JAPSC
Journal of Animal and Poultry Sciences, 2014, 3 (3): 95-104
Available online at http://www.JAPSC.com

Small Intestine Morphology, Growth Performance and Nutrient Digestibility of Young Broilers Affected by Different Levels of Dietary Putrescine

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Abstract

This experiment was conducted to investigate dietary putrescine effects on broiler performance. Treatments were 6 levels of putrescine (0, 0.01, 0.02, 0.03, 0.04 & 0.05 %) added to a basal diet and fed to 4-32 day old broilers. The weekly growth performance was recorded, and ileal digesta and small intestine samples were collected at the end of the 2nd and 3rd week of age. At 21 d, body weight and feed intake were increased with 0.03 and 0.01% dietary putrescine supplementation respectively, while feed conversion ratio was not affected. At younger age (14 d), decreasing effects of putrescence on BW and energy- protein digestibility were seen. Meantime, protein digestibility decreased at 0.04% putrescence at 14 days old chicks while at older age this effect was not seen, suggesting an interaction between the age and dietary putrescence effect. Duodenal villus height and crypt depth were increased significantly due to putrescine at both ages (14 and 21 days) as compared to control group. In conclusion, dietary putrescine may have an accelerating effect on the growth rate of birds since whilst body weight and feed intake improved feed conversion ratio remained unaffected. Dietary putrescine is positively effective on small intestine villus height and crypt depth, particularly at younger ages.

Key words: Polyamines, Growth rate, Intestinal morphology, Digestibility

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Introduction

The polyamines (PAs) including putrescine (PUT), spermidin (SPD) and spermine (SPM) are biological cationic compounds that are biosynthesized from amino acids methionine and arginine. The most important function of PAs is their effects on cell growth, differentiation and normal functioning (Pegg, 2009; Wallace, 1996; Yang et al., 1984). They are present in all cells at low levels influencing DNA, RNA and protein synthesis (Loser, 2000).It was shown that dietary PAs are involved in intestinal growth and maturation(Sabater-Molina et al., 2009). LuminalPAs which is affected by dietary resources is important local factors for growth and the development of small intestinal (Bardocz, 1993). It has been reported that colonic mucosa and cessation of cellular growth is correlated with reduced concentrations of PAs (Heby et al., 1990). Supplementing soy protein isolate diets with PAs may enhance intestinal absorption and enterocyte proliferation, and this was due to PUT impact on greater crypt depth(Grant et al., 1990). Furthermore, it has been shown that 0.4% PUT in the chicks fed pure diets caused faster growth rate than controls (Smith, 1990), but higher levels resulted in reduced growth rates. In pre-ruminant calves (Grant et al., 1989) and neonatal pigs (Grant et al., 1990), supplementing the milk replacers with PUT or ethylamine, a putrescine analogue, may enhance intestinal absorption through increment of villus height and proliferation of enterocyte. The positive effects of PUT supplementation to increase efficiency of feed utilization have been documented (Mogridge et al., 1996). In contrast, it has been reported that supplementation of practical broiler diets with PUT failed to promote the growth of birds fed a low protein diet (Colnago and Jensen, 1992). This information showing that the potential benefits of dietary PAs are mostly achieved when the normal functioning of the intestinal tract is inadequate or interrupted.

Although the effects of PUT on the morphology of the small intestine have been investigated, there is still a lack of information for the digestibility of the main nutrients affected by dietary PUTin broiler production. The objective of this study was to study the effects of dietary PUT levels on performance and ileal apparent metabolisable energy (iAME) and digestibility of crude protein (CP), calcium (Ca) and phosphorus (P) in broiler chickens as well as intestinal morphology.

Materials and Methods

Experimental Birds and Diets

A total of 468 male day-old chicks (Cobb 500) were individually weighed, wing-banded and randomly distributed into 36 battery cages, each containing 13 chicks. Six levels of pure PUT (T1=0, T2=0.01, T3=0.02, T4=0.03, T5=0.04 and T6=0.05%) considered as treatments were added to the diet (Table 1). Pure PUT was purchased from Merck Company (CAS number 333-93-7, Darmstadt, Germany). The experimental diets were

Table 1. Compositions of experimental diets

Ingredients (%)		T1	T2	T3	T4	T5	T6
Corn		48.60	48.59	48.58	48.57	48.56	48.55
Soybean meal (48%)		41.00	41.00	41.00	41.00	41.00	41.00
Palm oil		6.20	6.20	6.20	6.20	6.20	6.20
$CaCO_3$		1.50	1.50	1.50	1.50	1.50	1.50
Monocalcium Phosphate		2.00	2.00	2.00	2.00	2.00	2.00
Salt		0.40	0.40	0.40	0.40	0.40	0.40
Vitamin premix[1]		0.05	0.05	0.05	0.05	0.05	0.05
Mineral premix[2]		0.05	0.05	0.05	0.05	0.05	0.05
DL-Methionine		0.20	0.20	0.20	0.20	0.20	0.20
PUT		**0**	**0.01**	**0.02**	**0.03**	**0.04**	**0.05**
Calculated Nutrients							
ME	Kcal/ Kg	3036	3036	3036	3036	3036	3036
Crude protein	%	22.00	22.00	22.00	22.00	22.00	22.00
Ether Extract	%	8.23	8.23	8.23	8.23	8.23	8.23
Fiber	%	4.08	4.08	4.08	4.08	4.08	4.08
Calcium	%	1.00	1.00	1.00	1.00	1.00	1.00
Avail. Phos.	%	0.47	0.47	0.47	0.47	0.47	0.47
Arg	%	1.54	1.54	1.54	1.54	1.54	1.54
Lys	%	1.22	1.22	1.22	1.22	1.22	1.22
Met+Cys	%	0.86	0.86	0.86	0.86	0.86	0.86
Met.	%	0.53	0.53	0.53	0.53	0.53	0.53
Threonine	%	0.85	0.85	0.85	0.85	0.85	0.85
Tryptophan	%	0.29	0.29	0.29	0.29	0.29	0.29
Putrescine[3]	**%**	**0.06**	**0.07**	**0.08**	**0.09**	**0.10**	**0.11**

[1]Supplied per kilogram of diet: vitamin A, 1,500 IU; cholecalciferol, 200 IU; vitamin E, 10 IU; riboflavin, 3.5 mg; pantothenic acid, 10 mg; niacin, 30 mg; cobalamin, 10 μg; choline chloride, 1,000 mg; biotin, 0.15 mg; folic acid, 0.5 mg; thiamine 1.5 mg; 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; iodine, 0.18 mg.

[3]Putrescine content of corn and soybean meal samples were analyzed (in the average PUT content of corn=1 mg/g and soybean meal=0.3 mg/g)

Table 2. Polyamines content of corn and soybean meal used

Ingredient	PUT (Mg/g)	SPM (Nano g/g)	SPD (Nano g/g)
Corn	1.01	ND	ND
SBM[2]	0.31	ND	ND

[1]ND= not detectable
SBM=soybean meal

fed from day 1 to 21. The polyamines content of corn and soybean meal used in this experiment were measured (Table2).

Parameters measured

Body weight (BW) and feed intake (FI) were measured weekly. Other parameters such as feed conversion ratio (FCR), protein efficiency ratio (PER), energy efficiency ratio (EER) and body weight gain (BWG) were calculated accordingly. FCR was calculated as daily Feed Intake / daily BWG. PER and EER were calculated as daily BWG (g) / daily protein intake (g); and daily BWG (g) / daily 1000 Cal intake, respectively.

Ilealdigesta and intestine samples were collected from 6 chicks per cages at the end of the second and third week of age. Titanium oxide (TiO_2) was used as an indigestible marker at the level of 0.3%. Samples of digesta and feeds were subjected to analyzing gross energy (by oxygen calorimetric bomb), crude protein (by kjeldahlapparatus), calcium (by flame atomic absorption spectrophotometer), phosphorus (by colorimetric method) and TiO_2 (Myers et al., 2004). The following formula was used for iAME calculation (Scott et al., 1998):

$iAME = GE_{diet} - (GE_{digesta} \times Marker_{diet} / Marker_{digesta})$

Minerals digestibility was calculated based on the formula:

$Minerals\ Digestibility = 100 - [100 \times (\%Marker_{diet} / \%Marker_{digesta}) \times (\%Mineral_{digesta} / \%Mineral_{diet})]$

Samples of small intestine (duodenum, jejunum and ileum) kept in 10% formalin were dehydrated, embedded into paraffin, cut and stained with haematoxylin and eosin. VH and CD were measured based on Thanh et al. (2009).

Polyamine analysis of corn and soybean meal samples

PAs concentrations of corn and soybean meal (SBM) sample were measured using benzoylation method by HPLC as described by Hwang et al. (1997).

Statistical analysis

Data were analyzed by GLM procedure of SAS software (S.A.S, 1999). Duncan multiple range test was used for comparing the means. The P values less than 0.05 was considered significantly different. For those parameters measured at two different ages, the interaction between age and PUT levels were analyzed.

Results

At 7 d of age, 0.02% dietary PUT had significantly (P<0.05) greater BW and body weight gain (BWG) as compared to the control group (Table 3). BW and BWG of 14 d old broilers were decreased significantly (P<0.05) at 0.03% and 0.04% PUT when compared to the control group. At 21 d of age, 0.01, 0.02, 0.03% and 0.05% PUT supplementation improved BW and BWG significantly (P<0.05) and FI was significantly (P<0.05) increased due to 0.01% PUT supplementation when compared to the control.

At 14 days of age (as shown in Table 3), supplementation of broiler diet with 0.04% PUT lowered the iAME rate (P=0.06) when compared to the control group. At day 14, CP digestibility was decreased significantly (P<0.05) at 0.04% PUT when compared to the control. Interaction between PUT and age was significant on CP digestibility (P<0.05). In all treatment groups, CP digestibility was increased by age significantly (P<0.05), except for 0.02% PUT and control group. PUT was not effective on Ca digestibility but higher age caused greater Ca digestibility. At 0.02% PUT, older chicks showed a decreased P digestibility, and at the 0.04% PUT level, P digestibility was increased in older chicks. The age significantly affected the VH and CD in every part of the small intestine, except in the Jejunum CD. PUT levels produced greater duodenal and ileal VH and CD. The interaction between PUT and age was significant (P< 0.05) on jejunum and ileum VH. At 14 d of age, duodenal VH was increased significantly (P< 0.05) in 0.04% and 0.05% PUT level as compared to the controlgroup, while in 21 d old chicks, 0.02, 0.03 and 0.04% PUT increased VH. At 14 d of age, the duodenal CD was

Table 3. Effects of PUT levels on performance parameters of broilers over 21 d of age

Treatment	PUT%	BW (g)	BWG (g)	BWG (g/b/d[1])	FI (g/b/d)	FCR[2]	PER[2]	EER[2]
				7 d				
T1	0	152[b]	110[b]	15[b]	22.77	1.45	3.08	0.2
T2	0.01	155[ab]	112[ab]	16[ab]	23.7	1.47	3.02	0.19
T3	0.02	158[a]	116[a]	16[a]	23.85	1.44	3.1	0.2
T4	0.03	154[ab]	112[ab]	16[ab]	23.51	1.47	3.05	0.2
T5	0.04	149[b]	107[b]	15[b]	21.88	1.43	3.11	0.2
T6	0.05	152[b]	110[ab]	15[ab]	22.39	1.42	3.13	0.2
N		498	498	498	36	36	36	36
Pooled SEM		0.85	0.83	0.11	0.32	0.01	0.04	0.002
P value[3]		S	S	S	NS	NS	NS	NS
				14 d				
T1	0	314[a]	272[a]	19.47[a]	30.53	1.58	2.83	0.18
T2	0.01	316[a]	273[a]	19.51[a]	32.73	1.67	2.66	0.17
T3	0.02	312[a]	270[a]	19.32[a]	31.98	1.66	2.69	0.17
T4	0.03	291[bc]	249[bc]	17.82[bc]	31.46	1.77	2.55	0.16
T5	0.04	283[c]	241[c]	17.24[c]	31.44	1.82	2.46	0.16
T6	0.05	305[ab]	263[ab]	18.85[ab]	31.33	1.67	2.67	0.17
N		282	282	282	36	36	36	36
Pooled SEM		2.64	2.6	0.18	0.47	0.03	0.04	0.003
P value		HS	HS	HS	NS	NS	NS	NS
				21 d				
T1	0	601[c]	559[b]	26.65[b]	38.7[b]	1.45	2.94	0.20
T2	0.01	641[ab]	583[ab]	28.04[ab]	42.24[a]	1.48	2.88	0.20
T3	0.02	643[ab]	600[ab]	27.81[ab]	40.26[ab]	1.41	3.04	0.21
T4	0.03	661[a]	613[a]	30.76[a]	40.23[ab]	1.37	3.14	0.22
T5	0.04	613[bc]	570[ab]	27.17[b]	38.42[b]	1.42	3.03	0.21
T6	0.05	655[ab]	601[ab]	28.40[ab]	40.06[ab]	1.39	3.1	0.21
N		66	66	66	36	36	36	36
Pooled SEM		5.98	5.95	0.28	0.39	0.02	0.04	0.003
P value[3]		S	S	S	S	NS	NS	NS

Means with different superscripts in each column are significantly different ($P < 0.05$)

[1]/b/d= gram/bird/day

[2]FCR=feed conversion ratio, PER=protein efficiency ratio, EER=energy efficiency ratio

[3]*P* values are shown as not significant (NS) and significant at $P<0.05$ (S) and $P<0.01$ (HS). The interaction effects of PUT × age on performance parameters were not significant.

significantly greater ($P< 0.05$) in 0.02% and 0.04% PUT groups compared with the control. However, 0.02, 0.03, 0.04 and 0.05% PUT caused higher duodenal CD significantly ($P< 0.05$) as compared with control group at 21 d. At 14 d of age, Ileal VH, was significantly increased ($P< 0.05$) in 0.04 and 0.05% PUT as compared with the control. Ileal CD was negatively affected ($P< 0.05$) by 0.02 PUT levels at 14 d while in older age (21 d), 0.03% PUT increased the ileal CD as compared to the control (table 5).

The interaction between PUT and age was significant ($P< 0.05$) on jejunum and ileum VH. At 14 d of age, duodenal VH was increased significantly ($P< 0.05$) in 0.04% and 0.05% PUT level as compared to the control group, while in 21 d old chicks, 0.02, 0.03 and 0.04% PUT increased VH. At 14 d of age, the duodenal CD wassignificantly greater ($P< 0.05$) in 0.02% and 0.04% PUT groups compared with the control. However, 0.02, 0.03, 0.04 and 0.05% PUT caused higher duodenal CD significantly ($P< 0.05$) as compared with control group at 21 d. At 14 d of age, Ileal VH, was significantly increased ($P< 0.05$) in 0.04 and 0.05% PUT as compared with the control. Ileal CD was negatively affected ($P< 0.05$) by 0.02 PUT levels at 14 d while in older age (21 d), 0.03% PUT increased the ileal CD as compared to the control (table 5).

Polyamine content of corn and soybean meal: SPD and SPM were not detected in the samples, but PUT was found in corn and SBM.

Table 4. Effects of PUT × Age[1] on ileal digestibility of energy, protein, Ca and P

Treatment	PUT%	iAME[2]		CP		Ca		P	
		d14	d21	d14	d21	d14	d21	d14	d21
T1	0	2116	2167	48.97 ^{abx}	50.15 ^{bx}	29.28^{x}	57.28 ^{y}	61.91 ^{x}	54.74 ^{x}
T2	0.01	2214	1993	40.75 ^{bcx}	50.44 ^{aby}	19.09^{x}	45.39 ^{y}	51.14 ^{x}	53.15 ^{x}
T3	0.02	2480	2041	55.56 ^{ax}	48.80 ^{bx}	42.33^{x}	60.54 ^{y}	63.58 ^{x}	49.59 ^{y}
T4	0.03	1858	1999	41.32 ^{bcx}	55.43 ^{aby}	20.59^{x}	61.76 ^{y}	56.03 ^{x}	57.25 ^{x}
T5	0.04	1556	1953	31.12 ^{cx}	56.17 ^{aby}	38.96^{x}	64.91 ^{y}	53.21 ^{x}	62.98 ^{y}
T6	0.05	1913	2415	45.63 ^{abx}	58.61 ^{ay}	33.94^{x}	59.04 ^{y}	60.95 ^{x}	55.26 ^{x}
N		71		71		63		71	
Pooled SEM		54.9		1.26		2.62		0.94	
P value[3]									
PUT		0.06		S		0.06		NS	
Age		NS		HS		HS		NS	
PUT×Age		0.08		HS		NS		S	

means with different superscripts in each column ($^{a, b, c,...}$) and row ($^{x, y, z,...}$) are significantly different (P< 0.05)
[1]d14=day 14, d21= day 21
[2]iAME= Ileal apparent metabolisable energy$_{(\text{Kcal/Kg})}$
[3]*P* values are shown as not significant (ns) and significant at P<0.05 (S) and P<0.01 (HS)

Table 5. Effects of PUT ×Age[1] on small intestine villus height (VH) and crypt depth (CD)-µm

Treatment	PUT%	Duodenal VH		Duodenal CD		Jejunal VH		Jejunal CD		Ileal VH		Ileal CD	
		14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d	14 d	21 d
T1	0	674 ^{cx}	898 ^{by}	190 ^{cx}	218 ^{bx}	620^{x}	865 ^{aby}	252	215	283 ^{dx}	483 ^{by}	184 ^{abx}	216^{bx}
T2	0.01	855 ^{bcx}	1005 ^{aby}	212 ^{bcx}	235 ^{bx}	743^{x}	1035 ^{ay}	239	248	373 ^{cdx}	536 ^{by}	158 ^{bcdx}	211^{bx}
T3	0.02	868 ^{bcx}	1115 ^{ay}	285 ^{abx}	300^{ax}	798^{x}	1024 ^{ay}	220	235	357 ^{cdx}	547 ^{by}	108 ^{dy}	174^{bx}
T4	0.03	932 ^{bcx}	1162 ^{ay}	189 ^{cdy}	316 ^{ax}	649^{x}	728 ^{bx}	212	246	393 ^{cdx}	818 ^{ay}	195 ^{aby}	251^{ax}
T5	0.04	1038 ^{abx}	1148 ^{ax}	307 ^{ax}	306 ^{ax}	703^{x}	779 ^{bx}	235	255	572 ^{abx}	488 ^{bx}	187 ^{abx}	209^{abx}
T6	0.05	1088 ^{ax}	1043 ^{abx}	240 ^{abcx}	295 ^{ax}	754^{x}	746 ^{bx}	202	200	625 ^{ax}	588 ^{bx}	146 ^{bcdx}	183^{bx}
N		72		72		72		72		72		72	
Pooled SEM		38		8		31		7		23		7	
P value[2]													
PUT		S		HS		NS		NS		HS		HS	
Age		HS		HS		HS		NS		HS		HS	
PUT×Age		NS		NS		S		NS		HS		NS	

means with different superscripts in each column ($^{a, b, c.}$) and row ($^{x, y, z}$) are significantly different (P<0.05)

[1]day 14 (d14) and day 21 (d21).

[2] P values are shown as not significant (NS) and significant at P<0.05 (S) and P<0.01 (HS).

Discussion

In poultry nutrition, biogenic amines and PAs have been shown to decrease feed efficiency (Brugh and Wilson, 1985; Stuart et al., 1985) and to induce proventricular enlargement (Harry et al., 1975). Meantime Girdhar et al.(2006) found that dietary PUT (0.1, 0.2 and 0.3%) can improve FCR and weight gain of turkeys that challenged with a mixed coccidial infection at the age of 24 d. In the current study, BWG, EER and PER of broilers were negatively affected by 0.04% PUT at 14 d of age and this effect was not seen in 21 d old chicks. This may imply that age is a moderator for the dietary PUT effects on performance. This is in agreement with Smith (1990), who reported that 14 d old chicks fed purified diets with small doses of orally administered PUTshowed better body growth.The effects of dietary PUT on tissue polyamine concentrations have been reported to be variable (Mogridge et al., 1996). Since biosynthesis of PAs is dependent on the age (Holt and Luk, 1990), the beneficial level of dietary PAs is variable. The factors such as age, diet ingredients (Mogridge et al., 1996; Smith, 1990), and disease occurrence (Girdhar et al., 2006) influence the beneficial levels of dietary PAs.

Mogridge et al.(1996) stated that dietary PUT improves nutrient uptake of the birds fed raw soybean. In the current study, 0.04% PUT caused the lowest CP digestibility at 14 d old chicks but had no effect in olderchick indicating that PUT effect on CP digestibility is age-dependent. Similarly, Sunder et al.(2008) reported that age intervals have a significant influence on nutrient digestibility in broilers. The evidenceprovided by the current study indicates that uptake of energy and protein through the intestinal wall at a younger age is more responsive
to dietary PUT supplementation.

In laying hens, PUT effects on Ca metabolism depends on dietary calcium concentration (Chowdhury and Smith, 2001). In the current study, we found no changes on Ca digestibility due to PUT, most likely because of lower dietary Ca level in broiler feed. Since vitamin D deficiency regulates duodenal biosynthesis of PAs (Imanishi et al., 1996; Shinki et al., 1981), the exact connections between dietary PAs and Ca and P metabolism need more investigation.

The evidence provided by this study suggests that dietary PUT is effective on the small intestinal VH and CD. This finding is in agreement with Girdhar et al.(2006). Grant et al. (1990) reported an enhancement in the intestinal absorption and enterocyte proliferation by dietary amines. Loser et al. (1999) reported that long term feeding of polyamine deficient diets would resulted in a significant hypoplasia of the small intestinal. At 21 d old chicks, all levels of added PUT (0.01, 0.02, 0.03, 0.04 and 0.05 %) increased VH in duodenum and jejunum part. However, in the younger age (14 d), 0.04 and 0.05% PUT were effective. This result indicates that higher dietary PAs is required for mucosal development in younger ages.

Conclusion

This study showed that significant improvement in BW was achieved in 0.01, 0.02, 0.03 and 0.05% PUT supplemented chicks at 21 d. Moreover, PUT levels may have an accelerating effect on the growth rate of birds since whilst BW and FI improved FCR remained unaffected. At 14 d old chicks, 0.04% PUT had an adverse effect on CP digestibility, but in older age this effect was not observed, suggesting an interaction between the age and PUT. The evidence provided by this study, prove that dietary PUT has positive effects on the small intestinal VH and CD, particularly at younger ages.

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