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Effect of Different non Starch Polysaccharides in Semi Purified Diets on Performance and Intestinal Microflora of Young Broiler Chickens

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Abstract: An experiment was conducted with broiler chickens to study the effect of different non starch polysaccharides; NSPs (cellulose, pectin and carboxymethyl cellulose; CMC) on growth performance and intestinal microflora. 96 day-old male chickens were allocated to 4 experimental diets (a semi purified control diet or diets containing 3% of above mentioned NSPs) in a completely randomized design for two weeks. On day 10, one bird from each replicate was slaughtered to remove the intestinal contents for microbial analyses. According to the results, total feed intake over 2 weeks was increased (P<0.0001) as a result of cellulose addition to the diet. The final body weight and also feed conversion ratio of chickens on this diet were improved (P<0.0001). Growth performance parameters were dramatically declined by CMC (P<0.0001). Pectin significantly (P<0.001) increased the number of total anaerobes in the duodenum. CMC resulted in higher caecal number of Enterobacteriaceae (P<0.001). Compared to the control diet, all NSPs decreased the number of lactic acid bacteria in the intestinal segments. Under the conditions of this study, it was concluded that different NSPs are acted differently on performance and intestinal microflora when imposed to broiler chickens.

Key words: Pectin, cellulose, carboxymethyle cellulose, microflora, broiler

Introduction

Non starch polysaccharides (NSPs) as components of several poultry feed ingredients have been the interest of researches in recent years. These polysaccharides include a large variety of molecules and are classified into three main groups; cellulose, non cellulosic polymers and pectic polysaccharides (Choct, 1997). Smits and Annison (1996) reviewed the physico-chemical property of NSPs and concluded that their antinutritional effect in poultry diets, are arisen from their solubility and viscosity. It is also reported that the antinutritional effects of water soluble NSP on broiler performance and depression of nutrient digestibility are mainly mediated by intestinal microflora (Choct *et al*., 1996; Langout *et al*., 1999).

The microflora of gastrointestinal tract, especially in the posterior parts, plays an important role during the growth of broiler chickens. Bacterial densities in the ileum and caeca of day-old broiler chickens increase and reach to 10^9 and 10^{11} per gram of digesta, respectively, then remain relatively constant for the following 30 days (Apajalahti *et al*., 2004). The microbial population also changes by age. Enterobacteriaceae and enterococci are dominant in the caeca of 3 day-old broilers, but decrease when birds get older (van der Wielen *et al*., 2000). Lactobacilli are present in large numbers as well in 3 day-old chicks and retain stable during the growth of broiler and make up the major species present in the small intestine and caeca (van der Wielen *et al*., 2000; Amit-Romach, 2004). It is demonstrated that manipulation of diet as providing substrates can affect the composition and activity of gastrointestinal microflora in broilers (Wagner and Thomas, 1978; Choct *et al*., 1996; Danicke *et al*., 1999).

Although it is shown that inclusion of soluble NSP such as CMC (van der Klis *et al*., 1993; Smits *et al*., 1998), a non fermentable and viscus fibre, and pectin (Wagner and Thomas, 1978; Ricke *et al*., 1982 and Langout *et al*., 1999) in broiler diets can decrease the bird performance, there are limitted published reports on the effect of these fibres on intestinal microflora. Smits *et al*., 1998 reported that inclusion of CMC in the broiler diet increased total aerobic and anaerobic microbial counts in the digesta of proximal parts (duodenum plus jejunum) of intestine. Addition of high methylated citrus pectin also changed the intestinal microbial population and increased the microbial activity in the ileum especially those of Enterococci, Bacteroidaceae, Clostridia, *E. coli* (Langhout *et al*., 1999) and total counts of anaerobic bacteria in the small intestine (Wanger and Thomas, 1978). Unlike the soluble fibres, insoluble fibres effect on the composition and quantity of the microflora is relatively insignificant (Hetland *et al*., 2004). There is no report on the comparison of different fibres effect in any trial,

so the aim of this study was to evaluate the influence of some non starch polysaccharides, pectin, CMC and cellulose on growth performance and selected microbial population of intestinal segments in young broilers.

Materials and Methods

Birds and diets: Ninety six day-old Ross male broiler chickens, obtained from a commercial hatchery were used for the study. The chicks were distributed into 16 battery cages in groups of 6 with equal group weight. To insure that the experimental diets have minimum fibre, a semi purified basal diet (control) was used to meet the requirements of chickens (NRC, 1994). It is reported that the broiler diets roughly contain 3% of cellulose (Bach Knudsen, 1997). Therefore 3% of each cellulose, CMC and high methylated citrus pectin was replaced with the same part of corn starch in basal diet (Table 1). The diets were fed the birds from 1-14 days of age. The study was conducted in an environmentally controlled room. Feed and water were provided *ad libitum* and light was provided for 24 h/day while the temperature was gradually reduced from the initial 32°C to approximately 26°C by day 14. Body weight and feed intake were measured at the end of each week.

Sample collection: On day 10, one bird randomly selected from each cage (replicate) and was killed by cervical dislocation and immediately after dressing, samples were collected (Hubener *et al*., 2002). The complete intestinal tract was removed and transferred into anaerobic chamber. Sample sites were then ligated, separated with a sterile scalpel and transferred into fresh sheets of sterile aluminium foil. The digestive tracts between the gizzard and bile duct and from bile duct to Meckel's diverticulum were removed considered as duodenum and jejunum, respectively. The ileum was isolated as the section between Meckel's diverticulum and the ileocecal junction. The caeca also removed and the digesta of all above mentioned segments were obtained for microbial study.

Media and incubation: Homogenized digesta was diluted in physiological serum solution (0.9% NaCl). For microbial determination these media were used: Blood agar base (liofilchem, Italy) with 7% of defibrinated sheep blood for isolation of total anaerobic bacteria, MRS agar (Liofilchem, Italy) and 0.1% of Tween 80 for isolation of lactic acid bacteria and Violet Red Bile agar (Biotec Laboratories Ltd, UK) for isolation of Enterobacteriaceae. The number of total culturable anaerobic bacteria and lactic acid bacteria were counted after incubation in an anaerobic chamber at 37°C for 48 hours. Enterobacteriaceae were enumerated after aerobic incubation at 37°C for 24 hours.

Table 1: Composition of the control, Pectin, Cellulose and CMC diets (% of diet)

Ingredient	Control	Pectin	CMC[1]	Cellulose
Corn starch	56.24	53.24	53.24	53.24
Pectin	-	3	-	-
CMC	-	-	3	-
Cellulose	-	-	-	3
Corn gluten meal	29.52	29.52	29.52	29.52
Fish meal	8.47	8.47	8.47	8.47
Dicalcium phosphate	1.73	1.73	1.73	1.73
Limestone	1.37	1.37	1.37	1.37
Soybean oil	1	1	1	1
Salt	0.61	0.61	0.61	0.61
L-Lysine HCL	0.56	0.56	0.56	0.56
Vit. and Min. premix[2]	0.5	0.5	0.5	0.5
Total	100	100	100	100

[1]Carboxymethyl cellulose. [2]Supplying (per kilogram of diet) retinyl acetate, 11000 IU; cholecalciferol, 1800 IU; dl-α-tocopheryl acetate, 36 mg; menadion, 5 mg; Thiamin, 1.53; Riboflavin, 7.5 mg; calcium pantothenate, 12.40 mg; niacin, 3.04 mg; pyridoxine, 1.53 mg; folic acid, 1.26; cyanocobalamin, 1.6 mg; biotin, 5 mg; coline chloride, 1100 mg; antioxidant, 100 mg; Mn, 16.3 mg; Zn, 84.5 mg; Fe, 250 mg; Cu, 20 mg; I, 1.6 mg, Co.

Viscosity measurement: To simulate the *in vitro* pH and dilution of feed in broiler gastrointestinal tract, the procedure of Razdan *et al*. (1997) was employed with some modification. Viscosity of supernatants was measured by Brookfield DV II$^+$ viscosimeter (Brookfield Engineering laboratories) with a CP 40 cone.

Statistical analysis: A completely randomized design (CRD) with 4 replicates was employed. Data were analyzed by GLM procedure of Statistical Analysis System (SAS, 1998). Duncan's multiple range test was used when means were significant ($P<0.05$). Logarithmic ($\log_{10}$) transformation was applied for microbial colony forming unit (CFU).

Results

Viscosity: *In vitro* viscosity of experimental diets exposed with different pH to mimic the GIT, is shown in Table 2. CMC significantly ($P<0.01$) increased the viscosity of diet in all solvents. The viscosity of the diet containing cellulose was similar to the control. Pectin also significantly ($P<0.01$) increased the viscosity compared to the control diet, but had less viscosity than CMC. Changing pH by acidification and the neutralization numerically declined the viscosity of CMC and pectin diets.

Growth performance: The performance data are presented in Table 3. Feed intake, body weight and feed conversion ratio of broiler chickens were affected by the treatments. Compared to the control diet,

Table 2: Viscosity (m Pa.s) of experimental diets (n=6) suspended in distilled water, acidified (HCL) and neutralized ($NaHCO_3$) solvents

	Control	Pectin	CMC	Cellulose	SEM	P value
Distilled Water	1.07^{c}	3.08^{b}	9.031^{a}	1.09^{c}	0.242	0.001
Acidification	1.06^{c}	2.88^{b}	7.52^{a}	1.21^{c}	0.12	0.001
Neutralization	1.13^{c}	1.78^{b}	3.39^{a}	1.19^{c}	0.057	0.001

Means within rows with no common superscript differ significantly (P<0.001).

Table 3: Effect of dietary treatments on growth performance of broiler chickens

	Control	Pectin	CMC	Cellulose	SEM	P value
Feed intake						
Days 1-7	43.75^{ab}	49.58^{a}	23.04^{c}	37.71^{b}	2.229	0.0001
Days 7-14	67.06^{b}	54.91^{c}	35.20^{d}	86.07^{a}	2.338	0.0001
Days 1-14	110.81^{b}	104.49^{b}	58.24^{c}	123.78^{a}	1.75	0.0001
Weight gain						
Days 1-7	8.12^{a}	7.83^{a}	2.37^{b}	8.33^{a}	0.288	0.0001
Days 7-14	28.98^{b}	12.00^{c}	4.30^{d}	44.69^{a}	1.071	0.0001
Days 1-14	37.10^{b}	19.83^{c}	6.68^{d}	53.02^{a}	0.851	0.0001
Feed conversion ratio						
Days 1-7	5.38b^{c}	6.34^{b}	9.72^{a}	4.52^{c}	0.389	0.0001
Days 7-14	2.31^{c}	4.66^{b}	8.18^{a}	1.93^{c}	0.174	0.0001
Days 1-14	2.99^{c}	5.38^{b}	8.73^{a}	2.33^{d}	0.163	0.0001

Means within rows with no common superscript differ significantly (P<0.0001).

cellulose increased (P<0.01) feed intake at week two and the whole period of the experiment. CMC dramatically decreased (P<0.01) feed intake of the chickens. Pectin significantly (P<0.01) lowered feed intake at 7-14 days, but its effect was similar to that of control at the period of 1-14. With increasing feed intake, weight gain of the chickens fed on cellulose also increased and feed conversion ratio decreased significantly (P<0.01). CMC showed the least weight gain and the highest feed conversion ratio. Pectin didn't worsen as CMC did. It also adversely affected the bird weight gain and feed conversion ratio.

Microbial population: The results of the bacterial counts in different intestinal segments are showed in Table 4. A continuous increase in bacterial colonization from duodenum to the caeca was followed. The number of total anaerobes was higher in pectin fed birds (P<0.001). Cellulose significantly decreased the number of these bacteria in the ileum (P<0.0001) and CMC increased their counts in the caeca compared to the other treatments (P<0.01). The counts of Enterobacteriaceae were higher in the duodenum and jejunum of broilers fed cellulose (P<0.05), however in jejunum the difference was not significance between cellulose and pectin fed birds. The chickens fed with CMC and pectin had higher Enterobacteriaceae counts in their caeca (P<0.001). Lactic acid bacteria were affected by the treatments especially in the proximal segments. Cellulose fed birds had the least count of lactic acid bacteria in the duodenum, jejunum and ileum (P<0.01). There were no significant differences in caecal lactic acid bacteria among birds fed different dietary treatments.

Discussion

The decreased feed intake of the birds on the diets containing pectin and particularly CMC compared to the control diet probably is because of increasing the intestinal digesta viscosity (van der Klis *et al*., 1993; Langout *et al*., 1999) that causes increase of feed retention time in the gastrointestinal tract (van der Klis *et al*., 1993). Since, there is a relationship between rate of feed passage through the gut and feed consumption in young chickens (Almirall and Estive-Garcia, 1994), inclusion of these soluble fibres in the diet leads to less feed intake. Lower weight gain and higher feed conversion ratio of the birds on these diets are also predictable due to less feed intake and less nutrients utilization because of high viscosity of the chyme. The results are in agreement with the previous studies by semi-purified (van der Klis *et al*., 1993; Smits *et al*., 1998) and practical diets (Wanger and Thomas, 1978; Langhout *et al*., 1999). In contrast, addition of cellulose resulted in high feed intake and weight gain. It is noted that cellulose as an insoluble fibre can affect gut function by increasing digesta passage rate and modulating nutrient digestibility, especially starch (Hetland *et al*., 2004). So, higher feed intake of birds fed with cellulose diet may be related to faster gut emptying of these birds.

Biochemical conditions in the digesta, as a result of feed composition or physiological responses from the host will affect substrate availability and concentration and thus microbial product formation (Barrow, 1992). In this study, changing the feed composition by including different NSPs modified selected microbial population in different segments of intestine. Inclusion of pectin in the diet resulted the highest counts of total

Table 4: Effect of dietary treatments on the counts of selected microflora (log CFU/ gram digesta) of different intestinal segments of broilers (10 d of age)

	Control	Pectin	CMC	Cellulose	SEM	P value
Total anaerobes						
Duodenum	3.96^{bc}	5.74^{a}	4.60^{b}	3.77^{c}	0.191	0.0002
Jejunum	5.50^{a}	6.21^{a}	5.93^{a}	5.58^{a}	0.271	0.3649
Ileum	8.07^{a}	7.51^{a}	7.41^{a}	5.13^{b}	0.231	0.0001
Caeca	7.19^{b}	7.16^{b}	8.83^{a}	7.26^{b}	0.203	0.0031
Enterobacteriaceae						
Duodenum	2.48^{b}	2.34^{b}	2.87^{ab}	3.30^{a}	0.181	0.0318
Jejunum	2.42^{b}	4.24^{a}	2.07^{b}	4.39^{a}	0.231	0.0001
Ileum	6.91^{a}	5.18^{b}	6.90^{a}	3.85^{c}	0.234	0.0001
Caeca	5.74^{b}	7.58^{a}	8.25^{a}	5.96^{b}	0.235	0.0003
Lactic acid bacteria						
Duodenum	8.19^{a}	7.33^{b}	6.46^{c}	5.75^{d}	0.196	0.0001
Jejunum	8.95^{a}	8.11^{b}	8.43^{ab}	6.84^{c}	0.18	0.0001
Ileum	9.18^{a}	8.62^{a}	8.90^{a}	7.33^{b}	0.24	0.0011
Caeca	10.10^{a}	9.21^{a}	9.08^{a}	9.39^{a}	0.304	0.1543

Means within rows with no common superscript differ significantly ($P<0.05$).

anaerobic bacteria in the proximal parts. There are no clear reports of pectin effect on the microflora of duodenum and jejunum to compare the results obtained by this experiment. In the ileum, the result is in agreement with Langhout *et al.* (1999) who indicated that high methylated citrus pectin (3% of diet) had no effect on total anaerobic bacteria of this segment. The results obtained by CMC disagree with the earlier report (Smits *et al.*, 1998) indicating the role of CMC (1.5% of diet) to increase the number of anaerobic bacteria in the digesta of proximal parts (duodenum plus jejunum) of intestine. It is likely that high level of included CMC (3% of the diet) resulted in high viscosity in the posterior parts like crop (Table 2) and created a good environment by reducing digesta flow for bacterial activity in this part of the gut.

Increasing Enterobacteriaceae counts in the caeca of birds fed CMC, is probably because of viscous digesta. It has been shown that dietary cereals leads to high intestinal viscosity enhance enterobacteria (Danicke *et al.*, 1999; Hubener *et al.*, 2002).

Lactic acid producing bacteria are the dominant culturable bacteria in the small intestine of poultry (Engberg *et al.*, 2000). These bacteria are considered as beneficial microflora for the host. In this experiment compared to the control diet, soluble or insoluble fibres included into the diets didn't increase these beneficial bacteria.

Conclusion: Under the conditions of this study, the different effects of soluble and insoluble NSPs on broiler performance and intestinal microflora were observed. Soluble fibres decreased the performance of broiler chickens that may partly be contributed to changing the physico-chemical conditions of the gut and possibly the changing of the microflora. In contrast, insoluble fibre (cellulose) resulted in better performance of the chickens compared to the control.

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