Cholesterol	**mg/dL**				
Day 1		42.53 ± 1.44	43.03 ± 0.75	42.53 ± 0.29	0.91
Day 30		102.66^{a} ± 1.45	95.43^{b} ± 0.26	97.00^{b} ± 0.57	0.001
Creatinine	**mg/dL**				
Day 1		3.25 ± 0.02	3.25 ± 0.01	3.35 ± 0.12	0.58
Day 30		0.90 ± 0.08	0.96 ± 0.03	0.95 ± 0.05	0.11
IgG	**mg/dL**				
Day 45 *		1104.33^{c} ± 2.33	1169.33^{b} ± 2.60	1241.66^{a} ± 7.26	0.001

G1(control): calves suckled whole milk without additives, G2: calves suckled whole milk supplemented with 10 g of probiotics (2×10^9 CFU/g of *Bs* + 2×10^9 CFU/g of *Bl*), G3: calves suckled whole milk supplemented with 20 g of the same probiotics. *: 15 days post-treatment. a, b and c: Means in the same column with different letters are significantly (P<0.05) differ. ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, IgG: Immunoglobulin G.

There were no significant variations (P≥0.05) in all of serum biochemistry measurements among all treatments on the 1st day of the study.

On the 30th day of probiotics treatment, concentrations (conc.) of serum glucose and total protein, creatinine and activities of ALT and AST did not alter (P≥0.05). On the other hand, values of albumin were decreased (P= 0.02) by 13.86 and 8.26% in G2 and G3, respectively, while the conc. of globulin was elevated (P=0.02) by 20.30% in G2 compared with those in G1. Additionally, the triglycerides and cholesterol mean values were significantly (P=0.001) lower in G2 and G3 than those in G1 by 37.98 and 19.54% and 7.04 and 5.51%, respectively. However, 15 days after the end of probiotics treatment, serum immunoglobulin G (IgG) conc. were significantly (P=0.001) increased with calves that received the probiotics treatments (G2 and G3) earlier compared to that in G1.

Many authors agreed with our finding concerning the insignificant impact of probiotics treatment on glucose and/or total protein conc. in Holstein calves (**Riddell *et al.*, 2010; Noori**

***et al.*, 2016; Fouladgar *et al.*, 2016; Le *et al.*, 2016; Seifzadeh *et al.*, 2017).** The insignificant effect of the probiotics treatment on creatinine values is similar to those obtained by **Talha *et al.* (2009), Le *et al.* (2016) and Mousa *et al.* (2019).**

Despite the significant decrease in albumin conc. with the addition of probiotics to milk, its mean values remained within the normal range (from 2.0 to 2.7 g/dL) which was revealed by **Hussein *et al.* (2020)** for the suckling Holstein calves. The remarkable elevation of globulin conc. as a result of probiotics inclusion in G2 concurred with that observed by **Talha *et al*. (2009)** in buffalo calves fed milk supplemented with probiotics. Nevertheless, the globulin level in G2 is still within the normal range (from 3.0 to 4.6 g/dL) published by **Hussein *et al.* (2020)** on the suckling Holstein calves.

Results of triglycerides conc. in the present investigation are harmonized with that reported by **Le *et al.* (2016)** for the suckling Holstein calves. Likewise, **Talha *et al*. (2009) and Noori *et al.* (2016)** indicated that cholesterol values were significantly ($P<0.05$) reduced by the probiotics treatment, which agreed with our findings.

After 15 days of the probiotics treatment end, the significant rise of serum IgG conc. in G2 and G3 are similar to that noticed by **Chen *et al.* (2021)** who recorded that probiotics (*B. subtilis*, *B. licheniformis* and *Lactobacillus plantarum*) treatment increased the conc. of IgG in lambs. In the same trend, beef calves supplemented with *B. amyloliquefaciens/B. subtilis*, the serum IgG levels were increased ($P>0.05$). Serum IgG are produced by B-lymphocytes, which are the major impact factors of humoral immunity, to prevent and resist infection **(Du *et al*., 2018)**. Moreover, there was a tendency for increasing the IgG1 conc. with the probiotics (*B. subtilis* + *B. licheniformis*) addition in milk replacer on the 45^{th} day of life of suckling Holstein calves (**Riddell *et al.*, 2010)**. Supplementing *Bacillus* based probiotics to the diet would stimulate an increase in IgG1 levels as an anti-spore immune response **(Hong *et al*., 2005).**

Effect of Probiotics Supplements on Health Status

The lower dose of probiotics supplementation (G2) showed a lower incidence of diarrhea (ID) and duration of diarrhea (DD) and the incidence of pneumonia (IP) compared with the control (G1) and the higher dose of probiotics supplementation (Figs. 2 and 3). These results are consistent with no mortality problems; also no respiratory problems were detected all over the experimental period.

These results are similar to those obtained by **Mousa** and **Marwan** (2019) who found that pathogenic microbes number was decreased and the beneficial microbes number was increased which reflected in low diarrhea incidence. Also, **Agazzi *et al.* (2014) and Kowalski *et al.* (2009)** reported that using lactic acid bacteria in suckling animals lowered the incidence and frequency of diarrhea and showed slightly lower fecal score. In contrast, many authors noticed a significant reduction in health problem incidences when calves received the probiotics (**Abe *et al*., 1995; Abu-Tarboush *et al*., 1996; Timmerman *et al*., 2005**) which may be related to the reaction of calves on probiotics depends on the conditions in which the experiment is being conducted.

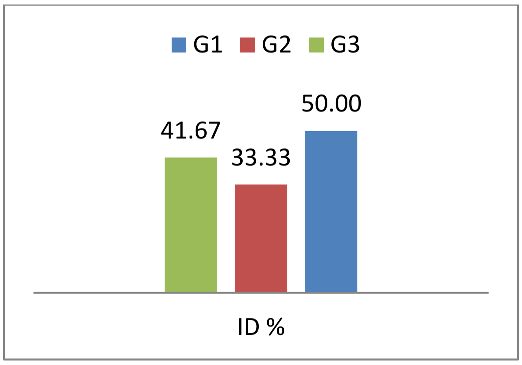
The differences between some previous studies and results in this study might be due to the feeding strategy, environmental conditions, diet composition, type and dose of the supplemented probiotics.

Economic Feed Efficiency

The economic evaluation was based on the current selling price of the tested diets (liquid and dry), the tested additives and the kilograms of live body weight had shown in (Table 5).

The price per each Kg of milk, starter mixture, probiotic and weight gain were 7.50, 4, 24, 240 and 127,5 E£, respectively. The relative profit = (average daily profit of G1,G2 and G3/ average daily profit of G1)×100.

The results showed that the adding the lower dose of probiotics (10g/head/day) increased the total return/head/day 120.70% compare with the total return of the control one (100%). However, adding the higher dose of probiotics (20 g/head/ day) increased the total return/head/day only by 101.22% compared with the control diet. There was no discernible difference between the higher supplemented dose and the control.



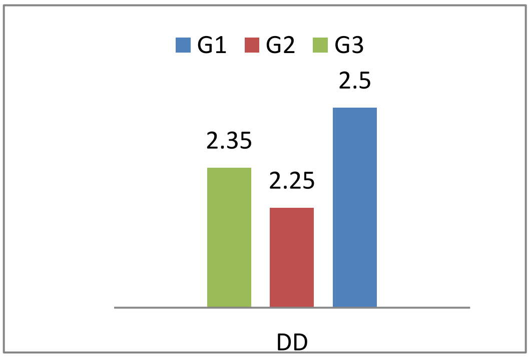


Fig. 2. Effects of probiotics supplementation on percentages of diarrhea incidence (ID %) and diarrhea duration (DD, in days)

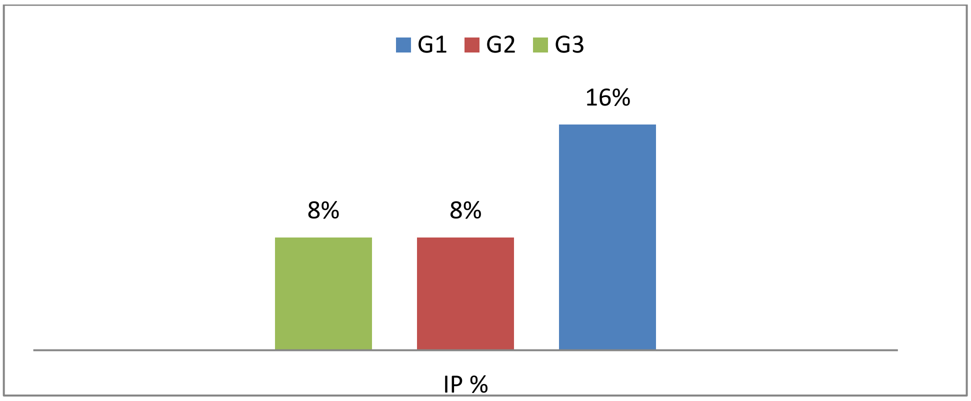


Fig. 3. Effects of probiotics supplementation on percentages of incidence of pneumonia (IP %) before weaning

Table 5. Effect of probiotics supplementation on the economic feed efficiency of the suckling Holstein calves.

Items		Groups		
		G_1	**G_2**	**G_3**
Total consumption:				
Milk	Kg/h	510	510	510
Starter mixture	Kg/h	18.60	21.58	18.93
Total cost:				
Milk	E£	3825	3825	3825
Starter mixture	E£	78.86	89.04	81.40
Probiotics	E£	0.00	72	144
Total d cost	E£	3903.86	3986.04	4050.40
Average daily cost	**E£**	65.06	66.43	67.50
Total gain:				
Total gain	Kg/ 60 day	39.16	47.25	39.62
Average daily gain	Kg/day	0.652	0.787	0.660
Average daily gain	**E£**	71.72	86.57	72.60
Daily gain %	**E£**	100	120.70	101.22
Average daily profit	**E£**	6.66	20.27	5.10
Relative profit	**%**	100	304.35	76.58

Conclusion

It can be concluded that the performance and health status of the suckling Holstein calves were improved by using 10 g/head/day of *Bacillus strains* (*subtillis* and *licheniformis*) spores as safe alternatives to antibiotics.

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أثر استخدام البروبايوتيك المتكون من (*Bacillus subtilis* و *Bacillus licheniformis*) على الأداء الحيوي وصحة العجول الهولشتين الرضيعة

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قسم الإنتاج الحيواني – كلية الزراعة – جامعة الزقازيق - مصر

كان الهدف من هذه الدراسة هو تقييم تأثير إضافة جراثيم *Bacillus subtillis* و*Bacillus licheniformis* على إجمالى المادة الجافة المأكولة ومعدل النمو اليومي والاستفادة من العلف وعمر الفطام وحالات حدوث الإسهال ومدته وحالات حدوث الالتهاب الرئوي وبعض مكونات مصل الدم في عجول الهولشتاين الرضيعة. تم تقسيم عدد 36 من عجول الهولشتاين النقية حديثة الولادة (عمر 5 أيام، ومتوسط وزن 44,27 ± 0,37 و 42,87 ± 0,55 كجم للذكور والإناث، على الترتيب) بشكل عشوائي إلى ثلاث مجموعات متجانسة (12 عجلاً/مجموعة ، 6 عجول من كل جنس). تم تغذية جميع المجموعات على اللبن الكامل ثلاث مرات يوميًا لمدة 30 يوماً متصلة بواسطة دلاء الرضاعة. وتمت إذابة صفر و10 و20 جرام من مسحوق البروبيوتيك في اللبن كامل الدسم لتمثل G1 (المجموعة الضابطة) وG2 و G3 على التوالي. ويحتـوي كـل جـرام مـن البروبيوتك على 2×10^{9} من جـراثيـم *Bacillus subtillis* وكذلك 2×10^{9} من جـراثيم *Bacillus licheniformis*. بدأ تقديم مخلوط العلف البادئ للعجول في اليوم الثامن من العمر. وتمت دراسة نفس المعالم المختبرة لجميع مجموعات العجول لمدة شهر آخر بعد انتهاء معاملة البروبيوتك. أظهرت النتائج المتحصل عليها أن قيمة إجمالى المادة الجافة المأكولة لم تتغير أثناء فترة المعاملة بالبروبيوتيك (30 يومًا) ، لكنها زادت بشكل ملحوظ ($P<0.001$) خلال الشهر التالى للمعاملة في G2 عن تلك الموجودة في G1 و G3. وخلال مرحلتي التجربة، كان معدل النمو اليومي للعجول فى G2 أفضل ($P<0.001$) من ذلك في المجموعات المختبرة الأخرى. وفي نهاية التجربة، كانت عجول G2 أثقل وزناً بمقدار 8,68 كجم من عجول المجموعة الضابطة. كانت جميع قياسات كفاءة الاستفادة من الغذاء أفضل ($P<0.001$) مع الجرعة الأقل من البروبيوتيك (G2). وقد ترتب على ذلك أن كان عمر الفطام في G2 أبكر منه فى بقية المجموعات المختبرة. وقد انخفض معدل حدوث الإسهال ومدته في عجول G2 مقارنة بالعجول فى G1 و G3، بينما لوحظت أعلى نسبة حدوث للالتهاب الرئوي مع عجول G1 وذلك خلال كامل فترة التجربة. ولم يكن هناك اختلاف معنوى ($P\geq0.05$) لتركيزات الجلوكوز والبروتين الكلي والكرياتينين ولأنشطة ALT و AST في سيرم الدم عجول المجموعات المختبرة عند نهاية المعاملة بالبروبيوتيك. ومن ناحية أخرى فقد انخفضت مستويات الألبومين ($P=0.02$) في G2 و G3 ، بينما ارتفع مستوى الجلوبيولين ($P=0.02$) في G2 مقارنة مع G1 ولكنهما ظلا فى معدلاتها الطبيعية. بالإضافة إلى ذلك ، كانت قيم الدهون الثلاثية والكوليسترول أقل بشكل معنوى($P=0.001$) في G2 وG3 من تلك الموجودة في G1. فى حين أنه قد ارتفعت معنوياً ($P=0.001$) تركيزات الجلوبولين المناعي IgG في مصل الدم في G2 و G3 بعد 15 يومًا من نهاية المعاملة بالبروبيوتيك مقارنةً بتلك الموجودة في G1. وقد أظهر التقييم الاقتصادي لتغذية عجول الهولشتاين الرضيعة عائداً أفضل مع المجموعة التى تلقت الجرعة الأقل من البروبيوتيك (G1) مقارنة بالمجموعات الأخرى. ويمكن أن نجمل بأن إضافة جراثيم *Bacillus subtillis + Bacillus licheniformis* إلى اللبن كامل الدسم كانت لها آثاراً مفيدةً على أداء وصحة عجول الهولشتاين الرضيعة.

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