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# INFLUENCE OF SUPPLEMENTAL, DIATARY LACTOSE ON LAYING HENS PERFORMANCE AND SOME INTESTINAL MICROFLORA.

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**Abstract:** *The current study aimed to investigate the effect of feeding different graded levels of dietary lactose on performance of laying hens, some intestinal microflora, some blood constituents and egg quality.*

*For this purpose 75 Hy line (W-36) white layers were sited from 70 to 79 week of age in individual cages and randomly distributed into five experimental groups of 15 layers each, the five experimental groups were fed on a basal diet (control) supplemented with four graded levels of lactose as 1%, 1.5%, 2% and 2.5%.*

*The results indicated that, egg production of layers fed 1% and 1.5% lactose were significantly higher by 5.6% and 6.2% respectively than the control group. Average egg weight was not affected by adding lactose into diets.*

*Feed consumption values didn't differ significantly. There were a non significant improvement in feed conversion ratio (g. feed/g. egg) due to enriching lactose into the diets by 1% and 1.5%.*

*The improvement effect of adding lactose on egg production and feed conversion may be related to its lowering pH value of small intestine and enhancing absorption rate of nutrients.*

*Eggshell percentage and eggshell thickness were enhanced due to adding lactose into diets.*

*Blood total protein and albumin values were lower than control while blood globulin was not affected by treatments. Blood total lipids increased by feeding lactose while blood cholesterol wasn't affected.*

*The microbiological examination revealed an increase in lactobacilli sp. and inhibition in count of salmonella due to adding lactose into the diets.*

*E. coli bacterial count was slightly inhibited by the levels of 1%, 1.5% and 2% lactose while the high level of lactose (2.5%) had no effect.*

*The results of intestinal microflora showed that lactose probably offer protection against salmonella by promoting, the growth of lactobacilli bacteria which compete with salmonella for colnisities.*

## INTRODUCTION

Prebiotics are a group of indigested oligosuccardies which are used as alternative to antibiotics that have been banned as poultry feed additives in developed countries (Van Immerseel, 2002).

Lactose sugar is well documented as a prebiotics for poultry (Kermanshahi and Rostami; 2006) due to its effect in eliminating E.coli bacteria, salmonella and other pathogens from gastro intestinal tract of poultry (Morishita et al, 1982; Tellez et al 1993; Terad et al, 1994; Chambers et al, 1997).

The inhibitory effect of lactose against pathogenic bacteria is related to its role in lowering intestinal pH creating acidic media unsuitable for the growth of these bacterial strains.(Hinton et al, 1991; Chamberes, et al 1997). In addition the presence of undigested lactose in the chyme enhances the proliferation of lactobacilli bacteria (Morishita et al, 1982). Lactobacilli bacteria compete with pathogens for colonisites, excluding it from mucosa of intestinal wall (Oyofo, et al 1989; Kermanshahi and Rostami, 2006).

The reduction of the growth of pathogenic intestinal bacteria, reduces the incidence of infectious processes, and the functions of secretion, digestion and absorption of nutrients can be appropriately performed by the mucosa and efficiency of feed utilization improved (Tellez et al 1993; Pelicano et al 2005). However the results of the previous studies are still contradictory, due probably to differences in experimental conditions..

Gleaves and Salim (1982) reported that, neither egg production nor egg weight was affected by adding 1%, 2% or 3% lactose into laying hen diets.

As well, Yamaguchi et al (1992) and Waldroup et al (1992) didn't show any significant effect on performance of broiler chicks by feeding dietary lactose.

In contrary, Pjescak (1970) found that the addition of lactose to rechitogenic diets improved egg production and eggshell quality. Douglas et al; (2003) and Terada et al (1994) observed a slight improvement in body weight gain of broiler chicks fed dietary lactose. Therefore the current study

aimed to investigate the effect of feeding different graded levels of dietary lactose on performance of laying hens as well as the effect on intestinal microflora, some blood constituents and egg quality were also studied under Egyptian conditions.

## MATERIALS AND METHODS

This study was carried out at (Layer Nutrition Research Unit) Faculty of Agriculture, Ain Shams University.

It was conducted using 75 Hy-Line (W-36) white layers which were randomly sited from 70 to 79 week of age in individual battery cages located in open sided laying house. The hens were randomly distributed into five treatment groups of 15 layers each. The individual hen was represented as experimental unit. For ten weeks experimental period the hens were fed on a basal diet (control) supplemented with four graded levels of lactose 1.0%, 1.5%, 2.0% and 2.5%. The basal diet was formulated (Table1) to meet all nutrient requirements of laying hens according to Hy-Line (2000) management guide.

Feed was provided *ad lib* in an individual feeders and water was supplied through automatic nipples. Lighting hours were 17 hours per day. Egg weight in grams was recorded daily for each hen throughout the experimental period. Average egg weight, egg production percentage and average egg mass (g/hen/day) were calculated for each hen and treatment group. Feed consumption in grams per hen was recorded weekly and average feed consumption per treatment group was calculated. Feed conversion ratio was calculated as gram feed consumed per gram egg produced (g. feed/g. egg). Body weight gain was calculated for each hen and treatment group by subtracting individual body weight of hen at 70 weeks from that at 79 weeks of age. Egg components percentages were assessed by using 12 eggs per treatment representing 6 hens as two consecutive eggs per hen. For this purpose, eggs were individually weighted, broken, yolk and albumin was separated weighed and related as percentage to whole egg weight. Egg shell with membrane were cleaned, dried, weighed and related as percentage to the whole egg.

Egg shell thickness (millimeter) was determined using a micrometer.

**Blood analysis and microbiological examination:**

At the end of the experiment five hens per experimental group were slaughtered, blood samples were collected and centrifuged for 15 minutes.

Plasma total protein was determined according to Biuret method (Henery 1964), albumin according to Doumas et al (1971**)**. Plasma globulin was calculated by subtracting albumin from total protein. Then albumin to globulin ratio was calculated. Plasma total lipid was determined according to Knight et al (1972) and total cholesterol according to Watson (1960).

For microbial examination, ileal content samples were collected by pressing the outer wall of ileum to push its content into clean, sterile glass bottle.

The pH values of ileal content were determined using pH meter.

Microbiological examination procedure was done as follows:

One gram of ileal content was adjustably weighed and transferred into test tube containing 9 ml of 0.1sterile peptone the samples were mixed well and serial dilutions were prepared.

1- ***coliform counts***:- the conventional dilution pouring MPN technique was followed for most propel number (MPN) using Macconky broth according to Roberts et al (1995).

**2-*Salmonella viable count***:- the conventional dilution pouring plate technique was followed for salmonella viable count in fecal samples using bismuth sulfite agar according to Roberts et al (1995). The plates were incubated at 37°c for 48h the color of salmonella colony black to dark green Brock and Madigan (1991).

***3- Lactobacilli viable count***:- using MRS agar De Man et al (1960) the plates were incubated using candle Jar Technique Vedemuthu et al (1992) at 37°c for 48h.

***Statistical analysis***

Statistical analysis was carried out using statistical program SAS (1988). Duncan's multiple range tests was used to separate means.

## RESULTS AND DISCUSSION

**Laying performance**

Egg production percentage of layers fed 1% and 1.5% dietary lactose (Table2) were significantly higher by 5.6% and 6.2% than the control treatment respectively. This result is in harmony with those of Pjescak (1970) who observed an improvement in egg production of hens due to feeding dietary lactose. However Gleaves and Salim (1982) didn't show any significant effect on egg production of laying hens fed 1%, 2%, or 3% lactose.

This improvement in egg production may be related to the direct effect of lactose in enhancing the digestion and absorption media, saving more nutrients for egg production (Balloun and Khajarern 1974 ; Gleaves and Salim 1982). In addition to the indirect role of lactose in stimulating the proliferation of lactobacilli bacteria which enhance egg production (Jernigan et al 1984).

Supplementation of lactose by 2 and 2.5% caused a non significant slight impairment in egg production. It may be suggested that, addition of lactose beyond 1.5% is not suitable for layers. Gleaves and Salim (1982) stated that, low lactose levels less than 2% are necessary to demonstrate its beneficial effect. Kermanshahi and Rostami (2006) suggested that, poultry are lacking lactose enzyme, therefore the high level of lactose in poultry diets may cause osmotic diarrhea.

Average egg weight was not affected due to adding lactose into diet. Gleaves and Salim (1982) didn't record any effect on egg weight due to adding lactose to laying hen diets.

Average egg mass results were parallel to those of egg production where the level of 1% and 1.5% lactose improved egg mass while the level of 2% and 2.5% caused a slight impairment.

**Feeding performance**

Feed consumption values didn't differ significantly (Table2) due to lactose supplementation. However there were a reduction in feed consumption of layers fed 2.5% lactose (102.2g) compared with control (106.8g). this result is in harmony with those of Gleaves and Salim (1982) who didn't find any clear effect of lactose on feed intake of laying hens. Waldroup et al (1992) observed a significant reduction in feed intake due to adding more than 2.5% lactose into broiler diets.

Feed conversion ratios of layers fed 1% (2.12) and 1.5% (2.21) lactose were better than those fed the control diet (2.26), however the differences lacked significance. This improvement in feed conversion may be achieved by two manners:-

1- inhibitory effect of lactose against harmful microflora which may irritate the intestinal wall causing malabsorption syndrome (Tortuero, 1973).

2- lowering pH value of small intestine which enhances the digestibility of protein and calcium in addition to delay the transit time of chyme saving more time for absorption process (Giesting et al 1991, El Afifi et al 2001). There was a slight impairment in feed conversion ratios of layers fed the high dietary lactose levels 2% and 2.5%. This finding is in harmony with

those of Kermanshahi and Rostami (2006) who stated that, feed was less digested in layers with high level (6%) of dried whey (67% lactose) in their diets.

Body weight gain of layers fed dietary lactose were higher than control group. Gleaves and Salim (1982) observed more body gain of layers fed dietary lactose.

**Egg shell and egg components**

Egg shell percentage (Table3) was increased due to adding lactose into diets. In parallel egg shell thickness was increased by 1.7% to 5% as percentage to control due to supplementing lactose into diets, (Fig. 1), however the differences lacked significance. These results are in a good agreement with those of Gleaves and Salim (1982) who observed a significant improvement in egg shell quality due to adding lactose by 1%, 2% and 3% into laying hen diets. They related this influence to improve calcium utilization by feeding dietary lactose. The mode of action of lactose in enhancing calcium utilization may be depended up on its lowering effect on pH value of small intestine (Fig. 1) which increase calcium solubility and encourage its absorption (Tullett 1987, Haddading et al 1996).

Egg yolk and egg albumin percentage weren't affected by feeding dietary lactose.

**Effect on some blood constituents**

Blood total protein was 4.16g/100ml for control and reduced to 3.77, 3.89 and 3.63g/100ml due to adding lactose by 1%, 1.5% and 2% into diets respectively. However the reduction lacked significance (Table 4 ). This reduction in blood total protein of lactose feeding groups may be related to the effect of lactose in inhibiting salmonella and other harmful pathogenic bacterial strains those secrete inflammatory agents irritating the intestinal wall causing an increase in protein synthesis in liver and blood.

Klasing and Austic (1984) observed an increase in protein synthesis in liver, bursa and thymus gland of chicken infected with harmful bacteria.

The levels of blood albumin were parallel to those of blood total proteins.

There is no effect of lactose on blood globulin or albumin to globulin ratio which considered as an indicator for immune response of chickens. So it can be suggested that lactose addition have no effect on immune response of laying hens.

Blood total lipids increase from 4.22mg/ml of control to 5.49, 5.58, 5.22 and 4.69mg/ml due to adding lactose into diets by 1%, 1.5%, 2% and 2.5% respectively. This elevation in lipid concentration may be related to the role of lactose in increase the amount of non glycogenic (lipogenic) volatile fatty acids in intestinal tract of layers that may be transfer into blood stream via intestinal pores. Terada et al (1994) observed a significant increase of cecal citric and butyric fatty acid (non glycogenic fatty acid) of chicks fed lacto sucrose diet.

Blood concentration of cholesterol was not affected by adding lactose into laying hen diets.

**Effect on some ileal bacteria**

There was obvious increase in lactobacilli bacteria by enriching lactose into diets (Table5 and Fig. 2). This is expected result since lactose is the preferential substrate for the growth and proliferation of lactobacilli bacteria (Naughton et al 2001, Morishita et al 1982).

The results indicated that salmonella was inhibited due to adding lactose into laying hen diets. This result is in a good agreement with the finding of Oyofo et al (1989) and Terada et al (1994) who stated that lactose probably offers protection against salmonella by promoting the growth of lactobacilli bacteria which compete with salmonella for colonization sites, in addition to promote lactose-fermenting bacteria that produces volatile fatty acids which are toxic to salmonella.

E. coli bacterial count was reduced by adding lactose into diets at levels 1%, 1.5% and 2% while the high level of lactose 2.5% had no effect.

It can be suggested that E. coli didn't obviously suffer from lactose addition since lactose can be utilized and fermented by E. coli bacteria (Kermanshahi and Rostami 2006).

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**Table 1.** Composition and calculated analysis of basal diet

| **Ingredients** | **Percentage (%)** |
|---|---|
| Yellow corn | 59.93 |
| Soybean meal (48%) | 24.23 |
| Corn gluten meal | 2.0 |
| Calcium carbonate | 9.16 |
| di-calcium phosphate | 1.84 |
| Oil | 2.0 |
| Common salt | 0.364 |
| Methionine | 0.076 |
| Premix* | 0.4 |
| **Total** | **100** |
| **Calculated analysis** | |
| ME (kcal/kg) | 2806 |
| Protein % | 17.39 |
| Calcium % | 3.97 |
| Av. Phosphorus % | 0.465 |
| Meth. + Cyst % | 0.66 |
| Lysine % | 0.86 |

*Vitamins and minerals Premix: each 1kg supplied the following per kilogram of diet; vit. A; 12000 Iu, vit. $D_3$, 3000 Iu, vit E 12mg, vit $B_{12}$ 0.02mg, vit $B_1$ 1mg, Choline chloride 0.16mg, Copper 3mg, Iron 30mg, Manganese40mg, Zinc 45mg, and Selenium 3mg

**Table (2)** Effect of feeding dietary lactose on performance of laying hens

| **Items** | **Lactose levels** | | | | |
|---|---|---|---|---|---|
| | **0.0%** | **1.0%** | **1.5%** | **2.0%** | **2.5%** |
| **Egg production %** | $74.1^{b}$ | $79.7^{a}$ | $80.3^{a}$ | $71.1^{b}$ | $73.1^{b}$ |
| **Av. Egg weight(g)** | 64.2 | 64.3 | 61.4 | 64.5 | 62.3 |
| **Egg mass (g/h/day)** | $47.5^{ab}$ | $51.2^{a}$ | $49.3^{ab}$ | $45.8^{b}$ | $45.3^{b}$ |
| **Feed intake(g/h/day)** | 106.8 | 108.3 | 108.9 | 104.4 | 102.2 |
| **Feed conversion (g feed/g egg)** | 2.26 (100%) | 2.12 (96%) | 2.21 (93%) | 2.32 (89%) | 2.29 (99%) |
| **Body weight gain (g.)** | 21.5 | 38.9 | 77.4 | 30.0 | 49.4 |

a,b means with different superscript are significantly different ($p \leq 0.05$

( ) the value as percentage of control values

**Table (3)** Effect of dietary lactose on egg component and eggshell thickness

| Items | Lactose levels | | | | |
|---|---|---|---|---|---|
| | **0.0%** | **1.0%** | **1.5%** | **2.0%** | **2.5%** |
| **Egg yolk %** | 27.3[ab] | 26.9[ab] | 27.0[ab] | 28.6 [a] | 26.5[b] |
| **Egg albumen%** | 63.7 | 63.3 | 63.1 | 62.1 | 63.9 |
| **Egg shell%** | 9.08 | 9.79 | 9.85 | 9.36 | 9.57 |
| **Egg shell thickness(mm)** | 0.423 | 0.435 | 0.448 | 0.430 | 0.432 |
| | (100%) | (102.8%) | (105.9%) | (101.7%) | (102.1%) |

a,b means with different superscript are significantly different ($p \leq 0.05$)

( ) the value as percentage of control

**Table (4) Effect of dietary lactose on some blood constituents**

| Items | Lactose levels | | | | |
|---|---|---|---|---|---|
| | **0.0%** | **1.0%** | **1.5%** | **2.0%** | **2.5%** |
| **Total proteins (g/100ml)** | 4.16 | 3.77 | 3.89 | 3.63 | 4.12 |
| **Albumen (g/100ml)** | 1.93 | 1.77 | 1.69 | 1.68 | 1.95 |
| **Globulin (g/100ml)** | 2.23 | 2.00 | 2.21 | 1.95 | 2.17 |
| **Albumen/globulin ratio** | 0.889 | 0.89 | 0.778 | 0.865 | 0.933 |
| **Cholesterol (mg/100ml)** | 149 | 131.7 | 136.8 | 145.4 | 136.4 |
| **Total lipid (mg/ml)** | 4.22 | 5.49 | 5.58 | 5.22 | 4.69 |

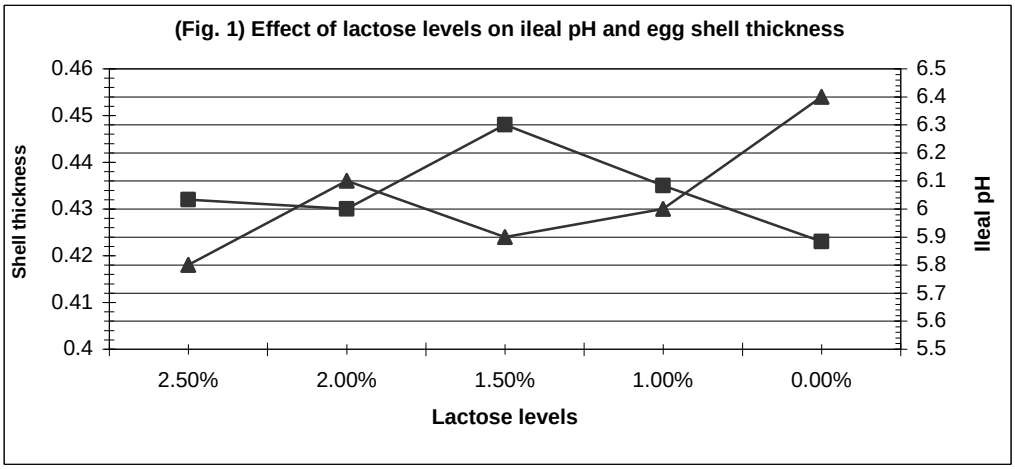
no significant differences were detected

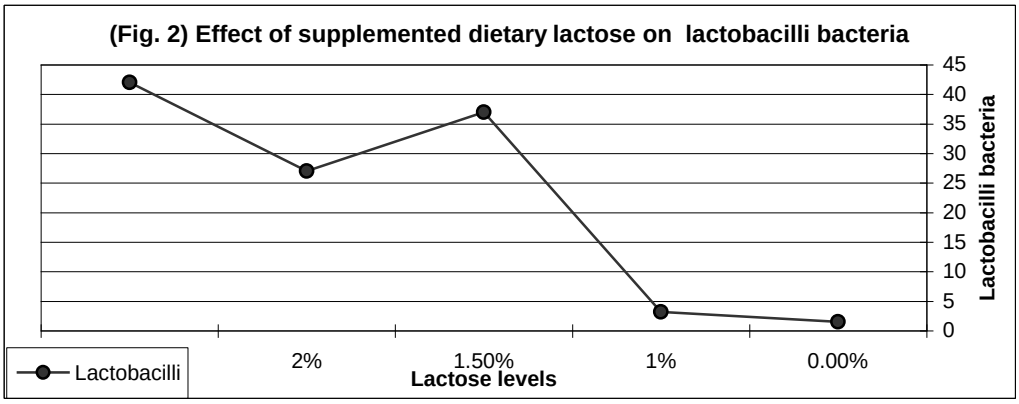
**Table (5)** Effect of dietary lactose levels on ileal PH and some bacterial strains

| Items | Lactose levels | | | | |
|---|---|---|---|---|---|
| | **0.0%** | **1.0%** | **1.5%** | **2.0%** | **2.5%** |
| **Ileal PH** | 6.4 | 6.0 | 5.9 | 6.1 | 5.8 |
| **Lactobacilli sp.** | $1.5\times10^{6}$ | $3.2\times10^{6}$ | $3.7\times10^{7}$ | $2.7\times10^{7}$ | $4.2\times10^{7}$ |
| **E. coli** | $3.1\times10^{4}$ | $2.1\times10^{4}$ | $1.7\times10^{4}$ | $2.8\times10^{4}$ | $3.6\times10^{4}$ |
| **salmonella** | $3.1\times10^{2}$ | $1.1\times10^{2}$ | N.d | $1.7\times10^{2}$ | N.d |

no significant differences were detected

N.d = non detected

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## الملخص العربى

## تأثير إضافة اللاكتوز لعلائق الدجاج البياض على الأداء الإنتاجي وبعض ميكروبات الأمعاء



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*مركز البحوث وتكنولوجيا الإشعاع- ج م ع



أجريت هذه الدراسة لمعرفة تأثير التغذية على مستويات مختلفة من اللاكتو ز على الأداء الإنتاجي للدجاج البياض وبعض السلالات البكتيرية بالأمعاء وبعض خصائص الدم وكذلك التأثير على جودة قشرة البيض.

استخدمت في الدراسة عدد 75 دجاجة بياضة من سلالة الهاي لاين الأبيض (w 36) مرباة في أقفاص مفردة، وتم توزيعها على خمس مجموعات بواقع 15 دجاجة لكل معاملة، تم تغذية المجاميع علي عليقة قاعدية (كنترول) مضاف إليها أربع مستويات مختلفة من اللاكتوز وهي 0.01٪، 1.5٪، 2٪ و 2.5٪ وأشارت النتائج إلى الأتي:-

تحسنت معدلات إنتاج البيض معنوياً للمجموعتين المغذاة على 1٪ و1.5٪ لاكتوز بواقع 5.6٪ - 6.2٪ على التوالي عن الكنترول بينما لم يتأثر متوسط وزن البيضة.

لم يكن هناك أي تأثير معنوي علي معدلات الاستهلاك الغذائي بينما تحسنت معدلات التحويل الغذائي (جرام عليقة/جرام بيضه) نتيجة لإضافة اللاكتوز إلي العلائق بنسبة 1٪ و1.5٪ .

ويمكن تفسير التحسن في معدلات إنتاج البيض والتحويل الغذائي إلي تأثير اللاكتوز الخافض لدرجة الحموضة (pH) الأمعاء وتحسين معدلات الامتصاص للعناصر الغذائية.

أشارت النتائج إلى زيادة النسبة المئوية لقشرة البيضة وسمك القشرة نتيجة للمعاملة بينما لم يكن هناك تأثير واضح على باقي مكونات البيضة.

تحليل مكونات الدم أشارت إلى انخفاض في نسبة بروتينات الدم و الألبيومين بينما لم يتأثر تركيز الجلوبيولين بالدم. ازداد تركيز الليبيدات الكلية بالدم بينما لم يتأثر مستوى الكولسترول نتيجة لإضافة اللاكتوز للعلائق.

الفحص الميكروبيولوجي أشار إلى زيادة في عدد بكتيريا اللكتوباسلاى وانخفاض ملحوظ في أعداد بكتيريا السالمونيلا بينما انخفضت أعداد بكتيريا القولون E coli بصورة طفيفة نتيجة المعاملة.

هذه النتائج تشير إلى أن اللاكتوز ربما يثبط نمو السالمونيلا عن طريق زيادة أعداد بكتيريا اللكتوباسلاى التي تتناحر مع السالمونيلا وتقلل من أعدادها.