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Role of CDX2 up regulation in Intestinal Development and Homeostasis in Neonatal Rats Fed with *Lactobacillus Acidophilus*

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***Abstract:*Back ground;** *Lactobacillus acidophilus (LBA) effects on physiological aspects of intestinal in regard to intestinal mucosal cells proliferation and differentiation were attract research interest but the specific molecular mechanism not well studied. The present study designed to study the influence of LBA on intestinal CDX2 expression .**Material and methods:**Ten pregnant female rats were divided into two groups, 1st group (A) and second group (B), At the day of parturition mothers of (A) were daily given 1ml MRS broth containing ($5x10^8$ CFU) of LBA , and mothers of group (B) were given 1ml of D.W , for 30 days ,as lactation period. At the day of weaning , 12 male kids from each group (A) and (B) were isolated and translated in to another cages , during the next 32 days they were daily given by oral cavage as the following:A1 (T1) – received 1 ml of MRS (5*108 CFU)) of LAB, A2(T2)- received 1 ml of D.W, B1 (T3) – received 1 ml of MRS (5*108 CFU)) of LAB, B2(C)- received 1 ml of D.W. At the end of the experiment blood and inte4stinal samples were collected. **Results:**The results of relative CDX2 gene expression showed significant increase ($p<0.05$) to many folds in experimental treated groups,T1,T2, T3 than control ones.Also results revealed that the Intestinal weight/ body weight ratio (%)was significantly($p<0.05$) increased in rats of T1 and T3 as compared with C and T2. Length measurement of total and different parts of intestine (cm) was increasedstatistically ($p<0.05$) in groups T1,T2 and T3 as compared with C group. The analysis of the photomicrograph of illume showed that T1,T2 and T3 group reported significant ($p<0.05$) increase in villus height, villus /crypt ratio and in size and density of goblet cell in villi and crypt of duodenum and decrease in depth crypt compared with the control groups. **Conclusion** results of the present study indicated that pups fed and sucked from mothers fed on Lactobacillus Acidophilus showed significant changes in intestinal mucosa cells proliferation , differentiation and CDx2 gene expression ,suggesting a positive interaction between Lactobacillus Acidophilus and host at sub cellular level.*

Key word:*CDX2, Lactobacillus Acidophilus, Villus high, growing rats, Intestinal wall*

I. Introduction

There is a mutual interaction between intestinal cells and microorganisms present in the gastrointestinal tract. Intake probiotic along with normal micro flora is a part of the commensal microorganisms in the gastrointestinal tract have multidirectional effects on digestion, absorption, barrier function, and secretory functions (Abass, 2013), also it can affect the postnatal maturation of intestinal mucosa (Bodera and Chcialowski, 2009). Furthermore, it has been known that they introduce their affect via changes in gene expression in intestinal cells (Hooper et al., 2001). Lactobacilli are the most important bacteria gaining more consideration in the area of probiotics (Tannock, 2004).Lactobacilli species are non-pathogenic gram-positive lactic acid production bacteria found in the normal intestinal microflora of animals and humans and are classified as probiotic agents(Bleau et al., 2005). Intestinal epithelial protection , development and homeostasis are altered by intestinal commensal microbiota (Bik,2009). The development and function of intestinal microbiota improvement influenced by different nutrient and medical factors(Amy and William, 2012). These intestinal microbiota develops rapidly after birth and how much they develop they influenced the later life health (Heyman and Menard, 2002).Commensal bacterium modulates expression of genes related to several important intestinal functions, including nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, angiogenesis, and postnatal intestinal maturation.(Macdonald et al.,2011). Among the many factors involved in intestinal mucosa, the Caudal-related homeobox transcription factor 2 (CDX2) is critical in early intestinal differentiation and has been implicated as a master regulator of the intestinal homeostasis ,and permeability. Intestine-specific transcription factors Cdx1 and Cdx2 are candidate genes for directing intestinal development, differentiation, and maintenance of the intestinal phenotype. The relative expression of Cdx1 to Cdx2 protein may be important in the anterior to posterior patterning of the intestinal epithelium and in defining patterns of proliferation and differentiation along the crypt-villus axis(Silberg et al.,2000).The intestine-specific home domain protein CDX2 is one of the transcriptional factors (TF) occupies more than 12,000 sites in the genome of adult mouse intestinal villus cells (Verzi et al., 2011). The Cdx2 specifies embryonic intestinal

epithelium (Gao and Kastner, 2009), imparts intestinal character to stomach cells (Silberg et al., 2002), and maintains adult intestinal function and identity (Hryniuk et al., 2012). When expressed, CDX2 modulates the processes including cell proliferation, differentiation, cell adhesion, migration, and tumorigenesis(Coskun et al., 2011) , it has aregulatory role in transcription of several known and novel gens , that place CDX2 as akey node in atranscription factor network controlling the proliferation and differentiation of intestinal cells (Boyd et al., 2010) . CDX2 is a homeoboxprimaly found in all epithelial cells located in the crypt–villus epithelium of the small intestine and colon (Suh , 1994; james,1994).

A growing body of evidence now suggests that the host microbiota and probiotics can influence intestinal physiology often occur in a strain-dependent manner and age of recipient. But the molecular mechanisms by which intestinal microbiota inter act with intestinal mucosa and influence intestinal morphology and physiology in growing rats need to be understood. Accordingly the present study was designed to investigate the hypothesis that there is a connection between the effects of Lactobacillus acidophilus on proliferation ;absorption; and secretion activity of intestinal epithelia and CDX2 Gene expression in intestinal epithelia in growing rat's pups either sucked from mother received Lactobacillus acidophilus or not .

II. Material and Methods

Experimental animal and diet

Ten health Albino Wister pregnant female rats, were kept under suitable condition of (21-25 C) in an air condition room ,they were fed freely with standard pellet diet (table-1. At the day of parturition each dam isolated in a single cage . After parturition these dams with their pups were divided into two groups, A and B groups, mothers of A were received (5×10^8 CFU) of reference strains Lactobacillus acidophilus LA-K CHR-Hansen (USA), mothers of B received 1 ml per animal of distilled water by oral gavages tube for 30 days . At weaning time, 12 male pups from each group were chosen , isolated and placed in a another cages as two sub group to treated as follow: A-1(T1) group kept on (5×10^8 CFU) of Lactobacillus acidophilus. A-2(T2)and ,B1(T3) began to received (5×10^8 CFU) of Lactobacillus acidophilus B-2(C) group kept on distil water . This treatment continued for another 32 days.

Table-1. Nutrient Composition (%)of the Diet(BESLER/Turkey)

Nutrient Composition	Percentage
Dry matter	88%
Crude protein	14-16%
Crude fat	2.5-3.0%
Crude fiber	18%
Crude Ash	10%
Moisture	10%
Calcium	1-3%
Phosphorus	1%
Nacl	1%
Vitamin A	5000 IU/KG
Vitamin D3	700IU/KG
Vitamin E	30 mg/KG
Metabolizable Energy	2400 Kcal/KG

Blood and intestine samples collection

At the end of the experiment all the experimental animals were anesthetized by intraperitoneal injection with 35 mg/kg ketamine hydrochloride. After opening the chest cavity, blood was collected by acupuncture through the left ventricle.Immediately after animal sacrifice , intestine from each animal were isolated for more measurements.

Intestine Weight to Body Weight (gm/gm).

At the end of the experiment all rats were weighted and sacrificed . After the animal dissection ,the whole intestine was isolated from mesenteric ligament tissues from beginning of duodenum to the anal orifice cleaned from any attached tissues, and dried with Wittman filter paper ,then weighted (gm)

Total and paortions Length of intestine ((cm).

The isolated intestines were emptied of its content, and placed on the length of on paper runway in centimeters (1-100) for total and partial length mwasurement.

HistomorphometricMeasurements

1cm specimens were collected from small intestine (ileume) were fixed in 10% formalin in phosphate buffered saline , embedded in paraffin and cut into 4 mm section . Ileume sections were stained with haematoxyline / eosine, the length of the villi crypt depth and the wall thickness (µm) were measured in the

small intestine preparation at low magnification (10x) using ocular micrometer in 20 well of each intestinal sample/ animal.

Density and size of Goblet Cell.

Neutral and acidic mucin secreting cells were assessed by staining with (PAS), according to Burkholder et al. (2008). The number of periodic acid-Schiff positive cells along the villi was determined by light microscopy, as cell per 200(μm2). Goblet cell size was measured as the "cup" area of the goblet cells (μm2)(Smirnov &Uni, 2004) . Twenty measurements were made for each intestinal sample/ animal. Only perfect cross sections of the goblet cells were measured, i.e. the goblt cell had to show the cup and the tail area to be measured, and also had to touch the villi edge. Goblet cell density was determined as the number of goblet cells per 200μm2. Only goblet cells found on the edge of the villus were counted for the density determination

CDX2 Gene Expression.

RNA were extracted from a piece weighing 250mg of each experimental rat intestine tissue was homogenized by micropestlel in 1m of TRIzol® reagent kit. The extracted RNA were treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samples (DNase I enzyme kit) and done according to method described by promega company, USA. There are two quality controls were performed on extracted RNA. First one is to determine the quantity of RNA (ng/μL), the second is the purity of RNA by reading the absorbance in at 260 nm and 280 nm in nanodrop spectrophotometer (Thermo.USA). Total RNA samples were used in cDNA synthesis step using AccuPower ®RocktScript RT PreMix Kit that provided from Bioneer, Korea. Quantitative Real –Time PCR(qRT-PCR) master mix prepeared according to AccuPowerTM Green Star Real-Time PCR kit that depend on syber green dye detection of gene amplification in Real-Time PCR system using a housekeeping gene . The relative expression of target genes (cdx2) in rat intestine tissue was calculated by using Livak Method (2^-ΔΔCT) (Livak and Schmittgen, 2001). that dependent on normalization of RT-qPCR (CT values) of target genes with housekeeping gene (B actin) as reference gene in control and different treatment groups.

Statistical Analysis

The Statistical Analysis System- SAS (2012) was used to effect of different factors in study parameters. Least significant difference –LSD test was used to significant compare between means in this study.

III. Results

Results in table -2 show that total length if intestine was significantly increased in T1,T2&T3 when compare with control, in duodenum, and jejunum length (cm) were increased significantly($p<0.05$) in T1, T2General all of the small intestine portions were increased their length in different significant degrees with exception of illume showed no length increase. On the other hand. Intestinal weight/ body weight (%)was significantly ($p<0.05$) increased in rats of T1,T2 and T3 as compared with C .

Table-2: Effects of *Lactobacillus acidophilus*on Length of Total intestine, Duodenum , Jejunum , Ileum , Large Intestine and Intestinal Weight / Body Weight(%) of rat kids in Different Experimental Groups post weaning . Mean± SE, n=6

Animals group	Length (cm)				Weight (gm)		
	Total intestine	duodenum	jejunum	ileum	Total intestine	Body Weight	Intestine/BW
C	110.38^{c} ±1.21	7.9^{c} ±0.22	80.2^{b} ±0.65	6.45^{b} ±0.25	13.4 ^{b} 0.27	151.4^{ab} ±1.2	8.90^{c} ±0.16
T1	120.2^{a} ±1.68	9.2^{ab} ±0.20	85.41^{a} ±1.02	8.05^{a} ±0.25	16.65^{a} ±0.65	156.7^{a} ±2.76	10.60^{a} ±0.44
T2	115^{b} ±1.07	8.61^{b} ±0.22	82.8^{ab} ±0.98	7.06^{b} ±0.3	13.46^{b} ±0.97	149.4^{b} ±2.13	9.02^{ab} ±0.68
T3	120.6^{a} ±1.36	9.36^{a} ±0.27	85.4^{a} ±0.99	8.2^{a} ±0.27	15.9^{a} ±0.40	157.7^{a} ±3.32	10.05^{a} ±0.14

Table-3:Effects of Lactobacillus acidophilus on Vilus high, Crypt depth, Villus/crypt ratio, and wall Thickness **.**Mean ∓SE .n=6 animals

Animals groups	Villus High	Crypt depth	Villus/crypt ratio	Thickness wall
C	d**5.82 ± 195.6**	a**3.1± 59.37**	c**0.20 ± 3.45**	b**1.7± 41.0**
T1	a**8.76 ± 257.5**	b**2.7 ± 49.37**	a**36. 0 ± 5.53**	a**2.3± 45.4**
T2	c**8.07 ± 201.8**	ab**4.4 ± 52.50**	b**0.38 ± 4.36**	b**1.3 ± 42.2**
T3	b**7.4 ± 225.6**	ab**3.9 ± 48.12**	a**0.36 ± 5.23**	a**3.1 ± 44.0**

C control group received distill water,T1 sucked from dams received lactobacillus in dose (5 × 108 CFU)for 30dayes then received lactobacillus in dose (5 × 10^8 CFU) during post weaning for 30 days ,T2 sucked from dams received lactobacillus in dose (5 × 10^8 CFU)for 30dayes,T3 received lactobacillus in dose (5 × 10^8 CFU) **(**during post weaning(32days) . The small letter denote significant differences between groups(columns

Intestinal Histomorphometric changes

Analysis of illume sections revealed that Villus height elevated statistically ($p<0.05$)in T1 than T3 than T2 than control as showen in table - . While the results showed significant($p<0.05$) difference in crypt depth, were the lowest value was in T3,T1,T2 groups respectively as compared with C group . Villus height \ crypt depth ratio statistically($p<0.05$) increased in agraduated manner recording the highest ration in T1 then T3 , then T2 . Also the analysis of the imajes from illume sections revealed that the distances ( ) between sub mucosa to serosa of the illume wall were significantly increase ($P<0.05$) in T1 and T3 when compared with T2 and control

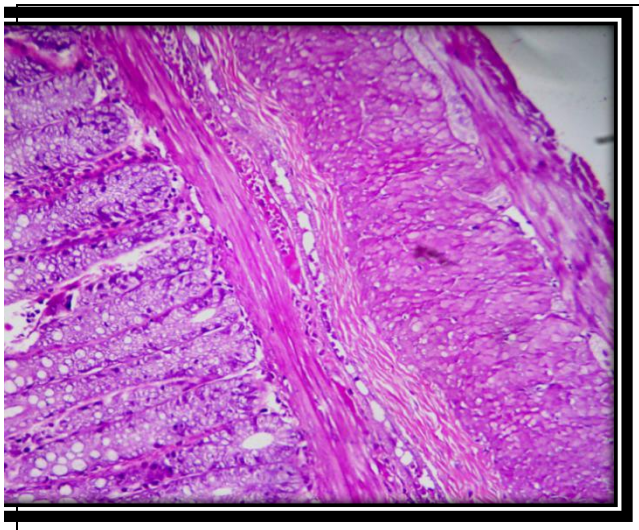

Figure-1 light microscopic photograph of ileum section of T1 show the increase in wall thickness and density of goblet cells.(H&E, 10X)

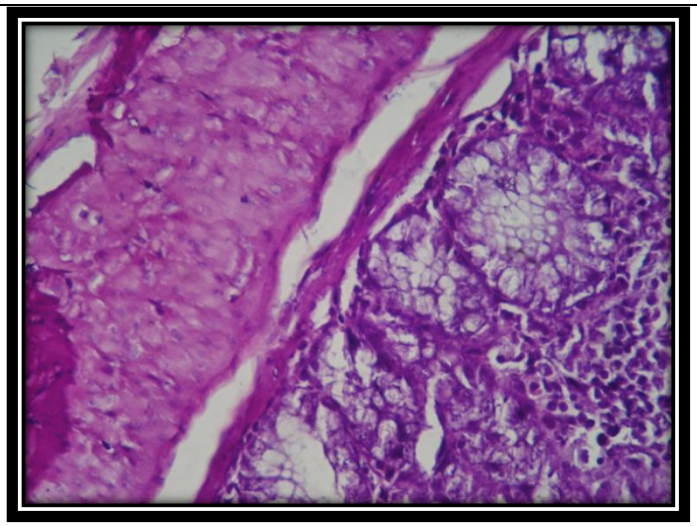

Figure-2 light microscopic photograph of ileum section of T3 show the increase in wall thickness and density of goblet cells.(H&E, 10X)

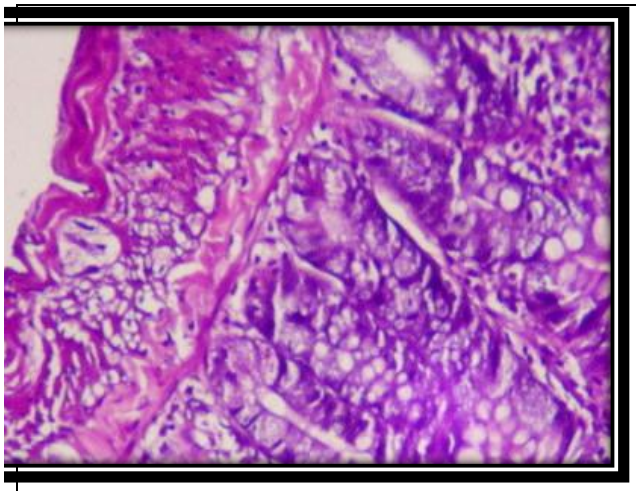

Figure-3 light microscopic photograph of ileum section of T2 show the slight increase in wall thickness and density of goblet cells.(H&E, 10X)

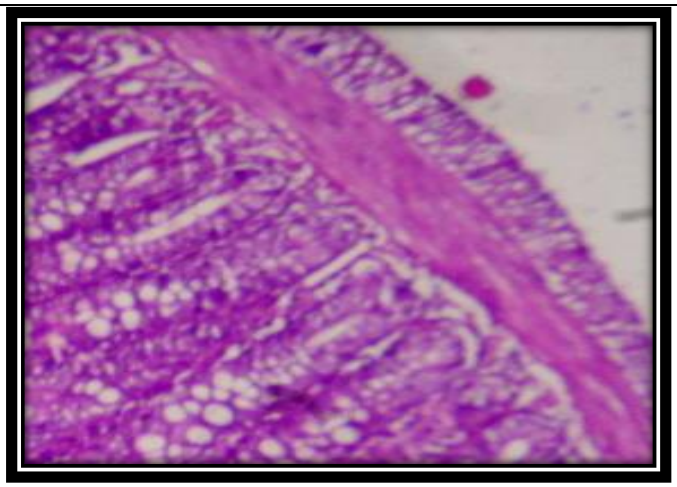

Figure-4 light microscopic photograph of ileum section of C show the normal wall thickness and density of goblet cells.(H&E, 10X)

Density(cellper200 µm2) and surface area (um^2) of goblet cells

Data represented by the figures-5,6 showed effects of *Lactobacillus acidophilus* on density and size of goblet cells in villi of duodenum . Larger goblet cell size were observed for the T1 and T3 group than for the rest of the and control groups. Results recorded that the number of goblet cell(um) was statistically increased ($p<0.05$) in groups T1,T2 and T3 as compared with C group .Goblet cell density in the treated groups ranged from 2 to 6 goblet cells per 200 µm2 villus area in c

ompare with control,1per200 µm2 (table-4) .

Table- 4: Effects of*Lactobacillus Acidophilus* on Density(Cells per 200 µm2 villus area) and Size (µm2) of goblet cells.

Animals groups	No. goblet cell / 200µm2 villus area	Average GC size (µm2)
C	1.81 ± 0.06^{d}	49.9 ± 0.51^{b}
T1	4.13 ± 0.19^{a}	69.4 ± 0.69^{a}
T2	2.15 ± 0.14^{c}	50.2 ± 0.25^{b}
T3	3.09 ± 0.18^{b}	67.9 ± 1.21^{a}

C control group received distill water,T1 sucked from dams received lactobacillus in dose (5×10^8 CFU)for 30dayes then received lactobacillus in dose (5×10^8 CFU) during post weaning for 30 days ,T2 sucked from dams received lactobacillus in dose (5×10^8 CFU)for 30dayes,T3 received lactobacillus in dose (5×10^8 CFU) during post weaning(30days) . The small letter denote significant differences between groups(columns)

Gene expression

All intestine tissue samples that used in the present study gave high concentrations of total RNA and appeared quantitatively and purity enough to proceed in quantitative reverse transcriptase real-time PCR as shown in figure-7. PCR quantification method in Real-Time PCR system was dependent on the values threshold cycle numbers (CT) of amplification plot of target gene and housekeeping gene. As shown in figure-8 there were no differences in CT value between different experimental animals for B-actin, the housekeeping gene. Otherwise CT values for amplification plot for CDX2 gene showed significant ($P<0.05$) up regulation in T1 , T3, and T2 respectively relative to control groups that is equal to 1 fold change of gene expression levels (figure-9) .

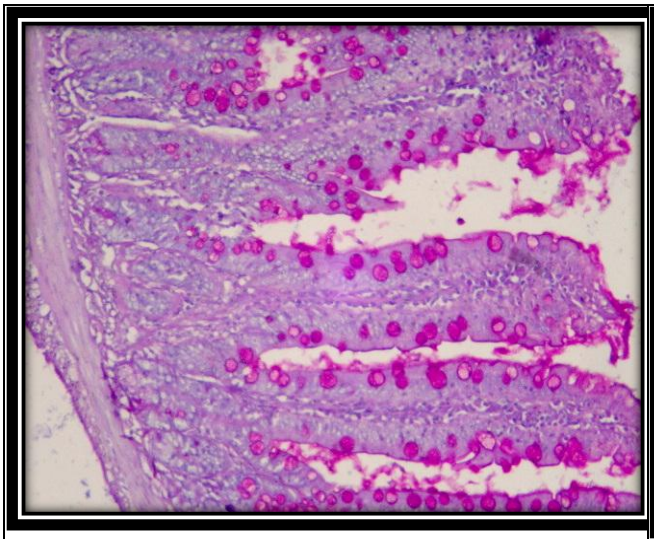

Figure-5. Light microscope photomicrographs of duodenum section of T1 group showing effects of L. acidophilus on distribution and size of goblet cells as PAS-positive cell along villi..400 X stained with PAS

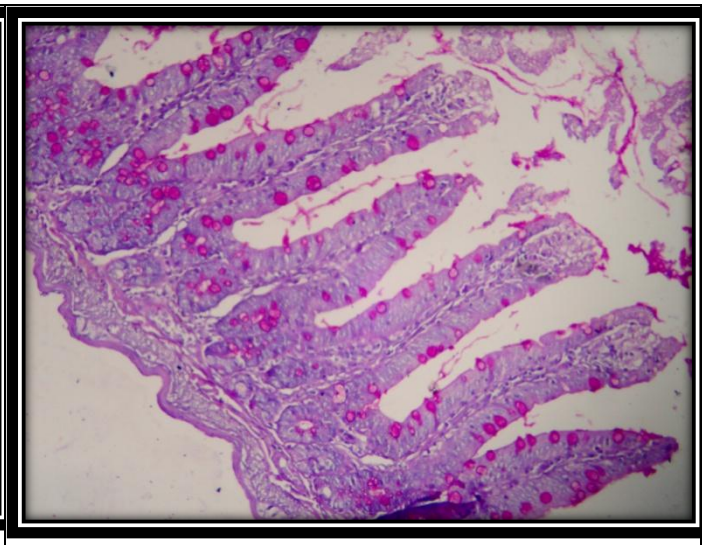

Figure-6. Light microscope photomicrographs of duodenum section of C group showing normal size of goblet cells as PAS-positive cell along villi. 400 X stained PAS

IV. Discussion

This study was based on the hypothesis that the addition of L. acidophilus will alter the intestinal development along with growth leading to changes intestinal morphology and will either increase the intestinal length and weight , which will project more villi numbers and will absorb more food.

In the present study, the administration of L. acidophilus to pups already sucked from mothers had A. bacillus had an increase in the mean of the intestinal weight / body weight and in the intestinal total and portions length rather than those pups who only sucked or only administered L. acidophilus . The denoted increase in the mass of intestines in the present study could be resulted from increase of the thickness of various layer of the bowel wall supported by the histological analysis match by increase in the mucosa ,sub mucosa thickness , such changes mainly contributed to the histological changes in birds(Awad et al., 2009; Fang et al., 2013). As for increasing the length of the intestine denoted in the present study could be suggested the direct interpretation for L. acidophilus on intestine epidermal growth factor (EGF) via activation of EGF- receptors pathway intestine (Chaet et al.,1994; Fang et al., 2013). Further more, the indirectly relaxant effect, of the lactobacillus on intestinal wall mediated by inhibiting prostaglandin biosynthesis may results in decrease in the resting tone of the intestine and therefore the increase in the length of intestine(Anil et al .,2011). The suggested mechanism for the decrease in the prostaglandins synthesis by L. acidophilus administration mediated via increased in the expression of Transforming Growth Factor-ß1(TGF-β1) downregulates COX-2 expression leading to decrease of PGE2 production (Steinberg et al.,2014;Erina et al .,2013).

In the present study the role of L. acidophilus in the evolution of intestinal absorption and secretion performances was very evident through the increase of villus height, and villus height to crypt depth ratio and low crypt depth. The height of villus and the depth of crypt are considered as the indicators of intestinal functions (Castillo et al., 2008) and is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes, and nutrient transport systems (Ijiet al., 2001, Hooge, 2004).These changes could be correlated with direct effects of L.acidophilus on increased epithelial turnover and longer villi are associated with activated cell mitosis (Samanya and Yamauchi ,2002), cell proliferation (Awadet al. (2010; Kim et al., 2011). These results are consistent with the results of the ileum wall thickness as highlighted by th e histomorphological analysis representitave by increased area of the mucosa , submucosa and muscle . Feeding probiotcs ,specialy LBA or food enhance intestinal LAB growth associated with increase of small intestine wall thickness(Felicianoet al., 2010; DahyunHwangaet al .,2014). Other possible mechanism for the wall thickness increase may be explained by the action of probiotics in increased blood supply to intestinal wall, where the intestinal microbiota contribute to the development of the capillary network in the small intestinal villi(Thaddeus et al .,2002). In vivo and in vitro studies focused on the advantages of administration of Lactobacillus acidophilus in improvement the morphological and physiological performance of intestinal mucosasuchas villus high and the number of goblet cells via events induce changes in intestinal mucosal cells proliferation, differentiation, and enzymes activity (Miremadi and Shah, 2012).

In our experiment, there was a tendency for an increase in goblet cells density and single cell size when pups were either sucked and /or fed L. acidophilus . Probiotics, such as Lactobacillus, adhere to the epithelial layer, there is an up regulation of mucin genes that are found in the GI goblet cells, which further enhances the mucus barrier (Mack et al. 2003). Goblet cell size and density was affected by treatment, with larger and greater numbers of goblet cells observed in all treatment groups in comparison to the control groups. Enlarged goblet cells indicate an increase in mucus storage in the goblet cells (Smirnovet al., 2005).

The proliferation and differentiation of the intestinal epithelium occurs in a sequential and spatially organized manner and is highly regulated at the transcriptional level. The CDX2 gene regulates the expression of MUC2 which is present in the cytoplasm of goblet cells of the small intestine and colon, CDX2 have been reported to up-regulate MUC2 transcription (Yamamoto et al., 2003; Ikedaet al., 2007; Gopal et al 2014).The intestine-specific transcription factors Cdx1 and Cdx2 are candidate genes for directing intestinal development, differentiation, and maintenance of the intestinal phenotype, therefore CDX2 expression is indicative of intestinal differentiation along the crypt-villus axis((Sliberget al .,2000).In the present study the analysis of the RT-qPCRout come confirmed the existence of an increase in CDX2 factor production which suggest a direct interaction of L. acidophilus with intestinal epithelial cells gene expression

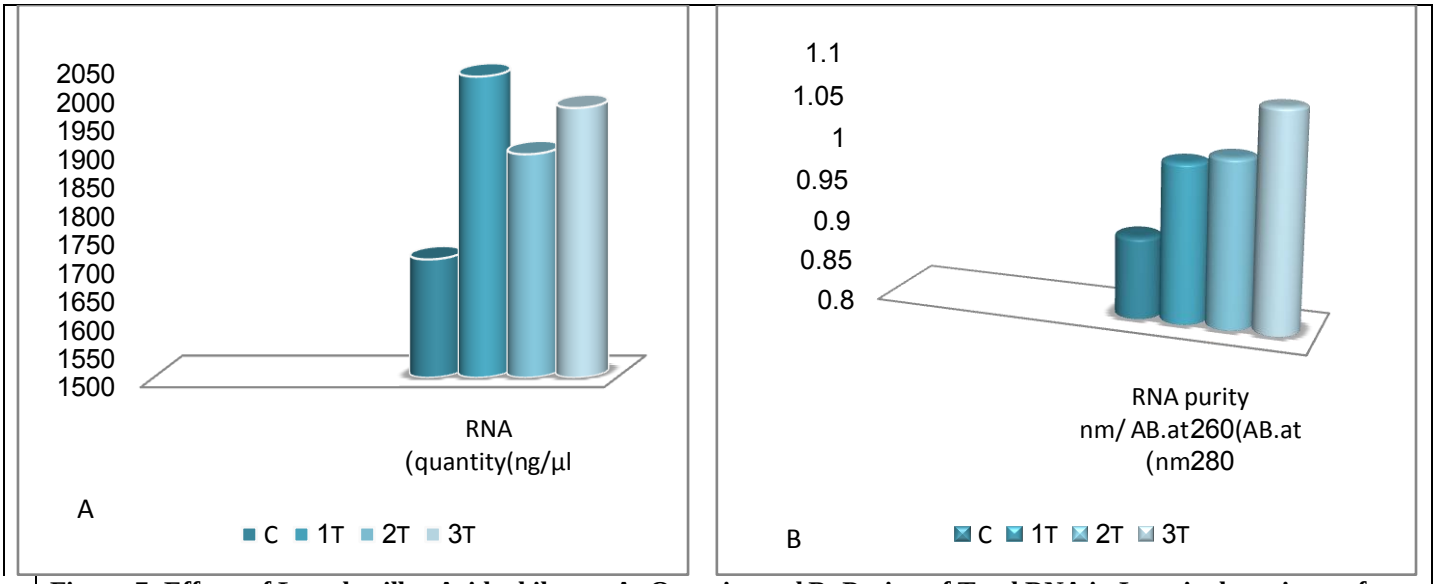

Figure-7: Effects of Lactobacillus Acidophilus on A- Quantity and B- Purity of Total RNA in Intestinal specimen of growing Rats ,Mean ±SE n=6 animals.

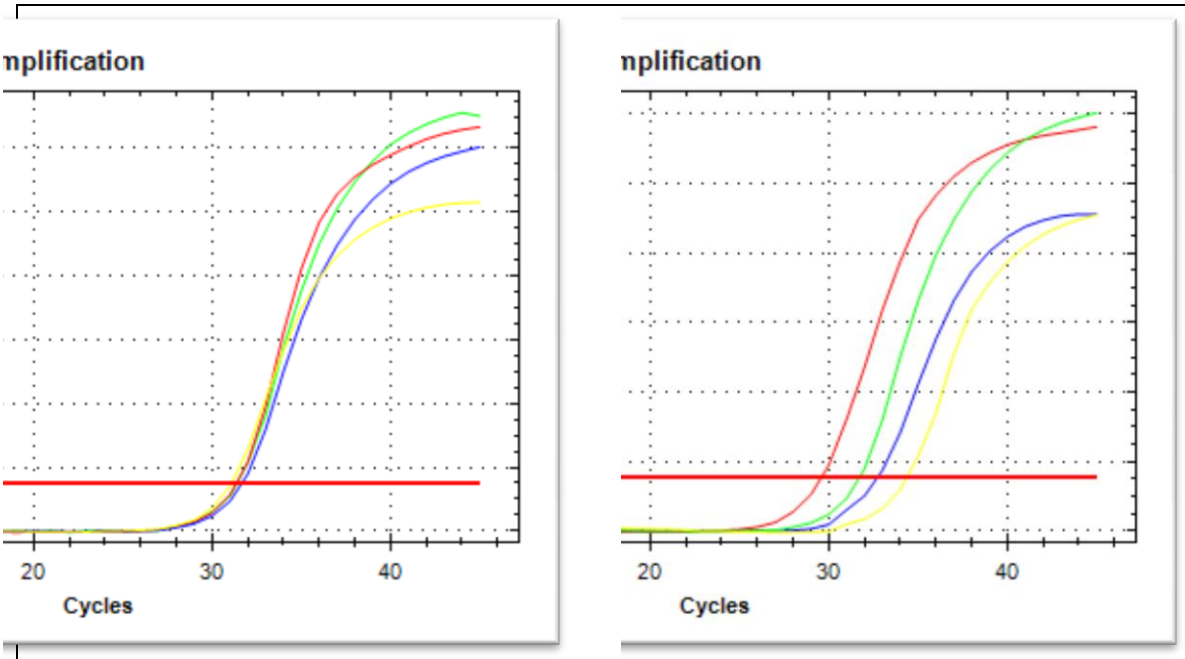

Figure-8: Amplification plot CDX2 gene of treated and control groups .
C control group received distill water//,T1 sucked from dams received lactobacillus in dose (5×10^8 CFU)for 30dayes then received lactobacillus in dose (5×10^8 CFU) during post weaning for 30 days //,T2 sucked from dams received lactobacillus in dose (5×10^8 CFU)for 30dayes//T3 received lactobacillus in dose (5×10^8 CFU) during post weaning(30days).

Intestinal microflora affect the function of the digestive system ultimate can change gene expression in the intestinal mucosa , gut microbiota affecting patterns of the expression of many genes in the human or mouse intestinal tract, including genes involved in nutrient absorption ,energy metabolism, immunity, and intestinal barrier function (Larsson, et al .,2012). In the same vein, the molecular mechanisms of the microbiota interaction with intestinal cells found to be directly via activation of TLR pathways mediated mechanisms (Julio et al., 2014), other wise via activation of ERK1/2 pathway resulting in crease the protein as well as mRNA levels of DRA protein levels in the colon in mice(Anoop et al .,2014). L.acidophilus posses strong induction of IL-12, IFN-β , (Weiss et al ., 2010). Other researcher found an indirect mechanisms via MEK/ERK and PI3K pathways in intestinal epithelial cells.Takuyaetal .,(2011) demonstrated that in inflammatory bowel disease , the elevated IL-6 increase in CDX2 transcription . Also IL-6 induced an increase in the mRNA level of Cdx2. Members of the CDX family of transcription factors have been linked to enterocyte lineage specification (Freund et al., 1998), CDX2 is an intestinal specific transcription factor which is essential for the intestinal development by regulating expression of some intestinal genes transcript specific enterocyts function such asSucrase-isomaltase, Lactase-phlorizin hydrolase, Calbindin-D9K, and Hephaestin genes(Suh,1996,Troelsen, 1997, Colnot 1998, Hinoi ,2005)

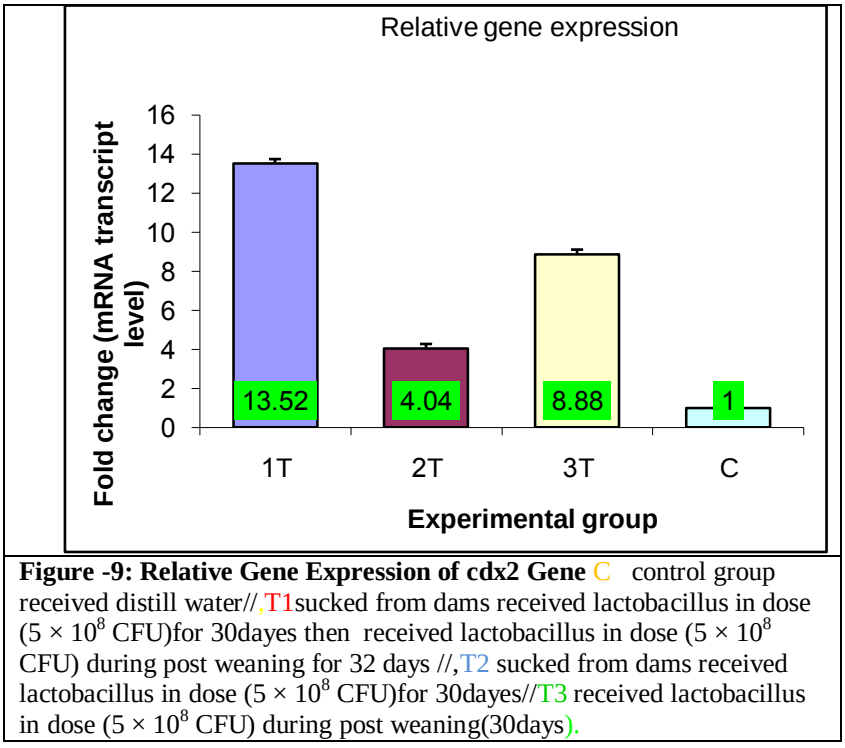

Figure -9: Relative Gene Expression of cdx2 Gene C control group received distill water//,T1sucked from dams received lactobacillus in dose (5×10^8 CFU)for 30dayes then received lactobacillus in dose (5×10^8 CFU) during post weaning for 32 days //,T2 sucked from dams received lactobacillus in dose (5×10^8 CFU)for 30dayes//T3 received lactobacillus in dose (5×10^8 CFU) during post weaning(30days).

As Lactobacilli and other lactic acid bacteria seem to be important components of the breast milk microbiota,Martineet al.,(2007)found that Lactobacillus group were found in breast milk where studies done by Rinneet al .,(2005) demonstrated that LAB transmitted with breast milk during lactation. Thomas et al.,(2009) reported that Lactobacillus treatment to pregnant and lactating mothers and their offspring inhibit spathogenic bacteria without inhibiting normal residents of the gastrointestinal tract. The mechanical transmission for Lactobacillus acidophilus to breast milk via dendritic cells (DCs) ,where the suggest study that Probiotic bacteria reachthe mammary gland through the entero-mammary pathway. It ha s been demonstrated that (DCs) can penetrate the gut epithelium to take up non-pathogenic bacteria from the gut lumen.(Martinet al .,2012). The bacteria inside the DCs or macrophages, could spread to other locations via mucosal associated lymphoid system or bloodcirculation to colonize distant mucosal surfaces such as respiratorytract and genitourinary tracts, salivary and lachrymal glands andmost significantly to the mammary gland (Roitt.,2001).

In conclusion when reviewing all the positive effects of L..acidophilus on intestinal epithelial physiology and morphology in different experimental growing rats of the present study , we found that there were a significant differences between groups in CDX2 expression and intestinal histomorphological changes and the high grad results were in pups that had sucked and received L.acidophilus. The clearly up regulated CDX2 expreesion concrescence with increase of cells proliferation as increase in vilus high and more goblet cells differentiation in intestinal epithelial highlight the direct interaction of L. acidophilus with host cells, in addition the differences in these changes among different experimental groups suggest that L.acidophilus produce accumulative effects and successfully interact with intestinal mucosa proliferation and differentiation in different life periods.

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