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Effect of Mannanase on Broiler Performance, Ileal and *In-vitro* Protein Digestibility, Uric Acid and Litter Moisture in Broiler Feeding

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Abstract: Protein digestibility and uric acid excretion may play the vital role in feed efficiency and consequently effect on broiler performance. Five hundred unsexed Arian day old chickens were placed after pre-starter feeding (10 days) in 22 pens with 23 chickens in each. This study was modulated in two levels of Mannanase (Tread mark of Hemicell enzyme) enzyme (0 and 5 kg/ton) and three levels of metabolizable energy (3000, 2900 and 2850 kcal/kg). Six treatments were arranged and statistical status was in complete random design (CRD) by management in factorial (2 x 3) in this experiment. There is no any significant effect on Ileal protein digestibility by different levels of enzyme. Where this digestibility was significantly higher (P<0.05) by 2900 kcal/kg metabolizable energy (ME) compared to other level of energy (Table 5). Ileal protein digestibility was significantly higher (P<0.05) by interaction between 2900 Kcal/kg ME and different levels of enzyme in comparison to 2850 kcal/kg ME with zero level of enzyme. Dry matter *in-vitro* digestibility (DMID) was greater significant (P<0.05) by using enzyme compared with no enzyme treatment Increased significantly *in-vitro* dry matter digestibility (P<0.05) was observed by 2850 kcal/kg ME compared to other level of energy. Otherwise increasing significant (P<0.05) dry matter digestibility was found by 2850 kcal/kg ME energy and high level of enzyme compared to other treatment with exception of 2850 kcal/kg ME and zero level of enzyme. The similar trend was indicted on *in-vitro* protein digestibility (CPID) regarding enzyme reaction. In addition this item was significantly decreased (P<0.05) by 3000 kcal/kg ME in comparison to other levels of energy. The highest significant rate (P<0.05) was appeared on *in-vitro* protein digestibility by interaction between of 2850kcal/kg ME and high level of enzyme with exception of 2900 kcal/kg ME and high amount of enzyme. Although no significant reaction was observed in uric acid excretion by energy but this parameter significantly decreased (P<0.05) by high level of enzyme. In their interaction huge declining was shown by 3000 kcal/kg ME and high level of enzyme in comparison to 2900 kcal/kg ME and low level of enzyme. No significantly response was shown regarding litter moisture by all treatments. No response has been appeared in feed intake (FI) regarding enzyme level but higher significant (P<0.05) body weight (BW) was indicated by high level of enzyme in the end of experiment (42 days of age). Growth rate (GR) was greater significant (P<0.05) by using high level of enzyme but no differences was obtained in feed conversion ratio (FCR) in concern to different levels of enzyme in the end of this study. The results of this study have shown that hemicell enzyme could improve performance and increased Ileal protein digestibility, *in-vitro* DM, CP digestibility and reduction in uric acid excretion which may lead to reduce protein utilization in broiler feeding

Key words: Mannanase, Ileal protein, *In-vitro* protein, digestibility

Introduction

Most scientific evidence has demonstrated that cereal and fibre content diets are not accurately utilized by poultry particularly in broiler feeding. Since their ability of gastrointestinal tract are not processed more fibre and cores ration such as ruminant. Therefore some contributions need to be employed to solve this disability. In the view of this fact, enzyme could improve nutritional availability and digestibility (Voragen *et al*., 2001). Nutrient quantity could be recognized by chemical composition but nutrient quality in feedstuff which is essential for useful consumption, may achieved by availability and digestibility. In the case of availability, digestibility plays a major role and leads to benefit production in poultry industry. Therefore not only chemical composition but availability and digestibility of nutrient will improve by enzyme utilization (Chesson, 1993). Measuring digestibility by *in-vivo* methods such as Ileal digestibility may eliminate the bacterial fermentation rather than fecal digestibility (Nesheim and Carpenter, 1967; Salter and Coastes, 1971). Since reduction this bacterial fermented may decline digestibility to lower rate (Zanella, *et al*., 1999). They also noted that ILeal protein digestibility of diets based corn and soybean with and without enzyme were (79.1 and 86.5%) respectively. Apparent Ileal protein digestibilities in heated and extracted soybean meal were (87.5 and 82.2%) respectively (Ghazi *et al*., 2002 and Marsman *et al*., 1997). *In-vivo* method is more accurate rather than *in-vitro*, but it is much expensive and procedure is longer and takes more time. Digestibility could be estimated by *in-vitro* method in the shorter time and cheaper way

(Moughan, 1999). The effect of various enzymes such as cellulose, xylanase, pectinase, hemicellulase, glucanase, phytase and protease have been tested in dry matter and protein *in-vitro* digestibility by (Saleh *et al.*, 2003). They have found that there was the significant increasing effect of hemicellulase and multi enzyme without protease on *in-vitro* protein digestibility, but cellulase, xylanase, phytase and glucanase increased protein digestibility by separate procedure. Malathi and Devegowda, 2001 have stated that not only *in-vitro* digestibility increased by pentosanase, pectinase and cellulase enzymes but decreasing viscosity were appeared in sunflower meal, soybean meal, rice bran and starter diet in this respect. Releasing sugar and degrading polysaccharides bandings were demonstrated by enzyme reaction (Chesson, 1993). Increasing endogenous losses of amino acid and 20% of nitrogen are well documented by none-starch polysaccharides (Angkanaporn and Coct, 1994). Boiler performance includes body weight (BW), growth rate (GR), feed intake (FI) and feed conversion ration (FCR) increased by enzyme supplementation (Chesson, 1987; 1992; 1993; Choct and Annison, 1990; Dierick, 1989). Most enzymes were considered in cereal (particularly in wheat and barley) Chesson, 1993, therefore limited work was carried out to using enzyme in soybean meal with lots of inhibitors, particularly in comparison of *in-vivo* and *in-vitro* methods in protein digestibility. Manann is major polysaccharides in soybean meal and need to be elucidating (ChemGen, 2002). Consequently, this study was focused to examine the effect of hemicell (Mannanase) enzyme on *in-vitro* dry matter, protein and ILeal protein digestibility and broiler performance in response to different levels of energy. Excreting of uric acid and litter moisture also were considered in this study.

Materials and Methods

Five hundred unsexed Arian broiler chickens were kept as a group in pre-starter diet in the first 10 day of age. After 10 days they were placed in 22 pens with 23 chickens in each to 42 days of age. Rearing condition such as temperature, humidity, vaccination, lighting regime were similar and have been arranged based on recommendation in guideline of rearing Arian broiler (Supporting Arian poultry Centre, 2000). Two levels enzyme (0 and 5 kg/ton) and three levels of metabolize energy (ME) 3000, 2900 and 2850 kcal/kg were used as six treatments for starter and also grower diets. These rations were modulated by NRC (1994) recommended (Table 1, 2, 3 and 4). Complete random design with factorial management (2 x 3) were used in statistical procedure of this experiment. *In-vitro* dry matter digestibility (IDMD), protein digestibility (IPD) and Ileal protein digestibility (IIPD) were determined by fuller methods (Fuller, 1993). Uric acid and litter moisture were tested by Marquardet Method (1983). Feed intake (FI), body weight (BW), feed conversion ratio (FCR) and growth rate (GR) were measured regarding enzyme and energy levels. Data was analysed by (SAS, 1996) program and means were examined by employed Duncan multiple test.

Results

There is no any different reaction by enzyme levels on Ileal protein digestibility. Where that Ileal protein digestibility increased significantly ($P<0.05$) by 2900 kcal/ kg ME compared with other levels of energy. In the interaction between ME and enzyme levels, 2900 kcal/kg ME and different levels of enzyme was dramatically significant higher ($P<0.05$) than 2850 ME and zero level of enzyme in Ileal protein digestibility (Table 5). As illustrated in Table 6, greater significant increased ($p<0.05$) was appeared in dry matter *in-vitro* digestibility by enzyme compared with no enzyme treatment. In the case of ME, 2850 kcal/kg ME was in the significant higher rate ($P<0.05$) compared to other levels of energy. In concern to interaction of these both factors high level of enzyme and 2850 kcal/kg ME have shown significant reaction ($P<0.05$) compared to other treatments with exception in 2850 in kcal/kg ME and zero level of enzyme. The similar trend was observed in the case of *in-vitro* protein digestibility regarding enzyme reaction (Table 6). Significant extreme rage of ($P<0.05$) *in-vitro* protein digestibility was found by 2900 and 2850 kcal/kg ME in comparison to 3000 kcal/kg ME. In related to interaction between these both parameters predominant significant ($P<0.05$) reflection was indicated in 2850 kcal/kg ME and high level of enzyme among of treatments. Although this was in the similar range by 2900 kcal/kg ME and high level of enzyme (Table 6). Uric acid excretion was significantly reduced ($P<0.01$) by enzyme supplementation (Table 7). In contrast no differences were shown by various level of ME on this item. In comparison huge decreased uric acid excretion was approached by interaction 3000 kcal/kg ME and high level of enzyme among of treatments (Table 7). No responses were observed related to energy, enzyme and their interaction effects on litter moisture in the end of experiment (Table 7). In feed intake (FI) higher significant response ($P<0.05$) was observed by 2850kcal/kg ME compared with other levels of energy in 21 day chicken. This consumption also was reduced significantly ($P<0.05$) by high level enzyme compared with low level. High significantly ($P<0.05$) feed intake were presented by Interaction of 2850 kcal/ME and different levels of enzyme in comparison to other treatments (Table 8). No differences were indicated in all these items in feed intake of 42 day old chicken. Body weight (BW) was not changed by enzyme and energy levels in 21 day old chicken but the highest rate of this character was observed in 3000 kcal/kg ME and high

Table 1: Ingredient of starter rations in different treatments (%)

Ingredient	1	2	3	4	5	6
Corn	55.48	55.37	53.00	52.80	55.60	55.56
Soybean meal[a]	34.38	34.40	31.21	31.23	29.86	29.80
Wheat	-	-	8.00	8.00	8.00	8.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.06
Sunflower oil	3.66	3.7	1.32	1.41	-	-
D. C. P	1.34	1.34	1.36	1.36	1.37	1.37
$Caco_3$	1.19	1.19	1.19	1.19	1.20	1.19
Nacl	0.33	0.33	0.30	0.32	0.32	0.32
Mineral permix[b]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin permix[c]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.12	0.12	0.12	0.14	0.15	0.15
Hemicell[d]	-	0.05	-	0.05	-	0.05
Total	100	100	100	100	100	100

a, Soybean meal with 42% crude protein; b, Mn 64 gr, Zn 44gr, Fe 100 gr, Cu 16 gr, I 0.64 gr and Se 8 gr (per 1 kg premix); c, A 7.7 gr, D_3 7 gr, E 14.4 gr, B_{12} 14.4 gr, K_3 1.6 gr, B_2 3.3 gr, B_5 12 gr and Colin Chloride 440 mg (Per 1 Kg Permix); d, Hemicell enzyme; 1 to 6, are treatments, without and with enzyme and includes metabolizable energy in, 3000, 2900 and 2850 Kcal/kg respectively

Table 2: Chemical composition of different rations in starter of broiler feeding (%)

Nutrients	1-	2+	3-	4+	5-	6+
ME	3000	3000	2900	2900	2850	2850
CP	21.51	21.96	20.59	20.61	20.37	20.28
ME/CP	139.40	136.60	140.80	140.70	139.90	140.50

1 to 6, are treatments, without (-) and with (+) enzyme and includes metabolizable energy in, 3000, 2900 and 2850 Kcal/kg respectively; ME, Metabolizable energy; CP, Crude protein.

Table 3: Ingredient of grower rations in different treatments (%)

Ingredient	1	2	3	4	5	6
Corn	55.77	57.77	60.92	60.92	60.36	60.35
Soybean meal[a]	29.72	28.20	27.30	27.30	27.93	27.93
Wheat	8.00	8.00	8.00	8.00	8.00	8.00
Sunflower oil	2.82	2.50	-	-	-	-
D. C. P	1.39	1.30	1.40	1.40	1.39	1.35
$Caco_3$	1.39	1.30	1.41	1.41	1.41	1.41
Nacl	0.32	0.31	0.36	0.31	0.31	0.31
Mineral permix[b]	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin permix[c]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.09	0.07	0.11	0.11	0.10	0.10
Hemicell[d]	-	0.05	-	0.05	-	0.05
Total	100	100	100	100	100	100

a, Soyabean meal with 42% crude protein; b, Mn 64 gr, Zn 44gr, Fe 100 gr, Cu 16 gr, I 0.64 gr and Se 8 gr (per 1 kg premix); c, A 7.7 gr, D_3 7 gr, E 14.4 gr, B_{12} 14.4 gr, K_3 1.6 gr, B_2 3.3 gr, B_5 12 gr and Colin Chloride 440 mg (Per 1 Kg Permix); d, Hemicell enzyme; 1 to 6, are treatments, without and with enzyme and includes metabolizable energy in, 3000, 2900 and 2850 Kcal/kg respectively.

levels of enzyme (638.96 g) compared with 2900 kcal/kg ME and high level of enzyme (Table 8). No response was shown by different level of energy in BW in 42 days of age. Where this factor was increased significantly (P<0.05) by high level of enzyme. The highest body weight in this period was shown by interaction of 3000 kcal/kg ME and high level of enzyme in comparison to 2850 kcal/kg ME and low level of enzyme, (Table 8). Although no reaction was indicated in feed conversion ratio by enzyme in 21 days of age but desirable significant (P<0.05) rae of this character was obtained in 3000 and 2900 kcal/kg ME (Table 9). Most highlighted significant (P<0.05) FCR was shown by interaction of 3000 kcal/kg ME and high level of enzyme with other treatment, with exception in 3000kcal/kg ME and low level of enzyme. In contrast no differences have found in 42 days of age regarding this parameter (Table 9). Growth rate (GR) was the similar by all treatments in the end of 21 day of ages (Table, 9). No reflection was illustrated by different levels of energy in GR by 42 days but this factor was significantly greater (P<0.05) by high level of enzyme. Highest GR was approached by 2900

Table 4: Chemical composition of different rations in grower of broiler feeding (%)

Nutrients	1-	2+	3-	4+	5-	-6
ME	3000	3000	2900	2900	2850	2850
CP	18.29	18.34	18.08	18.02	17.82	17.82
ME/CP	164.00	163.55	160.30	160.90	145.50	161.20

1 to 6, are treatments, without (-) and with (+) enzyme and includes metabolizable energy in, 3000, 2900 and 2850 Kcal/kg respectively; ME, Metabolizable energy; CP, Crude protein.

Table 5: ILeal protein digestibility in response to different levels of energy and enzyme In 42 days of age

Effects	Amount	Protein digestibility (%)
Energy levels	3000*	61.69[b]
	2900	63.79[a]
	2850	60.94[b]
Enzyme levels	-	61.80 [a]
	0.5**	62.48 [a]
Interaction energy and enzyme	I-ME- E	61.32± 0.98[ab]
	2-ME +E	62.07 ±0.98 [ab]
	3-ME- E	63.77 ±0.84[a]
	4- ME+E	63.82 ±0.84 [a]
	5-ME- E	60.33± 0.84 [b]
	6- ME+E	61.56 ± 0.84 [ab]

1 to 6, are treatments, without (–E) and with enzyme (+E) and includes metabolizable energy (ME) in, 3000, 2900 and 2850 Kcal/kg respectively. * Kcal/kg; ** (%); Means in the same column with no common superscript are different significantly (P<0.05).

Table 6: *In-vitro* dry matter and protein digestibility in response to different levels of energy and enzyme in 42 days of age

Effects	Amount	Dry matter (%)	Crude protein (%)
Energy levels	3000*	68.29[b]	60.63[b]
	2900	67.00[b]	64.12[a]
	2850	74.13[a]	64.95[a]
Enzyme levels	-	68.30[b]	62.49[b]
	0.5**	71.31[a]	64.96[a]
Interaction energy and enzyme	I-ME- E	67.21 ± 1.88[c]	60.14 ± 0.97[d]
	2-ME +E	69.38 ± 1.88[bc]	61.13 ± 0.97[d]
	3-ME- E	64.53 ±1.62[c]	64.40 ± 0.84[bc]
	4- ME +E	69.48 ±1.62[bc]	65.25 ± 0.84[ab]
	5-ME- E	73.18 ±1.62[ab]	62.35 ± 0.84[dc]
	6- ME +E	75.09 ± 1.62[a]	67.56 ± 0.84[a]

1 to 6, are treatments, without (–E) and with enzyme (+E) and includes metabolizable energy (ME) in, 3000, 2900 and 2850 Kcal/kg respectively. *Kcal/kg; ** (%); Means in the same column with no common superscript are different significantly (P<0.05).

Table 7: Uric acid and Litter moisture in response to different levels of energy and enzyme in 42 days of age

Effects	Amount	Uric acid (%)	Litter Moisture (%)
Energy levels	3000*	70.95[a]	4.49[a]
	2900	75.11[a]	5.31[a]
	2850	73.12[a]	4.13[a]
Enzyme levels	-	79.94[a]	4.68[a]
	0.5**	66.61[b]	4.64[a]
Interaction energy and enzyme	I-ME- E	74.90 ± 6.10[ab]	4.10 ± 1.33[a]
	2-ME +E	64.90 ± 7.4[8b]	4.88 ± 1.33[a]
	3-ME- E	84.48 ±6.10[a]	5.50 ± 1.15[a]
	4- ME +E	68.08 ±5.29[ab]	5.12 ± 1.15[a]
	5-ME- E	80.24 ±5.29[ab]	4.30 ± 1.15[a]
	6- ME +E	65.99 ± 5.29[ab]	3.97 ± 1.15[a]

1 to 6, are treatments, without (-E) and with enzyme (+E) and includes metabolizable energy (ME) in, 3000, 2900 and 2850 Kcal/kg respectively. * Kcal/kg; ** (%); Means in the same column with no common superscript are different significantly (P<0.05).

Table 8: Broiler feed intake and body weight in response to different levels of enzyme and energy

Effects		FI		BW	
		21 days	42days	21 days	42 days
ME	3000	630.72b	4298.77a	633.43a	2233.36a
	2900	651.79 b	4309.45a	622.40a	2238.12a
	2850	707.84a	4394.58a	223.52a	2218.90a
E	0.00	676.31a	4424.28a	629.38a	2198.97b
	0.5	654.11b	4223.56a	622.30a	2258.99a
ME x E,	1 - E	649.69b	4375.99a	632.01ab	2238.00ab
	2+ E	611.73c	4156.48 a	638.96a	2268.13 a
	3 - E	662.71b	4441.07a	630.38ab	2213.33ab
	4+ E	640.86bc	4177.84a	614.45b	2261.79 a
	5 - E	716.54a	4452.79a	628.15ab	2170.88b
	6+ E	699.13a	4336.38a	619.17ab	2265.09a

FI, Feed intake; BW, body weight; ME, Metabolizable energy, E, Enzyme; -, without enzyme; +, with enzyme; Treatments from 1 to 6 are without and with enzyme includes 3000, 2900 and 2850 kcal/kg ME respectively; Means in the same column with no common superscript are different significantly (P<0.05)

Table 9: Broiler feed conversion ratio and growth rate in response to different levels of enzyme and energy

Effects		FCR		GR	
		21 days	42days	21 days	42 days
ME	3000	0.99 b	1.89a	455.68a	534.04a
	2900	1.04 b	1.92a	441.59a	595.82a
	2850	1.13a	1.98a	440.80a	544.00a
E	0.00	1.07a	2.00a	449.35a	535.45b
	0.5	1.05a	1.86a	440.94a	589.71a
ME x E,	1 - E	1.03bc	1.95 a	451.39a	572.53ab
	2+ E	0.96c	1.83 a	459.97a	513.53ab
	3 - E	1.05b	2.00 a	450.55a	572.63ab
	4+ E	1.04b	1.84 a	432.64a	619.01a
	5 - E	1.12a	2.05 a	446.64a	470.45b
	6+ E	1.12a	1.91 a	434.96a	617.55a

FCR, Feed conversion ratio; GR, Growth rate; ME, Metabolizable energy, E, Enzyme; -, without enzyme; +, with enzyme ; Treatments from 1 to 6 are without and with enzyme includes 3000, 2900 and 2850 kcal/kg ME respectively; Means in the same column with no common superscript are different significantly (P<0.05).

kcal/kg ME and high level enzyme with compared to 2850kcal/kg ME and low level of enzyme in 42 of age (Table 9).

Discussion

In spite of considerable progress in chemical composition methods, there is no any doubt that availability and digestibility of nutrients play the major role in poultry feedstuffs to lead a desirable production. Numerous studies have elucidated the enzyme influence in increasing protein digestibility (Delang *et al.*, 1998; Gdala, *et al.*, 1997; Papadopoulos, 1998; Wright, 1995; Yaser, 2002). Although in this investigation no response was found in related to enzyme effect as a single factor on Ileal protein digestibility, but, in combination of different levels of enzyme and 2900 kcal/kg ME significant greater effect (P<0.05) was recognized on Ileal protein digestibility. This was agree with above results as general achievements. In addition in the particular case Baidoo *et al.*, 1998 and Zanella *et al.*, 1999 have noted that the influence of enzyme increased Ileal protein digestibility. This study has resulted to the increasing effect of enzyme and ME combination on *in-vitro* dry matter and protein digestibility which is confirmed by (Saleh *et al.*, 2003). In the case of declining uric acid by enzyme supplementation could be related to more availability and digestibility of protein and therefore eliminated nitrogen excretion as a main material of uric acid production. Consequently reduction in uric acid excretion may reduce the environmental contamination. In future this could lead to the lower rate of crude protein in broiler ration which treated by enzyme supplementation. *In-vivo* techniques are expensive to carry out for feed evaluation, require personal with special skill and frequently call for large amounts raw materials with taking more time. In the feed standard assay for predicts the nutritive value of feeding stuffs accurately without experimental animal, will be focused in the coming up investigation (Fuller, 1993). Increasing digestive capacity by using enzyme may also affect broiler performance particularly in feeds intake (Chesson, 1987 and 1992). Growth rate, FCR and BW have been changed by enzyme and these finding have proved by (Choct and Annison, 1990; Diertick, 1989). These results were corresponding by obtaining of current study in regarding to use Mannaanse enzyme for improving broiler performance.

Conclusion: Soybean meal as a main source of plant protein in broiler nutrition could be more useful by Mannanse enzyme supplementation. Since with improving protein digestibility and decreasing uric acid excretion may reduce ration price and save environmental aspects by nitrogen contamination. On the other hand improving broiler performance may lead to financial benefit by the results of this study. The details in mechanism of this enzyme should be clarified by further scientific investigations.

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