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Effects of Amino Acid, Enzyme Mixture and Phytase Added to Low Protein and Low Phosphorus Diet on Performance and Excretion of Nitrogen and Phosphorus in Broilers.

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In the purpose to reducing excretion of nitrogen (N) and phosphorus (P) without the influence of the growth performances and abdominal fat in broilers, a feeding trial and balance test were conducted. Four diets were prepared. The control diets (C) contained 23% crude protein (CP) for 0 to 21 days of age and 19% CP for 22 to 49 days of age, respectively. In the second diets (L), CP were reduced to 19 and 16% for both phases and crystal amino acids were added to be 110% of requirements at both phases. In the third diets (LE), an enzyme mixture (cellulase, xylanase and pectinase) was added to L. In the fourth diets (LEP), non-phytate P were reduced to 0.32% and 0.28% for both phases, and phytase was added. Eight hundred one-day-old broiler chicks, equal number of male and female, were divided into 4 treatments and given free access to one of the diets for 49 days of age, and body weight (BW), feed intake and mortality were recorded. At the end of experiment, 50 chicks per each treatment were slaughtered by bleeding through a jugular vein and eviscerated for the determination of abdominal fat. Using another 10 male broiler chicks per each treatment, excretion of dry matter (DM), N and P were determined by the balance test. There were no significant differences in BW gain, feed intake, feed conversion ratio and mortality among four treatments. Abdominal fat of broilers fed L was significantly heavier than that of other three treatments, and no significant differences were found in abdominal fat among other three treatments. Excretion of DM and N in L, LE and LEP decreased to 91, 84 and 82%, and 73, 70 and 70% of C, respectively. Excretion of P of broilers fed LEP decreased to a half of other three treatments, and there were no differences in P excretion among other three treatments.

Key words : broiler, excretion of nitrogen, excretion of phosphorus, amino acid, phytase

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Introduction

Recently, the scale of livestock producers has been getting intensive, and the number of livestock has increased in these farms. The amount of excreta, feces and urine excreted by livestock amounts yearly 92.9 million tons, in which 0.7 million tons nitrogen (N) and 0.5 million tons phosphorus (P) are included (Takemasa and Takagi, 2001). The amount of excreta is exceeding the demand of excreta as composts. In these circumstances, it is urged to develop a technique to decrease not only N and P, but also

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the total amount of waste.

It is recognized that N excretion is possible to be reduced by using the low-protein diet supplemented amino acids without reducing performance of broilers. However, it is well known that the depression of dietary CP levels causes the increment of abdominal fat (Summers *et al.,* 1992 ; Yamazaki *et al.,* 1996, 1998 ; Kerr and Kidd, 1999 ; Blair *et al.,* 1999). Therefore, the low CP diet was not acceptable practically in broiler industry. However, Zanella *et al.* (1999) showed that supplementation of the enzyme mixture containing amylase, protease and xylanase to the corn-soybean meal diets with 18% CP improved performance without increments of abdominal fat in broilers.

In the studies to reduce excretory P of broilers, Takemasa *et al.* (1996) and Yonemochi *et al.* (2001) reported that phytase supplementation improved the utilization of phytate P derived from plant feedstuffs, and decreased excretory P by approximately one-third without depressing performance. However, these studies were conducted at only the starter phase at seven to 21 days of age. Practically the data at the finishing phase are needed, but there are few reports which covered all of the feeding phase in broilers.

In this study, therefore, the effects of enzyme mixture (cellulase, xylanase and pectinase) added to low CP and low non-phytate P diet on performance, accumulation of abdominal fat and excretion of dry matter (DM), N and P were determined in broilers from the start to marketing.

Material and Methods

1. Experimental diets

Four starter diets for 0 to 21 days of age and four finisher diets for 22 to 49 days of age were formulated as shown in Table 1. In the control diet (C), CP, ME, calcium (Ca) and nonphytate P were adjusted to be the same as the commercial feeds for broiler in Japan, 23.0 and 19.0% CP, 3.10 and 3.20 Mcal ME/kg of diet, 0.94 and 0.83% Ca, 0.50 and 0.42% non-phytate P for starter and finisher diets, respectively. In other six diets (L, LE and LEP), the dietary CP levels were reduced to 19.0 and 16.0% for the starter and finisher diets, respectively. To the diet L, crystalline amino acids, L-arginine, L-lysine HCl, DL-methionine, L-threonine, L-tryptophan and L-valine were added to be 110% of recommendation by Japanese Feeding Standard for Poultry (JSFP, 1997). The LE diets were supplemented with 0.03% Cellulase MEIJI® which contained 1,000 units/g cellulase from *Aspergillus aculeatus* (Meiji Seika Kaisya Co Ltd., Tokyo, Japan), and 0.03% Aspelase® which contained 400 units/g xylanase and 1,200 units/g pectinase from *A. usamii mut. Shiro-usami* (Sankyo Co Ltd., Tokyo, Japan). The LAEP diets, non-phytate P was reduced to 0.32 and 0.28% for starter and finisher diets, and supplemented with 0.1% Phyatase Kyowa® which contained 500 units/g phytase from *A. niger* (Kyowa Hakko Kogyo Co Ltd., Tokyo, Japan).

2. Feeding trial

Eight hundred one-day-old Chanky chicks were individually weighted, and were divided into 16 groups of 50 chicks with 25 males and 25 females each. The mean body

Table 1. Composition of experimental diets (%)

Experimental period (days of age)	0 to 21			22 to 49		
Diets	C	L and LE	LEP	C	L and LE	LEP
Corn	43.96	53.86	54.10	53.41	61.61	61.77
Soybean meal	26.00	19.50	19.50	17.50	11.70	11.70
Corn gluten meal (CP 60%)	6.00	2.00	2.00	5.00	2.00	2.00
Feed grade animal fat	2.80	2.35	2.35	3.15	2.80	2.80
Calcium carbonate	0.80	0.76	1.52	0.77	0.73	1.32
Dicalcium phosphate	1.40	1.55	0.55	1.15	1.25	0.50
L-Arginine	0.11	0.35	0.35	0.21	0.42	0.42
L-Lysine HCl	0.14	0.32	0.32	0.14	0.30	0.30
DL-methionine	0.23	0.40	0.40	0.12	0.25	0.25
L-Threonine	—	0.12	0.12	—	0.13	0.13
L-Tryptophan	0.01	0.06	0.06	—	0.05	0.05
L-Valine	—	0.18	0.18	—	0.21	0.21
Others[1]	18.75	18.75	18.75	18.75	18.75	18.75
Calculated analysis						
CP (%)	23.0(110)[2]	19.0(91)	19.0(91)	19.0(112)	16.0(94)	16.0(94)
AME (Mcal/kg)	3.10(100)	3.10(100)	3.10(100)	3.20(103)	3.20(103)	3.20(103)
Ca (%)	0.95(105)	0.95(106)	0.95(106)	0.83(104)	0.83(104)	0.83(104)
Total Phosphorus (%)	0.68	0.68	0.49	0.60	0.60	0.45
Non-phytate Phosphorus (%)	0.50(111)	0.50(111)	0.32(71)	0.42(105)	0.42(105)	0.28(70)
Arginine (%)	1.33(110)	1.33(110)	1.33(110)	1.17(110)	1.18(110)	1.18(110)
Available Lysine (%)	1.08(110)	1.08(110)	1.08(110)	0.88(110)	0.88(110)	0.88(110)
Available methionine+cystine (%)	0.99(110)	0.99(110)	0.99(110)	0.77(110)	0.77(110)	0.77(110)
Available threonine (%)	0.76(117)	0.71(110)	0.71(110)	0.62(113)	0.61(111)	0.61(111)
Tryptophan (%)	0.24(110)	0.24(110)	0.24(110)	0.19(112)	0.19(112)	0.19(112)
Valine (%)	0.98(113)	0.95(110)	0.95(110)	0.82(104)	0.86(109)	0.86(109)

[1] Others contains (% of diet) : 15.0 ; Grain sorghum, 3.0 ; Fish meal (CP65%), 0.20 ; Sodium chloride, 0.20 ; Vitamin premix, 0.10 ; Mineral premix and 0.05 ; Coccidiostat.

[2] Values in parenthesis are percentage to recommended nutrient requirements of broiler chicks (JSFP, 1997).

weights of both sexes and each group were arranged to be as uniform as possible. Each treatment consisted of 4 replication groups, and chicks were given free access to one of the experimental diets and water for 49 days of age on the floor pens covered with wood shavings. Body weight, feed intake and mortality to 49 days of age were determined for each pen. At the end of experiment, 50 chicks (25 males and 25 females) were randomly chosen from each treatment. The chicks were slaughtered by bleeding through a jugular vein and eviscerated for determination of carcass and abdominal fat weight.

3. Balance test

Forty one-day-old male Chanky chicks hatched at the same days for the feeding trials were assigned to 4 dietary treatments with 5 replications of 2 chicks each. Chicks were given free access the same experimental diets and water for 49 days of age in metabolism cage. Chromic oxide (Cr_2O_3) was added to all experimental diets at a level of 0.2% as an indigestible marker. Excreta were collected twice a day for 4 days from 18 to 21 and 46 to 49 of age, and dried at 60℃ in a ventilated oven. Moisture, N, P and Cr_2O_3 in the diets and excreta were determined according to the methods described by AOAC (1990).

4. Statistical Analysis

The data were analyzed using the General Linear Models procedure of analysis of variance (SAS® Institute, 1990). Significant differences among mean values of treatments were calculated by Tukey's multiple range test (Yoshida, 1983).

Result

1. Growth performances

As shown in Table 2, there were no differences in BW gain, feed intake, feed conversion ratio and mortality among four treatments.

Table 2. Influence of enzyme addition to low protein and low phosphorus diets on growth performance of broiler chicks from 0 to 49 days of age

Diets	Body weight gain (g)	Feed intake (g)	Feed conversion ratio	Mortality (%)
C	2900	5470	1.89	2.5
L	2838	5377	1.90	2.5
LE	2881	5440	1.89	3.0
LEP	2886	5420	1.88	2.0
Polled SE	43	81	0.02	1.5
Probability	>0.05	>0.05	>0.05	>0.05

Each value is mean for 4 replicates of 50 chicks each.

2. Abdominal fat

The amount of abdominal fat as a percentage of the carcass weight are shown in Table 3. The amount of abdominal fat of L was significantly more than that of other three treatments, but there were no differences among other three treatments. The amount of abdominal fat of female was significantly more than that of male, but there were no interactions between treatments and sexes.

3. Excretion of DM, N and P

The excretion of DM, N and P are shown in Table 4. Compared to the data of C, DM excretion in L, LE and LEP decreased 10 to 20% at both phases. N excretion in L, LE and LEP decreased 26 to 37% of C at both phases. Although there were no differences in P excretion among three dietary treatments, C, L and LE, P excretions in LEP decreased 46 to 50% of C at both phases.

Table 3. Influence of enzyme addition to low protein and low phosphorus diets on abdominal fat of broiler chicks at 49 days of age

	Abdominal fat (% of carcass)
Diets	
C	2.6^{b}
L	3.0^{a}
LE	2.7^{b}
LEP	2.7^{b}
Sexes	
Male	2.6^{Y}
Female	3.0^{X}
Polled SE	0.6

Each value is mean for 25 chicks.
[a] and [b] Means within diet with different superscripts in the same row are significantly different ($p<0.05$).
[X] and [Y] Means within male and female with different superscripts in the same row are significantly different ($p<0.05$).

Discussion

In this study, when L was given to broilers, the excretory N decreased without reducing growth performance. However, the amount of abdominal fat was increased significantly. Because the abdominal fat is not a valuable as foods, the increment of abdominal fat is not desirable in the broiler industries.

The reasons why the abdominal fat increased on the low CP diets in which limiting amino acids are adjusted have been discussed as follows.

By reducing dietary CP, the energy used for degradation of protein was spared and stored as fat (Summers *et al.,* 1992) and/or the low dietary CP accelerated fat synthesis (Yamazaki *et al.,* 1996, 1998 ; Kerr and Kidd, 1999 ; Blair *et al.,* 1999)). In addition, it was reported that the accumulations of abdominal fat was accelerated by decreasing dietary essential amino acid (EAA) : non-essential amino acid (NEAA) ratio (Yamazaki *et al.,* 1996).

By adding enzyme mixture, abdominal fat and excreta were decreased, and N excretion was also depressed. These results suggested that the utilization of CP was improved. By the increment of digestibility of cellulose, the time of passing through the digestive tract might be fastened, the CP masked by cellulose was liable to be digested and contaminated proteases in the enzyme preparations also might have a role to digest CP.

In LEP, non-phytate P was reduced from the recommended requirement by JFSP (1997) and phytase was supplemented. As a result, the performance of broiler was not

Table 4. Influence of enzyme addition to low protein and low phosphorus diets on excretion of dry matter, nitrogen and phosphorus in broiler chicks

Experimental period (days of age)	Intake (g)		Excretion ratio (%)		Excretion (g)		
	0 to 21	22 to 49	0 to 21	22 to 49	0 to 21	22 to 49	0 to 49
Dry matter							
C	1027	4442	28.5[a]	22.4[a]	292.8[a]	966.6[a]	1259.4[a]
L	1010	4367	26.0[b]	20.2[b]	262.8[b] (90)[1]	882.9[b] (91)	1145.8[b] (91)
LE	1004	4437	23.8[c]	18.5[c]	239.1[c] (82)	819.0[ab] (85)	1058.1[c] (84)
LEP	1005	4415	22.9[c]	18.1[c]	229.9[c] (79)	800.1[c] (83)	1030.0[c] (82)
Polled SE			2.4	1.8	26.3	78.7	98.3
Nitrogen							
C	37.8	135.0	40.9[a]	41.0[a]	15.5[a]	55.3[a]	70.8[a]
L	30.7	111.8	36.0[b]	36.4[ab]	11.1[b] (72)	40.7[b] (74)	51.8[b] (73)
LE	30.5	113.6	32.2[c]	35.3[b]	9.8[c] (63)	40.0[b] (72)	49.8[b] (70)
LEP	30.5	113.0	31.9[c]	35.1[b]	9.8[c] (63)	39.7[b] (72)	49.5[b] (70)
Polled SE			4.1	3.6	2.4	7.3	9.4
Phosphorus							
C	5.1	18.7	54.5[a]	57.0[a]	2.8[a]	10.6[a]	13.4[a]
L	5.1	18.3	58.0[a]	57.4[a]	2.9[a] (104)	10.5[a] (99)	13.5[a] (101)
LE	5.0	18.6	55.1[a]	55.8[a]	2.8[a] (100)	10.4[a] (98)	13.2[a] (99)
LEP	3.2	12.4	44.7[b]	46.0[b]	1.4[b] (50)	5.7[b] (54)	7.1[b] (53)
Polled SE			3.5	5.3	0.6	2.1	2.7

Each value is mean for 5 replications of 2 chicks.
[a–c]Means within diet with different superscripts in the same row are significantly different ($p < 0.05$).
[1]Values in parenthesis are percentage to C.

affected, and P excretion decreased drastically from 46 to 50% of C. These results agreed well with those in the previous report, in which equivalent to 0.14% of phytate P was released by supplementation of 500 unit of phytase/kg of diet. It is reported that the utilization of not only phytate P but also CP were improved by supplementation of microbial phytase (Yonemochi *et al.,* 2000). Moreover, the efficiency of supplemented microbial phytase in pigs decreased with advancing age (Kemme 1997). In this study, the same efficiency of phytase was observed in both phases, which might indicate that 500 unit/kg of diet was enough or excess for broilers.

As a conclusion, it is possible to reduce not only N and P, but also total amount of excreta is reduced without the influence of the growth performance and abdominal fat in broilers by addition of enzyme mixture and microbial phytase. The usage of those enzymes will increase importance for the sustainable poultry industry in future.

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