

## Effect of NSP Degrading Enzyme Supplement on the Nutrient Digestibility of Young Chickens Fed Wheat with Different Viscosities and Triticale

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### ABSTRACT

To study the effect of cell-wall degrading enzymes on the nutrient efficiency of young chickens fed different grains, an *in vitro* experiment was conducted to determine the range of viscosities of seven local wheat varieties. From these, the highest (Flaat) and the lowest (Ghods) were selected for an *in vivo* study. 288 day-old Arian chickens were kept in cages and fed one of four grains (Flaat, Ghods, Triticale, and Corn) with or without a dietary NSP degrading enzyme in a 4×2 factorial arrangement with six replicates per treatment. An indigestible marker (chromic oxide) was used for digestibility measurements. Feed and water were provided *ad libitum*. Excreta samples were collected from 18-21 days of the experiment. Apparent metabolizable energy corrected for nitrogen (AME<sub>n</sub>) was improved (P<0.05) in all diets by enzyme supplementation except the corn diet. The apparent lipid digestibility (ALD) and apparent crude carbohydrate digestibility (ACCD) of all treatments were significantly improved by adding an enzyme (P<0.01). This improvement was highest in the Flaate variety (highest viscosity) of wheat. The results of this experiment clearly indicate the positive effects of supplemental NSP degrading enzymes on nutrient digestibilities and on AME<sub>n</sub> especially for the grains with the highest viscosity (wheat cultivar Flaate and triticale).

**Keywords:** Broilers, NSP degrading enzymes, Nutrient digestibility, Viscosity.

### INTRODUCTION

Young birds have a gastrointestinal tract with a limited capacity. In order to maximize the growth rate of young broilers at the starter period, diets with high energy and protein contents are recommended (NRC, 1994). The main sources of providing energy in such diets are grains like corn and wheat (Friesen *et al.*, 1992). Wheat can supply up to 70% of metabolizable energy and 40% of the protein requirements of broilers. Compared to other grains, there is a wider variability in wheat composition (Hew *et al.*, 1998). The variability in wheat energy (AMEn) for broiler chickens and the lack of relationship between the amount of

starch and the AMEn of wheat has been reported (Rogel *et al.*, 1987). A negative correlation between AME and the amount of soluble non starch polysaccharides (NSP) of wheat has been reported (Annison, 1991). The addition of wheat NSP to a diet containing sorghum decreased the AME (Choct and Annison, 1990, 1992). However, adding xylanase enzyme to a diet containing wheat improved its AME (Annison, 1992). These data suggest that a lower AME in wheat is related to its NSP content.

Smits and Annison (1996) studied the physico-chemical properties of NSP and suggested that the measurement of soluble NSP and the viscosity of ingredients in *in vitro* conditions would be important and can be used for their nutritional evaluation. Dif-

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ferent methods for the measurement of extract viscosity of wheat by water or buffers have been suggested (Fengler *et al.*, 1990; Scole *et al.*, 1993; Van der Klis *et al.*, 1995a). However, it seems methods that mimic the GIT conditions of the young birds, can be more practical to evaluate nutritional values of different wheat varieties. The two phase method of Bedford and Classen (1993) is a practical method but it is time-consuming and expensive.

The addition of commercial enzymes to the diets containing high levels of wheat can improve AME, lipid, protein (Friesen *et al.*, 1992; Steinfeld *et al.*, 1998), and other nutrient digestibility (Friesen *et al.*, 1992; Hew *et al.*, 1998). There is little research pub-

## MATERIALS AND METHODS

Seven wheat varieties and one triticale sample from local sources (three replicates of each) were prepared and their viscosities measured by water (Van der Klis *et al.*, 1995a) and acetate buffer, pH=5, 0.1M extraction (Scole *et al.*, 1993) using a Hopler BH2 viscometer. Varieties with the highest and the lowest viscosities, Flaate and Ghods, along with triticale were selected for further studies.

In an *in vivo* study, 288 day-old meat type male broiler chickens of the Arian strain were used. In a completely randomized experiment with 4×2 factorial arrangement, treatments using 6 replicates of six birds

**Table 1.** Chemical composition and calculated metabolizable energy of the selected grains (dry matter basis).

Grain	DM <sup>a</sup> %	GE Kcal/kg	CP %	EE %	Ash %	CF %	NFE %	AMEn <sup>b</sup> Kcal/kg
Flaat wheat	90.54	4000	13.68	2.74	1.84	3.31	78.43	3506
Ghods wheat	91.83	4030	9.19	3.21	1.54	3.32	82.74	3535
Triticale	90.92	4090	13.15	2.78	1.87	3.24	78.96	3437

<sup>a</sup>DM, dry matter; GE, gross energy; CP, crude protein; EE, ether extract; CF, crude fiber; NFE, nitrogen free extract; AMEn, apparent metabolizable energy corrected for nitrogen.

<sup>b</sup> AMEn (apparent metabolizable energy corrected for nitrogen) for wheat obtained from AMEn= 34.92 × CP + 63.1 × EE + 36.42 × NFE and for triticale obtained from AMEn= 34.49 × CP + 62.16 × EE + 35.61 × NFE (NRC, 1994).

lished on broiler chickens fed triticale that has more protein and lysine than either wheat, sorghum and corn (McGinnis *et al.*, 1985). Triticale like its parents, wheat and rye, contains pentosans that reduce its nutritional value (Pettersson and Aman, 1988). The addition of pentosanase enzymes to diets containing this grain and fed to young broiler chickens can increase the performance of the birds (Flores *et al.*, 1994; McGinnis *et al.*, 1985). Therefore, the objective of this experiment was to determine the effect of a NSP degrading enzyme supplement on AMEn, and nutrient digestibilities of diets containing wheat with different viscosities and triticale in young broiler chickens.

(group weight = 241 ± 0.36 g) housed in 48 cages and fed experimental diets containing 60% of each grain (Flaat and Ghods wheat cultivars, triticale and commercial yellow dent corn) with or without an NSP degrading enzyme supplement (0 and 0.06%, Endofeed W, GNC Bioferm Inc., Canada). The minimum β-Glucanase and xylanase activity of used enzyme was 440 and 1200 units per gram respectively. Feed and water were provided *ad libitum*. The chemical composition and estimated metabolizable energy content of the selected grains and the experimental diets are given in Tables 1 and 2.

In order to measure digestibility of the nutrients and the AMEn of the diets, 0.3% chromic oxide as an indigestible marker was

**Table 2.** Nutrient composition of experimental diets (%).

Ingredients	Corn	Ghods wheat	Flaat wheat	Triticale
Triticale	60	-	-	-
Flaat wheat	-	60	-	-
Ghods wheat	-	-	60	-
Corn	-	-	-	60
Soybean meal	28.02	25.57	26.74	28.75
Fish meal	6.34	8.00	3.56	2.61
Vegetable oil	2.41	3.27	4.9	5.03
Dicalcium phosphate	1.58	1.88	2.65	1.51
Oyster shell	0.67	0.31	1.17	1.04
Vit + Min. Suppl. <sup>a</sup>	0.50	0.50	0.50	0.50
Salt	0.30	0.21	0.28	0.39
D-L Methionine	0.10	0.18	0.13	0.11
Enzyme <sup>b</sup>	0.06	0.06	0.06	0.06
Calculated analysis				
AMEn (kcal/kg)	3000	3000	3000	3000
Crude protein (%)	21.56	21.56	21.56	21.56
Ca (%)	0.94	0.94	1.27	0.94
Available P(%)	0.42	0.42	0.57	0.42
Met + Cys (%)	0.84	0.84	0.84	0.84
Lys (%)	1.23	1.25	1.10	1.14

<sup>a</sup> Supplied per kilogram of diet: vitamin A, 11000 IU; vitamin D<sub>3</sub>, 1800 IU; vitamin E, 36 mg; vitamin K<sub>3</sub>, 5 mg; thiamin, 1.53 mg; riboflavin, 7.5 mg; Ca pantothenate, 12.240 mg; niacin, 30.4 mg; pyridoxine, 1.53 mg; folic acid, 1.26 mg; vitamin B<sub>12</sub>, 1.6 mg; biotin, 5 mg; choline chloride, 1100 mg, anti oxidant, 100 mg; Mn, 161.3 mg; Zn, 84.5 mg; Fe, 250 mg; Cu, 20 mg; I, 1.6 mg; Co, 0.47 mg; Se, 20 mg.

<sup>b</sup> Endofeed W (Xylanase activity= 1200 Ug<sup>-1</sup> and  $\beta$ - Glucanase activity = 440 Ug<sup>-1</sup>); 4 diets without enzyme had the same amount of sand.

added to each diet and fed to chickens from 18-21 days of the experiment. Performance of the chickens was measured from 1-14 days of age. Samples of excreta were collected for three days, two times per day and kept in a freezer (-20 °C). Feed, and excreta samples were oven dried (60 °C, 72 hours) and ground prior to analysis. Gross energy was measured by a bomb calorimeter (Schimazu Calorimeter), protein by Kjeldahl (Kjeltec Auto Analyzer 1030), fat by Soxhlete (Soxtec System HT6) using standard procedures of AOAC (1980) and chromic oxide by the method of Fenton and Fenton (1979). Apparent metabolizable energy corrected for nitrogen (AMEn) was measured by the Sibbald and Slinger formula (1963), digestibility of nutrients by the formula of Saha and Gilbreath (1993), and apparent crude carbohydrate digestibility (ACCD) by the formula suggested by Steinfeldt *et al* (1998). Data were analyzed using the Gen-

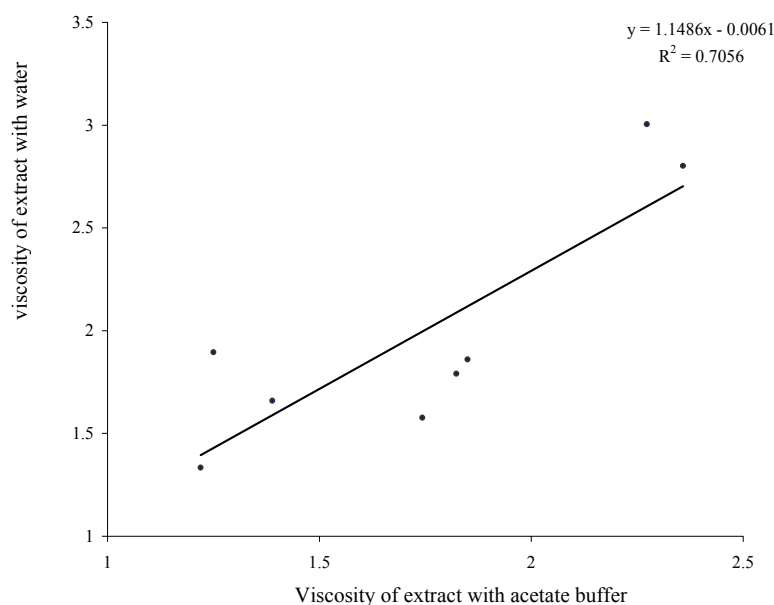
eral Linear Model of SAS (1986) and, when appropriate, the Duncan multiple range test was used to compare means.

## RESULTS AND DISCUSSION

The viscosity of extracts from wheat varieties and triticale are shown in Table 3.

**Table 3.** Viscosity values of extracts from different wheat varieties and triticale with water and acetate buffer, pH=5, 0.1 M (centi poase).

Grain varieties	Viscosity with water	Viscosity with acetate buffer
Flaat	2.802	2.358
Roushan	1.860	1.850
Alamout	1.791	1.823
Chamran	1.577	1.743
Nick Nejad	1.659	1.389
Mahdavi	1.895	1.250
Ghods	1.334	1.220
Triticale	3.005	2.273
(wheat * rye)		



**Figure 1.** Correlation between two methods of viscosity measurement.

Except for Alamout and Chamran, the viscosity of water extracts was greater than those of the acetate buffer. Due to a constant ratio of wheat to water or acetate buffer (1:2) and similar *in vitro* conditions, it seems that the higher viscosity values obtained with the water method probably relates to the increased pH of the media (Fengler *et al.*, 1988), greater solubility of viscous compounds in water and higher solubility of specific wheat proteins (gliadins) that increases the viscosity of extracts (Van der Klis *et al.*, 1995b). Since Flaar had a higher protein level than that of Ghods, it is possible that the higher viscosity value of Flaar in comparison to Ghods is related to its protein content. Due to the high positive correlation between the two methods ( $r^2 = 0.70$ ), one method can be chosen (Figure 1). However, there are some other methods (Fengler *et al.*, 1990; Bedford and Classen, 1993) and it seems important that a fast method for assessing the NSP content of grains be established. Measuring grain viscosities under situations that mimic the gastrointestinal conditions of young chickens can be of value.

AMEn, apparent lipid (ALD), and crude carbohydrate (ACCD) digestibilities and nitrogen retention (NR) of the experimental diets from 18-21 days of age are shown in Table 4. AMEn of treatments were affected by diet, enzyme ( $P < 0.01$ ) and diet  $\times$  enzyme ( $P < 0.05$ ). The addition of enzyme to diets containing all grains except corn improved their AMEn values. For Flaar, Ghods and triticale this improvement was 9.24, 3.15 and 3.32% respectively. ALD and ACCD were also improved by the addition of enzyme ( $P < 0.01$ ). This improvement was highest for Flaar followed by triticale. There was no diet /enzyme interaction for NR, but the addition of an enzyme to the diets significantly improved their NR ( $P < 0.01$ ). Results from other experiments with young broiler chickens indicate that adding enzyme to diets containing high levels of wheat improves AME, ALD (Annison, 1992; Friesen *et al.*, 1992; Van der Klis *et al.*, 1995b), NR (Scott and Hall, 1998), and ACCD (Steenfeldt *et al.*, 1998). Similar to the results of this study, other reports emphasize that enzyme addition to diets containing soluble NSP can improve lipid digestibility more than that of

**Table 4.** Effects of dietary treatments on AMEn (kcal/kg) and nutrient digestibilities (%) in broiler chickens from 18-21 days of age (dry matter basis).

Diet	Enzyme <sup>1</sup>	AMEn <sup>2</sup> (kcal/kg)	ALD <sup>3</sup> (%)	ACCD <sup>4</sup> (%)	NR <sup>5</sup> (%)
Corn	-	3211 <sup>f6</sup>	76.59 <sup>bc</sup>	83.58 <sup>b</sup>	65.43
	+	3276 <sup>ef</sup>	80.05 <sup>a</sup>	84.68 <sup>a</sup>	68.34
Ghods wheat	-	3393 <sup>cd</sup>	70.9 <sup>d</sup>	82.11 <sup>c</sup>	61.27
	+	3500 <sup>b</sup>	74.63 <sup>c</sup>	83.54 <sup>b</sup>	64.69
Flaat wheat	-	3354 <sup>a</sup>	65.53 <sup>e</sup>	80.39 <sup>e</sup>	66.43
	+	3664 <sup>cde</sup>	78.19 <sup>ab</sup>	83.33 <sup>b</sup>	70.24
Triticale	-	3344 <sup>de</sup>	69.56 <sup>d</sup>	79.53 <sup>f</sup>	61.44
	+	3455 <sup>bc</sup>	76.03 <sup>c</sup>	81.13 <sup>d</sup>	66.58
± SEM		31.3	0.632	0.209	0.378
Source of variation		probability			
Diet	<0.01	<0.01	<0.01	<0.01	<0.01
Enzyme	<0.01	<0.01	<0.01	<0.01	<0.01
Diet×enzyme	<0.05	<0.01	<0.01	<0.01	NS

<sup>1</sup> +, with enzyme; -, without enzyme.<sup>2</sup> AMEn, apparent metabolizable energy corrected for nitrogen.<sup>3</sup> ALD, apparent lipid digestibility.<sup>4</sup> ACCD, apparent crude carbohydrate digestibility.<sup>5</sup> NR, nitrogen retention.<sup>6</sup> Means with different superscripts in each column are significantly different, NS, not significant

other nutrients (Friesen *et al.*, 1992; Steinfeldt *et al.*, 1998). Wheats with higher extract viscosity increase the viscosity of the intestinal chyme more than those with lower extract viscosity (Van del Klis *et al.*, 1995a). In this study, addition of an enzyme to the diets containing Flaate wheat with the highest extract viscosity improved the digestibility of ALD more than that of Ghods wheat, which had the lowest extract viscosity (19.32 vs 5.26%). As shown in Table 4, addition of an enzyme to the diets containing Flaate wheat also improved the digestibility of AMEn, ACCD and NR more than those of Ghods wheat (9.24, 3.66, 5.74% vs 3.15, 1.74, and 5.58% respectively). Similar results were obtained when a commercial xylanase enzyme was added to a wheat based diet (Carre *et al.*, 1992). Given the increase in AMEn, they concluded that the improvement in AMEn is mostly related to the increased lipid, starch and to a lesser degree, protein digestibility. The release of pantose sugars from NSP cannot increase the amount of AMEn as increased by lipid, starch and protein (Carre *et al.*, 1992).

The feed intake, body weight gain, and feed-to-gain ratio of the chickens fed four different grain-based diets, with or without a dietary enzyme, from 1-14 days of age are presented in Table 5 that confirms the other data.

Amongst the several hypothesis relating to the mechanism of enzyme action, the decrease of digesta viscosity by NSP degrading enzymes is more highly validated. Based on this hypothesis, enzymes can hydrolyse the glycoside bonds of large molecules that are responsible for increasing the digesta viscosity. This action alleviates the antinutritional effect of soluble NSP (Bedford and Classen 1992). In addition to the effect of NSP on increasing viscosity, other physico-chemical properties of NSP such as the stirred water layer of the gut, bonding capacity, etc., are involved in digestibility and nutrient absorption (Smits and Annison, 1996).

Under the conditions of this experiment, it was concluded that the addition of this enzyme to diets containing wheat and triticale increases their AMEn, ACCD and especially

**Table 5.** Effects of dietary treatments on feed intake, body weight gain, and feed to gain ratio in broiler chickens from 1-14 days of age.

Diet	Enzyme <sup>a</sup>	Feed intake (g)	Weight gain (g)	Feed to gain ratio
Corn	-	438	260	1.69
	+	416	253	1.65
Ghods	-	417	249	1.68
wheat	+	406	250	1.62
Flaat	-	416	234	1.78
wheat	+	427	239	1.80
Triticale	-	427	227	1.89
	+	430	249	1.73
± SEM		8.36	6.33	0.042
Source of variation		Probability		
Diet		0.18	0.0068	0.0005
Enzyme		0.43	0.23	0.05
Diet×enzyme		0.21	0.18	0.23
Main effects				
Diet				
Corn		427	257 <sup>a b</sup>	1.67 <sup>b</sup>
Ghods wheat		411	249 <sup>a</sup>	1.65 <sup>b</sup>
Flaat wheat		422	236 <sup>b</sup>	1.79 <sup>a</sup>
Triticale		428	238 <sup>b</sup>	1.80 <sup>a</sup>
± SEM		5.91	4.48	0.029
Enzyme				
-		420	242	1.76 <sup>a</sup>
+		424	448	1.70 <sup>b</sup>
± SEM		4.18	3.16	0.021

<sup>a</sup> +, with enzyme; -, without enzyme.

<sup>b</sup> Means with different superscripts are significantly different (P<0.05).

ALD. The greatest improvement can be achieved for those grains with the highest extract viscosities (Flaat and triticale).

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## اثر مکمل آنزیمی بر قابلیت هضم مواد مغذی جیره های حاوی گندمهای با ویسکوزیته متفاوت و تریتیکاله در جوجه های گوشتی جوان

م. د. شکوری و ح. کرمانشاهی

### چکیده

با هدف بررسی اثر آنزیمهای تجزیه کننده پلی ساکاریدهای غیر نشاسته ای (NSP) محلول موجود در دیواره سلولی غلات بر روی قابلیت بهره وری مواد مغذی، ارقام مختلفی از آنها تهیه شده و ویسکوزیته آنها در آزمایشگاه تعیین گردید. پس از انتخاب ارقام دارای بیشترین کمترین مقدار ویسکوزیته آزمایشگاهی (فلات و قدس) از بین ۷ رقم گندم، به منظور بررسی اثر مکمل آنزیمی بر انرژی قابل متابولیسم ظاهری تصحیح شده برای ازت (AMEn) و قابلیت هضم مواد مغذی جیره های حاوی ۶۰٪ از ارقام انتخاب شده، تریتیکاله و ذرت، آزمایشی با ۲۸۸ جوجه خروس یکروزه آرین در قالب طرح کاملاً تصادفی با آزمایشات فاکتوریل ۲×۴ با ۶ تکرار در هر تیمار به اجرا درآمد. به دنبال تغذیه جوجه های آزمایشی با جیره های حاوی ۰/۳٪ اکسید کروم، جمع آوری نمونه های مدفوع در روزهای ۱۸ تا ۲۱ صورت گرفت. AMEn همه جیره ها به جز جیره حاوی ذرت تحت تاثیر مکمل آنزیمی افزایش یافت (P<۰/۰۵). قابلیت هضم ظاهری لیپید (ALD) و کربوهیدرات خام (ACCD) همه جیره ها با لحاظ کردن آنزیم بهبود معنی داری نشان داد. (P<۰/۰۱). در جیره حاوی گندم فلات بهبودهای مشاهده شده تحت تاثیر مکمل آنزیمی در همه شاخص ها بیشترین مقدار را دارا بود. نتایج موجود نشان دهنده اثر مثبت مکمل آنزیمی بر قابلیت هضم ظاهری مواد مغذی و AMEn جیره های آزمایشی و خصوصاً جیره های حاوی غلات با ویسکوزیته بالا (فلات و تریتیکاله) می باشد.