

RESEARCH ARTICLE

Effectiveness of Dietary *Pediococcus* on Growth and Some Vital Biochemical Parameters in Calves

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Abstract

The oral use of *Pediococcus* (23×10^6 CFU/calf/day) in drinking milk once a day for one month from January till February 2020 in Holstein's calves, induced a highly significant effect on metabolic hormones, protein electrophoresis and bodyweight. Moreover, it improved the future reproductive performance of the female calves. Albumin and Gamma globulin percentages showed a significant increase in *Pediococcus* treated group compared to the control one. Also, there was a significant increment in insemination body weight and milk production in the treated female group compared to the control groups. On the other hand, there was a significant decrease in levels of triiodothyronine (T3) and thyroxin (T4), and alpha globulin % in groups treated by *Pediococcus*. Besides, there was a significant decrease in insemination age and service conception in the treated female group compared to the control female groups. Moreover, there were non-significant changes in beta globulin % in the same groups compared to the control groups. Our results concluded that the use of *Pediococcus* (23×10^6 CFU/calf/day) orally in drinking milk once daily for one month has highly significant effects on metabolic hormones, protein electrophoresis, body weight gain in calves; moreover, it improves the future productive and reproductive performance of the female calves.

Keywords: *Pediococcus*, Calves, Immunity, Bodyweight.

Introduction

In the light of population inflation in Egypt and increase the need for animal protein and milk and keep pace with global trends of the utilization of probiotics to improve the overall health of the animal, resulting in increased productivity and reproductive efficiency. Improving productivity of the animal depends mainly on the improvement of the health status of the rumen, which leads to raising feed digestibility, thus increasing milk production. Direct-fed microbial preparations (DFM) either specific or non-specific (yeast, fungi, bacteria, cell fragments, and filtrates)

enhance animal performance, the health status of the intestinal villi and decrease the use of antibiotics [1].

Probiotics are cultures of live and safe bacteria or yeast species that improve the functionality and health status of the intestinal microflora [2]. Probiotics maintain the intestinal ecosystem and enhance animal health. So, probiotics are the best alternative methods with their many benefits for dairy or fattening animals [3].

The healthy animal gut has abundant numbers of lactic acid bacteria, which are

found also in most probiotic preparations and have no harmful effect on the health status of the animals like Lactobacillus, Bifidobacteria, Streptococci, and Pediococcus [4].

Pediococcus acidilactici and *Pediococcus pentosaceus* are sorts of Gram-positive cocci, which inhibit the colonization of pathogens which have a great influence on the treatment of digestive disorders. *Pediococcus* secret medicines (bacteriocins) have an inhibitory effect on harmful microorganisms, including intestinal pathogen. *Pediococcus* stimulates a humoral immune response against infectious parasitic pathogens. It inhibits pathogenic bacteria and other Gram-positive spoilage by possible competitive inhibition and medicines production [5]. This work was planned to evaluate some pharmacological effects of *Pediococcus* on level of metabolic hormones [triiodothyronine (T3) and thyroxin (T4)] and protein electrophoresis. Also, reproductive performance of females (first cycle, number of insemination, number of repeat breeder (service/breeder), and pregnancy diagnosis and body weight gain in cow calves) was studied.

Material and Methods

Drugs

Sachet 100 g, powder prepared for oral administration, which contains:

-*Pediococcus acidilactici* 1×10^6 CFU (colony forming units)/g

-*Pediococcus pentosaceus* 1.3×10^6 CFU/g

-Dextrose Carrier

Manufacture: Biosen for chemical and feed additives, Alexandria

Factory : Elnubaria ^{3rd} industrial area, **Reg. No: 4686**

Dose : 10 g / head / daily for 30 days orally that give 23×10^6 CFU /10 g [6].

Experimental calves

Twenty Holstein calves at Damietta Governorate in the special dairy farm were

divided into four groups including control (five males and five females) and experimental (five males and five females) groups; all groups under the same hygiene management and the same nutrition (starter ration 21% + milk before weaning, while after weaning the ration was 18% until reach 200-220 kg BW then 16% till reach over 350 kg BW from the same company). A calf under our experiment (10 calves) fed 10 gm. *Pediococcus* was prepared after fermentation in 100 mL water for 2 -3 hours then was added to the milk one time per day for one month. After one month, the preparation was given for three successive days every month by a dose of 20gm/head/once daily in drinking water after fermentation for 2-3 hours for five months. The bodyweight was determined at the beginning of the experiment and every week for four weeks then every two months for 10 months to all groups, moreover the general health conditions were observed.

Sampling

Two blood samples were collected from each animal in the four groups at zero-days and after 15, 30, and 45 days post-*Pediococcus* preparation administration [7].

Serum biochemical analysis

Biochemical serum analysis of metabolic hormones (T3 and T4) using Mini VIDAS and protein electrophoresis (albumin, prealbumin, alpha globulin, beta globulin, and gamma globulin) using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS- PAGE).

Mini VIDAS®

It is a minimal form of the VIDAS® system with a built-in computer, console, and printer. Two autonomous areas each acknowledge six tests and can process up to 12 tests at the same time. We used for assay T3, T4, and growth hormone.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS- PAGE): (Bio-

Rab), it was used for protein fractionation into its types using electric charge as described previously [8].

Statistical analysis

Data were analyzed by one-way ANOVA (Tables 1, 2, 4) and independent T-test (Table 3), using SPSS computer program (version. 20) [9].

Results and Discussion

The effect of oral administration of *Pediococcus* (23×10^6 CFU/animal) in drinking milk once daily for one month on T3 and T4 in cow calves was investigated and the results are illustrated in Table (1). Our findings revealed non-significant changes in T3 in all groups at zero day, while demonstrated a significant decrease in treated groups compared to control groups at second, fourth, and 6th weeks. Also, T4 showed non-significant changes in all groups at zero weeks, while the second week showed a significant elevation in control groups in comparison to treated groups and a significant decline in the

treated female group compared to the other groups. Our data revealed a significant increase in control groups compared to the treated ones and a huge lessening in the treated male group compared to the other groups at the 4th week. Also it demonstrated a huge decrease in treated groups at the 6th week compared to control groups.

This is the first report to study the effect of probiotics on the rate of thyroid hormones in Holstein's calves. The same results have been recorded in the blood plasma in developing germ-free rats when compared to conventional rats [10]. Raise in the rate of hepatic conversion of thyroxin into Triiodothyronine in the liver of the conventional rats and decreased from the bile salts [11]. Probiotics were considered as an alternative to antibiotics growth promoters, their competitive exclusion of intestinal naturalistic microflora, a favorable microbial balance, to various metabolic activity, to modified breakdown the bile acids and their interaction with the mucosal immune system [12-16].

Table (1): The effect of oral administration of *Pediococcus* (23×10^6 CFU/animal) in drinking milk once daily for one month on Triiodothyronine (T3) and thyroxin (T4) in cow calves.

Items	Time (Week)	Control male	Treated male	Control female	Treated female
T3(ng/ml)	0	2.35±0.20 ^a	2.55±0.20 ^a	2.84±0.13 ^a	2.62±0.22 ^a
	2 nd w	2.62±0.15 ^a	1.95±0.05 ^b	2.58±0.06 ^a	1.76±0.26 ^b
	4 th w	2.38±0.06 ^a	1.29±0.14 ^b	2.20±0.08 ^a	1.26±0.06 ^b
	6 th w	2.66±0.21 ^a	0.69±0.11 ^b	2.64±0.09 ^a	0.78±0.18 ^b
T4(µg/dl)	0	10.69±0.89 ^a	9.81±0.21 ^a	10.70±0.81 ^a	11.14±0.81 ^a
	2 nd w	12.16±0.71 ^a	9.54±0.24 ^b	11.71±0.71 ^a	6.59±0.48 ^c
	4 th w	12.06±0.60 ^a	4.86±0.23 ^c	11.79±0.47 ^a	6.42±0.40 ^b
	6 th w	11.89±0.52 ^a	4.76±0.48 ^b	12.57±0.62 ^a	4.27±0.10 ^b

The different litters in the same raw means significance at (p< 0.05) (Mean ± S.E) n= 5

Herein, Table 2 showed the effect of oral administration of *Pediococcus* (23×10^6 CFU/animal) in drinking milk once daily for one month on albumin, alpha globulin,

beta globulin, and gamma globulin percentages in cow calves. Our results revealed a significant increase in albumin %in the treated male group compared to

the other groups at zero weeks, while a significant increment was recorded in the treated female group compared to the treated male group at the sixth week.

Work mirrored a non-significant change at zero weeks in alpha, beta, and gamma globulin % in all groups, but at the sixth week showed a significant diminishing in treated groups compared to control groups in alpha globulin and non-significant changes in beta globulin % in all groups. Results detected a significant increase in treated groups compared to control groups.

Our outcomes were agreed with Patterson and Burkholder study [17] who proved that probiotics and prebiotics improve the immune system response, vitamin B synthesis, regulate the inflammatory reactions, and antagonize pathogen colonization. However, more

researcher agreed with our data and reported the beneficial effects of probiotics on the immunity of the intestine by enrichment the intestinal mucosa with a huge amount of IgA and other immunoglobulin producing cells. Moreover, they elevate the production of bacteriocins, which decrease the colonization of harmful microorganisms on the intestinal villi thus lead to pathogen exclusion [18, 19].

More confirmation to our results by a previous work [20] indicated that rabbits received a mixture of pre and probiotics to improve cell-mediated immune response. Also in agreement with Bajaga and coauthors [21] who showed that probiotics containing *L. fermentum* and *S. cerevisiae* increase serum immunoglobulin levels increased serum levels of IgA and IgM in broiler compared with untreated chickens.

Table (2): The effect of oral administration of *Pediococcus* (23×10^6 CFU/animal) in drinking milk once daily for one month on albumin and globulin parameters globin cow calves.

Items	Time (Week)	Control male	Treated male	Control female	Treated female
Albumin (%)	0	62.60±0.90 ^b	65.46±0.54 ^a	61.70±0.50 ^b	61.56±1.24 ^b
	6w	65.91±1.18 ^{ab}	60.41±3.09 ^b	65.01±1.64 ^{ab}	66.83±1.16 ^a
Alfa Globulin (%)	0	11.67±0.25 ^a	10.77±0.12 ^a	10.83±0.34 ^a	11.34±0.66 ^a
	6w	11.01±0.14 ^a	9.33±0.66 ^b	10.58±0.33 ^a	9.31±0.60 ^b
Beta Globulin (%)	0	12.93±0.23 ^a	11.60±0.67 ^a	11.63±0.69 ^a	12.60±0.87 ^a
	6w	11.96±0.35 ^a	11.53±0.93 ^a	12.96±0.64 ^a	10.94±0.31 ^a
Gamma Globulin (%)	0	13.32±0.14 ^a	12.77±0.23 ^a	12.37±0.66 ^a	13.03±0.36 ^a
	6w	12.95±0.64 ^b	15.91±0.88 ^a	13.91±0.33 ^b	16.80±0.93 ^a

The different litters in the same raw means significance at ($p < 0.05$)

(Mean ± S.E)

n= 5

Table (3) presented the impact of oral administration of *Pediococcus* (23×10^6 CFU/animal) in drinking milk once daily for one month on reproductive performance (insemination age, insemination body weight, service conception, and milk production) in female cows. Our result demonstrated a significant decrease in insemination age in the treated female group compared to the control female

groups and a significant increase in insemination body weight in the treated female group contrasted to control ones. Whereas, it demonstrated a significant reduction in service conception in the treated female group contrasted to control female groups and a significant increase in milk production in the treated female group compared to control female one.

Probiotic bacteria (pediocin-producing) was introduced previously [22] into the vaginal tract of postpartum cows as novel prophylactic interventions against metritis. However, an earlier study [23] reported that the application of probiotic supplement (*Lactobacillus sakei* and two strains of *Pediococcus acidilactici*) intra-vaginal before delivery accelerate uterine involution and the treated cows had low open days. Moreover treated cows exhibited greater milk production. On the other hand, another study [24] proved that

probiotic supplementation in cow feed especially after delivery not only increase milk production but also increase milk fat concentration. An increase in milk production began after one week from probiotic supplementation but the maximum elevation in milk production was in the 4th week after probiotic supplementation [4]. All these data confirm our results that the probiotic is essential to improve the reproduction and production of dairy animals [25, 26].

Table (3): The effect of oral administration of *Pediococcus* (23×10^6 CFU/animal) in drinking milk once daily for one month on reproductive performance and milk production in female cows.

	Control animal	Treated animal
Insemination age/month	15.6±0.2 ^a	12.8±0.2 ^b
Insemination BW/kg	348±2.5 ^b	372±5.8 ^a
Service conception	2±0.3 ^a	1.4±0.2 ^b
Milk production/kg	20.8±0.3 ^b	26.4±0.6 ^a

The different litters in the same raw, refers to significance at ($p < 0.05$). (Mean ± S.E) n= 5

Table (4): The effect of oral administration of *Pediococcus* (23×10^6 CFU/animal) in drinking milk once daily for one month on body weight in cow calves.

Items	Time (Week)	Control male	Treated male	Control female	Treated female
Body weight(kg)	0	83.2±1.39 ^a	76.6±5.98 ^a	79.6±2.18 ^a	76.2±6.37 ^a
	2nd w	82.2±2.83 ^a	92.4±8.70 ^a	87±.836 ^a	92.6±4.61 ^a
	4th w	100.2±3.28 ^b	122±8.45 ^a	100±1.70 ^b	116±5.099 ^a
	6th w	105±1.61 ^b	134.2±9.91 ^a	103.8±2.00 ^b	133.6±6.70 ^a
	14th w	175±2.98 ^b	196±9.66 ^a	163.6±2.69 ^b	180±6.71 ^{ab}
	22th w	271±2.68 ^b	329±3.31 ^a	224.8±5.54 ^c	260.6±3.85 ^b
	37th w	359.6±1.75 ^c	419.6±9.17 ^a	333.8±6.64 ^d	387±2.53 ^b
47th w	402.4±4.58 ^b	477.6±8.35 ^a	359.4±2.54 ^c	399±9.00 ^b	

The different litters in the same raw means significance at ($p < 0.05$) (Mean ± S.E) n= 5

Table (5): Comparison the healthy status between groups of another reared *Pediococcus* treated male calves groups and another reared male calves group in the farm.

	Untreated								treated					
	1	2	3	4	5	6	7	Average	1	2	3	4	5	Average
Diseased animals	P*	P	P+E	P+E	P+E	P+E	E**	5	-	-	-	-	-	zero
Death	√	√	-	-	-	-	-	2	-	-	-	-	-	zero
B.wt. at zero day (kg)	-	-	110	113	89	87	92	98.2	85	87	87	61	63	76.6
B.wt. after one month(kg)	-	-	132	140	110	120	145	129.4	130	140	135	95	110	121.4
Growth rate (kg/d)	-	-	0.7	0.9	0.7	1.1	1.76	1.03	1.5	1.76	1.6	1.1	1.5	1.5
Treatment drugs	√	√	√	√	√	√	√	√	-	-	-	-	-	zero

*P=Pneumonia

**E=Enteritis

The effect of oral administration of *Pediococcus* (23×10^6 CFU/animal) in drinking milk once daily for one month on BW in cow calves was considered (Table 4). The outcomes of the current demonstrated non-extensive changes in BW in all groups at zero and second week, while demonstrated a huge increment in treated groups compared to control groups at the fourth and sixth weeks. But in the 14th week, there is a significant increase in the treated male group compared to control groups. Also, there is a significant increase in the treated male group compared to other groups and a significant decrease in the control female group compared to other groups at 22th and 47th week. Whereas the results showed a significant increase in the treated male group compared to other groups and a significant increment in the treated female group compared to other control groups at 37th week.

Therefore, supplementation of probiotics in the feed of animals increases forage intake and improves live weight gain moreover improves the quality of meat [25-27]. Abundant of researchers confirmed our results not only in ruminant but also in different species as a piglet, rabbits, and broiler chickens that the probiotics improve digestibility and utilization of nutrient, body weight gain and feed conversion ratio [28-32].

Conclusion

From this study it was concluded that the use of *Pediococcus* (23×10^6 CFU/calf/day) orally in drinking milk once daily for one month has highly significant effects good on metabolic hormones, protein electrophoresis, body weight gain in calves. Moreover, it improves the future productive and reproductive performance of the female calves.

Conflict of Interest

The authors declare no conflict.

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الملخص العربي

فاعلية بكتيريا البيديوكوكس الغذائية علي النمو وبعض العوامل البيوكيميائية الحيوية في العجول

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وجد ان استخدام البيديوكوكس بجرعة (23×10^6) وحدة تشكيل المستعمرة/عجل/يومية) عن طريق الفم في اللبن مرة واحدة يوميا لمدة شهر واحد من يناير إلي فبراير 2020 للعجول البقر من سلالة الهولشتين له كفاءة عالية علي هرمونات التمثيل الغذائي والمناعة ووزن الجسم علاوة علي ذلك فانه يحسن الأداء الإنتاجي و الإنجابي المستقبلي للعجول الانثوية.

وجد انخفاض معنوي في مستويات هرمون الثيروكسين T3 وهرمون تراي أيدوثيرونين T4 في المجموعتين المعاملة بالبيديوكوكس مقارنة بالمجموعتين الضابطين. اظهر نسبة الألبومين والجاما جلوبيولين زيادة معنوية في المجموعتين المعاملة بالبيديوكوكس مقارنة بالمجموعتين الضابطين ولكن نقص معنوي في الألفا جلوبيولين وعدم حدوث تغير معنوي في نسبة البيتا جلوبيولين في المجموعتين المعاملتين بالبيديوكوكس مقارنة بالمجموعات الضابطة. كانت هناك زيادة معنوية في الوزن الكلي للجسم وأيضا متوسط الزيادة الاسبوعية في الوزن في المجموعتين المعاملتين بالبيديوكوكس مقارنة بالمجموعتين الضابطين. لوحظ كفاءة عالية في الأداء التناسلي للعجلات والتي تمثلت في نقص معنوي في عمر التلقيح ومعدل الإخصاب وزيادة معنوية في الوزن عند التلقيح ومعدل إنتاج اللبن في المجموعة الانثوية المعاملة بالبيديوكوكس مقارنة بمجموعة الإناث الضابطة.