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Liquid Metabolite of *Lactobacillus plantarum* and Putrescine Effects on Growth, Tissue Polyamine, Blood Lipids and Intestine Morphology of Broiler Chickens

Research Article

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ABSTRACT

This experiment aimed to investigate the effects of liquid metabolite (LM) produced by *Lactobacillus plantarum* and dietary putrescine (PUT) on growth, blood lipids, villus height (VH), crypt depth (CD) and polyamines (PAs) content of intestinal tissue and ileal digesta in chickens. Six treatments, replicated six time each, were factorial arrangements of two levels of LM (0 and 0.3%) and three levels of dietary putrescine (0, 0.03 and 0.05%). Growth performance and PAs content of digesta and excreta (at 21 d) were measured, as well as small intestine VH and CD. Blood cholesterol, triglyceride and glucose were measured at 24, 33 and 40th d. Putrescine (0.05%) negatively affected body weight, feed conversion ratio and protein and energy efficiency ratio while increased duodenal VH significantly ($P<0.05$) as compared to the 0.03% putrescine. Aging was effective on blood cholesterol, triglyceride and glucose. Blood triglyceride decreased by 0.03% putrescine ($P<0.05$). Faecal spermidine was increased significantly ($P<0.05$) by 0.05% putrescine. Duodenal PAs declined in the chickens fed LM, whereas dietary putrescine had no effects on intestinal tissue polyamine. In conclusion, luminal PAs content was not affected by treatments. LM influenced intestinal tissue PAs but had no effects on growth and ileal digesta polyamine content. However, putrescine (0.05%) was harmful to the growth but increased duodenal VH.

KEY WORDS blood lipids, intestinal morphology, luminal polyamines, microbial metabolite, putrescine.

INTRODUCTION

The effects of probiotics and prebiotic are mediated by microbial metabolites such as short chain fatty acids, lactate, polyamines (PAs) and bacitracin (Choudhari *et al*. 2008). It is shown that production of PAs by probiotic microbes boost intestinal enzyme expression (Buts *et al*. 1994) and some lactobacillus fermented food like soy yogurt augments the PAs concentration of intestinal mucosa in rats (Takagi *et al*. 2010). In contrast, it is reported that feeding a probiotic mixture to rats caused a significant decrease in colonic PAs levels, ornithine decarboxylase (ODC, a key enzyme in PAs biosynthesis) activity and cell proliferation as compared with controls (Linsalata *et al*. 2005; Singh *et al*. 1997). This information implies that there is a connection between PAs and probiotics. Probiotic supernatant called as liquid metabolite (LM) produced by *L. plantarum* possess probiotic properties (Foo *et al*. 2005; Thanh *et al*. 2009; Thu *et al*. 2011b) and is effective on improvement of luminal mucosal growth, villous height (VH) and growth performance in piglets (Thu *et al*. 2011a). While improvement of growth performance and VH due to probiotics and

LM have been documented (Bradley *et al*. 1994; Thanh *et al*. 2009), the lack of positive effects of probiotics and LM are also reported (Loh *et al*. 2009; Mutus *et al*. 2006).

PAs including putrescine (PUT), spermidine (SPD) and spermine (SPM) are biological active compounds involving in cell proliferation process in normal and malignant cells (Larque *et al*. 2007; Pegg and McCann, 1982). PAs are important growth promoters of six species of lactobacilli (Guirard and Snell, 1964) and are clearly acting as luminal mucosal growth promoter (Loser *et al*. 1999). In pre-ruminant calves (Grant *et al*. 1989), neonatal pigs (Grant *et al*. 1990) and chickens (Hashemi, 2013) the dietary supplemental PUT improved the VH and crypt depth (CD).

Current evidences don't support strongly whether the effects of probiotics and LM on growth and intestinal morphology are mediated by biological compounds, like PAs or not. Therefore, the association between LM and the concentration of luminal and dietary PAs needs to be further explored.

Reduction of plasma cholesterol (Chol) and triglyceride (TG) by probiotics and microbial metabolite have been documented earlier (Foo *et al*. 2003a; Ignatova *et al*. 2009; Loh *et al*. 2009; Loh *et al*. 2013). Chol reducing effects of probiotics is still controversial since there are reports revealing that administration of probiotics do not influence blood Chol and TG profiles (Hatakka *et al*. 2008; Simons *et al*. 2006). Additionally, PUT is able to decrease serum glucose (Glu), TG and Chol concentrations because of controlled insulin secretion through protective role of PAs over the pancreatic beta-cell (Mendez and Hernandez, 2005).

The objective of the current study was to determine the effects of dietary LM and PUT on PAs concentration of ileal digesta and intestinal tissue, as well as growth performance, intestinal morphology and blood Chol, TG and Glu in chicken.

MATERIALS AND METHODS

Experimental birds and diet

Total number of 216 wing banded day old male chicks (Cobb 500) was allocated to dietary treatments with three PUT levels (0, 0.03 and 0.05%) and two LM levels (0 and 0.3%) with factorial arrangements. The treatments included T1) control diet with no PUT and LM supplementation, T2) control diet with 0.03% PUT supplementation, T3) control diet with 0.05% PUT supplementation, T4) control diet with 0.3% LM supplementation, T5) control diet with 0.03% PUT and 0.3% LM supplementation and T6) control diet with 0.05% PUT and 0.3% LM supplementation. Dietary treatments (LM and PUT) were added to a basal diet and each treatment replicated 6 times, containing 6 chicks per replicate.

All experimental diets were formulated iso-caloric and iso-nitrogenous as shown in Table 1. LM was produced according to the procedures as described previously (Foo *et al*. 2003b) and added to the diets by spraying on the complete feed while mixing. Analysed concentration of PAs in mixed diets is shown in Table 2. No SPM and SPD were detected. Pure PUT was purchased from MERCK Inc. (CAS number 333-93-7, Darmstadt, Germany). The chickens were fed *ad libitum* with experimental diets for 42 days. This study was conducted in accordance with the ethical standards of the Ethics Committee of Animal Utilization of the University Putra Malaysia (UPM).

Growth performance

Individual body weight (BW) and group feed intake (FI) were measured weekly and mortality of the chicken was recorded daily. Other parameters such as feed conversion ratio (FCR), protein efficiency ratio (PER), energy efficiency ratio (EER) and body weight gain (BWG) were then calculated accordingly. FCR was calculated as daily feed intake per daily BWG. PER and EER were calculated as daily BWG (g) per daily protein intake (g) and daily BWG (g) per daily 4184 J energy intake, respectively.

Villous height and crypt depth:

At the age of 21 days, six chickens per treatment were chosen randomly and slaughtered for samples of small intestine segments and ileum digesta. The content of ileum up to ileocecal junction was collected into a small container. The samples were then kept frozen at -20 ˚C for future polyamine analysis. Whole intestine flushed with distilled water properly. Samples of small intestine were taken from 3 parts including: 1) the middle part of the duodenal loop, 2) midway between the end point of duodenal loop and Meckel's diverticulum (jejunum) and 3) midway between the Meckel's diverticulum and the ileocecal junction (ileum). Intestinal tissue samples were kept frozen at -20 ˚C for future polyamine analysis.

In order to measuring VH and CD, intestine samples were longitudinally excised and dehydrated for 16 h in a tissue processing machine (Leica ASP 3000, Japan) followed by embedding in paraffin. Using a microtome (Leica RM 2155, Japan), embedded samples was cut with 4 µm thick and then fixed on the glass slides and dried at 57 ˚C. Haematoxylin and eosin were used for staining, thereafter mounted with cover slips. The VH was determined by measuring the distance between the crypt mouth and the tip of villous. The CD was measured based on the distance between the basement membrane and the mouth of crypt using microscopes and read by PC Life Science Olympus software (Olympus Soft Imaging Solutions) (Thanh *et al*. 2009).

Table 1 Experimental feeds composition for starter and grower period with different LM and PUT supplementation

Ingredients %		Starter					
		T1	T2	T3	T4	T5	T6
Corn		47.38	47.35	47.33	46.68	46.65	46.63
Soybean meal		42	42	42	42.2	42.2	42.2
Palm oil		6.5	6.5	6.5	6.7	6.7	6.7
Wheat pollard		0	0	0	0	0	0
L-lysine		0.05	0.05	0.05	0.05	0.05	0.05
DL-methionine		0.19	0.19	0.19	0.19	0.19	0.19
Mono calcium phosphate		1.9	1.9	1.9	1.9	1.9	1.9
$CaCO_3$		1.6	1.6	1.6	1.6	1.6	1.6
Salt		0.28	0.28	0.28	0.28	0.28	0.28
Vitamin permix[1]		0.05	0.05	0.05	0.05	0.05	0.05
Mineral permix[2]		0.05	0.05	0.05	0.05	0.05	0.05
LM		0	0	0	0.3	0.3	0.3
PUT		0	0.03	0.05	0	0.03	0.05
Calculated nutrients							
Metabolizable energy	MJ/kg	13.25	13.25	13.25	13.25	13.25	13.25
Crude protein	%	22	22	22	22	22	22
Ether extract	%	8.4	8.4	8.4	8.4	8.4	8.6
Fiber	%	4.12	4.12	4.12	4.12	4.12	4.12
Calcium	%	1	1	1	1	1	1
Total P	%	0.814	0.814	0.814	0.814	0.814	0.813
Avail. Phos.	%	0.45	0.45	0.45	0.45	0.45	0.45
Arg	%	1.57	1.57	1.57	1.57	1.57	1.57
Lys	%	1.32	1.32	1.32	1.32	1.32	1.33
Met + Cys	%	0.89	0.89	0.89	0.89	0.89	0.89
Met	%	0.54	0.54	0.54	0.54	0.54	0.54
Threonine	%	0.87	0.87	0.87	0.87	0.87	0.87
Tryptophan	%	0.29	0.29	0.29	0.29	0.29	0.29
PUT	%	0.06	0.09	0.11	0.06	0.09	0.11
Ingredients %		**Grower**					
		T1	T2	T3	T4	T5	T6
Corn		49.7	49.67	49.65	49.2	49.17	49.15
Soybean meal		29.5	29.5	29.5	29.5	29.5	29.5
Palm oil		6.5	6.5	6.5	6.7	6.7	6.7
Wheat pollard		10.3	10.3	10.3	10.3	10.3	10.3
L-lysine		0	0	0	0	0	0
DL-methionine		0.11	0.11	0.11	0.11	0.11	0.11
Mono calcium phosphate		1.8	1.8	1.8	1.8	1.8	1.8
$CaCO_3$		1.7	1.7	1.7	1.7	1.7	1.7
Salt		0.29	0.29	0.29	0.29	0.29	0.29
Vitamin permix		0.05	0.05	0.05	0.05	0.05	0.05
Mineral permix		0.05	0.05	0.05	0.05	0.05	0.05
LM		0	0	0	0.3	0.3	0.3
PUT		0	0.03	0.05	0	0.03	0.05
Calculated nutrients							
Metabolizable energy	MJ/kg	13.11	13.11	13.11	13.11	13.11	13.11
Crude protein	%	18	18	18	18	18	18
Ether extract	%	8.4	8.4	8.4	8.4	8.4	8.63
Fiber	%	4.1	4.1	4.1	4.1	4.1	4.0
Calcium	%	1	1	1	1	1	1
Total P	%	0.812	0.812	0.812	0.812	0.812	0.81
Avail. Phos.	%	0.45	0.45	0.45	0.45	0.45	0.45
Arg	%	1.17	1.17	1.17	1.17	1.17	1.17
Lys	%	1	1	1	1	1	1
Met + Cys	%	0.7	0.7	0.7	0.7	0.7	0.7
Met	%	0.4	0.4	0.4	0.4	0.4	0.4
Threonine	%	0.66	0.66	0.66	0.66	0.66	0.66
Tryptophan	%	0.22	0.22	0.22	0.22	0.22	0.22
PUT	%	0.06	0.09	0.11	0.06	0.09	0.11

[1] Supplied per kilogram of diet: vitamin A: 1500 IU; vitamin E: 10 IU; Cholecalciferol: 200 IU; Riboflavin: 305 mg; Pantothenic acid: 10 mg; Niacin: 30 mg; Cobalamin: 10 µg; Choline chloride: 1000 mg; Biotin: 0.15 mg; Folic acid: 0.5 mg; Thiamine: 1.5 mg and Pyridoxine: 3.0 mg.

[2] Supplied per kilogram of diet: Copper: 8 mg; Selenium: 0.15 mg; Iron: 80 mg; Zinc: 40 mg; Manganese: 60 mg and Iodine: 0.18 mg.

LM: liquid metabolite; PUT: putrescine (putrescine content of corn and soybean meal samples were analyzed (in average PUT content of corn=1 mg/g dry matter and soybean meal=0.3 mg/g dry matter)).

Table 2 Analyzed polyamines content of experimental mixed diets in grower period

Diet[1]	Putrescine (mg/g)	Spermidine (ng/g)	Spermine (ng/g)
T1	0.45	ND[2]	ND
T2	0.57	ND	ND
T3	1.33	ND	ND
T4	0.53	ND	ND
T5	0.78	ND	ND
T6	0.96	ND	ND

[1] T1, T2 and T3 contain 0% liquid metabolite (LM), supplemented with 0, 0.03 and 0.05% putrescine (PUT), respectively and T4, T5 and T6 contain 0.3% LM, supplemented with 0, 0.03 and 0.05% PUT, respectively.
ND: not detectable.

Blood samples

Six blood samples per treatment were collected at the ages of 24, 33 and 40 days in order to study the blood cholesterol (Chol), triglyceride (TG) and glucose (Glu). Six the blood parameters were measured using spectrophotometer and commercial diagnostic kits (RANDOX Laboratory Ltd, UK) based on an enzymatic method.

Polyamine analysis of excreta, digesta, feed and intestinal tissue

Fresh droppings of excreta were collected at 21 and 42 days of age and kept frozen at -20 ˚C. At the age of 42 days, 6 birds per treatment were slaughtered for small intestine samples. Samples of intestine, excreta and digesta (collected at 21th day) were subjected for PAs analysis. PAs concentration was measured by HPLC method (Hwang *et al*. 1997). Pure PAs was purchased from MERK Inc. and the standard solution of PAs (1000, 750, 500 and 250 ppm) was prepared by dissolving determined amount of pure PUT, SPM and SPD in 0.1 *M* HCl. Intestine tissue samples were grounded and dried excreta, digesta and feed samples were mashed finely. PAs content of samples were extracted by homogenizing with 20 mL of 6% trichloroacetic acid (TCA) and 1 *M* $HClO_4$ (1:1) for 3 min. The homogenate was centrifuged (8000 g, 10 min, 4 ˚C) and filtered through Whatman No. 2 filter paper. The filtrate was placed in a volumetric flask and made up to 50 mL. Extracts and standard PAs solutions subjected to benzoylation by benzoyl chloride. The final product was extracted by diethyl ether, evaporated and dissolved in methanol. 20 µL aliquots were then injected into HPLC for analysis. Agilent HP1100 system (Agilent Technologies Inc) and Nova-Pak HR C18 reversed-phase column (5 µm, 300×3.9 mm waters, W21141A 005) was used for the analyses of PAs.

Statistical analysis

Data were statistically analyzed with the GLM procedure of SAS software (SAS, 1999). The statistical model used was:

$$Y_{ijk} = \mu + P_i + LM_j + PLM_{ij} + e_{ijk}$$

Where:

Y_{ijk}: response variables observed from each replicate or individual birds.
µ: overall mean.
P_i: effect of dietary PUT.
LM_j: effect of dietary liquid metabolite.
PLM_{ij}: effect due to interactions between dietary PUT and LM.
e_{ijk}: statistical error.

Duncan multiple range test were used for comparison between means. The normality test was performed in each of data set.

Non-normalized data (mortality and PAs concentration) data were analyzed using Chi-Square and Kruskal-Wallis tests.

RESULTS AND DISCUSSION

Performance data

Growth performance of broilers at starter (0-21 days), grower (21_42 days) and whole period is shown in Table 3. At starter period, 0.05% PUT negatively affected BWG, FCR, PER and EER significantly (P<0.05). For the whole period, 0.05% PUT decreased PER and EER significantly (P<0.05).

LM had no effects (P>0.05) on the performance in any stages of rearing. No interaction effects (P>0.05) between LM and PUT were observed.

Villous height and crypt depth

Table 4 shows the effects of LM and PUT on VH and CD at the age of 21 days. 0.05% PUT increased duodenal VH and decreased duodenal CD significantly (P< 0.05) as compared with the 0.03% PUT significantly (P<0.05). Duodenal VH in 0% LM and 0.05% PUT was higher than the control (no LM, no PUT). 0.03% PUT increased duodenal CD significantly (P<0.05), whereas 0.05% PUT was not effective. LM decreased jejunum CD significantly. There was significant (P<0.05) interaction between LM and PUT on jejunum CD. The highest jejunum CD was found in control. However, the lowest was observed in 0.03% LM and 0% PUT.

Table 3 Effects of liquid metabolite (LM) and putrescine (PUT) on broiler performance at different ages

Items			BWG (g)	BWG (g/b/d)[1]	FI (g/b/d)	FCR	EER[2]	PER[2]	Mortality (%)
	LM (%)	0.00	674.28	30.98	50.86	1.65	0.19	2.74	6.67
		0.30	705.33	33.07	52.21	1.59	0.20	2.84	3.33
	PUT (%)	0.00	723.40^{a}	34.44^{a}	52.19	1.55^{b}	0.20^{a}	2.89^{a}	5.00
		0.03	693.80^{ab}	33.03^{ab}	51.54	1.61^{ab}	0.19^{ab}	2.79^{ab}	5.00
		0.05	652.20^{b}	31.05^{b}	50.89	1.68^{a}	0.18^{b}	2.68^{b}	5.00
0-21 d	N		170	170	36	36	36	36	36
	Pooled SEM		10.01	0.47	0.80	0.02	0.002	0.04	-
	P-value								
	LM		NS	NS	NS	NS	NS	NS	NS
	PUT		*	NS	NS	*	*	*	NS
	LM × PUT		NS	NS	NS	NS	NS	NS	-
	LM (%)	0.00	1469.78	69.37	130.38	1.88	0.15	2.12	1.39
		0.30	1355.94	65.21	125.87	1.94	0.14	2.07	9.28
	PUT (%)	0.00	1477.31	70.34	134.27	1.93	0.15	2.18^{a}	4.17
		0.03	1405.80	66.94	127.51	1.89	0.14	2.03^{b}	2.08
		0.05	1355.47	64.54	122.58	1.92	0.14	2.05^{b}	9.75
21-42 d	N		128	128	36	36	36	36	36
	Pooled SEM		26.02	1.23	2.30	0.02	0.002	0.03	-
	P-value								
	LM		NS	NS	NS	NS	NS	NS	NS
	PUT		NS	NS	NS	NS	NS	*	NS
	LM × PUT		NS	NS	NS	NS	NS	NS	-
	LM (%)	0.00	2180.42	47.22	84.51	1.79	0.17	2.35	7.78
		0.30	2102.47	46.65	82.97	1.78	0.17	2.37	10.00
	PUT (%)	0.00	2229.59	49.19	87.07	1.77	0.17^{a}	2.45^{a}	8.33
		0.03	2135.33	47.12	83.86	1.78	0.16^{b}	2.32^{b}	6.67
		0.05	2059.42	44.48	80.30	1.81	0.16^{b}	2.30^{b}	11.67
0-42 d	N		128	128	36	36	36	36	36
	Pooled SEM		29.52	0.70	1.37	0.01	0.001	0.02	-
	P-value								
	LM		0.06	NS	NS	NS	NS	NS	NS
	PUT		NS	NS	NS	NS	*	**	NS
	LM × PUT		NS	NS	NS	NS	NS	NS	-

[1] g/b/d: gram/bird/day.
[2] EER= daily BWG (g)/daily 4184 J energy intake and PER= daily BWG (g)/daily protein intake (g).
BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; EER: energy efficiency ratio and PER: protein efficiency ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05) and ** (P<0.01).
NS: non significant.
SEM: standard error of the means.

Blood component

Table 5 shows the blood lipids and glucose changes in different ages of chicken. Blood Chol, TG and Glu were significantly (P<0.05) affected by age. The blood Chol and TG for 40 days of age chicken were significantly (P<0.05) lower than 33 days. In contrast, blood Glu was significantly lower (P<0.05) at 33 days than 40 days of age.

LM and PUT effects on blood Chol, TG and Glu are shown in Table 6. LM supplementation had no significant (P>0.05) effect on blood components in any ages. However, 0.03% and 0.05% PUT supplementation significantly (P<0.05) decreased blood TG and increased blood Glu in 33 days old chicks.

Polyamines analysis

No changes were found on excreta PAs due to dietary LM, but at the age of 42 days, fecal SPD increased significantly (P<0.05) in 0.05% dietary PUT (Table 7).

None of the PAs concentration of ileal digesta (P>0.05) were affected by dietary LM and PUT (Table 8). In the meantime, the chicks received LM had significant lower (P<0.05) duodenal tissue SPD and SPM as compared with the non-supplemented birds (Table 9). Dietary PUT at any level had no effect (P>0.05) on intestinal tissue PAs content. In this study, no effect of LM was observed on overall performance. This could be due to the single strain of metabolite (TL1) used in current study.

Table 4 Effects of liquid metabolite (LM) and putrescine (PUT) on intestinal villous height (µm) and crypt depth (µm) at the age of 21 days

Items		Duodenum		Jejunum		Ileum	
Main effects		VH	CD	VH	CD	VH	CD
LM (%)	0	1472	156	890	182^{a}	532	124
	0.3	1558	154	836	126^{b}	594	111
PUT (%)	0	1487^{ab}	148^{b}	1003	173	617	124
	0.03	1364^{b}	178^{a}	782	160	568	118
	0.05	1692^{a}	138^{b}	805	131	505	110
Interaction							
LM	PUT						
0	0	1275^{c}	164	1085	235^{a}	540	135
	0.03	1308^{c}	163	860	176^{ab}	523	123
	0.05	1833^{a}	140	725	136^{bc}	532	113
0.3	0	1700^{ab}	133	920	110^{c}	693	113
	0.03	1420^{bc}	193	703	143^{bc}	612	113
	0.05	1552^{abc}	136	885	125^{bc}	478	106
N		36	36	36	36	36	36
Pooled SEM		62.17	6.49	48.32	11.40	40.05	5.26
P-value							
LM		NS	NS	NS	*	NS	NS
PUT		*	*	NS	NS	NS	NS
LM × PUT		*	NS	NS	*	NS	NS

VH: villus height and CD: crypt depth.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
NS: non significant.
SEM: standard error of the means.

Table 5 Effects of age on blood cholesterol, triglyceride and glucose

Items		Cholesterol (mg/dL)	Triglyceride (mg/dL)	Glucose (mg/dL)
Age (day)	24	135^{ab}	103^{b}	185^{a}
	33	161^{a}	146^{a}	82^{c}
	40	104^{b}	63^{c}	124^{b}
N		63	63	63
Pooled SEM		8.30	6.91	5.86
P-value		*	*	*

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
NS: non significant.
SEM: standard error of the means.

This result is in agreement with other finding as only one strain of metabolites applied in rats (Foo *et al*. 2003b). However, using multi strain of lactic acid bacteria (LAB) metabolite resulted in better performance (Thanh *et al*. 2009). Despite of the significant function of the PAs in regular physiology and potential association with abnormal conditions (Pegg, 1986), it is reported that PAs decrease feed efficiency in poultry (Brugh and Wilson, 1986; Stuart *et al*. 1986). Moreover, in particular situations like methionine deficiency, dietary PAs can depress body growth (Hashemi *et al*. 2014). Biogenic amines and PAs have the capability to change metabolism synergistically (Bjeldanes *et al*. 1978; Lyons *et al*. 1983), suggesting that the total level of dietary amines are more important than the level of any single amine.

Luminal nutrients stimulate gut mucosal growth (Jenkins and Thompson, 1994). However, it is stated that (Bradley *et al*. 1994) no positive effects of *Saccharomyces cerevisiae* on VH were found in chickens.

Controversial effects of probiotics on VH and CD implies that there must be many available biological components affecting intestine morphology and luminal system is so diverse. In contrast, current results showed that PUT supplementation positively affected duodenal VH and CD. PAs are required for cell division processes in intestinal tissue (Johnson and McCormack, 1999), so, it is concluded that dietary PAs are beneficial for mucosal growth of small intestinal tissue. LM increases duodenal VH (Loh *et al*. 2010; Thanh *et al*. 2009; Thu *et al*. 2011a), which is contradicted with our result. This is mainly due to single strain of LM used in current study compared to multi strain of LM in other studies. Nevertheless, the interaction between LM and PUT on duodenal VH was significant. Chicks received 0.3% LM with no supplemental PUT and those chicks received 0.05% PUT with no LM fortification had greater duodenal VH as compared to control (0% LM and 0% PUT). These results shows the duodenal VH was not benefited from the feeding of LM and PUT together.

Table 6 Effects of liquid metabolite (LM) and putrescine (PUT) on blood cholesterol, triglyceride and glucose at different ages

Items			Cholesterol (mg/dL)	Triglyceride (mg/dL)	Glucose (mg/dL)
24 days	LM (%)	0	138	116	183
		0.3	132	91	186
	PUT (%)	0	133	110	187
		0.03	155	85	185
		0.05	116	115	182
33 days	LM (%)	0	181	152	79
		0.3	140	140	83
	PUT (%)	0	198	194[a]	60[b]
		0.03	138	122[b]	87[a]
		0.05	145	123[b]	96[a]
40 days	LM (%)	0	99	68	129
		0.3	110	58	117
	PUT (%)	0	106	65	124
		0.03	94	53	127
		0.05	112	71	119
N			63	63	63
Pooled SEM			8.3	6.91	5.86
P-value					
LM			NS	NS	NS
PUT			NS	**	*
LM × PUT			NS	NS	NS

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05) and ** (P<0.01).
NS: non significant.
SEM: standard error of the means.

Table 7 Effects of liquid metabolite (LM) and putrescine (PUT) on polyamine content of excreta at the ages of 21 and 42 days[1]

Items		PUT (m g/g DM)	Spermidine (nano g/g DM)	Spermine (nano g/g DM)
21 days				
LM (%)	0.00	3.62	236.41	0.00
	0.30	5.73	149.77	0.00
P-value		NS	NS	NS
PUT (%)	0.00	3.62	129.21	0.00
	0.03	3.69	175.23	0.00
	0.05	6.68	274.82	0.00
P-value		0.08	NS	NS
42 days				
LM (%)	0.00	5.37	496.28	4420.62
	0.30	4.09	579.76	9234.14
P-value		NS	NS	NS
PUT (%)	0.00	3.24	336.14[b]	12730.54
	0.03	3.88	578.14[ab]	2161.31
	0.05	7.07	699.79[a]	5590.29
P-value		NS	*	NS

[1] The zero value of PAs content means "not detectable". Because of non normal distribution of polyamine values, Kruskal Wallis Test used for difference significances.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
NS: non significant.
SEM: standard error of the means.

However it seems that PUT effects on duodenal VH and CD might be dose responsive.

In current study LM had no impacts on blood lipids and glucose. This is in agreement with previous reports (Capcarova *et al*. 2011; Hatakka *et al*. 2008). Besides, there are evidences showing the reduction effects of probiotics and microbial metabolite on plasma lipids (Foo *et al*. 2003b; Ignatova *et al*. 2009; Loh *et al*. 2009).

It is indicating that there are some unknown factors interacting with microbial product on plasma components. The discrepancy of results could be due to the strain of LAB metabolite used in different studies.

Evidences suggest that decreased PAs level increases the insulin secretion (Tersey *et al*. 2013) through affecting on pancreatic beta-cell, resulting in decreased serum Glu, TG and Chol concentrations (Mendez and Hernandez, 2005).

Table 8 Effects of liquid metabolite (LM) and putrescine (PUT) on polyamine content of ileal digesta at the age of 21 days[1]

Items		PUT (mg/g DM)	Spermidine (ng/g DM)	Spermine (ng/g DM)
LM (%)	0.00	2.36	132	0
	0.30	5.34	251	0
P-value		NS	NS	-
PUT (%)	0.00	1.71	85	0
	0.03	2.71	145	0
	0.05	7.12	344	0
P-value		NS	NS	-

[1] The zero value of polyamines content means "not detectable". Because of non-normal distribution of polyamine values, Kruskal Wallis Test used for difference significances.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
NS: non significant.
SEM: standard error of the means.

Table 9 Effects of liquid metabolite (LM) and putrescine (PUT) on polyamines content of duodenum and Jejunum + Ileum at the age of 21 days[1]

Items		Duodenum			Jejunum + Ileum		
		PUT (mg/g)	Spermidine (ng/g)	Spermine (ng/g)	PUT (mg/g)	Spermidine (ng/g)	Spermine (ng/g)
LM	0	0.22	20[a]	363[a]	0.7	0	45
	0.3	0.23	1[b]	105[b]	0.3	0	1912
P-value		NS	*	*	NS	-	NS
PUT	0	0.18	13	251	0.2	0	0
	0.03	0.25	13	228	0.8	0	2889
	0.05	0.24	6	223	0.5	0	46
P-value		0.06	NS	NS	NS	-	NS

[1] The zero value of polyamines content means "not detectable". Kruskal Wallis Test used for difference significances.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
* (P<0.05).
NS: non significant.
SEM: standard error of the means.

However, in the current study, dietary PUT increased blood Glu in 33 days old chickens. Since PUT worsened the EER in current study, it is reasonable that blood Glu get increased due to dietary PUT supplementation. However, the relationship between dietary and blood PAs and their effects on Glu metabolism and insulin secretion need further investigation. Likewise, the effect of PUT on blood lipids is still uncertain, because while in this study no changes of blood lipids, due to dietary PUT, were observed, it is reported that elevated PUT level due to PAs catabolism, reduced blood Chol level through more bile acid synthesis (Pirinen *et al*. 2010).

Excessive PUT in tissue is known to be toxic, whereas small dosage can improve growth (Santos, 1996). Duodenal SPM and SPD were lower in those birds supplemented with LM. Similarly, it is reported (Linsalata *et al*. 2010) that probiotic homogenate is able to decrease ODC activity and PAs content in gastric tissue. According to present study, LM has the ability to reduce the concentration of PAs of intestinal tissue in which the metabolism rate is high. This is inconsistent with (Takagi *et al*. 2010) who stated PAs concentration of intestine in rats fed LAB fermented food was higher. Although probiotics and yeasts are able to produce PAs (Buts *et al*. 1994; Tabor and Tabor, 1985; Tovar *et al*. 2002) but in current study, PAs content of digesta and faeces was not affected by LM and PUT. SPM was not detectable in both excreta and digesta in young broilers (21 days).

It is reported that the concentrations of luminal SPM and SPD is low to undetectable (Osborne and Seidel, 1990) and PUT is the main endogenously generated PAs secreted into the lumen of gut (Noack *et al*. 1996). Determination of fecal PUT is important due to high correlation between fecal PUT and nutrient malabsorption (Forget *et al*. 1997). Since dietary PUT had no effects on intestinal tissue PAs content, it is concluded that tissue PAs biosynthesis is highly regulated and is not affected by dietary polyamines.

CONCLUSION

Single strain of LM was not effective on broiler performance. Meantime, 0.05% PUT had negative effects on performance and negatively affected on PER and EER. Interaction between LM and PUT increase duodenal VH. LM had no effect on blood lipids and glucose, but PUT supplementation have the ability to increase blood glucose. Duodenal SPM and SPD may be reduced by LM supplementation while PAs content of digesta and feces is not affected by dietary LM and PUT supplementation, meaning that intestinal tissue polyamines contents is strictly regulated and dietary polyamines cannot change it.

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