

The influence of digesta viscosity on the development of the stomach, on *in vitro* small intestinal motility and on digestion of nutrients in broiler chickens*

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ABSTRACT

The aim of the study was to investigate the effect of digesta viscosity on the development of the digestive tract, motility of the small intestine and digestion of nutrients in the early stages of life of broiler chickens. At the age of 1 week sixty-six broiler Hybro females were allocated to 3 groups, 22 per group. The birds were caged individually and fed *ad libitum* a control, wheat-based diet supplemented with xylanase (1g/kg), or rye-based diets: R, non-supplemented or RE, supplemented with xylanase (3g/kg). Diets were cold pelleted. Body weight and feed intake were registered in weekly intervals. In the fourth week of life apparent fat and protein digestibility and metabolizable energy value of the diet were estimated in a balance study on 8 birds from each group. In the fifth week of life all birds were sacrificed and the extent of stomach distention was scored from 1 (normal) to 5 (most distended). Duodenum and jejunum fragments were taken and their *in vitro* motility was measured in 7 chickens per group. From the remaining birds the content of the stomach, jejunum and ileum were collected for viscosity measurements. Due to enzyme supplementation of the rye-based diet, the viscosity of digesta decreased significantly from 2.6, 17.1 and 35.4 in the stomach, jejunum and ileum digesta in group R, to 1.7, 4.7 and 8.2, respectively, in group RE ($P < 0.001$), the performance of chickens improved, and apparent fat (from 79 to 88%) and protein (from 86 to 87%) digestibilities and the AME_N value of the diet increased (from 13.3 to 14.5 MJ ME/kg DM). The contractile activity of the duodenum and jejunum in response to acetylcholine was higher in group RE

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than in R, but only in the jejunum was the difference significant. The mean score of stomach distention was 3.05 in group R, 2.32 in group RE, and 2.05 in the control group ($P < 0.05$). High digesta viscosity may negatively affect the development of the stomach and motility of the small intestine in young chickens; lowering viscosity by enzyme supplementation of diets containing viscous NSPs allows reduction of disturbances in digestive tract development.

KEY WORDS: rye, digesta viscosity, intestinal motility, stomach distention, broiler chickens

INTRODUCTION

It has been demonstrated in several studies that the addition of certain non-starch polysaccharides (NSPs) to broiler diets affects the ability of birds to digest starch, protein and lipids, with the digestion of saturated lipids being particularly depressed (Antoniou et al., 1981; Fengler and Marquardt, 1988; Choct and Annison, 1996; Smulikowska and Mieczkowska, 1996). It is well established that the depression is closely related to the *in vivo* gel-forming properties of these NSPs, as diminishing digesta viscosity by supplementation of the diets with NSP-degrading enzymes positively affected digestion of nutrients, particularly fat (Choct and Annison, 1996; Smulikowska and Mieczkowska, 1996). Although the association of digesta viscosity with depression of fat digestibility in chickens has been known for over twenty years, the mechanism by which viscosity affects digestion is still under discussion. In their review of this subject, Smits and Annison (1996) proposed several possible mechanisms: in a viscous environment the contact intensity between fats and digestive secretions (lipases, bile salts) is reduced and the diffusion and convective transport of lipase, oil and bile salt micelles to the epithelial surface is impaired; viscous NSPs may entrap bile salts and thereby reduce their effectiveness in solubilizing fat components; viscous NSPs may increase the secretion of mucus, giving rise to an increase in the thickness of the unstirred water layer, which reduces lipid absorption; the losses of endogenous fat may increase due to viscosity; viscous NSPs, by reducing the flow of digesta and increasing the amount of undigested material present in the distal small intestine, enhance the growth of bacterial populations; enhanced growth of bacterial populations may also affect gut morphology, which may reduce absorption of lipids.

All of the above-mentioned mechanisms may also alter digestive functions in both older and younger birds. However, in older broilers reduction of digesta viscosity by enzyme supplementation plays a lesser role, in laying hens its effect is negligible in terms of nutrient digestibility and performance (Almirall et al., 1995; Smulikowska, 1998a,b; Oloffs et al., 1999; Garcia et al., 2000; Chesson, 2001). It was assumed that the stronger negative reaction of young birds to digesta viscosity is caused by limited production of pancreatic enzymes (Almirall et al., 1995), or that the immature gastrointestinal tract of very young birds could not relocate

the viscous digesta as effectively as adult birds (Smulikowska, 1998b). It was reported that in broilers fed diets based on wheat or rye varieties containing viscous NSPs, the occurrence of proventriculus and gizzard distension was more frequent (Scott, 1993; Boros, 1997), which may point to disturbances in digesta relocation. There is a lack of information on the effect of digesta viscosity on small intestine motility in young birds.

The aim of this study was to investigate the effect of digesta viscosity on the development of the gastrointestinal tract and motility of the small intestine in the early stages of life of broiler chickens.

MATERIAL AND METHODS

Growth experiment

The experiment was conducted on 66 broiler Hybro females. All birds were fed a starter diet for the first week of life, after which they were deprived of food for 4 h, weighed and allocated to 3 groups, 22 birds per group. The mean initial body weight of the chickens was 137 ± 6 g. The birds were housed in individual balance cages, and fed cold pelleted diets (control, R or RE) *ad libitum* for the next 2 weeks. The composition of diets is given in Table 1. The control diet was based on wheat and was supplemented with 1 g/kg of an enzyme preparation (Avizyme 1300, Finnfeeds Int., containing xylanase and protease activity); in both experimental diets (R and RE) 400 g/kg rye was substituted for wheat, diet R was not supplemented, but diet RE supplemented with 3 g/kg of the same enzyme preparation as in the control diet.

Feed intake and body weight were measured in weekly intervals. On day 22 of life the birds in each group were randomly divided into 2 subgroups, 8 birds were chosen for the balance experiment and given balance diets, the remaining birds continued on the same diets fed *ad libitum*.

Balance experiment

The balance diets were of the same composition as in the growth experiment (Table 1), but contained 3 g/kg Cr_2O_3 added as a marker prior to pelleting. The balance diets were fed at the level of 90 g/bird/day, in three meals. After two days of preliminary feeding the birds were fasted for 17 h, then fed for 3 days and fasted for 17 h. Feed intake was recorded and excreta collected daily during the last 89 h of the balance experiment. Excreta were immediately frozen and kept at -18°C . After the balance period birds were again fed *ad libitum* their respective diets without Cr_2O_3 .

TABLE 1

Composition and nutritive value of diets*, g/kg

Component	Group		
	Control	R	RE
Rye (7.9% CP)	-	400.0	400.0
Wheat (13.1% CP)	477.2	5.5	2.5
Soyabean meal (43.1% CP)	322.2	393.9	393.9
Maize (8.1% CP)	100.0	100.0	100.0
Lard	60.0	60.0	60.0
Dicalcium phosphate	18.0	18.0	18.0
Limestone	5.0	5.0	5.0
NaCl	3.0	4.0	4.0
L-lys (74.8%)	2.2	1.8	1.8
DL-met (98%)	1.4	1.8	1.8
Mineral-vitamin premix**	10.0	10.0	10.0
Enzyme***	1.0	-	3.0
Crude protein, calculated	210	210	210

* in diets used in balance experiment 3g/kg of wheat was substituted by 3 g/kg of Cr₂O₃ added as a marker

** supplied per kg diet: vit. A 1300 IU; vit. D₃ 3500 IU; (mg) vit. E 40; vit. K₁ 4; vit. B₁ 3; vit. B₂ 7; vit. B₆ 5; vit. B₁₂ 0.02; niacin 45; Ca pantotheate 16; folic acid 1.5; biotine 0.1; choline 400; Na 270; Mn 80; Zn 60; Fe 70; Cu 10; J 1; Se 0.15; Ca 2,25 g; DL-met 1 g; Flavomycine 5 mg; Diclazuril 1 mg

*** Avizyme 1300 (Finnfeed Int.) containing (according to producer) 2500 U xylanase and 800 U protease activity

Measurements of contractile activity

From day 26 to 29 of life *in vitro* measurements of the contractile activity of the small intestine were done on 7 birds of each group according to the procedure described by Woliński et al. (2001). The birds were not deprived of feed before sacrificing. To avoid the influence of time of day on measurements, 2-3 birds of each group were killed each day at different times. The birds were weighed and sacrificed by decapitation. The entire gastrointestinal tract was removed for visual inspection and tissue sampling. The upper 6 cm of the duodenum and 6 cm of the jejunum (from 9 to 3 cm above Meckel's diverticulum) were immediately collected and placed in Krebs-Henseleit solution kept at room temperature. The duodenal and jejunal segments were rinsed with the solution to remove digesta and divided into 2 segments about 2.5 cm long. The segments were mounted in an 4-channel automatic organ bath equipped with isotonic pressure transducers (Letica Scientific Instruments, Spain) to Meckel and coupled to a data acquisition system (PowerLab/4e,

ADInstruments, Australia) and PC computer for measuring smooth muscle isotonic contraction activity. Following recording of spontaneous contractile activity, the investigated segments were stimulated with graded doses of acetylcholine in a cumulative manner (from 10^{-10} to 10^{-4} M every 3 min), next washed 3 times for 10 min with fresh buffer and finally relaxed for 3 min with a single dose of isoproterenol (10^{-4} M). Contraction and relaxation amplitudes were measured with Chart v4.04 (ADInstruments, Australia) software.

Measurements of digesta viscosity

From day 26 to 30 of life digesta viscosity was measured on twelve birds of each group. The birds were not deprived of feed before sacrificing and were killed by decapitation. The entire gastrointestinal tract was immediately removed for visual evaluation and digesta sampling. The digesta from the stomach (if present), jejunum (from the end of the duodenum to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileo-caeco-colic junction) was separately collected into centrifuge tubes placed on ice and immediately centrifuged at 10000 g for 10 min at 4°C. The viscosity of the supernatant was measured using a Brookfield Digital Viscometer DV-II+LV (Brookfield Engineering Lab. Inc., Stoughton, MA, USA) with a Cone-Plate 40 spindle at 40°C.

Score of stomach distension

The extent of stomach distension was evaluated in a 5-point scale (1- normal, 5 - most distended). The evaluation was done immediately after opening of the abdominal cavity in all birds except one from group R culled on 21 day of life. The digesta remaining in the proventriculus and gizzard was weighed.

Chemical analysis

Prior to analysis excreta collected in the balance experiment were dried in a forced-draft oven at 70°C for 24 h, then ground to pass through a 1 mm sieve. The chemical composition of balance diets and excreta was determined according to AOAC (1990), fat was extracted with diethyl ether after acid hydrolysis with 4 M HCl. Gross energy was determined using a Parr adiabatic oxygen bomb calorimeter (KL-11, Mikado, Precyzja, Bydgoszcz, Poland) and benzoic acid as a calibration standard. Chromic oxide was analyzed spectrophotometrically following wet ashing according to Hinsberg et al. (1953). Faecal N in excreta was determined according to Ekman et al. (1949).

Calculations and statistical analysis

Body weight gain (BWG) and feed conversion ratio (FCR) were calculated for 14 days of the growth experiment. Apparent digestibility of nutrients and AME value of the diets were calculated relative to the ratio of Cr_2O_3 to the content of nutrient or gross energy in feed and droppings. AME was corrected for zero nitrogen retention (AME_N) assuming a value of 34.4 kJ/g of nitrogen retained (Hill and Anderson, 1958).

Experimental data were subjected to one-way analysis of variance using Statgraphic plus ver. 7 Software. Treatment differences were compared by Duncan's multiple range test. Data of *in vitro* measurements of contractile activity of intestinal segments were analyzed statistically by GraphPrism v 2.0 (GraphPad Software, USA). The correlation between digesta viscosity and score of stomach distension was analyzed with the use of STATISTICA PL software (Stanisz, 2001).

RESULTS

All birds from the control and RE groups were healthy, except one bird from group R, which was culled on 21 day of life; this bird was not taken into account in any calculations. During 2 weeks of the growth experiment chickens from group R ate more feed, at the end they had lower body weight (BW), and their feed conversion ratio (FCR) was worse ($P < 0.001$) than in the control group. In group RE feed intake was lower, but BWG higher, and as a consequence FCR was better ($P < 0.05$) than in group R (Table 2).

TABLE 2
Performance of chickens between 8-22 day of life ($n=21$ to 22), viscosity of digesta in different segments of digestive tract (26-29 day of life, $n=8$ to 12) and score of stomach distention ($n=21$ to 22)

Dietary treatment	Performance			Digesta viscosity, mPas.s			The score of stomach distention
	BWG g	feed intake, g	FCR, g feed/g BWG	stomach	jejunum	ileum	
Control	591	850 ^A	1.44 ^{aA}	1.23 ^A	1.92 ^A	2.55 ^A	2.05 ^a
R	579	927 ^B	1.60 ^{bB}	2.63 ^B	17.11 ^B	35.45 ^B	3.05 ^b
RE	588	886 ^{AB}	1.51 ^{bAB}	1.66 ^A	4.70 ^A	8.16 ^A	2.32 ^{ab}
SEM	11.8	17.1	0.02	0.19	1.87	11.97	0.29

^{a,b,A,B} - means within column with different superscripts differ at: ^{a,b} $P < 0.05$; ^{A,B} $P < 0.001$

SEM - pooled standard error of the mean

The apparent protein and fat digestibility of diet R was lower than of the control diet, so were organic matter retention and AME_N (Table 3). After supplementation of the rye-based diet with feed enzyme, all of the measured parameters increased

significantly ($P < 0.05$), fat digestibility in group RE was higher than in groups R and control; protein digestibility was higher than in group R, but did not differ significantly from the control, organic matter retention and protein and AME_N values were higher than in R, but lower than in the control group ($P < 0.05$).

TABLE 3

Results of balance experiment (22-27 day of life, $n=8$)

Dietary treatment	Apparent fat digestibility, %	Apparent protein digestibility, %	Organic matter retention, %	AME_N , MJ/kg DM
Control	84.1 ^{hAB}	87.7 ^{uA}	74.2 ^A	14.96 ^{uA}
R	79.2 ^{cB}	85.9 ^{bb}	67.4 ^C	13.54 ^{cB}
RE	88.2 ^{uA}	87.1 ^{uAB}	70.7 ^B	14.49 ^{hA}
SEM	1.29	0.36	0.77	0.15

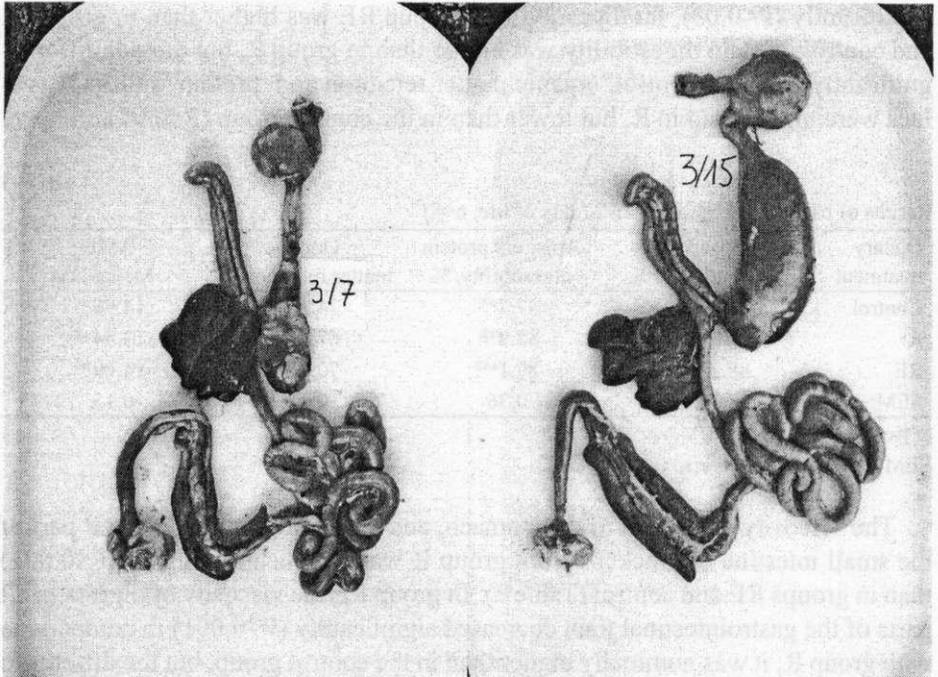
^{u,b} $P < 0.05$; ^{A,B} $P < 0.001$

SEM - pooled standard error of the mean

The viscosity of digesta in the stomach, and in the proximal and distal part of the small intestine of chickens from group R was significantly higher ($P < 0.001$) than in groups RE and control (Table 2). In group RE the viscosity of digesta in all parts of the gastrointestinal tract decreased significantly ($P < 0.001$) in comparison with group R; it was nominally higher than in the control group, but the difference was not significant. The viscosity of digesta in the stomach was relatively low and not significantly correlated with the viscosity of digesta in the jejunum and ileum, but there was a significant ($r=0.89$; $P < 0.05$) positive correlation between viscosity of the digesta in the jejunum and ileum.

The stomach distension score in the fourth week of life (Table 2) in group R was higher than in the control and RE groups ($P < 0.05$). Two chickens from group R were culled on day 25 of life because they showed symptoms similar to ascites syndrome; *post mortem* it was found that in both birds the proventriculus and gizzard were distended with a score of 5 points, one bird had fluid in the abdominal cavity, these 2 birds were, however, included in the calculation of the stomach distension score. Birds scoring 4-5 were found in all groups, in control group there were 4 (18%) such birds, in group R, 9 (41%), in group RE, 5 (23%). Stomachs of birds with a score of 1 (No 3/7 - control group) and 5 (3/15 - R group) are shown in Photograph 1. In the distended stomach an isthmus connecting the proventriculus and gizzard atrophied and both parts of the stomach formed a pouch, filled with digesta. The stomach content in birds with a score of 1-2 ranged from 0 to 15 g, in birds with a score of 3, from 28 to 40 g, in birds with a score 4-5, from 63 to 110 g. The stomach distension score was not significantly correlated with the viscosity of stomach digesta or viscosity of small intestinal digesta.

Results of the *in vitro* study on isolated intestinal segments are shown in Figures 1 and 2. Pharmacologically induced activity, after stimulation with acetylcho-



Photograph 1. The normally developed stomach scored 1 from chick fed with the control diet (left) and distended stomach scored 5 (note the lack of isthmus and distended proventriculus) from chick fed with R diet (right)

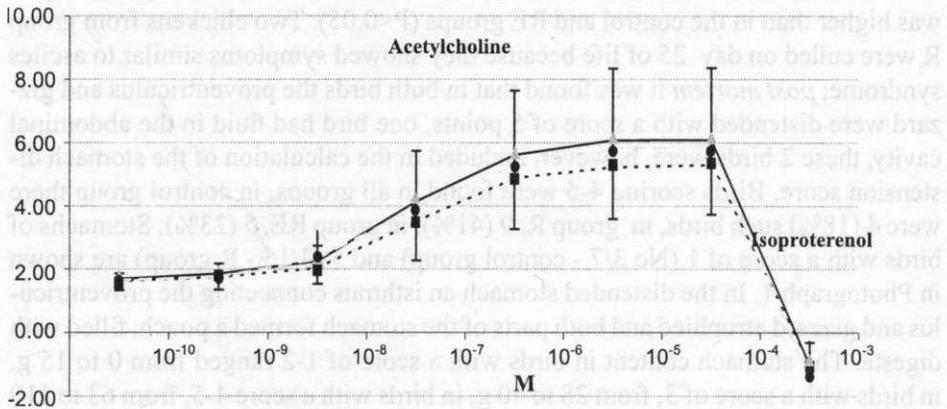


Figure 1. Mean contractile activity (\pm SD) *in vitro* (mm) of the duodenal segments from chickens fed with control diet (\blacktriangle), 40% rye diet without enzyme (\blacksquare) or 40% rye diet supplemented with xylanase (\bullet) in response to acetylcholine and isoproterenol. Differences between groups were not statistically significant

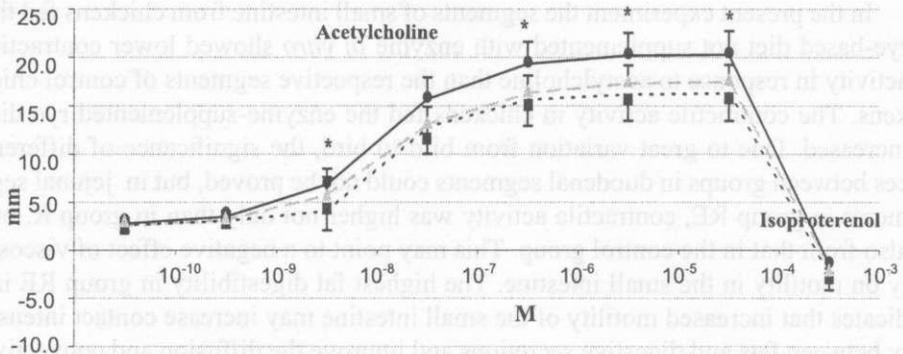


Figure 2. Mean contractile activity (\pm SD) *in vitro* (mm) of the jejunal segments from chickens fed with control diet (\blacktriangle), 40% rye diet without enzyme (\blacksquare) or 40% rye diet supplemented with xylanase (\bullet) in response to acetylcholine and isoproterenol.

* statistical differences between groups were significant at $P < 0.05$

line, was lowest in the chickens fed the rye diet not supplemented with enzyme (R group). In the duodenal segments, these differences were, however, non-significant, but in the jejunal segments there was a significant ($P < 0.05$) increase in contractile activity in response to acetylcholine in group RE in comparison with groups R and control (Figure 2).

DISCUSSION

The low digestibility of nutrients, fat in particular, from rye-based diets in broiler chickens as well as an increase of digestibility after supplementation of diets with xylanase were reported by many authors, including Bedford and Classen (1992), Smulikowska and Mieczkowska (1996), Boros (1997), Smulikowska (1998a), Oloffs et al. (1999). The results of the present study agree well with those of the studies cited above. There was a direct correlation of nutrient digestibility with intestinal viscosities, in the present experiment the rye-based diet not supplemented with enzyme induced on average about two-fold higher viscosity of digesta in the stomach, nine-fold higher in the jejunum and fourteen-fold higher in the ileum than the control, wheat-based diet supplemented with enzyme. After supplementation of the rye-based diet with feed enzyme, in group RE, digesta viscosity decreased and was not significantly different from that in the control group, and nutrient digestibilities increased (Table 2). In reviewing the subject, Smits and Annison (1996), Iji (1999) and Chesson (2001) proposed different mechanisms by which high digesta viscosity might negatively affect fat digestion, but little attention was paid to the effect of viscosity on the motility of the digestive tract and on its development.

In the present experiment the segments of small intestine from chickens fed the rye-based diet not supplemented with enzyme *in vitro* showed lower contractile activity in response to acetylcholine than the respective segments of control chickens. The contractile activity in chickens fed the enzyme-supplemented rye diet increased. Due to great variation from bird to bird, the significance of differences between groups in duodenal segments could not be proved, but in jejunal segments in group RE, contractile activity was higher not only than in group R, but also from that in the control group. This may point to a negative effect of viscosity on motility in the small intestine. The highest fat digestibility in group RE indicates that increased motility of the small intestine may increase contact intensity between fats and digestive secretions and improve the diffusion and convective transport of lipase, oils and bile salt micelles to the epithelial surface.

It may be hypothesized that lowering digesta viscosity positively affects the absorption of some nutrients, vital for proper development and functioning of the gastrointestinal tract. However, contrary to our assumption, the stomach distension score was not correlated with the viscosity of stomach- or small intestinal digesta.

The viscosity of the stomach content, even in chickens from group R, was relatively low in comparison with the viscosity of digesta from the jejunum and ileum (Table 2). This supports the results of Bedford and Classen (1992), who showed that the viscosity of digesta in birds fed rye- and wheat-based diets increased along the small intestine due to the increase in concentration of the high molecular fraction of NSPs, released slowly from both the soluble and insoluble fractions of dietary fibre and was highest in the distal part of the small intestine. The viscosity of stomach digesta was not correlated significantly with the viscosity of small intestinal digesta. This proves that the distension of the stomach is not simply a result of disturbances in expression of digesta from the stomach to the duodenum. However, the higher frequency of stomach distension in group R indicates that high digesta viscosity may indirectly adversely influence stomach development in very young chickens. Iji (1999) suggested that changes in the availability of digestion end products induced by viscosity may disturb functions of some hormones associated with nutrient metabolism and development of the digestive tract.

CONCLUSIONS

High digesta viscosity may negatively affect the development of the stomach and motility of the small intestine in young chickens, since lowering viscosity by enzyme supplementation of diets containing viscous NSPs enables reduction of disturbances in digestive tract development.

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STRESZCZENIE

Wpływ lepkości treści na rozwój żołądka, aktywność skurczową jelita *in vitro* oraz trawienie składników odżywczych u kurcząt brojlerów

Celem pracy było zbadanie wpływu lepkości treści na rozwój żołądka, aktywność skurczową jelita cienkiego i trawienie składników odżywczych we wczesnym okresie życia kurcząt brojlerów. Sześćdziesiąt sześć kurek Hybro w wieku 1 tygodnia przydzielono do 3 grup, 22 w grupie. Ptaki utrzymywano w indywidualnych klatkach i żywiono przez 2 tygodnie dietą kontrolną z pszenicą uzupełnioną ksylanazą (1g/kg) lub dietami z żytem, R - nieuzupełnioną lub RE - uzupełnioną ksylanazą (3g/kg). Diety były granulowane na zimno. W czwartym tygodniu życia na 8 ptakach z każdej grupy przeprowadzono doświadczenie bilansowe, w którym mierzono pozorną strawność tłuszczu, białka i energię metaboliczną diet. W piątym tygodniu życia wszystkie ptaki ubito i oceniono stopień deformacji (rozdęcia) żołądka w skali od 1 (normalny) do 5 (najbardziej rozdęty). Od 7 ptaków z grupy pobrano fragmenty dwunastnicy i jelita czczego i zmierzono w nich aktywność skurczową *in vitro*. Od pozostałych ptaków pobrano treść żołądka, jelita czczego i jelita biodrowego i zmierzono jej lepkość. Po uzupełnieniu diety żytniej ksylanazą lepkość treści żołądka, jelita czczego i jelita biodrowego obniżyła się z 2,6; 17,1 i 35,4 w grupie R do 1,7; 4,7 i 8,2, odpowiednio, w grupie RE ($P<0,001$), wykorzystanie paszy uległo poprawie, zwiększyła się również pozorna strawność tłuszczu (z 79 do 88%) i białka (z 86 do 87%) oraz AME_N diety (z 13,3 do 14,5 MJ/kg SM). Aktywność skurczowa dwunastnicy i jelita czczego w odpowiedzi na acetylocholinę była większa w grupie RE niż w grupie R, ale tylko w jelicie czczym różnice te były statystycznie istotne. Stopień rozdęcia żołądka w grupie R wynosił 3,05, w grupie RE 2,32, w grupie kontrolnej 2,05 ($P<0,05$). Duża lepkość treści ujemnie wpływa na rozwój żołądka i perystaltykę jelita cienkiego. Obniżenie lepkości treści pozwala na zmniejszenie zakłóceń w rozwoju przewodu pokarmowego u kurcząt brojlerów.