Non-starch Polysaccharide Degrading Enzymes in Corn and Wheat-Based Broiler Diets: Dual Activity for Major Substrates

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Abstract: This study consolidates the efficacy of a non-starch polysaccharide (NSP)ase enzyme-concept in corn and wheat-based broiler diets. The NSP building blocks of corn, wheat and soybean meal (SBM, 44% protein) were studied first. Cereal NSP primarily consists of arabinose, xylose and glucose molecules that form arabinoxylan, β -glucan and cellulose polysaccharides. In soybean meal (SBM), glucose (cellulose) predominates, next to galactose and uronic acids that form “pectins”. Broiler performance progress using in-feed NSPase enzymes depends on the concentration, complexity and solubility of the substrate and the genetic potential of the animal, mainly. A dual NSPase enzyme-concept for cereal and SBM NSP, predominantly being arabinoxylan, β -glucan and cellulose, was developed. Methods for measuring enzyme activities (endo-1,4- β -xylanase (EC 3.2.1.8) and endo-1,3(4)- β -glucanase (EC 3.2.1.6)) were developed (AVEVE Biochem UNITS) and preparations thereof (XG) standardized to meet requirements for most challenging NSP (corn, barely soluble). Feed intake (FI), bodyweight gain (BWG) and feed:gain ratio (F:G) were assessed in three zootechnical studies, each using 160 Ross 308 broilers split in two feeding groups with 80 birds/group (10 replicates of eight) for 42 d (starter/grower period). Respective corn-SBM, wheat-SBM and corn/wheat-SBM diets were used as negative control (NC) or added with the enzyme-concept (XG). In the total period, XG ameliorated BWG and F:G compared to NC in each study, where BWG increased best in diets with corn and F:G lowered most in diets with wheat. The dual NSPase enzyme-concept offers ingredient flexibility in present setting by enhancing the nutritional content of corn, wheat and SBM, expectedly from cleaving major NSP target molecules. Thereby, broiler professionals increase feed formulation liberty and safety and production result simultaneously.

Key words: Broiler, xylanase, glucanase, BWG, F:G, corn, wheat, SBM.

1. Introduction

Cereals, legumes and oil seeds (and their by-products) are basic broiler feed ingredients. Plant cell walls and associated structures are fibrous and rich in non-starch polysaccharide (NSP) [1]. Monogastric species physiologically lack endogenous NSPase enzymes. Therefore, intact NSP in the broiler gut can easily hamper digestion and consequently

disrupt the intestinal balance, leading to inferior productivity and economy. The anti-nutritional effects of NSP are diverse [2]. The most important effects are associated with the NSP molecular structure and complexity, which is generally classified as “soluble” or “insoluble”. More than 20 years ago, the development of highly efficacious enzymes that act on both soluble and insoluble NSP was considered the future challenge for sustainable agriculture [1].

Soluble NSP has a molecular structure that facilitates interactions with the environment [3]. In the

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gut, NSP-water interactions increase the viscosity of the digesta [4], which hinders proper nutrient digestion and absorption, prolongs the retention time of the intestinal contents and alters fore- and hind-gut microbial populations [5-8]. In wheat f.i., NSP accounts for 10.2%-11.9% of dry matter (DM) and is 19.5%-21% soluble [9-11]. The remaining “insoluble” wheat NSP creates a protective cell wall [12]. These structures render endogenous digestive enzymes inaccessible to the nutrient filled core [13, 14]. In corn f.i., NSP accounts for 9.7%-10.3% of DM and is 91% insoluble [9, 10]. Protein-rich plants and especially their by-products have a higher NSP content than cereals. Soybean meal (SBM) f.i. holds 19.6%-21.7% NSP of DM, with 29% solubility [9, 10].

The constitution of (soluble and insoluble) NSP can be examined by splitting these complex polysaccharides to their individual sugar building blocks: arabinose and xylose (pentoses), glucose, galactose and mannose (hexoses), rhamnose and fucose (6-deoxyhexoses) and uronic acids (glucuronic and galacturonic acid) [15, 16]. It is demonstrated that in grains as well as in SBM, arabinose, xylose and/or glucose are highly prevalent [9-11]. The corresponding cell-wall polysaccharides in cereals are arabinoxylans, β -glucans and cellulose (β -1,4-linked D-glucose) [3, 15-18]. In SBM, cellulose predominates in the hull-fraction, where the remaining NSP is rich in arabinose, galactose and uronic acid that form “pectic” polysaccharides [19, 20]. Therefore, NSP degrading enzyme activities targeted to the substrate polysaccharides arabinoxylan, β -glucan and cellulose, have major potential to degrade the viscous nature of NSP and encapsulated nutrients from corn, wheat and SBM, in the small intestine, for a more efficient use of nutrients.

To confirm and define predominant enzyme target substrates, this study is based on a raw material NSP analysis (corn, wheat and SBM, AVEVE Animal Nutrition, Merksem, Belgium). Two selected NSPase enzyme activities, xylanase and glucanase, were

standardized to their optimum in a dual concept (*in vitro* UNITS, AVEVE Biochem, Merksem, Belgium) to cleave insoluble and soluble NSP, with *in vivo* efficiency. Optimal UNITS were based on feed formulation and *in vitro* and *in vivo* enzyme research expertise (AVEVE Biochem, Merksem, Belgium). The action of the dual NSPase enzyme-concept on broiler performance was consolidated in three individual experiments at the same trial facility, to simulate production cycles with changing feed formulations. Corn-SBM, wheat-SBM and corn/wheat-SBM were used, with or without NSPase enzymes (100 ppm in each study). This dual NSPase enzyme-concept, applied at the same dose in different formula, would increase formulation liberty and smoothen the nutrient variability amongst and within ingredients for and efficient growth and limited environmental nutrient loss. The aim of this study therefore was to check the NSP content of various yet common feed ingredients corn, wheat and SBM and to test the effect on broiler performance of the dual NSPase enzyme-concept applied to diverse (high soluble versus high insoluble NSP) feeds.

2. Materials and Methods

2.1 Preliminary Raw Material Chemical and Fiber Analysis

Corn, wheat and SBM (44% protein) samples were provided by AVEVE Animal Nutrition (Merksem, Belgium). Samples were analyzed at the Department of Animal Sciences and Aquatic Ecology (Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium), as part of a research project in cooperation with AVEVE Group (1991), to investigate NSP target substrates for fiber degrading enzymes. A representative sample of each of raw materials was milled in a Tecator Sample Mill (0.50 mm) (Waltham, MA, USA). Techniques for total dietary fiber (TDF) and NSP analysis were first optimized at the Ghent University laboratory; the following analyses were performed: (1) DM, crude protein (CP), crude fat (EE),

crude fiber (CF) and crude ash were determined via the Weende analysis principles; (2) neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest *et al.* [21]; (3) TDF (with determination of the soluble and insoluble fraction) according to Prosky *et al.* [22]; (4) dietary fiber (NSP + Klason lignin); (5) NSP up to the monomer fraction (arabinose, xylose, glucose, galactose, uronic acid, mannose, rhamnose and fucose). NSP pentoses (arabinose and xylose), hexoses (glucose, galactose and mannose) and deoxy-hexoses (rhamnose and fucose) were determined using methods mainly based on the work of Englyst *et al.* [16] and Theander *et al.* [13]. The uronic acids analyses were determined via a colorimetric reaction with meta-hydroxyphenyl, mainly based on the work of Blumenkrantz and Asboe-Hansen [23], Ahmed and Labavitch [24] and Faulks and Timms [25].

2.2 Birds and Management

At the Experimental Station of the Department of Animal Nutrition of the University of Life Sciences (Poznań, Poland), three zootechnical experiments were conducted, each using 160 one-day old male Ross 308 broiler chickens obtained from Hama Plus (Dan Hatch Group, Wolsztyn, Poland). Their average initial body weight was 40 g in each experiment. In each of the experiments, the birds were randomly allocated to two feeding groups with 80 animals per group. Each group had 10 replicates, being 10 pens with each eight birds. Birds had *ad libitum* access to feed and water.

The three experiments were conducted for a total of 42 d each, using mash feed in all feeding phases. The first experiment on a corn-SBM diet was done with a starter (1-14 d) and grower (15-42 d) period, the second experiment on a wheat-SBM diet with a starter (1-10 d) and a grower period (11-42 d). The third experiment was done on a combined corn/wheat-SBM diet with a starter (1-14 d) and a grower period (15-42 d). Mortality was noted. Experiments were conducted in accordance with the principles and specific

guidelines presented in Guide for the Care and Use of Agricultural Animals in Research and Teaching (Champaign, Illinois, USA) [26].

2.3 Experimental Diets

Three diets were formulated, their composition and nutrient values are presented in Table 1. The basal diets (NC) were based on corn-SBM, wheat-SBM and corn/wheat-SBM and contained an adequate nutrient composition for broiler starters and growers according to typical broiler recommendations. Per experiment, NC was used as a negative control (NC1-3), the corresponding experimental groups were NC1-3 added with the same dose of the NSPase enzyme-concept (Exp. 1-3). Broiler diets were manufactured at the Experimental Station of the Department of Animal Nutrition (Miedzychód, Poland) and prepared in a 100 kg horizontal mixer (Zuptor 100, Leszno, Poland).

DM, CP and CF content were determined using standard procedures, being respectively methods 965.17, 976.05 and 920.39 of the Association of Official Agricultural Chemists (AOAC) procedures [27]. The nitrogen content was analyzed using the Kjeldal method (Kjel Foss Automatic 16210, A/S N. Foss Electric, Hillerød, Denmark). The fat content was determined using the Soxtec System (HT 1043, Foss Tecator, Hillerød, Denmark). Gross energy (GE) was determined by an adiabatic bomb calorimeter that was standardized with benzoic acid (KL 12Mn, Precyzja-Bit PPHU, Bydgoszcz, Poland). Data are presented in Table 2. Fiber analysis (TDF, ADF, NDF, Klason lignin and NSP) on the test diets was not performed.

2.4 Exogenous Enzymes

The NSPase enzyme-concept contained multi-enzyme activities (strain selection and fermentation optimization), but was standardized to contain two major activities, namely 40,000 endo-1,4- β -xylanase UNITS (XU/g, EC 3.2.1.8) and 9,000 endo-1,3(4)- β -glucanase UNITS (BGU/g, EC 3.2.1.6), further on referred to as “XG” (AveMix[®] XG

10, AVEVE Biochem NV, Merksem, Belgium). An XU is the amount of enzyme which releases 1 μ mol of reducing sugar per min from xylan of oat spelt under defined test conditions. A BGU is the amount of

enzyme which releases 1 μ mol of reducing sugar per min from β -glucan of barley under defined test conditions. The endo-1,4- β -xylanase and endo-1,3 (4)- β -glucanase UNITS were determined in the concept

Table 1 Composition (%) and nutritive value of experimental broiler diets (Exp. 1-3) per feeding phase (starter, grower).

Experiment	Exp. 1		Exp. 2		Exp. 3	
Period	Starter (1-14 d)	Grower (15-42 d)	Starter (1-10 d)	Grower (11-42 d)	Starter (1-14 d)	Grower (15-42 d)
Composition						
Maize	54.04	63.00	-	-	19.70	20.85
Wheat	-	-	53.15	62.00	30.00	35.00
SBM 45%	37.30	28.70	35.10	26.20	33.63	21.89
Rapeseed meal	-	-	-	-	2.50	5.00
Sunflower meal	-	-	-	-	2.77	5.00
Soybean oil	4.00	4.00	6.90	7.20	7.40	8.16
NaCl	0.35	0.35	0.35	0.30	0.15	0.15
Sodium bicarbonate	0.10	-	-	-	0.30	0.34
L-lysine 20%	0.30	0.35	0.60	0.70	0.21	0.30
DL-methionine 20%	1.00	0.80	1.00	0.80	0.27	0.23
L-treonine	-	-	-	-	0.14	0.09
Limestone	0.40	0.40	0.50	0.50	0.49	0.41
Monocalcium-phosphate	1.50	1.40	1.30	1.20	1.33	1.28
Premix Vit./Min. (Ca 31%)	1.00	1.00	1.00	1.00	1.00	1.00
Calculated nutritional value (% unless otherwise)						
Metabolizable energy (MJ/kg)	12.39	12.82	12.75	13.20	12.70	13.00
Metabolizable energy (kcal/kg)	2,959.6	3,062.8	3,047.0	3,152.0	3,033.34	3,104.99
N*6.25	22.50	19.50	22.50	19.50	22.70	20.00
Digestible Lys	1.28	1.06	1.26	1.07	1.18	1.05
Digestible Met + Cys	0.54	0.47	0.53	0.45	0.88	0.80

Feeds without acids or growth promoters. SBM = soybean meal.

Table 2 Nutritional value of experimental diets in three experiments (Exp. 1-3).

Table 2. Nutritional value of experimental diets in three experiments (Exp. 1-3).						
Diet (experiment)	Corn (Exp. 1)		Wheat (Exp. 2)		Corn/wheat (Exp. 3)	
Starter						
Nutritional value (% unless noted otherwise)	NC	Exp.	NC	Exp.	NC	Exp.
GE (MJ/kg)	17.08	17.20	17.58	17.62	17.93	17.91
CP	22.60	22.8	22.17	22.10	22.10	22.01
EE	6.80	6.64	8.16	8.2	9.40	9.30
CF	3.62	3.73	3.50	3.46	3.89	3.80
Grower						
Nutritional value (% unless noted otherwise)	NC	Exp.	NC	Exp.	NC	Exp.
GE (MJ/kg)	17.07	16.87	17.67	17.63	18.01	18.02
CP	19.57	19.64	19.59	19.70	20.10	20.07
EE	7.02	7.14	8.78	8.62	10.10	10.17
CF	3.27	3.30	3.27	3.28	4.05	4.01

GE = gross energy; NC = negative control; CP = crude protein; EE = crude fat; CF = crude fiber.

and verified in each basal feed + XG (in-feed enzyme analyses) according to the manufacturer's methodology (AVEVE Biochem Laboratories, Merksem, Belgium).

2.5 Statistical Analysis and Calculation

Body weight and feed consumption were recorded at the end of the starter period and at 42nd day to evaluate feed intake (FI, g), body weight gain (BWG, g) and to calculate the feed to gain-ratio (F:G). This resulted in average (standard error, SE) FI, BWG and F:G data for the starter, grower period and total period.

Explorative data analysis was performed to detect outliers by means of a distribution analysis procedure using the SAS Software (SAS Institute Inc., Cary, NC, USA). To test whether the means of groups NC1-3 and respective Exp. 1-3 can be considered statistically different, a *t*-test was performed using the SAS Software. The hypothesis of interest is:

$$H_0: \mu_1 = \mu_2 \text{ vs. } H_a: \mu_1 \neq \mu_2.$$

The test was applied to each of the variables of interest (BWG, FI and F:G) and was analyzed for each period (starter and grower) and for the total period (1-42 d). All data are presented as means with pooled standard errors of the mean (SEM).

3. Results

3.1 Raw Material Chemical and Fiber Analysis

The chemical analyses of the basal corn-SBM, wheat-SBM and corn/wheat-SBM feeds (Table 2) were consistent with the respective nutritional content estimations (Table 1). The *in vitro* analysis of XG and of the feed samples with XG, confirmed the presence of guaranteed enzyme activities (AVEVE Biochem UNITS) of endo-1,4- β -xylanase (EC 3.2.1.8) and endo-1,3(4)- β -glucanase (EC 3.2.1.6) for each diet. The Weende and fiber analyses of the individual ingredients corn, wheat and SBM are given in Table 3. The NSP sugar monomer composition of corn, wheat and SBM is presented in Figs. 1a-1c. In the cereal samples (Figs. 1a and 1b), arabinose, xylose and glucose are of major importance and summed they account for respectively 86% and 90% of the total NSP content in corn and wheat. In corn, glucose is predominant (37% of total NSP), whereas in wheat, xylose is most abundant (37% of total NSP). Remaining cereal NSP molecules are mannose, rhamnose and fucose, summed that is respectively 2% and 3% of the total NSP content in corn and wheat. In SBM (Fig. 1c), glucose is the most important (35%

Table 3 Chemical composition of raw materials corn, wheat and SBM (44% protein of DM)*.

Raw material	Corn	Wheat	SBM (44%)
Mono/dico	Mono	Mono	Dico
Weende analysis			
DM	95.29	91.60	91.21
CP	9.47	10.66	43.47
EE	5.18	2.42	2.87
CF	2.09	1.93	9.34
Crude ash	1.51	1.55	7.08
ADF	2.22	2.42	11.66
NDF	7.45	8.90	15.75
TDF	14.05	13.44	27.72
TDF insol.	11.55	10.81	23.54
TDF sol.	2.50	2.63	4.18
Klason lignin	0.65	1.04	0.85
Total NSP	8.74	9.92	22.00

*All samples were provided by AVEVE Animal Nutrition and analyzed at the Department of Animal Sciences and Aquatic Ecology (Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium), as part of a research project in cooperation with AVEVE Group (1991), to investigate NSP target substrates for fiber degrading enzymes. Mono = monocotyledon; Dico = dicotyledon; DM = dry matter; ADF = acid detergent fiber; NDF = neutral detergent fiber; TDF = total dietary fiber; NSP = non-starch polysaccharide.

Fig. 1 Mean content (expressed as % of DM and % on total NSP) and composition of NSP in (a) corn, (b) wheat and (c) SBM (44% protein of DM).

Figures are to be read clockwise beginning with arabinose (black with white inscriptions), xylose, glucose, galactose, uronic acid, mannose, rhamnose, fucose.

of total NSP), where the second and third most prevalent are galactose (18%) and uronic acid (16%), respectively. Arabinose and xylose together represent 21% of the total NSP in SBM. Remaining SBM NSP molecules are mannose, rhamnose and fucose, summed that is 8% of the total NSP content.

3.2 Broiler Experiments

3.2.1 Mortality

The mortality was generally low but variable. In Exp. 1 (corn-SBM), mortality in NC (basal feed, no enzyme treatment) was 5.0% and 0 in XG. In Exp. 2 (wheat-SBM), mortality was 1.25% in both experimental groups. No dead birds were recorded in Exp. 3 (corn/wheat-SBM).

3.2.2 Performance

Broiler BWG, FI and F:G are presented in Table 4. Considering BWG, in the starter period, XG was not different from NC (Exp. 1-3). From the grower period on, BWG differences were distinct. In the grower period, XG increased BWG in Exp. 1 (corn-SBM) and Exp. 3 (corn/wheat-SBM) ($p < 0.05$), where the difference was numerical (+23 g) in Exp. 2 (wheat-SBM). Hence, in the total period (1-42 d), XG resulted in a higher BWG ($p < 0.05$) in broilers fed corn-SBM (Exp. 1) and corn/wheat-SBM (Exp. 3). In the wheat-SBM fed birds (Exp. 2), in the total period,

there was a numerical improvement in BWG of +29 g in XG compared to NC.

Concerning FI, there were few differences amongst treatment groups and these differences were noticed only early in life. In starter birds fed corn-SBM (Exp. 1), FI was higher for XG than NC ($p < 0.05$), with an average difference of +28.7 g feed per bird. While in starter birds fed wheat-SBM, FI was lower for XG than NC ($p < 0.05$, Exp. 2), with an average difference of -34 g feed per bird. These differences did not persist in the grower period of Exp. 1 or 2 although FI tended to be higher in corn-SBM and lower in wheat-SBM fed birds. Therefore, no differences were demonstrated between XG and NC in the total period for Exp. 1 and 2, although FI tended to be higher in corn-SBM and lower wheat-SBM fed birds. In broilers fed corn/wheat-SBM (Exp. 3), FI tended to be lower in XG compared to NC in each period and consequently also in the total period.

Considering F:G, in the starter period using corn-SBM (Exp. 1), XG was higher than NC ($p < 0.05$) with an average difference of +0.09, corresponding with the higher FI for XG than NC in that feeding phase ($p < 0.05$) with a similar growth for XG and NC. In the grower period, XG tended to improve F:G using corn-SBM (Exp. 1) with an average difference of -0.07. Hence, in the total period, in broilers fed corn-SBM,

Table 4 Performance data of broilers in three experiments, given per experimental period and on the total period*.

	BWG (g)			FI (g)			F:G (kg FI/kg BWG)		
	Starter	Grower	Total	Starter	Grower	Total	Starter	Grower	Total
Corn (Exp. 1)									
NC (SE)	343.7 (6.3)	1,957.9 ^a (17.7)	2,301.7 ^a (21.3)	440.0 ^a (7.2)	3,543.4 (51.8)	3,983.4 (53.0)	1.28 ^a (0.02)	1.81 (0.02)	1.73 (0.02)
Treatment (SE)	343.7 (6.0)	2,045.0 ^b (29.2)	2,388.7 ^b (29.1)	468.7 ^b (13.1)	3,561.2 (12.6)	4,030.0 (16.0)	1.37 ^b (0.04)	1.74 (0.02)	1.69 (0.02)
<i>p</i>	NS	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05	NS	NS	<i>p</i> < 0.05	NS	NS
Wheat (Exp. 2)									
NC (SE)	304.0 (4.1)	2,185.0 (29.4)	2,489.0 (27.3)	504.0 ^a (8.2)	3,839.0 (49.4)	4,343.0 (51.0)	1.66 ^a (0.03)	1.76 ^a (0.01)	1.75 ^a (0.01)
Treatment (SE)	311.0 (4.7)	2,208.0 (19.5)	2,518.0 (22.2)	470.0 ^b (9.8)	3,735.0 (52.5)	4,205.0 (58.5)	1.51 ^b (0.03)	1.69 ^b (0.02)	1.67 ^b (0.02)
<i>p</i>	NS	NS	NS	<i>p</i> < 0.05	NS	NS	<i>p</i> < 0.05	<i>p</i> < 0.05	<i>p</i> < 0.05
Corn/wheat (Exp. 3)									
NC (SE)	359.0 (3.6)	2,119.3 ^a (19.1)	2,478.3 ^a (20.1)	391.0 (6.9)	3,839.3 (91.0)	4,230.3 (89.4)	1.09 (0.02)	1.81 ^a (0.03)	1.71 ^a (0.03)
Treatment (SE)	353.7 (5.2)	2,361.5 ^b (37.8)	2,714.2 ^b (38.6)	383.0 (7.5)	3,691.2 (61.7)	4,074.2 (64.7)	1.09 (0.03)	1.57 ^b (0.03)	1.50 ^b (0.02)
<i>p</i>	NS	<i>p</i> < 0.05	<i>p</i> < 0.05	NS	NS	NS	NS	<i>p</i> < 0.05	<i>p</i> < 0.05

* Studies were performed at the Department of Animal Nutrition, University of Life Sciences (Poznań, Poland).

^{ab} = Means within a column with no common superscripts differ significantly (*p* < 0.05). SE = standard error of the mean; NS = non-significant; BWG = body weight gain; F I= feed intake; F:G = feed to gain ratio.

XG tended to improve F:G compared to NC (-0.04), coming from an increased growth (*p* < 0.05) with a trend towards higher FI in XG compared to NC. The effects on F:G were most distinct in wheat-SBM fed broilers, where XG performed better than NC in all feeding phases (and in the total period) (all *p* < 0.05), corresponding with a numerically higher BWG and lower 42-d FI in XG compared to NC in all feeding phases (and in the total period). In the starter period of corn/wheat-SBM fed broilers, F:G of XG and NC did not differ, where from the grower period on, XG improved F:G compared to NC (*p* < 0.05) and this was also the case in the total period (*p* < 0.05). This comes from an improved growth in XG compared to NC in corn/wheat-SBM fed broilers, with a trend towards lower FI.

4. Discussion

Nutritionists close the gap between animal production demands and the availability and quality of feed ingredients. The use of NSP degrading enzymes to improve the nutritive value of feedstuffs is long-known,

with important basic research on fiber and enzymes performed in the 1980's and early 90's by Ghent University [2, 10, 28, 29] and others [4, 13, 15, 16, 30-33]. The first application (xylanase type) in broilers was to reduce soluble NSP, that is to decrease the viscosity of wheat or barley-based diets, with the ultimate aim to stop wet litter and improve production safety [4, 34]. Last decades, a tremendous amount of broiler data have been published, using various feed ingredients and different types and combinations of exogenous enzymes. Still, feeds with basic ingredients used in broiler nutrition corn, wheat and SBM have shown inconsistent outcome on performance using exogenous enzymes [35-41]. In the actual field, NSPase enzymes are commonly applied to reduce anti-nutritional effects (mainly viscosity) of broiler feedstuffs, where the power to ameliorate the dietary energy and/or protein (amino acid) content, remains a debate. It must not be forgotten that NSP degrading enzymes have been developed to unlock plant cell walls, for a better ileal digestibility of nutrients, ultimately aiming to reduce manure and environmental

contamination by excess nitrogen and undigested fraction. Present study renovates the image of NSPase enzymes, by selecting and standardizing appropriate activities in a dual NSPase enzyme-concept, based on well-described NSP research techniques, with consolidation of broiler result using corn, wheat and SBM-feeds.

The Weende analysis of corn, wheat and SBM and the results on dietary fiber (TDF, ADF, NDF), Klason lignin and total NSP analyses are in line with previous data performed by the same research group [10] and other data that were generated using the same, or adapted, analysis technique [9, 42, 43]. The NDF analysis of SBM is within the variation of 11.3% to 18.5% of DM reported by Grieshop and Fahey [44] and Grieshop *et al.* [45], which presently analyzed NDF was higher than 18.2% to 20.5% of DM [45]. The total NSP contents of corn, wheat and SBM here, were 8.7%, 9.9% and 22.0% respectively. Total SBM NSP was reported lower by Huisman *et al.* (14.5%) [20] and Meng *et al.* (14.8%) [14], though the latter used similar techniques as present study. There is complexity and diversity in SBM cell walls over different samples [18] and that can also explain differences in the dietary fiber and NSP content of SBM in different analysis. Corn, wheat and SBM NSP were cleaved to monomers of arabinose and xylose (pentoses), glucose, galactose and mannose (hexoses), rhamnose and fucose (6-deoxyhexoses) and uronic acids (glucuronic and galacturonic acid) to investigate the predominance of specific sugars (and corresponding NSP polysaccharides). Corn and wheat show abundant arabinose, xylose and glucose, when added up that is 86% and 90% of the total NSP content, respectively (Figs. 1a-1c). From these sugars, the respective cereal NSP polysaccharides, arabinoxylans, beta-glucans and cellulose, are generated [15]. The remaining NSP monomers in the investigated cereals are galactose (4%-5%) and uronic acid (3%-7%) and fewer mannose, rhamnose and fucose (Figs. 1a-1c).

Glucose is the predominant molecule in SBM, being 35% of the total NSP content in present analysis. Commercial SBM types are hull-less SBM ($\pm 49\%$ CP) containing less cellulose than the initially de-hulled SBM with hulls mixed in again ($\pm 44\%$ CP) [46], which is also the SBM type used for the *in vitro* and *in vivo* part of this study. Soybean hulls are rich in cellulose (β -(1,4) linked D-glucose), hemicellulose (cellulose heteropolymers f.i. with arabinoxylan) as well as pectic substances associated with mannans, galactans and xyloglucans [20]. Soybean cotyledons have a similar composition as the hull, though there is far less cellulose and additional arabinogalactan [19]. This is confirmed by the abundance of galactose (18% of total NSP) and uronic acid (16%) in present study, followed by arabinose (12%) and xylose (9%). Remaining SBM monomers are few, being mannose (5%), rhamnose (3%) and fucose (2%). Presented NSP monomer patterns are still in line with respective NSP analyses of corn, wheat and SBM published by the same research group (Dierick and Decuypere, Ghent University [10]) and also with others [11, 47]. From this feedstuff research, it is confirmed that common and ubiquitous ingredients for broiler feeds as corn, wheat and SBM have identical and abundant NSPase enzyme target sites over different studies using a similar technique [16]. This suggests that NSPase enzyme substrates in corn, wheat and SBM are omnipresent but may vary with the species and within different tissues of the source [48, 49]. Therefore, the dual NSPase enzyme concept was developed with (1) major enzyme activities fitting predominant substrates (endo-1,4- β -xylanase and endo-1,3(4)- β -glucanase) and (2) the optimal concentration of each individual activity to fulfill the needs to cut also insoluble substrates with animal performance benefits. NSPase enzymes have two concomitant effects: (1) solubilisation of insoluble NSP and (2) hydrolysis of “original soluble” NSP and NSP that was solubilized first from the “original insoluble” NSP [50]. However, to maximize (1) and (2) and to enhance feed

efficiency to its optimum, more complex NSPase enzyme activities are needed [51].

In diets using corn (corn-SBM and corn/wheat-SBM), XG improved the final body weight compared to NC. This difference in BWG due to XG was numerical in the wheat-SBM group, being on average 23 g heavier than NC. This agrees with the study of Munyaka *et al.* [52], using a xylanase and glucanase combination in broilers fed corn or wheat based diets. There, BWG increased using the NSPase enzymes in both diets compared to NC (on a total period of 21 d), where BWG for corn was higher than wheat. Also, using 54% of coarsely or finely ground corn, broiler BWG and F:G improved using an NSPase enzyme blend including xylanase and glucanase (in a total period of 21 d) [53]. NSPase enzyme combinations with xylanase and glucanase as main activities perform better than each activity on its own in order to improve F:G and ileal protein digestibility in broilers (5-18 d of age) [14]. The studies mentioned above were done on a limited time frame, early in life. Age can have impact on the performance outcome of enzymes, where young animals are suggested to be more responsive [17, 54, 55]. It is therefore suggested that the effect of NSPase enzymes determined on a 18-d to 21-d total period, does not represent the potential enzyme-action on a full production cycle.

Present study used a growth period of 42 d to represent the maximal broiler life span in field conditions (i.e., generally 35-42 d depending on the production standards, region and country). Here, the improvement of BWG using XG compared to NC was more distinct in the grower than the starter period, especially using corn-SBM and corn/wheat-SBM. The appearance of performance effects of NSP degrading enzymes at later age, was also seen in another study [56] applying xylanase to a wheat-based diet. There, broiler BWG and F:G improved first at 21-28 d in comparison to the same diet without enzymes. Presented 42-d FI in diets with corn or wheat was not affected by the XG treatment and this is also seen in

other studies using xylanase and glucanase [14, 52]. Maintaining FI while increasing growth indicates that XG supplemented diets with corn, had more nutritional content than NC. This is in line with Cowieson *et al.* [36] using an energy reduced corn-soy diet with a xylanase and glucanase combination, which restored F:G to the same level as the control diet (without energy reformulation) in a 42-d total broiler period. FI however tended to be higher in XG than NC when broilers were fed corn-SBM (+46.6 g on average). The higher FI using corn compared to wheat-based diets (or other “soluble” ingredients, barley and rye) was also seen by others [34]. It can be suggested that insoluble ingredients as corn, tend to increase FI compared to soluble ingredients because there is no gel effect that limits the ingestion of feed. On the other hand, enzyme supplementation could decrease FI due to the higher energy availability [41]. This is reflected in the trend towards lower FI in XG than NC when broilers were fed wheat-SBM or corn/wheat-SBM (-138.0 g, respectively -156 g on average), being the diets with higher NSP solubility.

Indeed, identical NSP degrading enzyme activities show a higher efficacy on “soluble” (as wheat) than “insoluble” NSP (as corn) [3, 34, 57] and the greater the effect on intestinal viscosity by the diet (e.g., wheat based diets), the higher the effect of NSP degrading enzymes [52]. This is reflected in XG that improved F:G in each broiler trial compared to NC, where the effect was more distinct in feeds with wheat (wheat-SBM and mixed corn/wheat-SBM, $p < 0.05$) than corn-SBM (numerical improvement of -2.4%). The latter is mediated by the trend towards increased FI and most likely also by the rather limited sample size (80 birds per treatment group). This is the first study to describe the effect of a dual NSPase enzyme concept in diets changing from “insoluble” to “soluble” or mixed, over a 42-d broiler production period. The dual NSPase enzyme concept was developed in such way, that the optimal combination of enzyme activities was done, and, the target concentration for

the most limiting NSP was achieved (insoluble, corn) for performance increase. In present study, a marked 42-d growth benefit of XG in the corn-SBM compared to NC was noted. The importance of a correct input of enzyme activities was also demonstrated by others, by measuring in the *in vitro* effect of an enzyme combination (with xylanase and glucanase) on a mixed soluble and insoluble substrate. At the highest concentration tested, the enzymes hydrolyzed both soluble and insoluble NSP best [50]. This suggests that the potential to release nutrients for soluble and insoluble feed ingredients depends on the level of the enzyme activity. Indeed, *in vivo*, there is a dose-response effect on broiler performance applying a xylanase in a wheat/rye-SBM diet (21-d total period) [58] and of an enzyme cocktail with xylanase as main activity in a corn-soy diet (21-d total period) [59]. Moreover, the specific combination of a xylanase and glucanase, at a distinct concentration, works sub-additive (that is: better than each enzyme on its own but less than the sum of the individual enzyme effects) on the performance and ileal starch and protein digestibility in broilers fed corn-soy [36, 60]. It is postulated that enzyme concentrations have a threshold value (*in vitro* UNITS) that has to be exceeded to improve animal performance (clinical outcome) and this is with the highest possible independence of the dietary composition (AVEVE Biochem and AVEVE Animal Nutrition Feed Formulation Research).

By cutting NSP polysaccharides to oligosaccharides, NSP-linked and entrapped nutrients are released in the small intestine, where they are further digested by endogenous broiler enzymes and absorbed by the enterocytes. This not only liberates the utilization of different types of ingredients, but also equalizes the variable nutritional content within whole grain cereals, plant-based protein sources and their by-products [11, 42, 52, 61-63]. The improved feed efficiency of corn-based diets by means of NSPase degrading enzymes was associated with a higher starch and protein availability in broilers [35, 60]. Also digestible

amino acid and total protein reformulation using XG was demonstrated previously, in a 42-d broiler experiment using corn/wheat SBM [64], showing the possibilities of lowering the N output by limiting the protein and digestible amino acid input. Besides improved nutrient utilization of soluble and insoluble type ingredients, XG may exert different other roles throughout the digestive tract. In the small intestine, XG prevents viscous digesta and improves nutrient digestibility, where remaining oligosaccharides enter the large intestine. Thereby, NSPase enzymes deliver molecules that are easily fermentable by the resident microflora and this enhances the production of volatile fatty acids that give additional energy [65-68]. The presence of fiber structures is indeed required for optimal gut function and physiology and more attention has been paid to gut effects of NSPase enzymes and the released oligosaccharides. F.i. xylanase and glucanase were associated with increased villus length and villus to crypt ratio and thus may improve the absorptive capacity in the small intestine [37, 69]. Presented dual concept, XG, improves feed formulation flexibility and animal performance, most likely from cleaving predominant NSP target substrates available in corn, wheat and SBM. Thereby, XG increases feed efficiency to an optimal level while decreasing emission/excretion of nutrients/gasses in the environment.

5. Conclusions and Practical Applications

Enzymes are proteins that are targeted in their action. This key-lock principle is the basis to identify proper enzymatic activities to cleave predominant corn, wheat and SBM substrates, arabinoxylan, glucan and cellulose polysaccharides that consist of arabinose, xylose and glucose monosaccharides as shown in present raw material analysis. The optimal level of each major enzyme concentration was standardized to act on both soluble and insoluble NSP, resulting in performance amelioration in each dietary composition. The dual XG concept that was developed enables feed formulation changes, over corn-SBM to wheat-SBM

and corn/wheat-SBM and improves animal production simultaneously. XG increased BWG best on corn (corn-SBM and corn/wheat-SBM), where XG decreased F:G best on wheat (wheat-SBM and corn/wheat-SBM)-based diets. Averaged over three studies, XG decreased F:G with 6.4% and broilers were 117 g heavier than NC. This study offers insight in the effects of XG to improve formulation flexibility (corn-wheat) and broiler production by increasing feed efficiency, ultimately aiming to reduce undigested nutrients and environmental burden.

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