

## Enzyme effect on intestinal viscosity in broilers fed with wheat and barley based compound feed

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### SUMMARY

The objective of this experiment was to determine the protocol of enzymatic products utilization according to the wheat and barley proportion in the compound feed used in broilers. We used this experiment to analyze the anti-nutritional NSP's effect of the wheat introduced in the compound feed in a proportion of 60 % and also of the compound feed including wheat in a proportion of 30 % and barley in a proportion of 30 %, on the digestive indices in broilers. To these, we added the variants with introduction of xylanase, in an amount of 100 ppm, and to counteract the anti-nutritional effect of the wheat and barley-based NSP, we introduced 50 ppm xylanase and 50 ppm beta glucanase. We also determined the coefficients of correlation between the digestive coefficient values (intestinal viscosity) and the soluble, insoluble and total NSP contents. The introduction of the wheat-specific enzyme in compound feed in a proportion of 60 % (LE3) or 30 % wheat and 30 % barley (LE5) determine viscosity reduction at duodenum and jejunum levels. The biggest correlation coefficient, at the age of 3 weeks at duodenum level, was recorded between viscosity and the NSPs content (0.762) and at jejunum level between viscosity and the NSPt content; at 6 weeks, the biggest coefficient at duodenum level was recorded between the NSPt content (0.987) and viscosity, and this was available in the case of jejunum, too (0.977).

Keywords: broilers, barley, wheat, enzymes, intestinal viscosity

### INTRODUCTION

Wheat has long been considered a superior poultry feed ingredient, but its feeding value can be highly variable and depends to a large extent on its content of high molecular weight, water soluble non-starch polysaccharides (NSP) (Andson, C., 1991, Bedford, M.R, et all, 1991, Choct, M. and G. Annison, 1992). Wheat contains non starch polysaccharides that reduce the utilization of nutrients. Arabinoxylans are the main NSP in wheat that increase viscosity of

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digestive content in small intestine and interfere with digestion and absorption of nutrients when fed to poultry. (Gao, F et al., 2008). Enzyme supplementation of diets based on wheat, rye, barley or oats can reduce the mentioned adverse effects (Bedford, 2000). Enzymes capable of degrading intestinal viscous polymers have been consistently beneficial in barley based diets (Jeroch, h. S., et al 1995). The performance of broilers fed wheat- and rye-based diets has been shown to be linearly related to the logarithm of intestinal viscosity (Bedford, M.R and H.L Classen, 1992), emphasizing the relevance of this measurement. Enzymes capable of degrading these polymers have been consistently beneficial in rye- and barley-based diets (Jemch, H., S. Danicke, and J. Brufau, 1995). Literature data suggest that the success of xylanase supplementation is not as dramatic in wheat-based diets as in rye-based diets, and in some cases there is no measurable benefit at all. Poor experimental design, particularly with respect to replication, can explain some of the inconsistencies observed, but inconsistencies may also be attributable to variation in the chemical composition of batches of wheat. The level of pentosans, which are the principal viscous NSPs in wheat, varies considerably (6, 7, 8, 9) from sample to sample, a factor that may partially explain the variability in reported results. Variety and environment appear to play a role in determining pentosan content.

#### MATERIAL AND METHODS

The experiment was performed for six weeks, on 150 chickens distributed in five experimental groups (LE1, LE2 and LE3, LE4, LE5). The chickens were fed as follows: during the first growth period, from eclosion until 3 weeks, the compound feed provided 3082.28-3173.01 kcal EM and a CP content of 22.25-22.97 %. In the second growth period, from 3 to 6 weeks, the compound feed provided 3131.21-3222.85 kcal EM and 19.49-20.11 % CP. We created five experimental groups, as follows: the experimental group LE1 fed with forage without wheat in its structure, the experimental group LE2 fed with compound feed including wheat in a proportion of 60%, the experimental group LE3, with wheat in a proportion of 60% and xylanase, in an amount of 100 ppm, the experimental group LE4, with wheat 30% and barley 30%, and the experimental group LE5, fed with compound feed including wheat 30%, barley 30%, 50 ppm xylanase and 50 ppm beta glucanase. At the age of 3 and 6 weeks, successive to chicken killing, we sampled the intestinal content and determined the intestinal viscosity. The compound feed's NSP content was determined according to the tabular data (Englyst H., 1989).

#### *Analysis of compound feeds' nutritive content*

To determine the nutritive value of the compound feed (CF) offered the broilers in our experiment, we applied the standard methods in concordance with the WEENDE scheme; the analyses were performed in the laboratory

belonging to the Department of Animal nutrition and alimentation, Faculty of Animal Science and Biotechnologies Timișoara, respectively:

- DM (%) – stove-drying at 105°C
- CP (%) – Kjeldahl method
- CF (%) – Soxhlet method
- CC (%) – Van Soest method.

*Analysis of viscosity at intestinal level and viscosity of the extract obtained from raw materials*

The intestinal content sampled from duodenum and jejunum level was centrifuged at 10,000 revolutions/minute for 10 minutes. The extract obtained was introduced in stove to determine the viscosity. This was determined with the Brookfield viscometer. The method used to determine raw material viscosity was the one elaborated by Dusel et al., 1997.

*Analysis of NSP content of the raw materials used during the execution of the six experiments*

The raw materials' NSP content determination was performed in the laboratory belonging to the University of Dublin.

*Processing methods of the results*

The primary data obtained successive to our researches were biostatistically processed. The methods of data processing were: variance analysis, calculation of simple and multiple correlation coefficients and determination of simple (polynomial 2nd and 3rd degree) and multiple regression equations, with the help of software (Minitab 14).

The testing of the significance of difference between groups, regarding the production indices, was carried out with the Mann Whitney (Wilcoxon) test, and with the software Minitab 14 and Duncan test as well.

Successive to these calculations, we drew the regression curves and studied their analytical shape, and also the errors given by the experimental data and by regression.

Finally, we decided which of the experimental variants proposed meets the best productive effects.

The experimental organization scheme is presented in table 1, and the nutritive characteristics of the compound feed used in this experiment are presented in table 2.

According to table 1, we may observed that during 0 and 6 weeks, the compound feed for broilers presented an energetic level comprised between 3204 – 3244 kcal and a protein level of 20.16 – 22.91 % in LE1, 3173.01 – 3222.85 kcal and 20.11 – 22.97 % in LE2 and LE3 and of 3082.28 – 3131.21 kcal EM and a protein level of 19.49 – 22.25 % in LE4 and LE5.

Table 1. Organization scheme of the experiment

Period 0-3 weeks				
LE1	LE2	LE3	LE4	LE5
CF (0 % wheat)	CF (60 % wheat)	CF (60 % wheat plus xylanase 100 ppm)	CF (30 % wheat 30 % barley)	CF (30 % wheat 30 % barley plus xylanase 50 ppm and 50 ppm beta glucanase)
EM (kcal/kg) 3204 CP 22.91%	EM (kcal/kg) 3173.01 CP 22.97%	EM (kcal/kg) 3173.01 CP 22.97%	EM (kcal/kg) 3082.28 CP 22.25%	EM (kcal/kg) 3082 CP 22.25%
Period 3-6 weeks				
LE1	LE2	LE3	LE4	LE5
CF (0 % wheat)	CF (60 % wheat)	CF (60 % wheat plus xylanase 100 ppm)	CF (30 % wheat 30 % barley)	CF (30 % wheat 30 % barley plus xylanase 50 ppm and 50 ppm beta glucanase)
EM (kcal/kg) 3244 CP 20.16%	EM (kcal/kg) 3222.85 CP 20.11%	EM (kcal/kg) 3222.85 CP 20.11%	EM (kcal/kg) 3131.21 CP 19.49%	EM (kcal/kg) 3131.21 CP 19.49%

Table 2. Nutritive characteristics of the compound feed used for feeding Broiler chicken

	LE1		LE2		LE3		LE4		LE5	
Specification	0-3 wks	3-6 wks								
ME (kcal/kg forage)	3204	3244	3173.01	3222.85	3173.01	3222.85	3082.28	3131.21	3082.28	3131.21
Crude protein (%)	22.91	20.16	22.97	20.11	22.97	20.11	22.25	19.49	22.25	19.49
Lysine (%)	1.27	1.04	1.15	0.94	1.15	0.94	1.17	0.96	1.17	0.96
Methionine + cystine (%)	0.95	0.73	0.907	0.72	0.907	0.72	0.90	0.72	0.90	0.72
Calcium (%)	1.03	0.84	1.08	0.89	1.08	0.89	1.10	0.91	1.10	0.91
Total phosphorus (%)	0.73	0.65	0.72	0.62	0.72	0.62	0.70	0.60	0.70	0.60

## RESULTS AND DISCUSSION

### *Compound feed content in non-starch polysaccharides (NSP)*

According to tabular data, we determined the compound feeds' content in soluble, insoluble and total NSP, and presented these values in table 3.

In table 3, regarding NSPs, we may notice that in the growth period 0-3 weeks they present a bigger value in the compound feed including 60 % wheat

in its structure, with 1.25 percentage points, and in the variants including 30 % wheat and 30 % barley, with 1.87 percentage points (p%). Regarding NSPi, these are bigger with 0.2 percentage points in the forages including 60 % wheat and with 1.08 percentage points in the groups fed with 30 % wheat and 30 % barley. As regards NSPt, these increase with 1.45 percentage points in the group with 60 % wheat and with 2.95 percentage points in the groups with 30 % wheat and 30 % barley.

During the growth period 3-6 weeks, we may notice that the group fed with compound feed including 60 % wheat presented a NSPs content bigger with 1.24 percentage points than the group fed with forage without wheat, and the groups fed with compound feed including 30 % wheat and 30 % barley presented a NSPs content that was bigger with 1.87 percentage points. NSPt was bigger with 0.92 percentage points in the group with 60 % wheat and with 2.28 percentage points in the compound feed including 30 % wheat and 30 % barley.

Table 3. Compound feeds content in non-starch polysaccharides (NSP) used for this experiment

Growth period	Specification	NSPs (%)	Percentage differences	NSPi** (%)	Percentage differences	NSPt*** (%)	Percentage differences
Period 0-3 weeks	0% wheat	0.85	-	9.3	-	10.15	-
	60 % wheat	2.1	1.25	9.5	0.2	11.6	1.45
	30 % wheat	2.72	1.87	10.38	1.08	13.1	2.95
	30 % barley						
Period 3-6 weeks	0 % wheat	0.79	-	9.35	-	10.14	-
	60 % wheat	2.03	1.24	9.03	-0.32	11.06	0.92
	30 % wheat	2.66	1.87	9.96	0.61	12.62	2.48
	30 % barley						

\* soluble non-starch polysaccharides

\*\* insoluble non-starch polysaccharides

\*\*\* total non-starch polysaccharides

There is a great variability of chemical content of forages in our country, and this is a reason to analyze the raw material for its nutritional content before its utilization in animal feeding.

The nutrients contained by maize and soy-bean grit are considered to present high-digestibility; the thermal treatment will disable the anti-nutritional factors, like protein and lectin inhibitors from soy-bean grit (Campbell et al., 1998). The maize contains approximately 0.9 % soluble and 6% insoluble NSP, while the soy-bean grit contains approximately 6 % soluble and 18 - 21 % insoluble NSP (Bach, 1997). Tables 4 and 5 present the results achieved successive to the determination of the NSPs, NSPi and NSPt contents, in a laboratory belonging to the University of Dublin. The tabular values are quoted from Englyst, 1989 and Choct, 1997.

Table 4. Nutritional characterization of the NSP content of the raw materials (cereals) used in broiler chicken feed

Specification	Maize		Percentage differences	Wheat		Percentage differences	Barley		Percentage differences
	TV	DV		TV	DV		TV	DV	
NSP t	8.1	6.12	132.35	11.4	11.3	100.88	16.7	10.5	159.04
NSP s	0.1	0.09	111.11	2.4	1.58	151.89	4.5	3.76	119.68
NSP i	8	6.03	132.66	9	9.72	92.59	12.2	6.74	181

TV – tabular values, DV – determined values

Table 5. Nutritional characterization of the NSP content of the raw materials (leguminous and subproducts) used in broiler chicken feed

Specification	Peas		Percentage differences	Soy-bean grit		Percentage differences	Good-quality sunfl. grit		Percentage differences
	TV	DV		TV	DV		TV	DV	
NSP t	29.1	12.2	238.52	17	18.77	90.57	26	27.59	94.23
NSP s	2.1	1.82	115.38	2.38	2.26	101.76	4.5	1.25	360
NSP i	27	10.38	260.11	14.62	16.51	88.55	21.5	23.14	92.91

Due to the high NSPs content, the raw materials generate high viscosity at digestive tract level. In correlation with the NSPs content, we carried out viscosity determinations of the raw materials used in compound feeds for broiler chickens. The viscosity values are presented in table 6.

Table 6. NSPs content and viscosity values of the raw materials used in compound feeds

Specification	NSPs values	Viscosity (cP)
Maize	0.09	1.02
Barley	4.5	1.77
Wheat	1.58	1.39
Peas	1.82	1.26
Soy-bean grit	2.26	1.24
Sunflower grit	1.25	1.19

Our results proved that there were differences between the experimental results and the tabular data presented by Englyst, 1992. In cereals, in the case of maize, the NSPs content, respectively the tabular data (0.1%) were 11.1-fold bigger than the experimental values (0.09%). Also bigger differences were observed in the case of barley and wheat; in barley, the tabular NSPs content was bigger with 19.68 % than the experimental values, and in the case of wheat with 51.89 %.

The experimental NSPi values differ from the tabular data as well. So, in the case of maize, the tabular data are bigger with 32.66% than the experimental values. In wheat, the situation is inverse, meaning that the experimental values are bigger than the tabular data with 7.41%. In barley, the differences are big; the tabular data are bigger than the experimental data with 81%.

In the case of legume species, the NSPs differences are not so big. In peas, the tabular values were bigger with 15.38% than the experimental values, and in soy-bean grit, with only 1.76%. An exception is represented by the sunflower grit, where the tabular data (4.5%) were bigger with 260% than the experimental values (1.25%).

Regarding the NSP<sub>i</sub> content, we may notice that this one presents variability, too; in peas, the tabular values are bigger with 160.11% than the experimental values, in soy-bean grit the tabular values are smaller with 11.45%, and in sunflower grit they are smaller with 7.09%.

Successive to viscosity determination in the case of raw material extracts, we observed that this is tightly correlated with the NSPs content. So, the biggest viscosity was recorded in barley (1.77cP), followed by wheat (1.39cP), peas (1.26 cP), soy-bean grit (1.24 cP), sunflower grit (1.19 cP) and maize (1.02) (table 6.).

The high and variable levels of NSPs content and viscosity require current laboratory analyses for the adaptation of a protocol of anti-nutritional effect prevention.

*Intestinal content viscosity at duodenum and jejunum level and the correlation between them and the NSP content of the compound feeds*

The way enzymes act is completely unknown. Their basic action seems to be the partial NSP hydrolysis in the superior digestive tract, important for the digestive viscosity reduction in the small intestine and for the elimination of the encapsulated nutrients (Meng et al., 2005 quoted by Gao, 2007).

When xylanase is added in the wheat-based feed, a great NSP proportion may be hydrolyzed, being able to affect the secretory function, and the organs' size may decrease. The xylanase may decrease significantly the viscosity, possibly by separating the big molecules into smaller fragments. Because the soluble arabinoxylans have a big molecular weight, they are responsible with the increase of digestive viscosity. Enzyme supplementation may influence significantly the intestinal microbial population, by reducing the fermentations within the ileonum (the volatile fatty acids production) and increasing it in the caecum (the AGV increase) (Choct et al., 1999). The enzymatic preparations reduce digestion viscosity in proventriculum and jejunum at the age of 21 days ( $P<0.05$ ) and in colon at the age of 49 days ( $P<0.05$ ) (Gao F., 2007).

Successive to chicken killing at the age of 3 weeks, we determined viscosity at intestinal level; we present viscosity values in table 7.

According to table 7, wheat incorporation in the compound feed structure determines viscosity increase at duodenum level with 20.61% and at jejunum level with 17.67%. The xylanase- supplementation determines viscosity reduction at duodenum level with 7.55 % and at jejunum level with 5.64 %.

Wheat substitution with barley in proportion of 50 % determines viscosity increase at duodenum level with 3.41 % and at jejunum level with 9.85%. The addition of the two enzymes, respectively xylanase and beta glucanase, in equal

amounts, respectively 50 ppm, determines viscosity reduction at duodenum level with 10.75 % and at jejunum level with 5.13 %.

Table 7 Intestinal viscosity in 3-week old broilers

Experimental variant	Percentage of participation (%)	Viscosity (cP) duodenum	Percentage difference	Viscosity (cP) jejunum	Percentage difference
LE1	0	1.94		1.81	
LE2	60% wheat	2.34	120.61	2.13	117.67
LE3	60% wheat plus xylanase 100ppm	2.14		2.01	
LE3/LE2			91.45		94.36
LE4	30% wheat			2.34	
	30% barley	2.42			
LE4/LE2			103.41		109.85
LE5	30% wheat			2.22	
	xylanase 100 ppm				
	30% barley beta				
	glucanase 50 ppm	2.16			
LE5/LE3			100.93		110.44
LE5/LE4			89.25		94.87

Viscosity determination at duodenum and jejunum level was performed at the age of 6 weeks, too; these values are presented in table 8.

Table 8 Intestinal viscosity at duodenum and jejunum level in 6-week old broilers

Experimental variant	Percentage of participation (%)	Viscosity (cP) duodenum	Percentage difference	Viscosity (cP) jejunum	Percentage difference
LE1	0	1.9		1.75	
LE2	60% wheat	2.06	108.42	1.96	112
LE3	60% wheat plus xylanase 100ppm	2.01		1.84	
LE3/LE2			97.57		93.87
LE4	30% wheat			2.24	
	30% barley	2.44			
LE4/LE2			118.44		114.28
LE5	30% wheat				
	xylanase 100 ppm				
	30% barley beta	2.39		2.2	
	glucanase 50 ppm				
LE5/LE3			118.9		119.56
LE5/LE4			97.95		98.21

At the age of 6 weeks, we may observe that maize substitution with wheat (LE2), in proportion of 60 %, determines viscosity increase at duodenum level with 8.42 %, and at jejunum level with 12 %. Wheat substitution with barley

(LE4) in proportion of 50 % determines viscosity increase at duodenum level with 18.44 %, and at jejunum level with 14.28 %.

The addition of the two enzymes fighting against the anti-nutritional NSP effect in the wheat and barley-based compound feed (LE5) determines viscosity decrease at duodenum level with 2.05 %, and at jejunum level with 1.79 %.

We may conclude that the supplementation of the enzymes specific to the cereals that participate in compound feeds in a proportion of 60 % (LE3) or 30 % wheat and 30 % barley (LE5) determines viscosity reduction at duodenum and jejunum level as well.

Soluble arabinoxylans in wheat are generally believed to be responsible for the majority of the anti nutritive activity of NSP in poultry by virtue of their capacity to increase intestinal viscosity (Choct et al 1996). Responses to enzymes supplementation depend on the birds age, which is apparently related to both the type of gut microflora present and the physiology of the bird. Old birds, because of the enhanced fermentation capacity of the microflora in their intestines, have a greater capacity to deal the effects of high viscosity (Choct et al, 1996).

The viscosity of intestinal content in both the jejunum and ileum was in general reduced ( $P < 0.05$ ) with enzyme supplementation, the xylanase preparations proving to be the most efficient. It was concluded that enzyme supplementation of wheat-based diets resulted in improved performance of broiler chickens, which was related to a concomitant reduction in intestinal viscosity. Sanna Steinfeldt et al, 1998

To determine the relationship between the two variables, respectively the compound feed content in NSPs, NSPi and NSPt and the intestinal viscosity, we established the simple correlation coefficients, presented in table 9.

Table 9 Simple correlation coefficients at the age of 3 weeks

Specification	Viscosity	
	Duodenum	Jejunum
PNA s	0.762 (p=0.0134)	0.944 (p=0.016)
PNA i	0.534 (p=0.354)	0.871(p=0.054)
PNA t	0.679 (p=0.191)	0.952 (p=0.012)

In table 9, we may observe that the biggest coefficient of correlation between viscosity and NSP content, at duodenum level, was recorded between the soluble NSP and viscosity, with the value of 0.762, followed by the one between NSPt (0.679) and between NSPi (0.534).

At jejunum level, the biggest correlation coefficient was recorded between NSPt and viscosity (0.952), followed by 0.944 (NSPs) and 0.871 (NSPi).

Figures 1 and 2 present the graphic representations of viscosity at duodenum and jejunum levels, according to the NSP content of the compound feeds, modelled with the help of the second degree polynomial regressions.

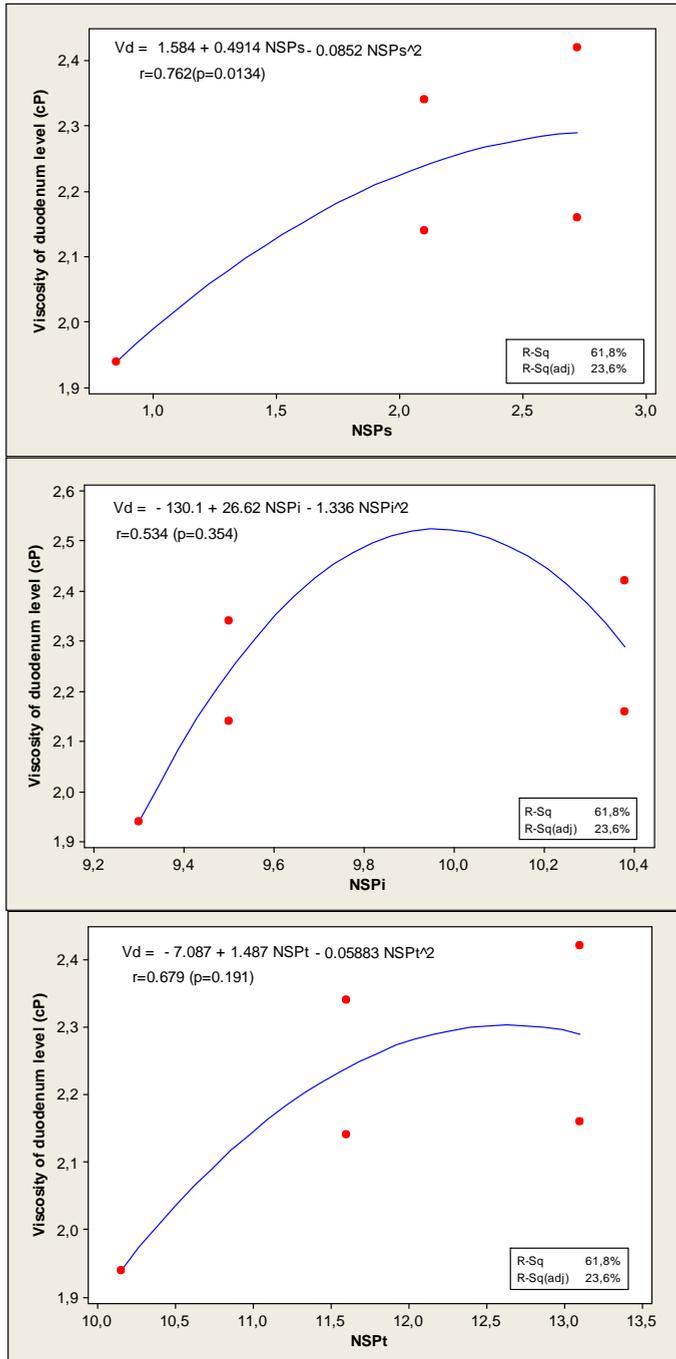


Fig. 1. Graphic representation of the duodenum viscosity function of the NSP content, modeled based on the second degree polynomial regression (at age of 3 weeks)

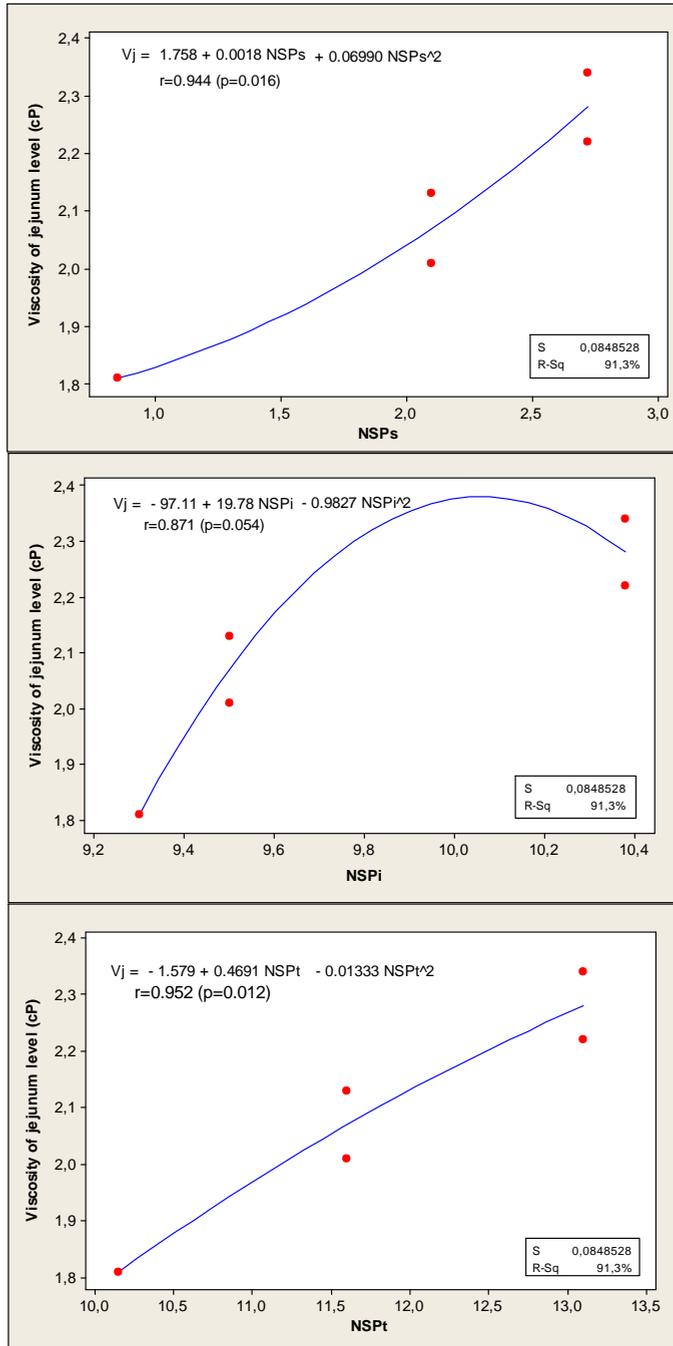


Fig. 2 Graphic representation of the jejunum viscosity function of the NSP content, modeled based on the second degree polynomial regression (at age of 3 weeks)

At the age of 6 weeks, we determined the simple correlation coefficients between the NSP content of the compound feed offered to broilers and the viscosity at duodenum and jejunum level; these coefficients are presented in table 10.

Table 10 Simple correlation coefficients – at age of 6 weeks

Specification	Viscosity	
	Duodenum	jejunum
PNA s	0.876 (p=0.051)	0.889 (p=0.044)
PNA i	0.866 (p=0.058)	0.821 (p=0.089)
PNA t	0.987 (p=0.002)	0.977 (p=0.004)

This table shows that the biggest correlation coefficient, at duodenum level, is recorded between NSPt and viscosity (0.987), followed by 0.876 (NSPs) and 0.866 (NSPi). At jejunum level, the situation is identical; the biggest correlation coefficient is recorded between NSPt and viscosity, followed by NSPs (0.889) and NSPi (0.821). The graphic representation of viscosity, function of the NSP content, modelled with the second degree polynomial regression, is presented in figures 3 and 4. This correlation may be explained by the fact that, the compound feed structure, beside the components that cause a big viscosity at duodenum and jejunum level, includes enzymes which determine viscosity reduction at intestinal level.

## CONCLUSIONS

Wheat substitution with barley in proportion of 50 %, in the period 0-3 weeks, determines the NSPs content increase with 1.87 pp, of NSPi with 1.08 pp, and in the period 3-6 weeks the NSPs content increases with 1.87 pp, and the NSPi with 0.61 pp. Successive to the determination of the NSP s, i and t content, we observed that there are big differences between the tabular data and the experimental ones, differences that represent even 260 % (sunflower grit), requiring current laboratory analyses for the adaptation of a protocol preventing the anti-nutritional effect.

There is a positive correlation between NSPs and raw material viscosity, so that the increase of the NSPs content determines the increase of raw material viscosity from 1.02 cP in maize to 1.77 cP in barley. The supplementation with the wheat-specific enzyme of the compound feeds where wheat participates in a proportion of 60 % (LE3) or 30 % wheat and 30 % barley (LE5), determine viscosity decrease at duodenum and jejunum level as well.

The biggest correlation coefficient at the age of 3 weeks at duodenum level was recorded between viscosity and the NSPs content (0.762), and at jejunum level between viscosity and the NSPt content. At the age of 6 weeks, the biggest correlation coefficient, at duodenum level, was recorded between the NSPt content (0.987) and viscosity, like in the case of jejunum (0.977).

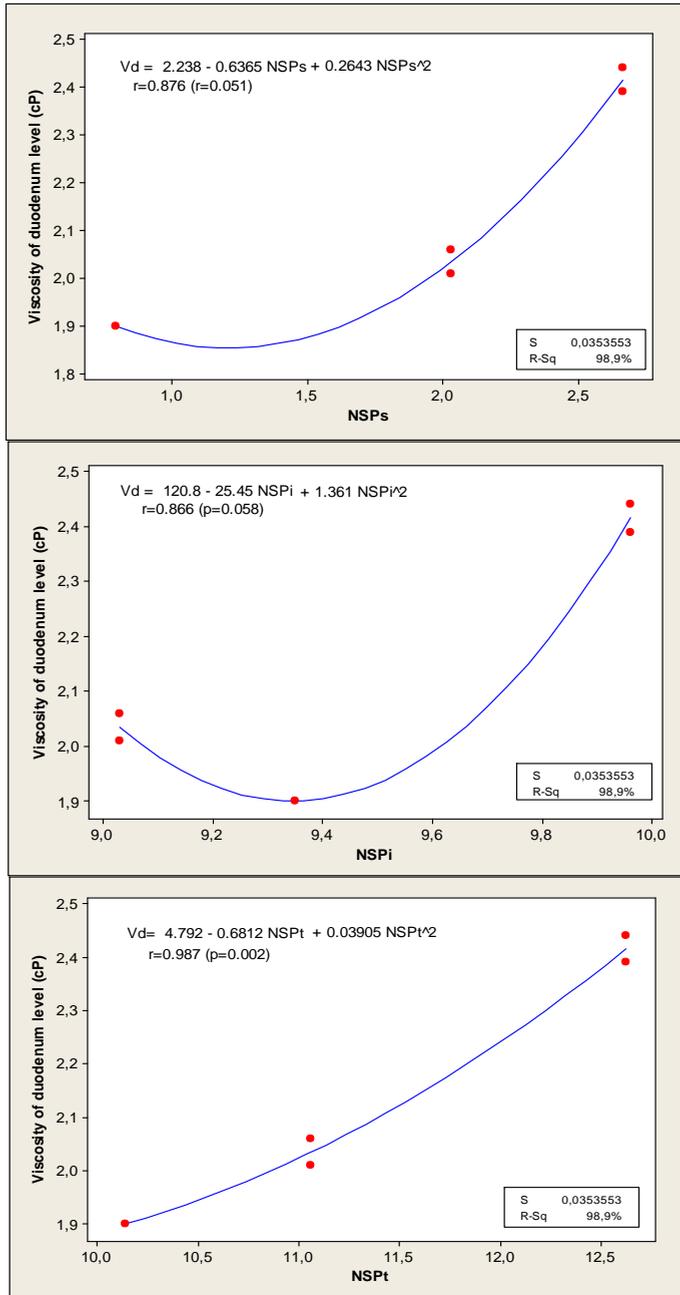


Fig. 3. Graphic representation of the duodenum viscosity function of the NSP content, modeled based on the second degree polynomial regression (at age of 6 weeks)

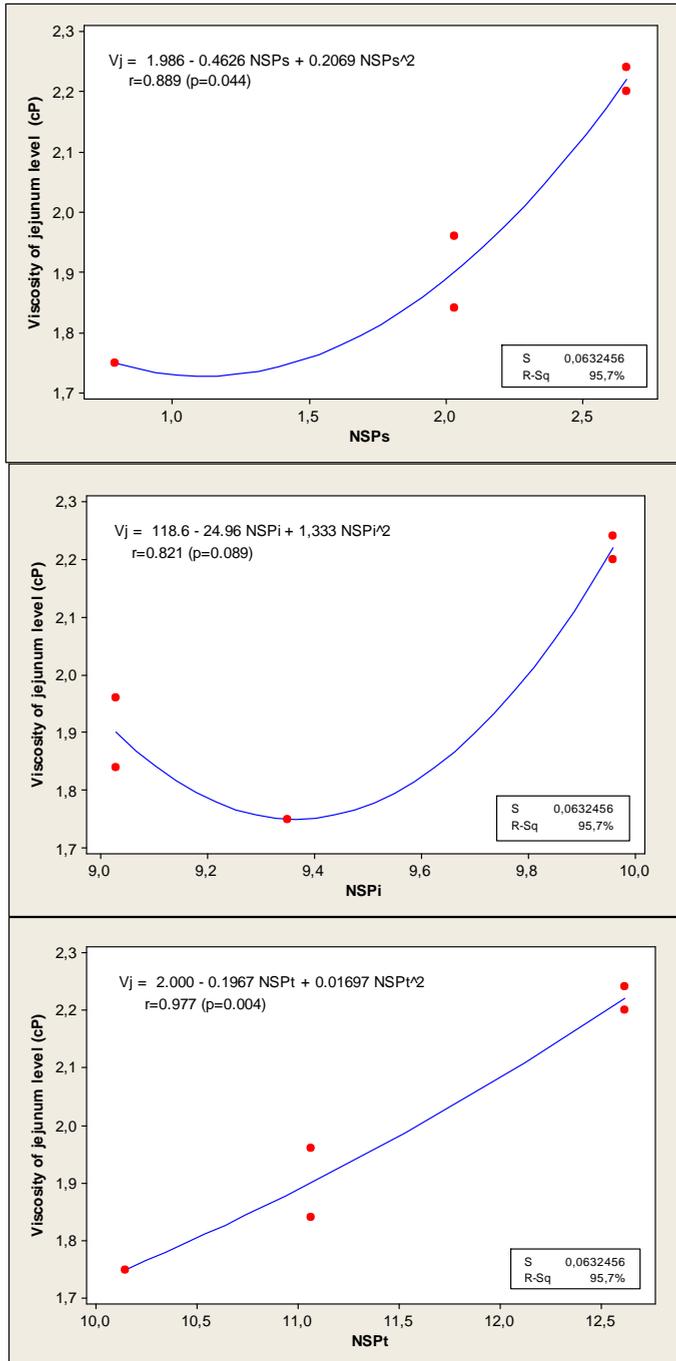


Fig. 4. Graphic representation of the jejunum viscosity function of the NSP content, modeled based on the second degree polynomial regression (at age of 6 weeks)

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