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The Effect of Prebiotic and Types of Feed Formulation on Performance, Intestinal Microflora and Cecum Gas Production of Laying Hens

Research Article

H. Jahanian Najafabadi[1*], A.A. Saki[1], Z. Bahrami[1], A. Ahmadi[1], D. Alipour[1] and M. Abdolmaleki[1]

[1] Department of Animal Science, Faculty of Agriculture, Bu Ali Sina University, Hamedan, Iran

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*Correspondence E-mail: hjahanian@yahoo.com

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ABSTRACT

In this experiment, the effect of prebiotic Bio-Mos® (MOS) and types of feed formulation (total and digestible amino acids) were evaluated on performance and the small intestine microflora in laying hens for a duration of 10 wks. A total of 168 Hy line W-36 laying hens, with an initial age of 73 wks, were randomly allocated to 4 dietary treatments, 7 replicates and 6 birds in each replicate. The study was conducted in a completely randomized design with 2 × 2 factorial arrangement of treatments including 2 preboitic levels (0, 0.5 kg/ton of diet) and 2 types of AA feed formulation (total and digestible). Egg production (EP), egg weight (EW), egg mass (EM), average daily feed intake (ADFI) and feed conversion ratio (FCR) were not affected significantly by MOS, types of AA feed formulation and their interaction. Egg quality parameters including, specific gravity (SG), shell thickness (ST), shell weight (SW), haugh unit (HU) and yolk color (YC) did not affected by P-BM and types of AA feed formulation (P>0.05). No significant effects were observed on ileum *Lactobacillus* count by MOS, types of AA feed formulation and their interaction (P>0.05). There were no significant differences in the rate and gas production volume of experimental treatments (P>0.05). In conclusion, feed additive used in this study did not significantly affect the laying hens performance; intestinal *Lactobacillus* count, cecum gas production and microbial population.

KEY WORDS amino acid (AA), *Lactobacillus*, laying hens, microflora, prebiotic.

INTRODUCTION

In recent years, more attention has been focused on antibiotics which have been used as therapeutic level in poultry production due to development of multiple drug resistance bacteria and their residuals in animal products. Many of the antibiotics in the poultry industry have been used in human medicine as well. Therefore, producers compelled to use alternatives of antibiotics in poultry industry as well as human. Alternatives to use of antibiotics such as probiotics, prebiotics, immunostimulants and medicinal plants are used as growth promoters. A prebiotic component is defined as non-digestible food ingredients which can be limited number of the intestinal bacteria by beneficially affect the host (Gibson and Roberfroid, 1995). It has been hypothesized that supplementation with a prebiotic, could improve the detrimental effects of low or high protein diets. Prebiotics are components non-digestible carbohydrates which more of these are short chains of monosaccharide (MOS), called oligosaccharides. Some oligosaccharides are seemed to improve the intestinal and its function as competition for sites of attachment to pathogenic bacteria. It has been concluded that MOS exert the beneficially effects by modification of the intestinal microflora, reduction in turnover rate of the intestinal mucosa and modulation of the immune system. These effects have the potential to enhance growth rate, feed efficiency and live ability in commercial broiler and turkeys, and egg production in layers (Shane, 2001).

Attempts have been made to improve performance and egg quality by supplementation diet with common performance enhancers, especially prebiotics (Zarei *et al*. 2011; Kim *et al*. 2011; Hajati and rezaie, 2012; Shahir *et al*. 2014).

Based on some reports (Berry and Lui, 2000; Shashidhara and Devegowda, 2003), improvement in egg shell quality observed by supplementing MOS to older breeder females diets. A positive effect of the prebiotics on some egg shell quality parameters in laying hens had been reported by Swiatkiewicz *et al*. (2010).

Amino acids are important components of poultry diets; recently there has been much interest in formulating diets based on a digestible amino acid in this respect. Formulating diets in this fashion can result in a decrease of excess nutrients being excreted into the environment. Feed safety margins (ei nutrient requirements) are commonly used in commercial feed formulations and reducing these safety margins can help reduce nutrient excretion into the environment. Reducing these feed safety margins can also decrease feed costs, which is an integral input in poultry production. However, there is utilization a lack of information regarding the amino acids content and digestibility in poultry industry (Garcia *et al*. 2007). On the other hand prebiotic MOS is a commercial product containing yeast cells of *Saccharomyces cerevisiae*.

This experiment was designed because of the lack of enough information to evaluate the effects of prebiotic MOS in diets based on total or digestible amino acids on performance, eggs qualities, small intestine microflora and the cecum gas production of laying hens.

MATERIALS AND METHODS

Birds, diets and experimental design

All procedures were used during this study approved by Animal Care Committee of Bu-Ali Sina University, Hamedan, Iran. In total, 168 Single Comb White Leghorn (SCWL) hens Hy Line W-36, with an initial age of 73 weeks allocated at random into 4 treatments, 7 replicates and 6 birds in each. This study was conducted in a 2 × 2 factorial arrangement including 2 levels of prebiotic (0, 0.5 kg/ton of diet) and types of AA feed formulation, (total and digestible). Experimental basal diet was formulated according to Hy-Line International (2011) recommendation (Table 1). Each experimental diet was offered for 10 weeks. The light was provided 16 h in daily and the temperature was maintained at 21-26 ˚C. All birds were maintained under similar management conditions throughout the experimental period in 73-83 weeks of age.

Egg production (EP) and EW were recorded daily and FI was recorded weekly.

This information was used to calculate ADFI, EP, EM and FCR. Egg mass were calculated by multiplying percentage EP and EW for each replicate. Egg quality traits were determined on a biweekly basis. These eggs were individually weighed and their external and internal qualities were tested.

To measure shell weight (SW), the shell was separated from the yolk and albumen weighed after drying overnight at 60 ˚C as indicated by Grobas *et al*. (2001).

Shell thickness (ST) was measured using a digital micrometer (Echometer 1061, Robotmation Company, Tokyo, Japan). Shape index (SI), egg surface area (ESA) and specific gravity (SG) were determined based on methods from Yannakopoulos and TserveniGousi (1986) and Paganelli *et al*. (1974). Haugh unit (HU) was calculated from egg weight and albumen height as indicated by Haugh (1973). It is shown by the following equation:

$$HU= 100 \text{ Log } (AH+7.57-1.7 \text{ } EW^{0.37})$$

Where:
HU: haugh unit.
AH: albumen height (mm).
EW: egg weight (g).

Determination of dietary total and digestible amino acid total amino acid profile ingredients (corn and soybean meal) were measured in Tehran Evonic Degosa Company by Infrared Spectrometer, then standardized ileal digestibility coefficients were calculated by Opapeju *et al*. (2006) and MacLeod *et al*. (2008) methods. It is shown by following equation:

Concentration of digestible amino acid (g/kg)= [(concentration of total amino acid of ingredients (g/kg)) × (standardized ileal digestibility coefficients of ingredients (%)] × 100

Lactobacillus population measurement was performed according to Mathlouthi *et al*. (2002) methods. Briefly, fresh samples of ileum were collected immediately from healthy laying hens at 83 wk after slaughter (2 birds per replicate). Samples were separately in normal saline tubes, kept on ice, and transferred to laboratory. Samples were serially diluted in normal saline (from 10^{-1} to 10^{-10}) plated onto Man Rogosa and Sharp (MRS) (Merck, Germany) medium and incubated anaerobically at 37 ˚C for 36 hour.

Gas production test

At the end of 83 weeks, two birds were randomly selected per replicate.

Table 1 Amino acids profile total and digestible of corn and soybean meal (%)

Amino acids profile	Corn (total)	Corn (digestible)	Soybean meal (total)	Soybean meal (digestible)
Methionine	0.156	0.147	0.592	0.539
Cysteine	0.175	0.152	0.661	0.542
Methionine + cysteine	0.333	0.299	1.250	1.075
Lysine	0.237	0.218	2.636	2.372
Threonine	0.283	0.241	1.710	1.453
Tryptophan	0.060	0.049	0.592	0.527
Arginine	0.357	0.332	3.096	2.879
Isoleucine	0.269	0.256	1.948	1.734
Leucine	0.938	0.882	3.267	2.908
Valin	0.369	0.340	2.064	1.816
Histidine	0.223	0.211	1.156	1.063
Phenylalanine	0.381	0.358	2.168	1.929

Table 2 Diet formulation and composition (%)

Ingredients (g/kg)	Diet 1	Diet 2	Diet 3	Diet 4
Corn	61.82	62.83	61.81	61.82
Soybean Meal	22.77	21.93	22.77	22.77
Soybean oil	2.64	2.45	2.64	2.64
Dicalcium Phosphate	1.37	1.38	1.37	1.37
Oyster shells	10.43	10.43	10.43	10.43
Common salt	0.34	0.34	0.34	0.34
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25
DL-methionine	0.13	0.13	0.12	0.13
L-lysine HCl	-	0.02	-	-
Prebiotic Bio-Mos®	0.05	0.05	-	-
Cost diets (IRR)	8930	8899	8740	8750
Total	100	100	100	100
Nutrient composition (%)				
Analyzed				
Metabolizable energy	2800	2800	2800	2800
Crude protein (N×6.25)	14.56	14.3	14.56	14.56
Calcium	4.37	4.37	4.37	4.37
Available phosphor	0.39	0.39	0.39	0.39
Sodium	0.17	0.17	0.17	0.17
Chlorine	0.24	0.24	0.24	0.24
Dietary cation anion balance (DCAB)	173.52	168.92	173.50	173.52
Arginine	0.92	0.83	0.86	0.92
Isoleucine	0.60	0.54	0.55	0.60
Methionine	0.36	0.33	0.33	0.36
Lysine	0.74	0.67	0.67	0.74
Methionine + cysteine	0.62	0.55	0.55	0.62
Threonine	0.56	0.47	0.48	0.56
Tryptophan	0.17	0.14	0.15	0.17
Valin	0.69	0.61	0.62	0.69

[1] Vitamin premix supplied per kg of diet: vitamin A: 8.8 IU; vitamin D_3: 2.5 IU; vitamin E: 6.6 g; vitamin B_1: 1.5 g; vitamin B_2: 4.4 g; vitamin B_3 (calcium panthotenate) 8 g; vitamin B_5 (niacin): 20 g; vitamin B_6: 2.5 mg; vitamin B_{12}: 0.08 g; Biotin: 0.15 g; Cholin chloride: 400 mg and vitamin K_3: 2.5 g.
[2] Mineral premix supplied per kg of diet: Phosphorus: 18.7%; Calcium: 22%; Sodium: 39%; Manganese (oxide): 64 g; Iron (sulfate): 100 g; Zinc (oxide): 44 g; Copper (sulfate): 16 g; Iodine (iodate calcium): 0.64 g and Selenium (1%): 8 g.
Prebiotic MOS is a mannan oligosaccharide (processed from cell wall of saccharomyces yeast) and product of ALtech American.
Diet 1: diet based on total amino acid with prebiotic MOS (0.5 kg/ton of diet).
Diet 2: diet based on digestible amino acid with prebiotic MOS (0.5 kg/ton of diet).
Diet 3: diet based on digestible amino acid without prebiotic Bio-Mos.
Diet 4: diet based on total amino acid without prebiotic Bio-Mos.

The contents of the cecum was removed under aseptic condition and placed in falcon tubes (50 mL) after slaughter. Samples were collected immediately, kept on ice, and transferred to laboratory.

Gas production test was carried out according to Menke and Steingass (1988) method.

Statistical analysis

All data were analyzed with prebiotic levels and amino acid (total and digestible) levels, as factorial 2 × 2 using a completely randomized design by the GLM procedure of SAS (SAS, 2004). Treatments means were compared with Duncan's multiple range tests (Duncan, 1955). All differences were considered significant at P ≤ 0.05. There was evaluated the normal distribution of data using Shapiro-Wilk test.

RESULTS AND DISCUSSION

Performance

The effects of dietary treatments on laying performance are shown in Table 3. Over the entire period, EW, EP, EM, ADFI and FCR were not affected (P>0.05) by MOS and types of feed formulation (total and digestible AA). The interaction between week with MOS and type of feed formulation on performance were not significant (P>0.05).

The possible reason for this, it may be age of hens, which with increasing of age, physiological conditions of the digestive tract were developed and, morphological conditions and gastrointestinal microbial were stable. In addition, it could be offered that prebiotics more effective in specific condition such as diseases, stress, density and poor environmental management which may occur in poultry industry. Furthermore, various responses to these different additives, may be due to the age and feed formulation, gut microflora, types of prebiotic dietary or others environmental conditions (Patterson and Burkholder, 2003; Hajati and rezaie, 2010).

This agrees with results of current study (Zarei *et al.* 2011) where found no significant effects on EP, FCR, FI and EM supplemented with two probiotics (Thepax and Yeasturer) and two prebiotics (Fermacto A-Max) and one synbiotic (Biomin) for six weeks.

Bozkurt *et al.* (2012) reports that production performance of laying hens were not affected by adding mannanoligosaccharides (MOS) and essential oils mixture (EOM) in the diet.

However, Chen *et al.* (2005) report that commercial prebiotics improved laying hens' performance. Berrin (2011), has reported that additives of probiotic and prebiotic to the quail breeder diets improved egg production and egg shell thickness and positively affected hatchability in quail breeders. Mostafa *et al.* (2015), indicated that MOS supplemented and forms of its inclusion in starter and grower diets were significantly affected chick performance.

It is reported that the body weight, body weight gain, feed consumption, FCR, mortality and percentage of carcass yield did not affected by the dietary inclusion of prebiotic, probiotic, and synbiotic compared with unsupplemented control in commercial broiler chicken (Sarangi *et al*. 2016).

Egg quality

The effects of dietary treatments on egg quality are shown in Table 4. Numerical differences in HU, ESA, USSR, SW, ST, SG and YC were apparent although not statistically significant (P>0.05). It seems that ST more influenced by the environment temperature, diet and age of birds. Based on the results in this study, the utilization of prebiotics can be a way to increase egg shell quality. The beneficial microorganisms increased absorption of vitamins and minerals especially calcium (Ca) and magnesium (Mg). This effect resulted to increased body weight and eggshell thickness (Roberfroid, 2000).

Also, Berrin (2011) suggested that the improvement in egg shell quality can be resulted from the increased mineral absorption. This agrees with earlier works (Nahashon *et al.* 1994; Mohan *et al.* 1995), which were reports a little improvement ST in hens supplemented with prebiotics for 10 weeks. Sharifi *et al.* (2011) have concluded that shell thickness significantly increased, it was due to the high Ca absorption and deposition and pH reduction of gastrointestinal tract by prebiotics which could have effect on egg shell, which this results in accordance with Swiatkiewicz *et al.* (2010) studies. In addition, it is demonstrated that some of the microbial species such as *Lactobacillus* sporogenes more increased absorption and concentration of Ca in the blood, therefore increasing of egg shell thickness (Panda *et al*. 2008).

This also agrees with Shahir *et al.* (2014) works; that found no significant effects on egg quality supplemented with commercial prebiotics. Zarei *et al.* (2011) reports that feed additives did have beneficial effects on egg quality characteristics in terms of egg shell weight and shell thickness.

However, Bozkurt *et al.* (2012) reports that egg quality except strength thickness were significantly affected by feed additives which additive, it is important or environmental temperature or both.

Intestinal *Lactobacillus* count

The effects of prebiotic MOS and types of feed formulation on ileum *Lactobacillus* count are shown in Table 5. In the present study, MOS, types of feed formulation and their interaction have no significant effects on population of lactic acid bacteria in the ileum (P>0.05). This is probably due to bird age, digestive tract evolution and absence of heat stress. Intestinal beneficial bacteria, especially lactic acid bacteria, reduced intestinal pH by the production organic acids. They weakened conditions for *Salmonella* and *Colibacillus* by alkaline pH for optimum their activity. They reduced proliferations of *Salmonella* and *Colibacillus* and their survival in the gastrointestinal tract (Nurmi *et al.* 1992).

Table 3 Effects of prebiotic Bio-Mos® and types of feed formulation on laying hens performance

Effect / index	ADFI (g/d)	EP (%)	EW (g)	EM (g/d)	FCR
Prebiotic					
0	100.26	81.47	52.56	43.72	2.42
0.5 (kg/ton)	101.37	78.92	51.25	41.28	2.62
SEM	0.30	0.84	0.58	0.84	0.083
Type formulation diet					
Total	100.72	78.57	50.72	40.79	2.68
Digestible	100.91	81.83	53.09	44.21	2.37
SEM	0.30	0.84	0.58	0.84	0/083
Prebiotic					
0.5 × total amino acid	101.42	78.12	50.24	40.05	2.74
0.5 × digestible amino acid	101.31	79.73	52.25	42.50	2.50
0 × total amino acid	100.50	83.92	53.92	45.92	2.23
0 × digestible amino acid	100.02	70.01	51.21	41.53	2.62
SEM	0.43	1.19	0.82	1.19	0.11
P-value					
Prebiotic	0.30	0.26	0.38	0.25	0.36
Types of feed formulation	0.85	0.16	0.13	0.11	0.16
Prebiotic × types of feed formulation	0.77	0.46	0.81	0.63	0.72
Week	< 0.0001	< 0.0001	0.0002	< 0.0001	< 0.0001
Prebiotic × week	0.28	0.34	0.25	0.35	0.16
Week × types of feed formulation	0.89	0.62	0.80	0.77	0.58
Prebiotic × types of feed formulation × week	0.76	0.42	0.55	0.47	0.53

ADFI: average daily feed intake; EP: egg production; EW: egg weight; EM: egg mass and FCR: feed conversion ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of prebiotic MOS and types of feed formulation on egg quality

Effect / index	SG	ST (mm)	SW (g)	HU	YC
Prebiotic					
0	1.07	0.31	5.40	79.47	5.08
0.5 (kg/ton)	1.07	0.75	5.56	79.17	5.20
SEM	0.001	0.31	0.06	0.66	0.003
Types of feed formulation					
Total	1.07	0.76	5.49	78.47	5.12
Digestible	1.07	0.30	5.47	80.18	5.16
SEM	0.001	0.31	0.06	0.66	0.003
Prebiotic					
0.5 × total amino acid	1.08	0.30	5.48	81.42[a]	5.21
0.5 × digestible amino acid	1.07	1.21	5.63	76.93[b]	5.18
0 × total amino acid	1.07	0.31	5.50	80.01[a]	5.13
0 × digestible amino acid	1.07	0.31	5.31	78.93[ab]	5.03
SEM	0/001	0/44	0.09	0.93	0.006
P-value					
Prebiotic	0.41	0.33	0.16	0.81	0.29
Types of feed formulation	0.47	0.32	0.87	0.19	0.72
Prebiotic × types of feed formulation	0.27	0.32	0.13	0.04	0.55
Week	0.005	0.43	< 0.0001	< 0.0001	< 0.0001
Prebiotic × week	0.31	0.43	0.002	0.18	0.0001
Week × type of feed formulation	0.32	0.44	0.28	0.13	0.01
Prebiotic × types of feed formulation × week	0.28	0.44	0.14	0.0001	0.28

SG: specific gravity; ST: shell thickness; SW: shell weight; HU: haugh unit and YC: yolk color.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

This agrees with Yang *et al.* (2007) reports that MOS was not significant affected on intestinal microbial composition.

Also, Ceylan *et al.* (2003) concluded that cecal microflora population did not affect by feed additives such as probiotics, organic acids, MOS and antibiotics.

Table 5 The effects of prebiotic MOS and types of feed formulation on ileum *Lactobacillus* count

Effect / index	Number of *lactobacillus* bacterial (Log10 cfu[1]/mL)
Prebiotic	
0	7.77±1.37
0.5 (kg/ton)	7.64±3.01
SEM	0.87
Types of feed formulation	
Total	7.86±2.19
Digestible	7.56±2.48
SEM	0.87
Prebiotic	
0.5 × total amino acid	7.36±2.72
0.5 × digestible amino acid	7.92±3.69
0 × total amino acid	8.35±1.77
0 × digestible amino acid	7.19±0.62
SEM	1.23
P-values	
Prebiotic	0.92
Types of feed formulation	0.81
Prebiotic × types of feed formulation	0.50

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

Table 6 Effects of prebiotic MOS and types of feed formulation on cecum microbial population of laying hens

Effect / index	Gas production rate (A)	Gas production volume (C)
Prebiotic		
0	0.046	281.93
0.5 (kg/ton)	0.043	255.83
SEM	0.003	10.02
Types of feed formulation		
Total	0.03	273.23
Digestible	0.05	264.53
SEM	0.003	10.02
Prebiotic		
0.5 × total amino acid	0.052	280.97
0.5 × digestible amino acid	0.040	282.90
0 × total amino acid	0.038	263.57
0 × digestible amino acid	0.048	248.10
SEM	0.005	14.18
P-value		
Prebiotic	0.58	0.10
Types of feed formulation	0.08	0.55
Prebiotic × types of feed formulation	0.93	0.64

A: potential ofgas production (mL/g) and C: rate of gas production (mL/h).
SEM: standard error of the means.

In addition, Fernandez *et al.* (2002) concluded that the bird intestinal microflora (*Bifidobacterium* spp. and *Lactobacillus* spp.) enhanced by supplementing diets with mannoseoligosaccharide (MOS) or palm kernel meal (PKM).

However, Donalson *et al.* (2008) report that laying hen cecal lactic acid bacteria enhanced with combination of fructooligosaccharide prebiotics with alfalfa or a layer ration. It is reported that additives of mannan-oligosaccharides (Bio-Mos or PKE) in diets did not affected population of the ileal *Lactobacilli*, *Enterococcus* or *Enterobacteriaceae* family (Bahman *et al.* 2015).

Gas production

There were no significant differences in the rate and gas production volume by experimental treatments (P>0.05). This is probably due to the number and low activity of cecum microbial population than the rumen of ruminants. In general, in total experimental period, the rate and gas production volume were not affected by MOS, types of feed formulation and their interaction (P>0.05). Although, Guo *et al.* (2003) shown that extract of polysaccharide increased gas production in cecum of chickens. Short chain fatty acid (SCFA) (such as acetate, propionate, butyrate and lactic acid) and gasses (CO_2, CH_4 and H_2) produced by prebiotic fermentation in hindgut. Acid production causes release of toxic NH_3 (and amines) to produce NH_4^+. This NH_4^+ is no-permeation and result to decreases the blood NH_3 level. *Lactoacillus* and *Bifidobacterium* are capable of using NH_3 as their N source and reduce its concentration both in the gut and in the blood.

Fecal pH and NH_3 concentration did not affected by supplementation of MOS in dog's diets (Pawar *et al*. 2008; Kore *et al*. 2009) but faecal lactate, propionate and butyrate concentrations tended to increase (Kore *et al*. 2009). Also, there was a significant increase (P<0.05) in the faecal lactate and SCFA contents with a reduction in the faecal NH_3 content in dietary of dogs (Samal *et al*. 2012). However, Zentek *et al*. (2002) found an increased faecal NH_3 concentration after lactulose supplementation.

Previous studies have demonstrated that additives of non starch polysaccharide (NSP) in poultry diets could decline detrimental gas production (Wang, 2009).

CONCLUSION

Prebiotic MOS and types of feed formulation did not significant effect on laying hen's performance, intestinal *Lactobacillus* number and gas production of cecum microbial population. Egg quality did not affected by P-BM and types of feed formulation based on total AA and ileal digestibility.

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