The effect of rice husk as an insoluble dietary fiber source on intestinal morphology and Lactobacilli and *Escherichi*a *coli* populations in broilers

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Abstract:

BACKGROUND: There are some reports on the positive effects of dietary insoluble fiber on the performance of broilers. **OBJECTIVES:** This study was carried out to determine the effect of inclusion rate and particle size of rice husk in the diet of broilers on the ileal and cecal bacteria populations and small intestine morphology. **METHODS:** The experimental diets consisted of a control husk-free diet and four diets containing 7.5 or 15 g/kg rice husk with particle sizes of less than 1 mm or between 1-2 mm. **RESULTS:** The dietary insoluble fiber did not affect feed intake of the experimental groups. The best body weight gain and feed conversion ratio was recorded in the broiler chickens fed the diet containing 15g/kg rice hulls with particle size of less than 1 mm ($p<0.05$). In the duodenum and jejunum, the crypt depth to villi height ratio in the control group was significantly lower than other groups ($p<0.05$). In the ileum, all the birds fed rice husk except the group fed the diet that contained 15g/kg rice husk with particle size of 1-2 mm, had higher *Lactobacilli* and lower *E. coli* and coliforms populations than the control group ($p<0.05$). **CONCLUSIONS:** The results of the present study suggest that the 7.5g/kg dietary inclusion and more coarse particles size of rice husk (1-2 mm) were more effective to promote broiler growth performance. The positive effects of dietary insoluble fiber on the growth performance of broilers in this study are probably a result of favorable changes in the bacteria populations of the gastrointestinal tract and not any improvement in small intestine absorptive capacity.

Introduction

To date, most attention has focused on fermentable carbohydrate components such as fructooligosaccharides and mannan oligosaccharides which have beneficial effects on intestinal microflora of broilers (Navidshad et al., 2015). However, the research conducted in recent years has shown that incorporation of low levels of different sources of insoluble fiber in diet improves development of digestive organs (Hetland and Svihus, 2007; Rezaei et al., 2011a,b, 2014) and increased secretion of bile acids, HCl and digestive enzymes (Hetland et al., 2003). These changes may improve the digestibility of nutrients, the health of the digestive tract (Montagne et al., 2003) and ultimately improve animal welfare (Van Krimpen et al., 2009). The crypt depth to villi height ratio is an indicator of digestion potential in the small intestine and a smaller ratio suggests an improvement on the intestinal mucosa (Hampson, 1986).

Lactic acid bacteria which are included *Lactobacilli* are the most commonly used probiotics (Brashears et al., 2003). Some strains of *E. coli* cause illness that can be fatal in domestic birds (Sackey et al., 2001).

Previous studies have shown that cellulose as an active component of the diet may increase the number of intestinal beneficial bacteria, particularly bifidobacteria and *Lactobacilli* and also affect potential pathogenic bacteria (Cao et al., 2003). It is generally accepted that the phenolic components of pure lignin have antimicrobial properties (Baurhoo et al., 2008). It has been shown that cellulose has an abrasive effect on the intestine and causes increased secretion of mucous (Montagne et al., 2004). Mucous layer has a dynamic nature (continually under construction and destruction) and is involved in protection, creating fluidity and nutrients absorption in small intestine. After creating friction by dietary cellulose, the secretion of mucin in the gastrointestinal tract by goblet cells is increased (Montagne et al., 2004).

Based on the literature review, there is a lack of information on the effect of the level and particle size of the dietary fiber on the microbial population of small intestine of broilers. This study was carried out to determine the effect of inclusion rate and particle size of rice husk in the diet of broilers on the ileal and cecal bacteria populations and small intestine morphology.

Materials and Methods

The rice husk (Hashemi cultivar) required in the trial was obtained from a commercial rice processing factory, Astara, Iran. After grinding with hammer mill with sieve sizes of 1 and 2 mm the husk was divided in two parts with particle sizes less than 1 mm and 1-2 mm, respectively. The dry matter, crude protein, fat, fiber and ash of the rice husk and experimental diets were determined (AOAC, 2000). The concentration of neutral detergent fiber (NDF), acid detergent fiber was determined based on Van Soest (1963). The lignin content of rice husk was considered 22.34% (Senthil Kumar et al., 2010).

Animal care guidelines were followed for animal use for experimental and other scientific purposes. The study lasted for 42 days, 200 broilers (Ross 308) of both sexes were used in a completely randomized design with 5 treatments, 4 replicates and 10 birds per replication. In this study, the experimental diets were isonitrogenous and isocaloric and were formulated based on Ross (308) broilers nutritional requirements tables (Table 1). The environmental conditions (temperature, humidity and light) were controlled based on the recommendation of the strain catalog. Access to water and feed was free from day 1 to the end of the experiment. The chicks with similar weights received one of the following experimental diets from 11 d of age:

1. The control diet without rice husk (C).
2. Diet containing 7.5 g/kg rice husk with particle sizes less than 1 mm (RSL).
3. Diet containing 15 g/kg rice husk with particle sizes less than 1 mm (RSH).
4. Diet containing 7.5 g/kg rice husk with particle size between 1 to 2 mm (RBL).
5. Diet containing 15 g/kg rice husk with particle size between 1 to 2 mm (RBH).

Feed intake and weight gain were measured periodically and the feed conversion ratio was calculated. Before the weighting, the birds were fasted for 7 hours to empty the gut contents. Mortality was recorded daily. European efficiency index (EEI) for each replication was calculated as follows,

EEI = (livability × live weight in kg/length of fattening period in days × FCR) × 100

At 42 d of age, two birds of each replication were randomly selected and slaughtered. Digestive tract was removed immediately. Digesta samples of the ileum (the boundary between Meckel's diverticulum and the branching point of the ceca) and ceca were immediately collected and transferred to sterile tubes containing 20% glycerol phosphate buffer saline and stored in -20°C until the tests.

Ileal and cecal bacterial populations were quantified using 'standard Koch's plate method (Merck Microbiology Manual, 2007). Samples were placed in buffer peptone 1% (w/v 1:9) and then the serial dilutions were prepared. The following bacterial species were quantified using specific culture media (Merck, Germany), lactic acid bacteria in MRS agar, *E. coli* on Chromocult TBX agar and coliforms on coliform Agar ES. All samples were plated in triplicate and incubated at 37°C for 4 h (Merck Microbiology Manual, 2007). All the collected data were analyzed using general linear model (GLM) of SAS statistical software14 and the comparison of means was done by Duncan procedure at 0.05 probability level.

Results

The chemical composition obtained for rice husks was as follows, 93% dry matter, apparent metabolizable energy of 834 kcal / kg, ash 16.8%, ether extract 3.49%, crude protein 3.23%, crude fiber 37.5% , NDF 76.0%, ADF 56.1%, calcium 0.8% and total P 0.27%.

The effect of dietary inclusion of rice husk on the production traits and EEI of broilers is shown in Table 2. The experimental diets had no effect on feed intake of birds. During the grower phase, the live weight gain of broilers fed diets containing 15g/kg rice husk (RSH and RBH treatments) was higher than the groups fed the lower level (7.5g/kg) rice husk (RSL and RBL) treatments ($p<0.05$). In the finisher phase, the differences observed in live weight gains were not significant, but numerically the diets with lower level of rice husk (RSL and RBL treatments) had higher weight gain. This effect resulted in an improved weight gain in the RBL group compared to the control group in the whole experiment period ($p<0.05$). During the experimental period, the best feed conversion ratio was also observed in RBL group such that the difference with the RSL group was significant ($p<0.05$).

European efficiency index in the experimental period was affected by the diets, such that as in all the rice husk fed birds except for RSL, EEI was higher than the control group ($p<0.05$). Table 3 shows the effect of rice husk on the intestinal morphology of broilers. Unexpectedly, dietary rice husk reduced the villi height compared to the control group in all parts of the small intestine ($p<0.05$), although this difference was not significant in the ileum. The small intestine epithelial thickness was not affected by the diets. In duodenum, the highest number goblet cells were observed in the control group and the lowest was in the RBH treatment ($p<0.05$). In the jejunum and ileum rice husk consumption resulted in increased numbers of goblet cells compared to the control group, such that in the jejunum the differences with RSL and RBH groups, and in ileum, the difference with the RSL group were significant ($p<0.05$). In duodenum, a dipper crypt was observed in RBH group rather than in the control and RSH groups ($p<0.05$). In the jejunum and ileum, crypt depth was not affected by diet. In the duodenum and jejunum, the crypt depth to villi height ratio in the control group was significantly lower than other groups ($p<0.05$), but this parameter was not affected in the ileum.

The bacterial flora of the gastrointestinal tract was also under the influence of rice husk in diets (Table 4). In the ileum, all the birds fed rice husk except RBH, had a higher lactic acid bacteria and lower *E. coli* and coliforms populations than the control group ($p<0.05$). Fewer numbers of *E. coli* were observed in the ileal

Table 1. Composition of experimental diets. 1Vitamin premix provided the following per kilogram of diet, vitamin A (retinyl acetate), 9,000 IU; vitamin D (cholecalciferol), 5,500 IU; vitamin E (dl-α-tocopheryl acetate), 68 IU; menadione, 9.0 mg; pyridoxine, 7.0 mg; riboflavin, 26.0 mg; Ca-pantothenate, 26.3 mg; biotin, 0.41 mg; thiamine, 3.66 mg; niacin, 75 mg; cobalamin, 0.03 mg; and folic acid, 3.70 mg. 2Mineral premix provided the following per kilogram of diet, Fe, 82 mg; Mn, 60 mg; Zn, 115 mg; Cu, 15 mg; I, 0.85 mg; and Se, 0.4 mg.

	Starter	**Grower**			**Finisher**		
		Control	**Rice hulls**		**Control**	**Rice hulls**	
Ingredient, %			7.5 g/kg	15 g/kg		7.5 g/kg	15 g/kg
Rice hulls	0	0	0.75	1.5	0	0.75	1.5
Corn	45.92	48.55	47.19	46.11	51.96	50.59	49.23
Soybean meal	43.83	40.29	40.44	40.53	37.34	37.49	37.63
soybean oil	5.88	7.19	7.67	7.91	6.96	7.44	7.91
DCP	2.09	1.72	1.72	1.72	1.6	1.6	1.6
Calcium Carbonate	1.19	1.07	1.05	1.04	1.02	1.01	1.02
Common salt	0.23	0.44	0.44	0.44	0.44	0.44	0.44
Vitamin premix1	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix2	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionin	0.32	0.25	0.25	0.25	0.18	0.18	0.19
HCl-Lysin	0.04	0	0	0	0	0	0
Chemical analysis							
Metabolisabld energy (Kcal/kg)	2980	3100	3100	3090	3130	3130	3130
Crude protein	23.27	22	22	22	21	21	21
Ca	1.033	0.9	0.9	0.9	0.85	0.85	0.85
AvP	0.52	0.45	0.45	0.45	0.42	0.42	0.42
Na	0.198	0.2	0.2	0.2	0.2	0.2	0.2
Lys	1.41	1.297	1.297	1.304	1.223	1.223	1.223
Met	0.69	0.5992	0.5992	0.599	0.524	0.524	0.524
Met+Cys	1.06	0.95	0.95	0.95	0.86	0.86	0.86
CF	4.7	4.49	11.45	11.86	4.39	11.54	11.92
NDF	11.61	11.44	11.87	12.31	11.45	11.88	12.30
ADF	5.01	4.80	5.19	5.59	4.6	5.05	5.44
Lignin	0.67	0.61	0.80	0.99	0.61	0.80	0.97

Table 2. Performance characteristics of broilers fed experimental diets contained rice husk. 1C=Control. RSL= The diet contained 7.5g/kg rice husk with particle size of less than 1 mm, RSH= The diet contained 15 g/kg rice husk with particle size of less than 1 mm, RBL= The diet contained 7.5g/kg rice husk with particle size of 1-2 mm, RBH= The diet contained 15g/kg rice husk with particle size of 1-2 mm. EEI= European efficiency index. Within a column not sharing a common superscript differ significantly at p<0.05.

	Daily feed intake (gram/bird/day)			**Daily weight gain (gram/bird/day)**			**Feed conversion ratio**			**EEI**
	11-24 d	25-42 d	11-42 d	11-24 d	25-42 d	11-42 d	11-24 d	25-42 d	11-42 d	
C1	81. 7	166.8	126.5	54.5ab	87.9	72.2b	1.50	1.89	1.75ab	292.5b
RSL	81.0	175.0	134.2	52.3b	92.5	74.0ab	1.54	1.90	1.79a	307.4b
RSH	85.6	169.9	131.7	56.3a	93.6	76.6ab	1.52	1.82	1.72ab	342.7a
RBL	82.9	174.3	131.6	55.0ab	101.3	79.6a	1.51	1.72	1.65b	345.8a
RBH	84.4	172.7	133.0	56.8a	96.1	78.4ab	1.49	1.80	1.69ab	346.0a
MSE	1.64	4.86	2.53	1.13	4.06	2.19	0.043	0.051	0.045	5.12
p value	0.34	0.51	0.82	0.04	0.32	0.03	0.19	0.33	0.02	0.03

Table 3. Small intestinal morphology of broilers fed experimental diets that contained rice husk. 1C=Control. RSL= The diet contained 7.5g/kg rice husk with particle size of less than 1 mm, RSH= The diet contained 15g/kg rice husk with particle size of less than 1 mm, RBL= The diet contained 7.5g/kg rice husk with particle size of 1-2 mm, RBH= The diet contained 15g/kg rice husk with particle size of 1-2 mm. Within a column, not sharing a common superscript differs significantly at p<0.05.

	Duodenum					**Jejunum**					**Ileum**				
	VH2	CD	CD:VH	ET	G	VH	CD	CD:VH	ET	G	VH	CD	CD:VH	ET	G
C1	1738^{a}	141.8^{c}	0.081^{b}	41.8	10.4^{a}	843^{a}	143.0	0.169^{b}	40.2	9.8^{c}	778	101.6	0.130	39.4	9.2^{b}
RSL	1713^{bc}	157.6^{ab}	0.092^{a}	42.0	7.2^{cd}	825^{b}	145.2	0.176^{ab}	39.0	12.4^{a}	769	101.0	0.131	38.8	10.8^{a}
RSH	1711^{bc}	152.0^{b}	0.089a	39.4	9.2^{bc}	823^{b}	143.6	0.174^{ab}	36.4	11.2^{abc}	767	102.1	0.131	38.7	10.0^{ab}
RBL	1706^{c}	155.8^{ab}	0.091a	39.6	8.4^{bc}	824^{b}	145.6	0.177^{a}	37.2	10.8^{bc}	766	103.2	0.135	40.4	10.4^{ab}
RBH	1717^{b}	158.2^{a}	0.092a	42.4	6.2^{d}	825^{b}	146.8	0.178^{a}	39.6	11.8^{ab}	868	102.6	0.133	38.6	11.8^{ab}
MSE	2.89	1.95	0.001	1.42	0.43	3.01	1.92	0.002	1.42	0.43	3.93	2.22	0.003	0.43	0.42
p value	0.02	0.02	0.01	0.66	0.03	0.01	0.12	0.04	0.15	0.02	0.42	0.36	0.61	0.59	0.03

Table 4. Ileum and cecum bacteria population of broilers fed experimental diets that contained rice husk. 1C=Control. RSL= The diet contained 7.5g/kg rice husk with particle size of less than 1 mm, RSH= The diet contained 15 g/kg rice husk with particle size of less than 1 mm, RBL= The diet contained 7.5g/kg rice husk with particle size of 1-2 mm, RBH= The diet contained 15g/kg rice husk with particle size of 1-2 mm. Within a column, not sharing a common superscript differs significantly at p< 0.05.

	Ileum (log 10 cfu/g)			**Cecum (log 10 cfu/g)**		
	Lactobacillus. sp	*E. coli*	Coliforms	*Lactobacillus*. sp	*E. coli*	Coliforms
C1	7.56b	9.95a	9.99a	9.60	8.60a	8.94a
RSL	8.25a	6.52c	6.55c	8.79	8.51ab	8.74a
RSH	8.54a	8.12b	8.53b	8.63	6.85c	7.55b
RBL	8.44a	6.64c	7.09bc	8.73	7.62bc	8.05ab
RBH	7.36b	7.74bc	7.94bc	9.44	8.30ab	8.62ab
MSE	0.22	0.46	0.48	0.52	0.34	0.42
p value	0.02	0.02	0.04	0.23	0.02	0.03

digesta of RBL and RSH groups (p<0.05) and the coliforms were also reduced in the BCL group. The total number of treatments in BCL (p<0.05). In ceca, the only significant difference was lower populations of coliforms and *E. coli* in RSH group compared with the control group (p<0.05).

Discussion

In this study, the use of the rice husk at 15 g/kg of the diet as a source of lignocellulose led to improved body weight gain of broilers at grower phase. However, this situation was changed at the finisher phase such that during the experimental period the birds fed the diet containing 7.5 g/kg rice husk with particle size of 1-2 mm showed better weight gain. This finding suggests that the dietary fiber effect on chick's performance will change with gastrointestinal development and a coarse and higher level of fiber may accelerate this development. These findings confirm the results of previous reports that the moderate levels of an insoluble fiber could improve the growth performance of broilers (Sarikhan et al., 2010; González-Alvarado et al., 2010; Mateos et al., 2012).

The insoluble dietary fiber speeds up the passage rate of digesta in the intestinal tract, which in turn may increase the feed intake (Hetland et al., 2003; Montagne et al., 2003). Such observations in broilers have also been reported using pure cellulose (Cao et al., 2003) and lignin (Mateos et al., 2012).

Increased goblet cell numbers in the jejunum and ileum of broilers in this study were reported in previous studies because of its abrasive effect that resulted in a change in mucin secretion (Montagne et al., 2003; Rezaei et al., 2011a,b, 2014). The mucus layer overlying the epithelium secreted by the goblet cells improves the elimination of gut contents and provides the first line of defense against physical and chemical injury caused by ingested food, microbes and the microbial products (Kim and Ho, 2010).

Increased crypt depth in the duodenum of chicks fed rice husk is in agreement with a report on chicks fed cellulose (Wils-Plotz and Dilger, 2013). Unexpectedly, in the present study, dietary rice husk decreased small intestine villi height compared to the control group. The crypt depth to villi height ratio in this study was reduced in all the experimental groups fed the diets that contained rice husk compared to the control group, which is not compatible with the more superior growth performance observed in the rice husk fed groups.

The results of the present study showed that adding rice husk as a lignocellulose source could promote the growth of beneficial Lactobacillus bacteria and reduce the population of pathogenic bacteria such as some *E. coli* in the ileum and cecum of broilers at 42 d of age. In the same report, it was shown that 4% alfalfa meal in the broiler diet increased *Lactobacilli* bacteria population and reduced the number of *E. coli* compared with the control group (Hampson, 1986).

The mechanism of lignocellulose action on bacterial populations in the gastrointestinal tract is not entirely clear. It can be assumed that this effect is due to different actions of cellulose and lignin in the intestinal environment. It has been found that the decomposition of insoluble dietary fiber (NDF) by chicken's bacterial enzymes is about 35% (Jamroz et al., 2001) but the effect on the microbial population is mainly due to fiber physicochemical properties. The friction effect of dietary insoluble fiber on the surface of gut, facilitates removal of pathogen bacteria from mucous layer (Mateos et al., 2012). This effect in turn is able to boost the growth of beneficial microflora. The positive effect of lignocellulose on gut Bifidobacterium and *lactobacilli* population has been confirmed in the previous reports (Cao et al., 2003). Some bacteria release bacteriocins which prevent further proliferation of pathogens in the gut (O'Shea et al., 2012). It has been reported that lignin Alcell decreased the growth of aerobic bacteria in mice in both in vivo and in vitro situations (Nelson et al., 1994).

The results of the present study suggest that a minimum amount of dietary insoluble fiber is needed to achieve the best growth performance of broiler chickens. So, diets of broilers should be formulated to provide enough insoluble fiber. The 7.5g/kg dietary inclusion and more coarse particles size of rice husk (1-2 mm) were more effective to promote broiler growth performance. The positive effects of dietary insoluble fiber on the growth performance of broilers in this study is probably a result of favorite changes in the bacteria populations of the gastrointestinal tract and not any improvement in small intestine absorptive capacity.

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اثر پوسته خارجی به عنوان یک منبع فیبر جیره‌های نامحلول بر ریخت شناسی و جمعیت لاکتوباسیلوس‌ها و اشریشیا کولی روده جوجه‌های گوشتی

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چکیده

زمینه مطالعه: گزارش‌هایی در مورد اثرات مثبت فیبر نامحلول جیره بر بازدهی جوجه‌های گوشتی وجود دارد. **هدف:** این مطالعه به منظور تعیین اثر سطح مصرف و اندازه ذرات پوسته برنج در جیره جوجه‌های گوشتی بر جمعیت باکتریایی ایلئوم و سکوم و ریخت شناسی روده کوچک انجام گرفت. **روش کار:** جیره‌های آزمایشی عبارت بودند از یک جیره شاهد فاقد پوسته برنج و چهار جیره حاوی ۷/۵ g/kg یا ۱۵ پوسته برنج با اندازه ذرات کمتر از ۱mm یا بین ۲mm-۱. **نتایج:** فیبر نامحلول جیره مصرف خوراک گروه‌های آزمایشی را تحت تأثیر قرار نداد. بهترین افزایش وزن و ضریب تبدیل غذایی در جوجه‌های گوشتی تغذیه شده با جیره حاویش ۵g/kg پوسته برنج با اندازه ذرات کمتر از ۱mm ثبت شد ($p < ۰/۰۵$). در دودنوم و ژژنوم، نسبت عمق کریپت به ارتفاع ویلی در گروه شاهد به طور معنی داری کمتر از سایر گروه‌ها بود ($p < ۰/۰۵$). در ایلئوم، تمام پرنده‌های تغذیه شده با پوسته برنج به استثناء گروه تغذیه شده با جیره حاوی ۱۵ g/kg پوسته برنج با اندازه ذرات ۲mm-۱، جمعیت لاکتوباسیلوسی بیشتر و جمعیت‌های ا.کولی و کولیفورم‌های کمتری نسبت به گروه شاهد داشتند ($p < ۰/۰۵$). **نتیجه‌گیری نهایی:** نتایج این تحقیق پیشنهاد می‌نماید که استفاده از سطح ۷/۵g/kg پوسته برنج با اندازه ذرات بزرگتر (۲ mm-۱) بطور موثری منجر به بهبود بازدهی رشد گردید. اثرات مثبت فیبر نامحلول جیره بر بازدهی رشد جوجه‌های گوشتی در مطالعه حاضر احتمالاً از بهبود در ظرفیت هضمی روده ناشی نشده ، بلکه در نتیجه ایجاد تغییراتی مطلوب در جمعیت باکتریایی مجرای روده بوده است.

واژه‌های کلیدی: جوجه گوشتی، جمعیت باکتریایی روده، ریخت شناسی روده، اندازه ذرات، پوسته برنج

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