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Inflection in nutrient transporter genes leads to potential changes in small intestine histomorphology with improved nutrient retention in chicken under dietary synbiotic supplementation

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Abstract

This study investigated the role of synbiotic supplementation on gut histomorphology, expression of nutrient transporters, and nutrient utilization in broiler chicken. Bacitracin methylene disalicylate (BMD), Probiotic *Lactobacillus acidophilus* (LBA), and prebiotic Mannan-oligosaccharides (MOS) were used to formulate total 6 dietary treatments viz. T1 (control; basal diet), T2 (BMD @ 20 mg/kg diet), T3/T4 (0.1% MOS with 10^6 and 10^7 cfu LBA/g diet), and T5/T6 (0.2% MOS with 10^6 and 10^7 cfu LBA/g diet). A total of 288 day old chicken were allocated at random among six treatments, each with six replicates of eight chicken (48 birds/treatment). Results revealed higher villus height, crypt depth, villus height: crypt depth ratio, and histological surface magnification ratio values in chicken fed a combination of MOS (0.2%) and LBA (10^6 or 10^7 cfu/g diet). BMD supplemented birds had higher values of these parameters compared to control birds. The villus width, villus bottom area, and mucosal unit bottom area were all increased in birds supplemented with BMD, but were similar to birds fed a combination of MOS (0.1 percent) and LBA (107 cfu/g diet). The villus width, villus bottom area, and mucosal unit bottom area were higher in BMD supplemented birds which were similar to the birds fed a combination of MOS (0.1%) and LBA (10^7 cfu/g diet). The birds fed a combination of MOS (0.2%) and LBA (10^6 or 10^7 cfu/g diet) revealed upregulation of SGLT1 and GLUT5 expression in jejunum but no significant effect was observed on the expression of PePT1 and EAAT3 gene. The AME_{Diet}, nitrogen, phosphorus, and calcium retentions were higher in birds fed a combination of MOS (0.2%) and LBA (10^6 or 10^7 cfu/g diet). However, the organic matter digestibility was higher in birds fed a combination of MOS (0.2%) and LBA (10^6 cfu/g diet) and BMD supplementation also improved the organic matter utilization compared to control. In conclusion, the supplementation of a combination of MOS (0.2%) and LBA (10^6 or 10^7 cfu/g diet) improves the intestinal architecture along with upregulation of SGLT1 and GLUT5 nutrient transporters and increase in nutrient digestibility in broiler chicken.

1. Introduction

Synbiotics refers to dietary supplements which positively influence the host by enhancing the mucosal surface attachment and survival of probiotics in gastrointestinal tract (GIT), hence improving host health (Gaggia et al., 2010). Dietary synbiotics benefit broiler chicken because of the enhancement of the growth and number of probiotics by the prebiotics in the environment of high temperature, oxygen, and low pH (Alloui et al. 2013). The development of new dietary synbiotic formulas is a continuous process which are mainly focussing on their functional benefits such as resistance to GIT infection, and enhancement of immunity in broiler chicken (Ghahri et al. 2013). However, the synergism/interaction between mannan-oligosaccharides (MOS) and probiotics (*Lactobacillus* and *Bifidobacterium*) is of particular interest. On the other hand, the advancement in molecular biotechnology field provides us the more opportunity to examine the interaction between gene expression and diet. Study of the effects of nutrients/bioactive food ingredients on the gene expression of an individual is called as nutrigenomics. In other words, nutrigenomics is the bridge that helps in

understanding the correlation between diet, health, and productivity of animal at genomic level. The nutrient absorption in the GIT lumen is influenced by digestion process and uptake by nutrient transporters. It has been reported that the synbiotic supplementation in chicken feed revealed improvement in the villi height (VH), villi width (VW), VH: crypt depth (CD) ratio, and total intestinal weight of broiler chicken (Kocher and Tucker 2005; Sharifi et al. 2012; Salim et al. 2013) which may have resulted in improved gut health, productivity, and immunity of birds.

The dietary supplementation of synbiotics positively alter the availability of nutrients which affect the nutrient transporter genes. Thus, the expression pattern of nutrient transporter genes can change in response to dietary supplements which potentially affects gut health along with improved nutrient uptake and assimilation. In the small intestines of chicken glucose transporter – GLUT5 mediates the uptake of fructose molecules across the membrane by facilitated diffusion (Gilbert et al. 2007) and is strongly influenced by dietary, developmental, hormonal, and circadian factors (Douard et al. 2008). Another Na+-dependent transporter termed SGLT1 transports glucose and galactose in the cell (Wright and Turk 2004). Furthermore, enterocytes absorb the breakdown products of protein digestion in the form of di/tri-peptides via the hydrogen ion-dependent peptide transporter 1 (PepT1) (Chen et al. 2002; Daniel et al. 2004). The neutral and cationic amino acids are effectively transported by PePT1 at a slightly acidic intestinal pH (Chen et al. 2002; Steel et al. 1997). However, the anionic amino acids, such as aspartic acid and glutamic acid, are transported by excitatory amino acid transporter 3 (EAAT3) (Kanai and Hedier 2004) which is predominantly expressed in the distal sections of the intestine (Iwanaga et al. 2005). The purpose of this study was to see how dietary synbiotics affected the gene expression profile of nutrient transporters in the chicken jejunum and the consequent alterations in the gut histology and nutrient absorption.

2. Materials and Methods

2.1 Dietary supplements: Probiotics, prebiotic, and antibiotic-BMD

ALPHARMA Company (New Jersey, USA) provided the antibiotic bacitracin methylene disalicylate (BMD) with 44% bacitracin activity. Kothari-Fermentation and Biochem Ltd. India supplied the the prebiotic Mannan-oligosaccharides (MOS). Probiotic *Lactobacillus acidophilus* (LBA: UBLA-34 MTCC 5401) was supplied by Unique Biotech Ltd. Hyderabad, India. It was of human faecal origin and characterised by whole-genome sequencing (DDBJ/ENA/GenBank; accession number: RBHY00000000) with no potential virulence factors, antibiotic-resistant genes, or plasmid. Furthermore, the Gram-positive LBA employed in this investigation had a water activity of one or less. Pathogens such as *Staphylococcus*, *E. coli*, *Salmonella*, and *Pseudomonas* were not found in the 10 g LBA powder, and there was no yeast mould count in LBA product.

2.2 Experimental birds and housing

From the experimental hatchery of the institute 288-day-old chicken of CARIBRO Vishal commercial strain were procured on uniform body weight basis. The trial lasted for six weeks (42 days) and the birds were kept in battery brooder cages randomly in groups of eight birds with equal male and female birds. The space provided to birds was 0.75 ft^2/bird and 24 hours of light was provided for first three days and then decreased by one hour each day until an 18-hour light period, that continued till 42 days.

2.3 Experimental diets and design

To meet the demand of all the necessary nutrients for broiler chicken, three iso-nitrogenous and iso-caloric basal diets were prepared as pre-starter, starter, and finisher diets (BIS 2007). The synbiotics (*Lactobacillus acidophilus* + MOS) were supplemented to the basal diets. The ingredients of basal diet used in the experiment and the nutrient composition are mentioned in Table 1. Total six experimental diets (treatments) were formed and each of the experimental diets were assigned six groups of birds with eight birds in each (48 birds/treatment) (Table 2). In a preliminary trial, the different levels of dietary supplementation of LBA, BMD, and MOS used in this investigation were standardised. In this study, birds were offered a weighed amount of respective diets *ad libitum* on a daily basis, and fresh water was always available to them. In this experiment, birds were given a weighed amount of feed *ad libitum* on daily basis, and fresh water as well.

2.4 Histology of jejunum

Jejunum samples were taken from six birds per treatment at the end of the experiment, and two cross-sections were made on the glass slide for each sample of jejunum. Approximately 2 cm length tissue section of the jejunum was dissected aseptically and fixed with 10% neutral buffered formalin after trimming and cleaning. Furthermore, following the standardised procedures tissue samples were subjected to sectioning by rotary microtome, mounting on clean glass slides, and staining by haematoxylin-eosin dye. Each slide was observed under light microscope fitted with a camera and an image analysis software (Motic Inverted microscope, Hongkong). The measurements evaluated were VH, VW, CD, and VH:CD ratio. Furthermore, the villus surface area (VSA), villus bottom area (VBA), mucosal unit bottom area (MUBA), and histological surface magnification ratio (M) of jejunum architecture were calculated as follows (Kisielinski et al. 2002):

$$VBA = \pi.r^2 = \pi \times (Villus\ width/2)^2$$

$$VSA = 2.\pi.r.h + 2\pi.r^2 = \pi.(Villus\ length\text{-}Villus\ width)$$

$$MUBA = \pi.R^2 = \pi.(r+a)^2$$

$= \pi. [(Villus\ width/2) + (crypt\ width/2)]^2$

$M = (VSA + UBA - VBA)/ MUBA)$

The description of villus dimensions of jejunum has been shown in Fig 1.

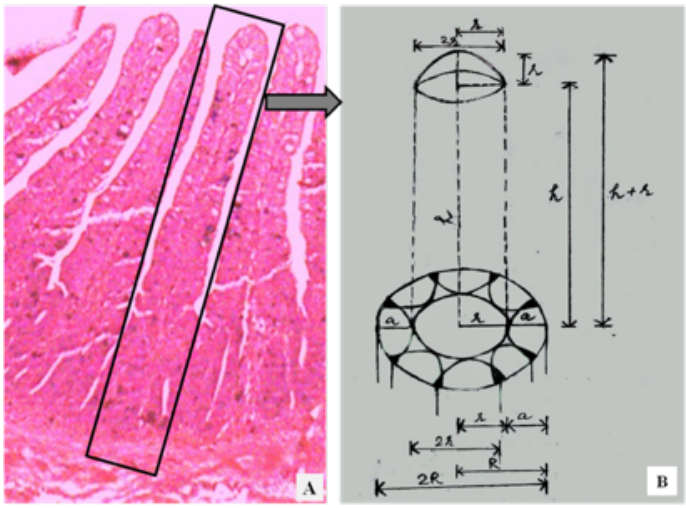

Figure 1 Measurement of small intestinal morphology at 42 days old age broiler chicken.
A: Showing histomorphology of jejunum
B: Geometrical representation of mucosal unit and the measurement of various parameters villus length (h + r), villus width (2r), crypt width (2a), r = radius of villus, a = radius of crypt, R = a+r = radius of mucosal unit

Table 2 Experimental diets and design of the study

Treatment	Diet	Replicates*	Total birds
T1	Basal diet: Control	6	48
T2	T1+ BMD @20mg/ Kg feed	6	48
T3	T1+ MOS @ 0.1% + *LBA* @ 10^6cfu/g	6	48
T4	T1+ MOS @ 0.1% + *LBA* @ 10^7cfu/g	6	48
T5	T1+ MOS @ 0.2% + *LBA* @ 10^6cfu/g	6	48
T6	T1+ MOS @ 0.2% + *LBA* @ 10^7cfu/g	6	48
Total		36	288

*Eight birds/replicate
BMD: Bacitracin methylene disalicylate
MOS: Mannan-oligosaccharides
LBA: *Lactobacillus acidophilus*

Table 1 Ingredients and nutrient composition of broiler chicken diets

Ingredients (g/Kg)	Prestarter (0-7 days)	Starter (8-21 days)	Finisher (22-42 days)
Maize	443	460	505
Soyabean	410	380	342
Rapeseed meal	30	30	30
Fish meal	50	50	30
Vegetable oil	42	55	65
Limestone	6.0	6.0	7.0
Di-calcium Phosphate	13.5	13.6	15.5
Salt	3.0	3.0	3.0
DL-Methionine	0.2	0.2	0.2
Trace Mineral premix[1]	1.0	1.0	1.0
Vitamin premix[2]	1.5	1.5	1.5
Vitamin B complex[3]	0.15	0.15	0.15
Choline chloride	0.50	0.50	0.50
Nutrient composition of diets (Analysed)			
Crude protein	231	220	200
M Energy (Kcal/ kg)	3001	3101	3200
Calcium	10.0	10.0	10.0
Available phosphorus	4.9	4.8	4.6
Lysine	13.3	12.1	10.6
Methionine	5.0	5.0	4.6

[1] Trace mineral mixture (100 g): $FeSO_4.7H_2O$ 8 g, $ZnSO_4.7H_2O$ 10 g, $MnSO_4.H_2O$ 10 g, $CUSO_4.5H_2O$ 1 g, KI 30 g
[2] Vitamin premix (1 g): Vitamin A 82.5 IU, Vitamin E 50% 160 mg, Vitamin D3 12000unit, Vitamin K 10 mg
[3] Vitamin B complex (1 g): Vitamin B1 8 mg, Vitamin B2 50 mg, Vitamin B6 16 mg, Vitamin B12 80 mcg, Niacin 120 mg, Calcium panthotheonate 80 mg, L-lysine 10 mg, and DL- Methionine 10 mg

2.5 Nutrient metabolism study

A metabolism trial for was conducted at 35th day of experimental period using six birds per treatment and birds were shifted to individual cages for three days. At 35th day feeding trail the birds were starved for the 2 hours (09 to 11 AM) for the evacuation of their gut of previous faecal matter. After that clean feeders and faecal trays were provided filled with weighed quantity of respective feed and fresh drinking water, respectively. The dropping of respective dietary group of each bird was collected separately once every day and transferred into pre weighed fresh aluminium dishes, weighed again to note fresh weight of faeces, and then placed in the hot-air oven at 60 °C through all the 3 days of collection. On the last day, the feeders were removed at 11 AM to estimate the net feed intake (FI), and the faecal trays were removed after 2 hours and excreta collection was done as usual. The faeces collected were dried for 4-5 days in oven at 60°C till a constant

Table 3 Primer sequence pairs used for the study of nutrient transporter expression

Gene [a]	Primer sequence (5`→3`)	Product size (bp)	Annealing temperature (^{O}C)	Gene Bank Accession number
SGLT1	F-TGTCTCTCTGGCAAGAAGTC R-TGTAAACCATGTAGTTCAGATCGA	71	59.4 ^{0}C	XM_415247
GLUT5	F-TTGCTGGCTTTGGGTTGTG R-GGAGGTTGAGGGCCAAAGTC	60	59.8 ^{0}C	XM_417596
Pep T1	F-CCCCTGAGGAGGATCACTT R-CAAAAGAGCAGCAGCAACGA	66	58.6 ^{0}C	NM_204365
EAAT3	F-TGCTGCTTTGGATTCCAGT R-AGCAATGACTGTAGTGCAGAAGTAATATAG	79	60 ^{0}C	NM_424930
SGLT1	F-TGTCTCTCTGGCAAGAAGTC R-TGTAAACCATGTAGTTCAGATCGA	71	59.4 ^{0}C	XM_415247
GLUT5	F-TTGCTGGCTTTGGGTTGTG R-GGAGGTTGAGGGCCAAAGTC	60	59.8 ^{0}C	XM_417596
Pep T1	F-CCCCTGAGGAGGATCACTT R-CAAAAGAGCAGCAGCAACGA	66	58.6 ^{0}C	NM_204365
EAAT3	F-TGCTGCTTTGGATTCCAGT R-AGCAATGACTGTAGTGCAGAAGTAATATAG	79	60 ^{0}C	NM_424930

[a]SGLT1: Na^{+}-D-glucose cotransporter 1; GLUT5: Fructose transporter; EEAT3: Excitatory amino acid transporter-3; Pep T1: H^{+}- dependent oligopeptide transporter

weight was reached that represented the net excreta output. The representative samples of dried test feed and excreta were properly ground and analysed for nitrogen, phosphorous (AOAC 1990), and calcium (Talpatra et al. 1940). The retention of nitrogen, phosphorus, calcium, total ash, and organic matter were expressed as percentage of intake. Gross energy (GE) of the dietary samples was estimated by using the adiabatic bomb calorimeter which was standardised with benzoic acid and the apparent metabolizable energy of diet (AME_{Diet}) was calculated as follows:

$$AME_{Diet}\ (Kcal/g) = [(Feed\ intake \times GE) - (Excreta\ weight \times GE)]/ Feed\ intake$$

2.6 Expression analysis of nutrient transporter genes

The expression pattern of nutrient transport genes in chicken intestine (jejunum) under different dietary treatments 42 days of age were investigated, using quantitative real time polymerase chain reaction (qRT-PCR). For normalization β-actin was used as housekeeping gene.

2.7 Sample collection, RNA extraction, and cDNA synthesis

A total of 1 g of tissue samples were obtained aseptically from six birds per treatment. The samples were placed immediately in labelled DEPC-treated and autoclaved 2 ml micro-centrifuge tubes containing 600 µl of RNA later solution. The tissue samples were then stored at -20° C until processed for RNA extraction. The tissue samples were homogenized to disrupt the cells using automated tissue homogenizer (Polytron). The RNA isolation from the samples was done by Trizol method. The air-dried sample RNA pellets were resuspended in 50 µl NFW and stored at -20^{o}C until complementary DNA (cDNA) synthesis. The characterization for of RNA samples purity as well the quantity was done by nanodrop-1000 (Thermo Scientific, Singapore) followed by purity checked using agarose gel electrophoresis. Using the RT-PCR, the first strand cDNA synthesis was done by taking the extracted RNA samples as template. The synthesis of cDNA was done with Revert Aid™ first strand cDNA synthesis kit (MBI, Fermentas, Hanover, MD, USA) using random hexamer primers and protocol was followed as described in Kit. The cDNA served as a template for the PCR amplification of genes in each sample. The size of PCR products was verified by comparison with GeneRuler™100 and 50 bp DNA ladder.

2.8 Gene expression of nutrient transporters

Table 3 lists the oligonucleotide primer sequences used to investigate the expression profile of nutrient transport genes. The cDNAs were amplified, and the relative expression of target gene mRNAs was measured using the IQ5 Cycler system (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. The results of gene amplification were expressed in Ct values and normalised against a reference gene β-actin, and fold expressions were calculated using the $^{\Delta\Delta}$CT method as follows (Pfaffl et al. 2002):

$$^{\Delta}Ct = Ct\ (target\ gene) - Ct\ (reference\ gene)$$

The $^{\Delta}$Ct values were then normalized using control group to obtain the $^{\Delta\Delta}$Ct.

$$^{\Delta\Delta}Ct = {}^{\Delta}Ct\ (experimental\ sample) - {}^{\Delta}Ct\ (control\ sample)$$

The relative quantity (in terms of fold change in expression) of

target genes were estimated by using the following formula:

Relative quantity (fold change in expression) = 2-ΔΔC

2.9 Statistical analysis

The data acquired in this study were analysed using the one-way ANOVA method by the GLM process of IBM-SPSS software-20, following a completely randomised design. Each sample bird was treated as an independent experimental unit. The Tukey post-hoc analysis was used to differentiate the significant mean differences between the treatments at a significance level of P<0.05.

3. Results

3.1 Histology of jejunum

This study revealed significant (P <0.05) dietary effects of LBA and MOS supplementation on the Jejunum histomorphology of birds (Table 4; Fig 2). A progressive increase in VH, CD, VH:CD ratio, and histological surface magnification ratio (M) was observed from T1 to T6, however, T5 was statistically similar to T6. When compared to other dietary treatments that did not differ substantially, the VW, VBA, and MUBA were considerably higher in T2 group birds, followed by statistically similar T4 group birds. The VSA was significantly (P <0.05) higher in T4 and was found statistically similar to treatment T2, T5, and T6 group.

3.2 Nutrient utilization

The effects of dietary LBA and MOS, as a synbiotic, supplementation on apparent nutrient utilization in broiler are shown in Table 5. The AME $_{Diet}$, nitrogen, phosphorus, and calcium retention values revealed an increasing (P<0.05) trend from T1 to T6 group with no significant differences between T5 and T6. Similarly, the retention of organic matter increased significantly (P <0.05) from T1 to T5 and then again decreased in treatment group T6, such that T6 was significantly different from T5 but statistically similar to T4. No significant differences were observed in total ash retention.

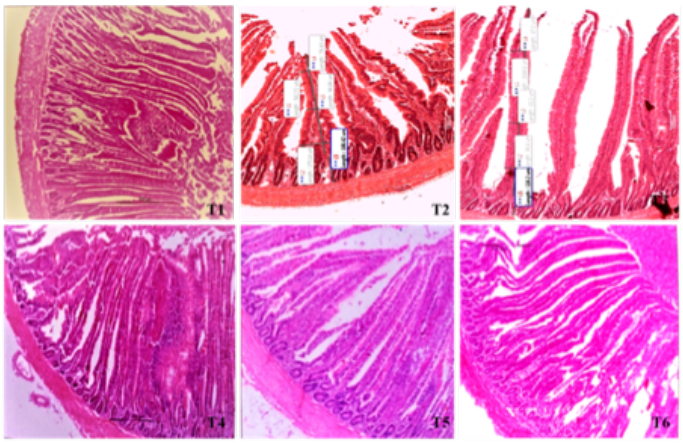

Figure 2 Small intestinal morphology (Jejunum part) of broiler chicken at 42 days of age under MOS and LBA supplementation

3.3 Nutrient transporter gene expression pattern in jejunum

The current study examined the effect of LBA and MOS supplementation on expression pattern of nutrient transporter related genes - SGLT1, GLUT5, PepT1, and EAAT3 in broiler chicken. The dietary treatments significantly (P<0.05) up-regulated the expression of the SGLT1 and GLUT5 genes at 21 days of age (Fig 3), whereas no effect was observed on the expression patterns of PepT1 and EAAT3. Higher (P <0.05) expression was observed in T5 and T6 treatment group followed by T4 and T3 compared to T1 and T2. But, the expression levels of T2 were found significantly higher compa-

Table 4 Intestinal histomorphology of broiler chicken at 42 days of age under the influence of MOS and LBA[1]

Parameters	Treatments						SEM	*P- value*
	T1	T2	T3	T4	T5	T6		
Villus height (VH)	1120^{a}	1288^{b}	1353^{c}	1395^{d}	1457^{e}	1451^{e}	19.9	< 0.05
Villus width (VW)	101.2^{a}	111.4^{c}	104.7^{ab}	108.9^{bc}	101.8^{a}	103.3^{a}	0.92	< 0.05
Crypt depth (CD)	216.5^{a}	229.3^{b}	238.5^{c}	244.7^{d}	249.9^{d}	247.5^{d}	2.10	< 0.05
VH/CD ratio	5.18^{a}	5.62^{b}	5.67^{b}	5.70^{b}	5.83^{c}	5.87^{c}	0.041	< 0.05
VSA	0.36^{a}	0.45^{bc}	0.44^{b}	0.48^{c}	0.47^{bc}	0.47^{bc}	0.008	< 0.05
VBA	0.008^{a}	0.010^{c}	0.009^{ab}	0.009^{bc}	0.008^{a}	0.009^{ab}	0.000	< 0.05
MUBA	0.020^{a}	0.023^{c}	0.021^{ab}	0.022^{bc}	0.020^{a}	0.021^{ab}	0.000	< 0.05
M	17.52^{a}	19.77^{b}	20.93^{c}	21.51^{d}	22.93^{e}	22.61^{e}	0.31	< 0.05

LBA: *Lactobacillus acidophilus*; MOS: mannan-oligosaccharides; BMD: Bacitracin methylene disalicylate; VSA: Villus surface area; VBA: Villus bottom area; MUBA: Mucosal unit bottom area; M: Histological surface magnification ratio. Measurement unit: µm

$^{a\ to\ c}$ Within a row in table, mean values bearing the different-superscripts differ significantly (P<0.05).

[1] Data is the mean of 6 birds/treatment

Table 5 Nutrient utilization of broiler chicken at 5th week of age under the influence of MOS and LBA[1]

Parameters	Treatments						SEM	*P- value*
	T1	T2	T3	T4	T5	T6		
AME_{Diet} (MJ/kg)	12.20[a]	12.44[a]	13.19[ab]	13.47[b]	14.01[c]	13.99[c]	0.724	< 0.05
N retention (%)	71.64[a]	71.96[a]	73.65[b]	73.97[b]	74.37[bc]	74.33[c]	1.145	< 0.05
P retention (%)	29.26[a]	30.17[b]	30.94[c]	31.87[c]	32.56[d]	32.45[cd]	0.684	< 0.05
Ca retention (%)	31.95[a]	32.45[a]	34.57[b]	34.45[b]	36.01[c]	35.92[c]	2.115	< 0.05
Total ash (%)	28.71	29.08	30.71	31.15	31.20	31.24	0.611	>0.05
Organic Matter (%)	66.52[a]	67.41[b]	68.57[b]	68.95[bc]	69.38[d]	70.35[c]	1.128	< 0.05

BMD: Bacitracin methylene disalicylate, MOS: Mannan-oligosaccharides, LBA: *Lactobacillus acidophilus,* AME: Apparent metabolisable energy; CP; Crude protein; P: Phosphorus; Ca: Calcium; SEM: Standard error of mean
[1] Data is the mean of 6 birds/treatment

-red to T1. Whereas, no significant differences observed between T5 and T6 group. Similarly, at 42 days of age the expression profile of SGLT1 and GLUT5 revealed significant (P <0.05) treatment effects, whereas, no (P>0.05) effects were observed on the PepT1 and EAAT3 expression (Fig 4). The SGLT1 expression increased progressively from T1 to T5 and then again decreased in T6. But, expression in T6 group was found statistically similar to both T4 and T5 groups. The expression of GLUT5 was (P <0.05) higher in treatment T5 and T6 followed by T2, T3, and T4 groups compared to T1. But the differences between T2, T3, and T4 and between T5 and T6 groups were non-significant.

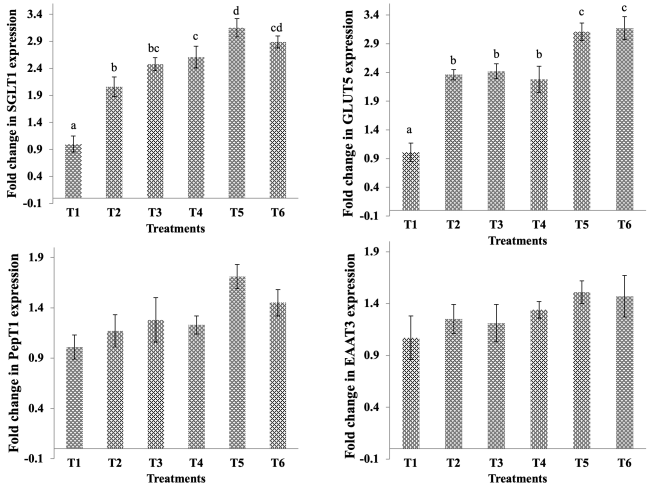

Figure 3 Nutrient transporter (SGLT1, GLUT5, PepT1, and EAAT3) gene expression at 21 days old chicken under the influence of MOS and LBA

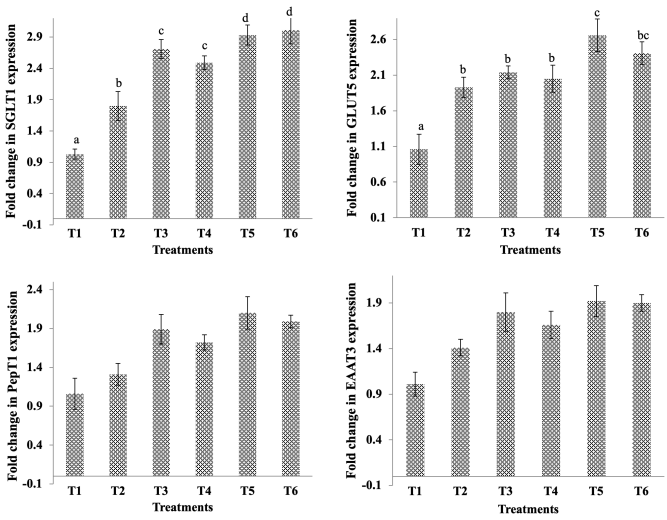

Figure 4 Nutrient transporter (SGLT1, GLUT5, PepT1, and EAAT3) gene expression at 42 days old chicken under the influence of MOS and LBA

4. Discussion

The important aspects of gut health include maintaining a healthy gut bacteria population, a chemical environment favourable to enzymatic digestion, and maintaining a normal morphology of the intestinal epithelium. Improved surface area for nutrient absorption has a positive impact on gut morphology (Kocher and Tucker 2005). Furthermore, adding prebiotics, probiotics, or synbiotics to chicken diets as a functional feed supplement improves the intestinal health and productivity of broiler chickens. In the present study, higher VH, CD, VH:CD ratio, and M values were observed in birds supplemented with a combination of MOS (0.2%) and LBA (10^6 or 10^7 cfu/g diet), whereas, in BMD supplemented birds these parameters had higher values compared to control birds. The BMD supplemented birds has higher values of VW, VBA, and MUBA, but similar to the birds fed a combination of MOS (0.1%) and LBA (10^7 cfu/g diet). On similar lines the thesupplementation of MOS and other commercial direct fed microbial additives in chicken diet improved VH, VW, and total intestine weight, VH: CD ratio compared to control or virginiamycin treatments (Kocher and Tucker 2005; Sharifi et

al. 2012; Salim et al. 2013). Antibiotic (bacitracin) supplementation decreased the small intestine length and weight in broiler chicken (Miles et al. 2006; Wang et al. 2016), whereas, the probiotic supplementation increased total small intestine length in chicken (Wang et al. 2016). Various previous studies have also reported that the dietary synbiotics improve the small intestinal histomorphology of chickens compared to BMD supplemented and control birds (Agboola et al. 2014; Vineetha et al. 2017). Larger intestinal villi could result in more absorptive surface, mucosal glands, and enzyme synthesis, all of which would improve digestion and nutritional absorption (Mohan et al. 1996). The potentially enhanced mucosal cell turnover in the intestinal epithelium of broiler chicken as a result of synbiotics feeding may have helped to preserve the mucosal integrity in the small intestine, avoiding harmful bacteria invasion (Fan et al. 1997; Begum et al. 2021). Also, the effects of dietary probiotic on villi morphology are dependent on the species of supplemented probiotic micro-organism (Vineetha et al. 2017). Furthermore, it has been depicted that the dietary fiber (such as oligosaccharides) supplementation improve feed efficiency as a result of improved villi architecture and associated glands in the jejunum part of chicken intestine (Gourbeyre et al. 2011).

The effectiveness of nutrient absorption is determined by the mucosal surface area of small intestine and the functional features of certain nutrient transporters found in the brush border epithelium (Amat et al. 1996). The nutrient transporters – GLTU5, SGLT1, PepT1, and EAAT3 are presented in the GIT of broiler chicken (Wright and Turk 2004). The current study revealed upregulation of SGLT1 and GLUT5 expression in chicken jejunum but the expression of PePT1 and EAAT3 was not influenced in response of dietary supplementation of a combination of MOS (0.2%) and LBA (10^6 or 10^7 cfu/g diet). These findings support recent research by Wang et al. (2016) and Biswas et al. (2020), who found that in broiler chicken synbiotic enhanced ($p<0.5$) the relative fold expression of nutrient transport genes. The changes in the nutrient transport expression have affected the nutrient assimilation process in this study. The supplementation of a combination of MOS (0.2%) and LBA (10^6 or 10^7 cfu/g diet) resulted in higher AME_{Diet}, nitrogen, phosphorus, and calcium retentions in birds. No effect of BMD supplementation was observed. However, the organic matter digestibility was higher in birds fed a combination of MOS (0.2%) and LBA (10^6 cfu/g diet) and BMD supplementation also improved the organic matter utilization compared to control. Corroborating the results of present study, the synbiotic supplementation in feed improved the nutrient utilization digestibility in broiler chicken (Yun et al. 2017; Nisar et al. 2021). Improved dry matter and crude protein digestibility have been observed in chicken supplemented with probiotics of different combinations (Shim et al. 2010; Kim et al. 2012). The supplementation of probiotics chicken diets increased nutrient utilization (Mountzouris et al. 2010) whereas, MOS supplementation in broiler chicken improved starch and protein digestibility (Yang et al. 2008). The probiotic supplementation has been reported to increase the beneficial microbial population in the gut which improves intestinal barrier function (Mountzouris et al. 2010) and oligosaccharide supplementation improves the gut health of broiler chicken (Tuohy et al. 2003). The digestive and absorptive activities of the gut in broiler chickens are improved by increasing the absorption surface, brush border enzymes, and nutrient transport expression (Awad et al. 2009).

5. Conclusion

This study revealed that the broiler chicken fed a combination of MOS (0.2%) and LBA (10^6 or 10^7 cfu/g diet) improves the intestinal architecture along with upregulation of SGLT1 and GLUT5 nutrient transporters. Whereas, a corresponding increase in the nutrient digestibility was also observed in broiler chicken compared to control group.

Declarations

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Conflicts of interest: None

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