APPLICATION OF THE EFFECTIVE MICRO-ORGANISMS (EM) IN LARGE DAIRY CATTLE FOR IMPROVEMENTS OF THE ANIMAL HOUSING CONDITIONS AND THE IMMUNE STATUS

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ABSTRACT

The effective micro-organism (EM) technology was applied in a private cattle farm through the current study, where 240 dairy Holstein cows were divided into two groups (120 animal/per group/ per yard). The EM-solution was sprayed by dilution of 1: 200 water on the floor of one yard (including bedde, manure and animal feed), the other yard not sprayed by the EM and considered as non-treated control yard. The EM-spraying was conducted one time per week for three consecutive weeks. The fly population per each trap of yards was counted, the total bacterial count, the pH-values and the percentages of carbon, nitrogen, phosphorus, potassium and sodium elements of the beddes were measured, all the above parameters were carried out before treatments and after each of the three treatments. Also, the frequencies of diarrhaea and pneumonia in cattle of each yard were recorded at the end of the experiment. The immunoglobulins were determined through serum protein electrophoresis for determination of the different protein fractions of the serum of cattle of EM-treated and control yards. The results indicated that the EM is of beneficial values in reducing the fly populations, total bacterial count of bedde, malodours (volutile fatty acids and toxic gases as natural toxius) of the treated yard and the frequencies of occurrence of either diarrhoea or pneumonia in cattle of treated yard, and increasing the fertility of manure of bedde (through reducing its pH-value and elevating its nitrogen, phosphorus and potassium percentages). Also, the immune status of cattle in EM treated yard was stimulated because of the elevation of gammaglobulins (immunoglobulins) of the serum of cattle in treated yards, so that the EM technology should be recommended for improving the cattle production.

INTRODUCTION

Flies are living on the manure that accumulates with animals of confined production (cows, horses, pigs and chickens). The flies irritate animals and people on these premises, as well as in the surroundings. The stable flies suck blood of cows and horses resulting that animals not feeding properly and gradually losing its weight. Flies also are potential vectors of various diseases that transmitting bacteria, ascarids, viruses, rickettsias, protozoa, ...etc (**Thomas and Skada, 1993**). So that for many years, the farmers have relied solely on chemical treatments by organochlorines, organophosphates, carbamates or pyrethroids insecticides to control fly population, but flies have rapidly developed resistance mechanisms against most of these chemicals (**Skovgard and Jespersen, 1999**). So that the use of these chemicals can be used as a detrimental to human and animal health and pollute also the environment, therefore the biological control of pests is needed (**Rutz, 1993**).

The biological control by the effective micro-organisms (EM) is established to control and regulate putrefactive bacteria and consequently the resultant malodours which are the predisposing important factor for flies accumulation, such biological control was applied to keep fly population below the threshold injury level, so that the house flies parasitism level have been reduced by spraying EM on the chicken manure (**Kapango and Galiomee, 2000**).

The current study by using the EM was conducted as one of the biological control of flies, total bacterial counts, malodours, improvement of manure quality, aiming at the end to improve the environmental condition of the animal housing in order to reduce the frequencies of diseases and improvement the immune status of these animals.

MATERIAL AND METHODS

Materials and animals:

1- Effective micro organisms (EM): they were a mixed culture inoculant of the beneficial micro-organisms including the following micro-organisms.

A- Lactic acid bacteria: included 5 species:

1- Lactobacillus plantarum, 2- Lactobacillus casei, 3- Lactobacillus fermentum, 4- Lactobacillus salivarius, 5- Lactobacillus delbrueckii.

B- Phototrophic bacteria: included 3-species:

1- Rhodopseudomonas palustris,

2- Rhodobacter sphaeroides,

3- Rhodobacter capsulatus.

C- Yeast: included one species called Sacharomyces cerevisiae. The effective microorganisms were symboled as AEM or EM-1 or EM-Bokashi for animals, they certificated by the Organic Material Review Institute (OMRI) March (2003) and produced by Emrousa Comp., USA as (Emro-USA-Effective micro-organisms).

2- Animals: 240 Holstein cows divided into two groups (120/per group/ per yard).

Methods:

240 Holstein cows were divided into two groups 120 per group, the animals were reared on two yards. Effective microorganisms (EM) were sprayed by a dilution 1: 200 water on the floor, animal bedde, on the manure outside the sprayed yard and on the animal feed of one yard, and this group considered as treated group. The other one on sprayed yard (manure, floor bedde and feed) was considered as non-treated control group. The treatment by spraying the EM-solution was conducted weekly for 3 weeks according to (**Kapango and Giliomee, 2000**). the following experimental studies were carried out before treatment and after each of the three treatments as: the fly populations per each trap of yard were counted on the different four periods (before and after treatments), the total bacterial counts of the beddes on the different four periods before and after treatments according to (American Public Health Association (**APHA), 1971**). the hydrogen ion concentration (PH-values) of the beddes before and after the three treatment periods using digital pH-meter. the percentage of some elements of the animals bedde before and after treatments such as: carbon. Nitrogen, phosphates, potassium and sodium were determined according to (Association of Analytical chemists (**AOAC**), **1980**). the frequencies of diarrhaea and pneumania on cow of the two groups were calculated, and the reflected influences of EM-treatment on immunoglobulins were determined by polyacrylamide gel immunoelectrophoresis on the serum of cows of the two groups by the end of the experiment according to **Gordon, (1980)** and the total serum protein was measured according to (**Doumas et al., 1971**). The data were statistically evaluated using the t-student test according to (**Snedecor and Cochran, 1969**).

RESULTS

A- Total bacterial counts in yards:

Effective microorganisms (EM) treated yards showed highly to very highly significant decrease of the total bacterial counts after the 1^{st}, 2^{nd} and 3^{rd} treatment trials than that of the corresponding non-treated control yard (table 1).

B- Fly population of yards:

EM-treated yards showed very highly significant ($P \leq 0.001$) decrease of fly population after the 1st, 2nd and the 3rd treatments than that of the corresponding non-treated control yard (table, 2).

C- the pH values and the changes of elements of the animal beddes after EM-treatments:

1- pH-change:

The pH-of the animal bedde was decreased significantly ($P \leq 0.05$) after the 1st EM-treatment, highly significantly ($p \leq 0.01$) after the 2nd EM-treatment and non-significantly decreased after the 3rd EM-treatment compared with that of the non-treated control animal bedde (table, 3).

2- change of carbon element:

There was non-significant increase of the percentages of carbon element in EM-treated animal bedde after the 1st, 2nd and the 3rd treatments compared with that of non-treated control animal bedde (table, 3).

3- Change of nitrogen (N) elements content:

There was significant increase ($P < 0.05$) of the percentage of nitrogen element in the animal bedde of EM-treated yard after the 1st, 2nd and the 3rd EM-treatments compared to that in the animal bedde of control yard (table 3).

4- Change in the phosphorous (P) content:

Only after the 2nd EM-treatment the phosphorus percentage of the animal bedde of the treated yard showed significant increase ($P \leq 0.05$) compared to the bedde of the non-treated yard, this increased of phosphorus percentage in the animal bedde of EM-treated yard become highly significant ($P \leq 0.001$) compared to its percentage in animal bedde of untreated control yard (table, 3).

6- Change of the sodium (Na) content:

No significant change in the sodium percentage of the animal bedde of EM-treated yard after the all of the three EM-treatment periods compared to that of the corresponding untreated control yard (table, 3).

D- Frequency of diarrhaea and pneumonia in cattle after EM-treatment:

The diarrhaea was highly significantly decreased ($P \leq 0.01$) when comparing with diarrhaea in cattle of untreated control yard. Also, the frequency of pneumonia of cattle was highly significantly decreased ($P \leq 0.01$) in the cattle of the EM-treated yard than that of cattle of the EM-non-treated control yard (table. 4).

E- Effects of effective microorganisms on the serum protein profiles and immunoglobulins of cattle:

1- Total protein: no significant change of the total protein of cattle of treated yard than that in cattle of untreated control yard.

2- Albumin: no significant change of albumin of cattle between treated yard and that of cattle of untreated control yard.

3- Alpha (α) globulin fractions: no significant changes between the serum γ-globulins of cattle of treated and untreated control yards.

4- Beta (β) globulin fractions: there was significant increase of beta globulins fraction ($P \leq 0.05$) in serum of cattle of the EM-treated yard than that of cattle of non-treated control yard.

5- Gamma (γ) globulin fractions (immunoglobulins): there was a significant increase ($P \leq 0.05$) of g-globulins (immunoglobulins) in serum of cattle of EM-treated yard compared to that in cattle of untreated control yard.

6- Total globulins: there was a significant increase ($P \leq 0.05$) of the total globulins of the serum of cattle of EM-treated yard when comparing to that of cattle of untreated control yard (table 5 and Fig. 1 and 2).

F- Malodours of EM-treated and untreated yards:

There was a significant and rapid reduction in sensation of malodours in the EM-treated yard and this sensation started after 2-3 days from the first EM treatment compared to EM-untreated control yard which showed pronounced malodours.

DISCUSSION

The use of the technology of the effective micro-organisms (EM) in large scale livestock operations in expanding manner at the present time, due to its acceptance by the farming community. This expansion cover all aspects of livestock including poultry, dairy, beef, swine and aquaculture. The current work aimed for the using of such technology for improving cattle production indirectly by reducing fly population, malodours and total bacterial counts from their housing environment which are considered as stress factors in animal production, in addition to improve the quality of fertilizer produced from the animal manure of bedde that used for agriculture.

The present result revealed significant reduction of both total bacterial count of the animal bedde and the fly population of EM-treated yard than that in the untreated control yard with the consequent reduction of malodours sensation in treated yard. The effective micro-organisms consumed the volatile fatty acids produced from the stool of animals that reducing the malodours which mainly as a result of these volatile fatty acids, which are the main attractive for house flies, hence the reduction of fly population was consequently resulted as recorded by **Kapongo and Gillomee (2000)** whose also suggested that the reduction of fly population after spraying of EM over the animal manure may reducing the decomposition of decayed organic matter into the malodour volatile fatty acids (isobutyric, valeric and caproic acids) as these volatile fatty acids are consumed by some microbes hence the reduction of the malodours producing bacteria (e.g., clostridia and enterobacteria).

The present study revealed that the EM-treatment significantly reduced the PH value of bedde (which in turn inhibit the growth of some pathogenic bacteria, this is one of the factors for reducing the total bacterial count as recorded by the present study) and significantly elevated the nitrogen, phosphorus and potassium percentages of the bedde of treated yard than that of bedde of untreated control yard. The contents of soil organic matters, nitrogen, phosphorus and potassium are of some important indicators of soil fertility with direct relationships between soil fertility and these parameters as reviewed by **Lynch (1998)**.

The present study revealed that the use of EM treatment significantly reduced the frequency of diarrhoeic cattle than that observed in cattle of untreated yard. The reduction of fly population in EM-treated yard may be helping in reduction of diarrhoea frequency through reduction of transmitting the enterobacteriaceae causing diarrhoea **(Radostits et al., 2000)**, and/or as a result of hypergammaglobulinemia in the serum of cattle of EM-treated yard as recorded by the present study, or as a result of reducing the total bacterial count (inclding the pathogenic ones) as recorded by the current study.

The current work also revealed that the EM-treatment reducing the frequency of pneumonic cattle in treated yard than that in non-treated one. The reduction of offensive volatile fatty acids and toxic gases from manures as significantly recorded in EM treated yards, such volatile gases may be of stress factor for respiratory diseases, such reduction of offensive gases in parallel with the reduction of fly population in treated yard may be the cause of reduction of pneumonia frequency among cattle of treated yard **(Radostits et al., 2000)**, and/or as a result of hypergammaglobulinemia in cattle of EM-treated yard as indicated by the current study, or as a result of reduction of total bacterial count including the pathogenic bacteria as recorded by our study.

The present study revealed that treatment with the EM inducing hyper β-globulinemia and hypergamma (γ) globulinemia and hyperglobulinemia in cattle of treated yard than that in cattle of untreated control yard. Gammaglobulins (immunoglobulins) are composed of the different immunoglobulins (e.g., IgM, IgG, IgA....etc) (**Grant and Kachman, 1976**) the γ-globulins are synthesized in the plasma cells which maturated from B-lymphocytes in the spleen, bone marrow and lymph nodes (**McPherson, 1984**). 20% of the circulating lymphocyte population are B-lymphocytes and the remainder are T-lymphocytes (**Jain, 1986**). So that, B- and T-lymphocyte assay should be needed for further confirmation.

Also, some γ-globulin fractions may migrate to the β fractions (**Grand and Kachman, 1976**), and this may be the cause of the significant increase of b-fraction as a result of hyper-γ-γ globulinemia in EM-treated cattle, so that the EM-treatment may induced immunostimulant action of the immune system indirectly through improving the environmental housing of the animal of treated yard compared with untreated yard, and the increased level of total globulins may be as a result of significant increase of both β-and- γ-globulins in the present study. Also the hypergamma globulinemia may be one of the causes in reducing the frequencies of both diarrhoea and pneumonia by immunostimulant property of such EM treatment as peviousely mentioned.

Based on the current study it could be concluded that the treatment of cattle yards with the EM may be of beneficial values in reducing fly populations, total bacterial count of the bedde, malodoures of treated yards and the frequencies of pneumonia and diarrhaea in cattle of treated yard, and increasing fertility of manure (through reducing pH and elevating nitrogen, phosphorus and potassium percentages of treated bedde), and inducing immunostimulant activity (perhaps indirectly) through elevating the immunoglobulins (γ-globulins) of serum of cattle of treated yard. So that EM-technology should be recommended for improving cattle production.

Table (1): Total Bacterial Count (10^6/gm dry bedde)in either EM-treated and control yards

Groups	Before treatment	After 1st treatment	After 2nd treatment	After 3rd treatment
Control yard	8.533 ± 0.867	10.933 ± 1.097	11.917 ± 0.803	9.767 ± 0.775
EM-treated yard	9.217* ± 1.079	8.067*** ± 0.778	7.233*** ± 0.542	5.817** ± 0.875

N.B 1- * = significant change between means (at $P \leq 0.05$). 2- ** = highly significant change between means (at $p \leq 0.01$).
3- *** = very highly significant change between means at ($P \leq 0.001$)4- NS = non-significant change between means

Table (2): Average Number of Fly Populations (per trap of yard) During Different Periods of EM-Treated Yard

Groups	Before treatment	After 1st treatment	After 2nd treatment	After 3rd treatment
Control yard	684.333 ± 14.336	728.00 ± 13.837	784.667 ± 15.315	734.33 ± 15.967
EM-treated yard	711.00NS ± 7.950	547.667*** ± 11.599	410.33*** ± 11.589	354.50*** ± 11.845

N.B 1- * = significant change between means (at $P \leq 0.05$). 2- ** = highly significant change between means (at $p \leq 0.01$).
3- *** = very highly significant change between means at ($P \leq 0.001$)4- NS = non-significant change between means

Table (3): Chemical Analysis of the Animal Bedde At Different Periods of EM-Treatments.

Analysts and group / Periods		Before treatment	After 1st treatment	After 2nd treatment	After 3rd treatment
PH-value	Control bedde	8.700 ± 0.104	8.515 ± 0.062	8.642 ± 0.060	8.472 ± 0527
	Treated bedde	8.808NS ± 0.040	8.270* ± 0.050	8.242** ± 0.040	7.968NS ± 0.089
Carbon (C) %	Control bedde	40.45 ± 0.521	40.117 ± 0.507	41.683 ± 0.327	40.767 ± 0.592
	Treated bedde	40.85NS ± 0.272	41.233NS ± 0.312	41.317NS ± 0.703	41.417NS ± 0.370
Nitrogen (N) (%)	Control bedde	1.870 ± 0.022	1.872 ± 0.014	1.825 ± 0.023	1.908 ± 0.038
	Treated bedde	1.885NS ± 0.023	2.068* ± 0.084	2.117* ± 0.073	2.200* ± 0.112
Phosphorus (P) (%)	Control bedde	0.603 ± 0.013	0.597 ± 0.023	0.602 ± 0.011	0.562 ± 0.017
	Treated bedde	0.605NS ± 0.010	0.732NS ± 0.057	0.773 ± 0.044	0.805*** ± 0.034
Potassium (K) (%)	Control bedde	3.992 ± 0.086	4.063 ± 0.092	3.897 ± 0.093	3.375 ± 0.050
	Treated bedde	3.922NS ± 0.121	4.337NS ± 0.112	4.15NS ± 0.225	4.102* ± 0.233
Sodium (Na) (%)	Control bedde	1.873 ± 0.047	1.922 ± 0.051	1.783 ± 0.052	1.775 ± 0.055
	Treated bedde	1.843NS ± 0.078	1.897NS ± 0.058	1.837NS ± 0.060	1.795NS ± 0.032

N.B 1- * = significant change between means (at $P \leq 0.05$). 2- ** = highly significant change between means (at $p \leq 0.01$). 3- *** = very highly significant change between means at ($P \leq 0.001$) 4- NS = non-significant change between means

Table (4): Frequencies of Diarrhoea and Pneumonia in Cattle of EM-Untreated (Control) Yard and EM-Treated Yard

Disease	Groups	1	2	3	4	5	6	Means ± SE
Diarrhoea	EM-untreated yard (control)	12	5	4	3	5	6	5.833 ± 1.302
	EM-treated yard	1	3	0.00	1	1	2	1.333 ± 0.422**
Pneumonia	EM-untreated yard (control)	7	3	6	9	3	8	6.000 ± 1.033
	EM-treated yard	2	1	2	0.00	1	0.00	1.000 ± 0.365**

N.B. ** = highly significant change between means (at $p \leq 0.01$).

Table (5): The Effect of Effective Microorganisms (EM) on the Serum Immunoglobulins and different protein fractions as Determined by serum protein Electrophoresis of Cattle of Treated and Untreated Control Yards

Group	Albumin (g/dL)	Alpha (α) globulins (g/dL)	Beta (β) globulins (g/dL)	Gamma (γ) globulins (g/dL)	Total globulins (g/dL)	Total protein (g/dL)
Control Cattle	4.106 ± 0.148	0.972 ± 0.061	0.572 ± 0.084	0.840 ± 0.042	2.384 ± 0.211	6.490 ± 0.164
Treated Cattle	3.684NS ± 0.211	1.297NS ± 0.190	0.791* ± 0.032	1.438* ± 0.214	3.526* ± 0.294	7.210NS ± 0.319

N.B: (1) NS = non significant change between means

(2) * = significant change between the two means (at $P \leq 0.05$).

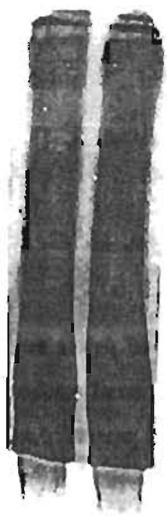

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Fig (1): Electrophoretically separted protein fractions, from bottom to top (prealbumin, Albumin, α_1- and α_2- globulins, β-globulins and gamma (immuno)-globulins) of the serum protein of control untreated (A) and EM-treated (B) cattle.

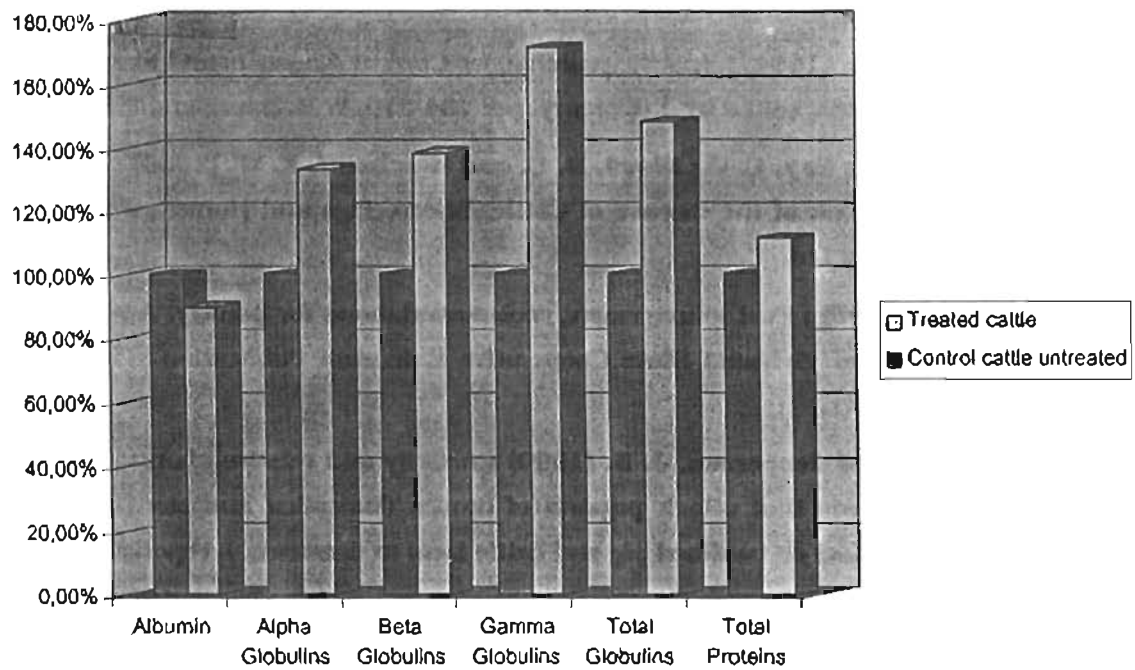

Fig (2): The percentages of the different protein fractions including the immunoglobulins (gamma globulins) of the serum of EM-treated and control (untreated) cattle.

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الملخص العربى

تطبيق إستخدام الميكروبات الفعالة فى مزارع الأبقار لتحسين الظروف البيئية للحيوان وتحسين حالته المناعية

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تم تطبيق تكنولوچيا الميكروبات الفعالة فى مزرعة خاصة للأبقار الهولستين الحلابة، حيث قسمت ٢٤٠ بقرة إلى مجموعتين (١٢٠ بقرة بكل مجموعة فى كل حوش تربية)، وتم رش محلول الميكروبات الفعالة (بتخفيف ١ : ٢٠٠ ماء) على أرضية أحد أحواش التربية بما عليها من فرش وروث وكذلك على عليقة الحيوانات (كمجموعة معالجة)، أما حوش التربية الآخر فبقى بدون رش الميكروبات الفعالة وأعتبر كضابط للتجربة، وتم رش حوش التربية المعالج مرة فى الإسبوع ولمدة ثلاث أسابيع متتالية، وقد تم عد الذباب بكل من حوشى التربية (المعالج والضابط) قبل التجربة وبعد كل معالجة). وكذلك تم عمل العد الكلى للبكتريا بالفرشة بكل من حوش التربية ٤ مرات (مرة قبل المعالجات وبعد كل معالجة)، وتم الاستشعار الشمى بدقة لمدى كثافة الروائح الكريهة فى كل حوش فى الفترات المختلفة، وكذلك تم قياس درجة حموضة الفرشة (PH) فى كل من الفترات السابقة، وتم قياس أيضاً نسبة عناصر الكربون والنتروچين والفوسفور والبوتاسيوم والصوديوم بالفرشة بكل حوش فى المراحل السابقة المختلفة، وكذلك تم تسجيل تكرارات حالات الإسهال والالتهاب الرئوى بحيوانات كل حوش تربية فى نهاية التجربة وحساب متوسطات نسب حدوثهما بكل مجموعة، وفى نهاية التجربة تم أخذ عينات دم لقياس مستوى الجلوبيولينات المناعية بمصل الأبقار فى المجموعتين عن طريق الفصل الكهربى لبروتين مصل حيوانات المجموعتين.

أوضحت نتائج الدراسة أن العلاج بالميكروبات الفعالة أحدث نقصاً فى عدد الذباب، ونقص ملحوظ فى الروائح الكريهة المستشعرة بالحوش المعالج وكذلك نقص معنوى فى العدد الكلى للبكتريا بالفرشة ونقص معنوى فى درجة حموضة الفرشة والروث وزيادة معنوية فى نسب عناصر النيتروچين والفوسفور والبوتاسيوم بالفرشة والروث، ونقص معنوى فى متوسطات نسب تكرارات حدوث كل من حالات الإسهال والالتهاب الرئوى بحيوانات حوش التربية المعالج بالميكروبات الفعالة، وزيادة معنوية فى مستوى الجلوبيولينات المناعية بمصل الأبقار المرباه فى الحوش المعالج بالميكروبات الفعالة - ولهذا يوصى باستخدام تكنولوچيا الميكروبات الفعالة فى مزارع الأبقار الحلوب لزيادة إنتاجيتها العامة.