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EFFECT OF SOYBEAN HULLS AND ENZYMES SUPPLEMENTATION ON DIGESTIVE TRACT LENGTHS AND CECUM MICROBIOLOGY OF BROILER CHICKS

N.M. El-Medany; F. Abd El-Azeem; M.A.M. Abdelaziz; A.I. El-Faham; Neamatallah, G. Ali and M.M. Hamed

Poult. Prod. Dept., Fac. of Agric., Ain Shams Univ., Egypt.

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SUMMARY

This study aimed to determine the effects of feeding different dietary levels of soybean hulls (SH) as a source of fiber in broiler diets on length of the digestive tract and cecum microbiology. A total of 210 one-day old chicks of the Indian River strain were randomly assigned to seven dietary treatments (7 treatments x 3 replicate groups x 10 chicks in each group). The experimental treatments were birds fed SH at levels 1.5, 3, and 4.5% without and with enzymes (T_{1-6}), and a control group without any supplement. The main results obtained can be summarized as follows: Noticeable effect in the lengths of digestive gut parts (cm), Using SH in broiler diets increased the total count of bacteria and *lactic acid bacteria* and decrease in the count of *coliforms.*

Keywords*: *Soybean Hulls; High levels of fiber, and Gut health.

INTRODUCTION

Dietary fiber (DF) is the edible parts of plants or analogous carbohydrates that are hardy to be digested and absorbed in the small intestine, with complete or incomplete fermentation in the large intestine. Dietary fiber contains polysaccharides, oligosaccharides, lignin, and associated plant materials. These fibers encourage useful physiological effects, including laxation, blood cholesterol attenuation, and/or blood glucose attenuation (AACC, 2000). To determine the crude fiber content of feed stuffs, a system for "detergent fibers" was instigated (Van Soest, 1963). This system includes two fractions: the neutral detergent fiber and the acid detergent fiber. This was devised in order to separate the more fermentable "hemicellulose" from the less digestible cellulose and lignin and was a much-improved measurement of fiber compared with crude fiber. However, commercial diet formulators have mostly ignored it, despite much research and refinement. (Van Soest, 1963).

Dietary fiber affects gut development in different ways, depending not only on the type of fiber used but also on its particle size. Jiménez-Moreno *et al.* (2011) reported that, in general, the effect of DF on the relative weight or length of the full gut was more evident with the inclusion of sugar beet pulp than with oat hulls (OH), probably because of the higher water-holding capacity of the pectin fraction of the sugar beet pulp.

Most researchers are more interested in intestinal histology than gut length in birds, which were fed many different sources of fiber.

Sittiya *et al.* (2019) conducted a study to determine the effects of rice hulls (RH) and soybean hulls (SH) levels on intestinal length of broiler chicks. One-day-old unsexed broiler chicks were fed comparable diets between control groups. The RH and SH experimental diets were as follows: 0% (control); 2.5% RH; 2.5% SH; 5% RH; and 5% SH, the length of the intestinal tract in birds fed on SH or RH was longer than in control groups. Also, Nursiam *et al.* (2021) recorded that using commercial sources of fiber in Cobb 500 broiler diets to increase levels of fiber in diets 5% from RH led to an increase in length of the digestive tract and small intestinal, but when they used RH in broiler diets, there were no effects on length of the digestive tract or small intestinal between all treatments. The similar results found in the study by Kurul *et al.* (2020) who found that increasing the levels of fiber from SH by levels 2, 4, and 6% in male (Ross 308) broiler diets had no effect on the total digestive tract or small intestinal tract between all treatments. Shang *et al.* (2020) found an increase in total digestive length and small intestinal size in broilers fed wheat bran by 3% compared to control groups.

On the other hand, Hikawczuk *et al.* (2023) found that the utilization of oat hull in the amount of 1 and 3% of the diet of broiler chickens reduced the total length of the gut and small intestinal tract compared to control groups.

On the other hand, DFs could modulate gut microbiota by increasing the proportion of *Bacteroidetes* and *Firmicutes* (Han *et al.,* 2018 and Li *et al.,* 2016). DFs could increase the abundance of *Bacteroides acidifaciens* and *Enterobacteriacea* (operational taxonomic units) associated with inflammatory bowel disease (Shibayama *et al.,* 2018), inhibit the growth of *Salmonella* (Kumar *et al.,* 2012), and stimulate the growth of butyrogenic bacteria and *Verrucomicrobia* (Li *et al.,* 2016). Additionally, wheat bran diets can increase the abundance of *Clostridiales* in the mice, which contain bacteria related to polysaccharide degradation and short-chain fatty acids (SCFAs) production (Matsuzaki *et al.,* 2020).

Akkermansia muciniphila is a dominant bacterium belonging to *Verrucomicrobia* that colonizes the outer layer of intestinal mucus (Ottman *et al.,* 2017). Recent studies have shown that *Akkermansia muciniphila* is a new important contributor involved in metabolic syndrome, immune function, and gut permeability (Cani and de Vos, 2017; Chelakkot *et al.,* 2018 and Depommier *et al.,* 2019). The abundance of *Akkermansia muciniphila* was higher in the colonic digesta of rats with wheat diets than that with rice diets (Han *et al.,* 2018).

Vasanthakumari *et al.* (2022) found that when using xylanase enzymes in broiler (Ross 308) chicks from 0 days to 36 days as hatch, the influence of supplemental xylanase added to diets with high fiber as grain and 5% dried distillers grains with solubles (CF 11.3%). All diets were steam-conditioned at 80°C on the microbiology of small intestinal cells. Xylanase supplementation had significant ($P < 0.05$) effects in modulating the intestinal microbiota, with a higher relative abundance of commensal bacteria such as Lactobacillus and a lower abundance of potentially non-beneficial bacteria such as *E. coli,* indicating a prebiotic mode of action.

Thus, the aim of this study was to evaluate the effects of high levels of fiber from SH without and with enzymes on gut length and cecum microbiology on broiler chicks.

MATERIALS AND METHODS

The present experiment was implemented in Poultry Nutrition Farm, Poultry Production Department, Faculty of Agriculture, Ain Shams University, Qalyubia, Egypt, in order to investigate the effect of high crude fiber levels from SH with and without enzymes on digestive tract lengths and cecum microbiology on broiler chicks.

Experimental design:

210 Indian River (IR) unsexed one-day old chicks were used in this study. All chick were randomly distributed into 7 treatments, each treatment contained 3 replicates each replicate included 10 chicks. The experimental design and composition and calculated analysis of the basal diet (starter from 1to21 days and grower from 21to35 days of age) are presented in Table (1).

Experimental birds and management:

Chicks were reared in electrics heated batteries under similar conditions of managements during the experimental periods. The lighting program was controlled to provide 23-hour light and one-hour dark daily by fluorescent tube lighting. The temperature was controlled and gradually reduced from 32°C to 20°C on day 35 of age.

Feeding and watering:

Feed was offered *ad-libitum* in mash form according to experimental diets in stainless steel feeders. Fresh water was accessible all the time by automatic nipple drinkers.

Measurements and procedures:

At 35 days of age, four birds from each treatment were randomly chosen, weighed and then slaughtered, after complete bleeding and feather removal, gut parts were removed and lengthen (total digestive tract, small intestine and cecum).

Table (1): Composition and calculated analysis of experimental diets.

Ingredients (%)	Starter (0- 21 days)				Finisher (22 – 35days)			
	Control	SH 1.5%	SH 3.0 %	SH 4.5%	Control	SH 1.5%	SH 3.0 %	SH 4.5%
Yellow corn	52.96	51.54	50.26	49.00	55.06	53.85	52.64	51.37
Soybean meal (46%CP)	33.00	34.00	34.00	34.00	32.10	32.10	32.10	32.10
Soybean hulls	0	1.50	3.00	4.50	0	1.50	3.00	4.50
Corn gluten meal (60% CP)	7.00	6.00	5.80	5.57	5.00	4.75	4.55	4.30
Soybean oil	2.40	2.40	2.40	2.40	3.70	3.70	3.70	3.70
Monocalcium phosphate	0.96	0.95	0.93	0.91	0.88	0.92	0.92	0.92
Limestone	2.50	2.45	2.45	2.45	2.18	2.08	1.99	1.99
HCL- Lysine	0.28	0.25	0.24	0.24	0.21	0.21	0.21	0.21
D-L Methionine	0.30	0.31	0.32	0.33	0.27	0.29	0.29	0.31
Salt (NaCl)	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated composition**								
Metabolizable energy (Kcal/kg)	3017	2970	2934	2870	3100	3065	3030	2994
Crude protein (%)	23.07	23.00	23.00	23.00	21.50	21.50	21.50	21.50
Crude fiber (%)	2.53	3.03	3.50	3.96	2.51	3.00	3.45	4.91
Calcium (%)	0.95	0.95	0.95	0.95	0.87	0.87	0.87	0.87
Available phosphors (%)	0.48	0.48	0.48	0.48	0.43	0.43	0.43	0.43
Lysine (%)	1.40	1.40	1.40	1.40	1.29	1.29	1.29	1.29
Methionine (%)	0.56	0.56	0.56	0.56	0.51	0.51	0.51	0.51
Methionine+ cysteine (%)	1.08	1.08	1.08	1.08	0.99	0.99	0.99	0.99

**Each 3Kg of premix containing: 15000000 I.U. VIT, A, 3000000 I.U VIT. D 50g. VIT E, 3000mg VIT. K3. 3000 mg VIT. B1, 8000 mg. VIT B2, 4000 mg. VIT B6, 20mg. VIT. B12, 15000 mg pantothenic acid, 60000 mg. niacin, 1500 mg. folic acid, 200mg. biotin, 200000 mg VIT C, 700 gm. choline chloride, 80 gm. Mn, 80 gm. zinc, 60 gm. iron, 10 gm. CU, 1 gm. Iodine, and 0.2 gm millennium, where CaCo3 was taken as a carrier up to 3kg, the inclusion rate was 3Kg premix/ton feed.*

*** Calculated analysis of the experimental diets was done according to Horacio, 2017 (Brazilian feed stuffs, 2017). Chemical composition of SH (Dry matter 88.90%, Ash 4.70%, Metabolizable energy 841 Kcal/Kg, crude protein 14.40% and Crude fiber 32.90%). Characteristics of enzymes as feed additives (B-mannanase 200U/g, Xylanase 9000 U/g, B-glucanase 250U/g and Celluase 750U/g). Control: basal diet, T1: feed with 1.5% SH (Soybean hulls), T2: feed with 3.00% SH, T3: feed with 4.50% SH, T4,T5 and T6 SH 1.50%, 3.00% and 4.50% with Enzymes.*

Cecum microbiology:

At the end of each experiment, one bird from each group was randomly selected for digesta sampling in the lower ileum (2 cm from Meckel's diverticulum to ileocecal junction). Content of cecum were collected to determine the microbiological flora in (microbiological laboratory, Faculty of Agriculture, Ain Shams University) for enumeration of total bacteria, *coliforms* and *lactic acid bacteria*. Samples were taken in plastic 20-ml tubes and cooled until incubation. The samples were processed quickly after collection.

Samples were weighed (1g) and serially diluted in 0.9% saline, and 1 ml of each sample was dispensed and spread on selective media in Petri dishes. After shaking, 10 mL of the extract was taken for further dilutions. From diluted extracts (10^1 to 10^6) plates were prepared with specific medium for each studied microorganism. Brilliant Green Agar media was used for Salmonella and MacConkey agar plates were used for *coil forms*.

Microbial suspension from each dilution of a sample was transferred through pour plate method (Quinn *et al.,* 1992) and incubated at 37ºC for 24 h. The pathogens were identified by growing on specific media and biochemical tests. Accordingly, the incubation medium, MRS agar, was used for lactic acid bacteria (LAB). LAB counts of the cecum contents were obtained at 30°C degrees following 3 days of incubation. After then, the colonies were counted through colony counter. The total colony count was (expressed as $\log^{10}$ cfu/g of contents) determined by multiplying reciprocal of the dilution factor and average numbers of colonies. The microbial counts were determined as logarithmic colony forming units (cfu) per gram of sample.

Statistical analysis:

Data were statistically analyzed using the General Linear Model Procedure of analysis (SAS, 2001). Duncan's multiple range test (Duncan, 1955) was used to test differences within means of treatments, while level of significance was set typically at minimum (P≤0.05). The statistical model used for analyzing data was as following:

$$Y_{ij} = M + T_i + e_{ij}$$

Where:
Y_{ij} = observation of the parameter measured. M = overall mean.
T_i = effect of treatment.
e_{ij}= Experimental random error.

RESULTS AND DISCUSSION

Effect of dietary treatments on digestive tract length:

The data presented in Table (2) showed that numerical differences between chicks fed different dietary treatments in total length of digestive tract between treatments compared with control were the lowest.

Values seen in control group (217.50), the highest value seen in broilers fed on diets with 4.5% SH without or with enzymes (236.25 and 235.00cm). While the results indicated a non-significant difference between treatments in length of small intestinal (Cm), they showed that using SH without or with enzymes (T_{1-6}) in broiler diets led to an increase in length of small intestinal (183.75, 191.50, 199.25, 189.67, 192.25, and 193.05 Cm) compared with control group (181.25 Cm). The results showed that there was no significant difference between treatments in length of cecum (Cm) and thickness (mm), but it was found that using SH without or with enzymes (T_{1-6}) in broiler diets led to an increase in length of cecum (Cm). Results presented in Table (2) recorded a non-significant increase in total digestive tract and small intestinal length in birds fed diets with SH without or with enzymes. This result agrees with Sittiya *et al.* (2019) who recorded that using 5% SH in birds' diets increased total digestive tract and small intestinal length. Generally, using high levels of fiber led to an increase in digestive tract length. This may be because birds respond rapidly to changes in DF content by changing the intestinal length and weight of the digestive organs and the rate of passage through the different segments of the gut (Svihus, 2011 and Hetland and Svihus, 2001). Because of their physical properties of fiber, they act to increase the water-holding capacity of the pectin fraction of the sources of fiber. Different types of fiber differ in structure, solubility, water holding capacity, viscosity, bulking capacity, and other physiochemical properties. On the other hand, chemical composition and fermentation capability, as well as the grade of lignification of the source of fiber, may affect this change in the gut, thus increasing the retention of feed to increase fiber and nutrient utilization (Jha *et al.,* 2019). In contrast, these results disagree with those of (Nursiam *et al.,* 2021 and Kurul *et al.,* 2020) who reported that there was no effect on the length of the digestive system in broilers fed high levels of fiber. The reasons may be due to the size of the sources or their manufacture in diets. Results, which are recorded in Table (2), proved that there was an increase in the size of the cecum in birds fed high levels of fiber (SH). The reasons for these results may be that DF components are not digested by digestive enzymes and thus are substrates for bacterial fermentation in the distal part of the gut. The main products of fermentation are (SCFAs), predominantly lactate, acetate, propionate, and butyrate. The SCFAs may assist in developing the size of the cecum as well as increasing the activity of epithelium cell production (Montagne *et al.,* 2003).

Effect of dietary treatments on cecum microbiology (CFU/G) of broiler chicks:

The obtained results in Table (3) showed that using SH in broiler diets (T_{1-6}) clearly gave higher counts for total count and *lactic acid bacteria* than control, while the counts of *coli forms* were highest in control group compared to other treatments.

Table (2): Effect of dietary treatments on some of lengths of digestive tract parts on broiler chicks.

Items	Treatments							S. E	Sign.
	Control	T1	T2	T3	T4	T5	T6		
Total digestive tract	217.50	226.62	228.25	236.25	231.00	234.01	235.00	3.49	N. S
Small intestine	181.25	183.75	191.50	199.25	189.67	192.25	193.05	3.65	N. S
Cecum length	17.18	18.14	18.56	18.63	18.83	19.06	20.25	0.30	N. S
Cecum thickness (mm)	9.35	10.39	10.23	10.44	10.15	10.38	10.59	0.41	N. S

N.S.: non-significant. Control: basal diet, T1: feed with 1.5% SH (soybean hulls), T2: feed with 3.00% SH, T3: feed with 4.50% SH, T4,T5 and T6 SH 1.50%, 3.00% and 4.50% with enzymes.

Table (3): Effect of dietary treatments on cecum microbiology (CFU/g) on broiler chicks.

Items	Treatments						
	Control	T1	T2	T3	T4	T5	T6
Total bacterium * 10^5	82	149	189	212	196	282	289
Coli forms *10^4	164	86	92	95	29	34	87
Lactic acid bacterium* 10^5	840	920	916	923	926	234	966

Control: basal diet, T1: feed with 1.5% SH (soybean hulls), T2: feed with 3.00% SH, T3: feed with 4.50% SH, T4,T5 and T6 SH 1.50%, 3.00% and 4.50% with Enzymes.

In general, the lowest figures of total count bacterium were seen when control group were incorporated into diets, and the corresponding values were 82 x 10^5, while another treatment (T_{1-6}) saw an increase in total count with an increase in the level of SH, as well as the addition of enzymes to diets containing SH (T_{4-6}), which led to an increase in total count bacterium but a decrease with an increase in the level of SH. While the results showed that the highest count of *coliform bacteria* was seen in the control group (164*10^4 CPU/g) compared to another treatment (T_{1-6}), and the addition of enzymes in diets with SH (T_{4-6}) decreases the count of *coliform bacteria*. On the other hand, it was recorded that the lowest count of lactic acid bacterium seen in control group was 840*10^5, while another treatment (T_{1-6}) mildly increased the count of *lactic acid bacterium* with an increase in SH and the addition of enzymes in diets with SH (T_{4-6}) improved the count of *lactic acid bacterium* to 920, 916, and 923*10^5, respectively, compared to SH without enzymes (T_{1-3}), which was 926, 934, and 966*10^5, respectively. This may be because fiber levels in diets may modify the growth and composition of the microbiota. Many researchers agree with this result (Sadeghi *et al.,* 2015; Yadav and Jha, 2019; Dunislawska *et al.,* 2017 and Rajendran *et al.,* 2017). They indicated that DF improved digestive microbiology as well as were used as prebiotics in feedstuffs, directly promoting the growth of beneficial gut microflora and the production of SCFAs. Similarly, insoluble fibers also act as a general nutrient diluent, which can be both helpful and harmful, potentially impacting the colonization of beneficial gut microbes (Jiménez-Moreno *et al.,* 2016). Inspection of bacterial values from the ceca of turkeys fed either a high or low fiber diet found that direct counts of microbes were significantly higher in high-fiber fed turkeys than low-fiber fed turkeys (Bedbury and Duke, 1983). Turkeys fed the high-fiber diet had a significantly greater number of *Peptostreptococcus* and facultative microorganisms in the cecum, while the number of *Escherichia coli* was significantly higher in low-fiber-fed turkeys. Similarly, Xu *et al.* (2003) observed that the number of *Bifidobacterium* and *Lactobacillus* significantly improved, while the number of *Escherichia coli* significantly decreased in the gut when broilers were fed with high fiber as compared to control groups. A similar result was also reported by Chen *et al.* (2015) when fed with an oligosaccharide. Similarly, the study of Abazari *et al.* (2016) also showed that adding rice husk as a fiber source could promote the growth of beneficial *Lactobacillus bacteria* and reduce the population of pathogenic bacteria such as some *Escherichia coli* in the ileum and cecum of broilers when fed with 7.5 g/kg and 15% of feed with a particle size of 1-2 mm and below 1 mm. Beneficial microbes desire a low pH environment, and variations or nutrient deficiencies can lead to colonization by detrimental microbes. DF has been seen to have an impact on the chemical composition of the gut (Akbaryan *et al.,* 2019). *Lactobacillus species* are particularly beneficial in maintaining the pH of the digestive tract, as they produce *lactic and acetic acids,* which help lower the overall pH (Dunislawska *et al.,* 2017). In a study, Wu *et al.,* 2011) showed that high dietary soluble fiber (7% of soy hull) inclusion in the broiler diets significantly increased acetic, propionic, isobutyric, butyric acids, lactic, and succinic acids in the cecum, leading to a decrease in coliforms. On the other hand, found (Matsuzaki *et al.,* 2020; Shang *et al.,* 2020; An *et al.,* 2022 and Elmasry *et al.,* 2017) Using high levels of fibers alone without treatments or supplementation decreases the benefit of cecum microflora and increases coliform bacteria. These results because feed ingredients affect bacterial populations differently depending on the type and size of carbohydrates.

CONCLUSION

It could be used soybean hulls as sources of fiber in broiler diets without or with enzymes due to their positive effect on gut lengths and cecum microbiology without negative effects on chick viability.

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تأثير استخدام قشر فول الصويا و الانزيمات علي اطوال القناة الهضمية و ميكروبات الأعور لكتاكيت اللحم

نبيل محمد حسن المدني ، وفتحي عبد العظيم، ، ومراون عبد العزيز محمود عبد العزيز، أحمد إبراهيم سليمان الفحام ونعمة الله جمال الدين ومحمد مصطفي حامد

قسم انتاج الدواجن – كلية الزراعة – جامعة عين شمس – مصر

أجريت هذه التجربة لتقيم تأثير التغذية على مستويات مختلفة من قشر فول الصويا كمصدر للألياف في علائق بدارى التسمين علي اطوال القناة الهضمية و ميكروبات الأعور. تم توزيع 210 كتكوت من عمر يوم من سلالة (Indian River IR) عشوائيا علي 7 معاملات كل معاملة 3 تكرارات لكل تكرار 10 طيور.

المعاملات الغذائية كالتالي: غذيت الكتاكيت علي عليقة قاعدية بدون أي إضافات (الكنترول) بينما المعاملات (T1-6) بالترتيب كتاكيت غذيت علي (1.5% و 3.0% و 4.5%) من قشر فول الصويا بدون و بإضافة انزيمات بالترتيب.

وتتلخص اهم النتائج التي تم الحصول عليها كما يلي:

1- لا يوجد تأثير معنوي على طول القناه الهضمية وطول الامعاء والاعور وكذلك سمك الأعور في الطيور التي غذيت علي علائق تحتوي علي قشر فول الصويا مقارنة بمجموعة الكنترول.

2- أشارت النتائج ان الطيور التي غُذيت علي علائق تحتوي علي قشر فول الصويا بزيادة العدد الكلي للبكتريا وزيادة عدد البكتريا المنتجة لحمض اللاكتيك وانخفاض في عدد البكتريا الضارة.