The Effect of Thermal Processing and Enzyme Treatments of Soybean Meal on Growth Performance, Ileal Nutrient Digestibilities, and Chyme Characteristics in Broiler Chicks

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ABSTRACT Effects of thermal processing (toasting or extrusion) of untoasted soybean meal on growth performance, apparent ileal nutrient digestibilities, and chyme characteristics were studied in broiler chicks fed diets with soybean meal as the main protein source. Effects of increasing shear forces during extrusion as well as enzyme treatments (protease and carbohydrase) were also studied. When compared with toasting, extrusion significantly improved feed conversion ratio (1.56 vs 1.62) and apparent ileal digestibilities of CP and nonstarch polysaccharides (87.5 vs 82.2% and 26.7 vs 11.4%, respectively). Enzyme treatment improved apparent ileal digestibility of CP and nonstarch polysaccharide compared with no enzyme treatment (85.2 vs 83.7% and 20.6 vs 14.5%, respectively); however, enzyme treatments did not result in a better growth performance of the chicks. Among the enzyme treatments, no differences were found in growth performance and apparent ileal CP digestibility, whereas the carbohydrase significantly improved apparent ileal nonstarch polysaccharide digestibility compared with the other enzyme treatments. Extrusion of SBM at the highest shear level caused a significant increase in the water-holding capacity, chyme viscosity, and concentration of soluble nonstarch polysaccharides in the chyme compared with extrusion of SBM at lower shear levels. The increase in chyme viscosity did not affect growth performance, nor did it influence apparent ileal nutrient digestibilities.

(*Key words*: soybean meal, toasting, extrusion, shear forces, enzymes)

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INTRODUCTION

The high content of proteins and a well balanced amino-acid pattern makes soybean meal (SBM) a valuable protein source in diets for livestock. However, the nutritional value of SBM is decreased by the presence of antinutritional factors (ANF). Thermal processing, e.g., toasting and extrusion cooking, is frequently used to increase the nutritional value of SBM (Liener, 1994). In general, process conditions like temperature, moisture content, screw-speed, shear forces, and duration of heating will determine the effectiveness of inactivation of the heat-labile ANF and the degree of denaturation of the storage proteins in SBM (Björck and Asp, 1983; Petres and Czukor, 1989). The extent to which trypsin inhibitors are responsible for the variation observed within the *in vivo* and *in vitro* protein digestibility in heat-treated SBM is uncertain. Other studies have shown that trypsin inhibitors are not the only factors determining the nutritional value of SBM (Naim *et al*., 1982; Molina *et al*., 1983; Marsman *et al*., 1993). At low trypsin inhibitor levels, the adjustment of process conditions like temperature, moisture content, and shear forces are more important than a further decrease in trypsin inhibitor activity (TIA) in order to achieve a SBM with an optimal nutritional value (Dale *et al*., 1987; Marsman *et al*., 1995a). Structural characteristics of the main storage proteins in SBM may explain the differences in nutritional value in SBM after toasting vs extrusion cooking (Camire, 1991). During toasting and, in particular, extrusion, the nutritional value of the compact folded proteins can be increased if both noncovalent interactions and disulfide bonds are broken, resulting in irreversible protein denaturation. This process increases the accessibility of proteins to enzymatic breakdown (Bhattacharya and Hanna, 1988). On the other hand, overprocessing may decrease the nutritional value of SBM due to, e.g., Maillard reactions (Araba and Dale, 1990; Marsman *et al*., 1995a).

In order to increase the nutritional value of SBM, several attempts were made by adding proteases and carbohydrases either before or after processing (Walsh *et*

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al., 1993; Bedford, 1995). Enzymatic breakdown of arabinoxylans and β-glucans are known to decrease the viscosity in the gastrointestinal tract of broiler chicks fed rye-, wheat-, or barley-based diets (Bedford, 1995). Multi-enzyme preparations designed to act on soybean nonstarch polysaccharides failed to induce any improvement in the growth performance of broiler chicks fed diets containing SBM as the main protein source (Irish and Balnave, 1993). In a previous study, broiler chicks fed diets containing extruded SBM showed no differences in BW gain, feed intake (FI), and feed conversion ratio (FCR) as a result of hydrolytic enzyme treatment; however, all birds had better FCR when compared with toasted SBM (Marsman *et al*., 1995b).

The objective of this research was to study the effect of toasting and extrusion cooking of SBM on growth performance, apparent ileal nutrient digestibilities, and chyme characteristics in broiler chicks. Secondly, chick performance was studied using different shear levels during extrusion cooking. A final objective was to determine whether extrusion cooking of SBM alters the *in vivo* susceptibility towards proteolytic and cell wall degrading enzymatic activity compared with toasted SBM.

MATERIALS AND METHODS

Birds and Management

Each of 520 1-d-old female Ross broiler chicks was randomly allocated to one of 10 dietary treatments. Each treatment consisted of four cages with 13 birds per cage. A cage was considered as a replicate experimental unit. Feed and water were consumed *ad libitum*. Ambient temperature gradually decreased from 32 C at Day 0 to 22 C at Day 25. A light regimen of 23 h light and 1 h dark was set to enable a continuous FI. All diets were fed in pelleted form.

Processing of the Experimental Diets

Commercial solvent-extracted, toasted (85 C, 20 min) SBM (tSBM) with a CP content of 51% (N × 6.25, as is) was supplied by Cargill.[1] Part of the solvent-extracted meal was not toasted, but air-dried resulting in the untoasted SBM (uSBM). Protein dispersibility indices of tSBM and uSBM, which are often used as a parameter to measure the protein quality of SBM, were 20 and 80, respectively. The protease preparation (Neutrase®) and the cell wall degrading enzyme preparation (Energex™) were obtained from Novo-Nordisk.[2]

Extrusion of uSBM (eSBM) was performed on laboratory-scale using an Almex Battenfeld single-screw extruder. The length to diameter ratio was 16 and the compression ratio 1.15. The trials were carried out with screws of a constant pitch of 32 mm and a diameter of 50 mm. The die diameter was 7 mm and the screw-speed 100 rpm. The uSBM with an initial moisture content of 25% was extruded with a torpedo screw containing zero (Ex-0), four (Ex-4), or eight (Ex-8) rows of flights on the screw, enabling an increase in shear forces (Marsman *et al*., 1995a). Moisturization of uSBM was performed with a Sunther-Papenmeier mixer. Temperatures in the different sections of the extruder were measured using eight thermocouples. With heaters on the barrel wall, the temperature of the product at the die was adjusted to 120 C. The throughput was about 12 to 14 kg/h. After extrusion the samples were dried for 2 d at 40 C.

TABLE 1. Composition of the experimental diets

Ingredients and analysis	Diets[1]
	(g/kg)
Soybean meal	382.5
Cornstarch	435.2
Sugars (meritose, dextrose)	100.0
Soya oil	50.0
Sodium chloride	3.8
Calcium carbonate	11.0
Monocalcium phosphate	14.5
DL-Methionine	3.0
Premix[2]	10.0
Chromic oxide	0.4
Calculated analysis	
CP (N × 6.25)	200
ME, kcal/kg	3,046
Total Lys	10.9
Total Met + Cys	9.0
Calcium	7.8
Available phosphorus	4.0

[1]Treatments 1 to 4: Toasted soybean meal (tSBM) and tSBM treated with Neutrase®, Energex™ and a combination of both enzymes, respectively. Treatments 5 to 7: Untoasted soybean meal (uSBM) extruded at different shear levels; 0, 4, and 8 rows of flights on the screw, respectively. Treatments 8 to 10: Extruded uSBM, 4 rows of flights, treated with Neutrase®, Energex™, and a combination of both enzymes, respectively.

[2]Premix contained per kilogram of diet: vitamin A, 10,000 IU; cholecalciferol, 2,000 IU; vitamin E, 20 mg E; riboflavin, 4 mg; niacin amide, 40 mg; pantothenic acid, 12 mg; choline chloride, 500 mg; vitamin B_{12}, 15 μg; vitamin K, 5 mg; folic acid, 0.75 mg; Biotin, 0.1 mg. Minerals: $CoSO_4 \cdot 7H_2O$, 1 mg; $Na_2SeO_3 \cdot 5H_2O$, 0.15 mg; KI, 5 mg; $FeSO_4 \cdot 7H_2O$, 300 mg; $CuSO_4 \cdot 5H_2O$, 100 mg; MnO_2, 100 mg; $ZnSO_4 \cdot H_2O$, 150 mg; and Ethoxyquin, 100 mg using cornstarch as carrier.

Experimental Design

The experiment comprised 10 treatments with four replicates per treatment. Treatments 1 to 4: tSBM (Treatment 1), and tSBM treated with Neutrase® (Treatment 2), Energex™ (Treatment 3), or a combination of both enzymes (Treatment 4), respectively. Treatments 5 to 7: eSBM at different shear levels; Ex-0 (Treatment 5), Ex-4 (Treatment 6), or Ex-8 (Treatment 7), respectively. Treatments 8 to 10: eSBM at shear level Ex-4 supplied with Neutrase® (Treatment 8), Energex™ (Treatment 9), or a combination of both enzymes (Treatment 10), respectively. The liquid enzyme preparates were sprinkled on the diets after pelleting. From Day 1 to 7, all broilers were fed the control diet with casein as the main protein source. At Day 7, chicks were weighed and the number of chicks

[1]Cargill B.V., Amsterdam, The Netherlands.
[2]NOVO Enzyme Process Division, Novo-Nordisk a/s, Bagsvaerd, Denmark.

per cage was decreased to 12. From Day 7 to 25 the chicks were fed the experimental diets. The composition of these diets is shown in Table 1.

Data Recording and Ileal Chyme Measurements

Body weight and FI of the birds were recorded at 7, 14, 21, and 25 d of age. From these data, FCR were calculated (grams of FI per grams of BW gain). At Day 25, all chicks were killed by an intravenous injection of T61, an aqueous solution containing 200 mg embutramide, 50 mg mebezoniumiodide, and 5 mg tetracainehydrochloride.[3] Chyme was collected for viscosity determinations from the anterior segment of the ileum, 15 cm starting from the junction jejunum. The posterior segment of the ileum, 15 cm backwards from the ileocecal junction, was used for the collection of chyme for chemical analysis. Chyme samples for chemical analysis were pooled per cage, freeze-dried, and grounded to pass through a 0.2-mm screen.

Viscosity was measured on two pooled chyme samples of three chicks per cage. Both observations were averaged for statistical analysis. The method of Bedford and Classen (1993) was used. Approximately 1.5 g of homogenized chyme was immediately placed in a microcentrifuge tube and centrifuged[4] at 12,000 × *g* for 1 min. The supernatant was directly used for the viscosity measurement using a Brookfield viscosimeter,[5] Model RVDV-II$^+$/CP with a CP40 spindle at 100 rpm. Viscosity was measured at 40 C and expressed in centipoise (cP). Nitrogen of the collected chyme and diets were analyzed with the semi-automated micro-Kjeldahl method using an auto-analyzer.[6]

Crude protein content was calculated by 6.25 × N. Trypsin inhibitor activity (TIA) of uSBM, tSBM, and eSBM extruded at different shear levels was performed with a modified Kakade method according to Smith *et al.* (1980). Benzoyl-DL-arginine-p-nitroanilide hydrochloride (DL-BAPA) was used as a substrate for trypsin. The TIA is expressed in milligrams inhibited trypsin per gram of SBM.

Starch in chyme and diets were determined enzymatically using a starch test kit.[7] Nonstarch polysaccharides (NSP) were determined as alditol acetates of their corresponding monosaccharides constituents using inositol as internal standard. In order to avoid the disturbing effects of high amounts of mono-, di- and small oligosaccharides, chyme was extracted with 80% ethanol or with water. After 2 h extraction at room temperature, the chyme was centrifuged for 10 min at 4,000 × *g*. The residue was washed three times with either 80% ethanol or water, air-dried (40 C), and used for the NSP determination, including pretreatment with 12 *M* H_2SO_4 (Englyst and and Cummings, 1984). Water-soluble NSP content was calculated by the difference between the results obtained after ethanol or water extraction. Although the starch content in the chyme samples was low and could be neglected, in the diets the starch was removed prior the NSP determination by dissolving the diets in dimethyl sulfoxide and incubation with α-amylase and pullulanase for 16 h at 40 C (Englyst and Cummings, 1984). To determine the amount of pectins, the uronic acid content of the chyme and diets after extraction with ethanol and water were determined as anhydro-uronic acid by the meta-hydroxydiphenyl assay (Thibault, 1979) using an auto-analyzer.[6] Sodium tetraborate (0.0125 *M*) was added to the 96% (wt/wt) H_2SO_4 in order to quantify glucuronic acid and galacturonic acid residues. Dry matter was determined by drying at a temperature of 105 C. The dried samples were used for chromium analysis using atomic absorption spectrophotometry.

Fatty acid content analysis was performed with a gas chromatography method as described by Anness (1984) with margaric acid as internal standard. Hydrolysis was performed in 6 *M* HCl at 60 C for 1 h. Samples were analyzed on a gas chromatograph equipped with a CPsil 88 column (50m × 0.25 mm). Standard fatty acid methyl esters were used for recognition of the different peaks.

The water-holding capacity (WHC) of the water-insoluble solids of the chyme samples was determined according to Robertson and Eastwood (1981). In a weighed tube (A), approximately 500 mg of sample was suspended in water (1:20) and stirred for 2 h. After centrifugation, excess water was decanted and the wet sample was weighed (B). After lyophilization, the sample was weighed again (C). The WHC was calculated as (B – A)/(C – A) and expressed in grams of water per grams of sample.

The molecular weight distribution of the soluble NSP fraction was determined using high-performance size-exclusion chromatography (HPSEC). Chyme samples were extracted with 80% ethanol and washed two times with 80% ethanol to remove mono- and small oligosaccharides. After adding TCA (to a final concentration of 12%) in order to precipitate the proteins and centrifugation (10 min at 4,000 × *g*), the supernatant was subjected to HPSEC analysis as described elsewhere (Schols *et al.*, 1991). Dextran standards with molecular weights (Mw) of 10 to 500 kDa were used to estimate apparent molecule masses.

For determination of the apparent ileal nutrient digestibility, chromic oxide was added (0.04%) to each diet as a marker. Apparent ileal nutrient digestibilities (percentage) of protein (DC_{CP}), NSP (DC_{NSP}), fatty acid (DC_{Fat}), and starch (DC_{Starch}) were calculated with the following formula:

$$DC_X = 100 - \left(\frac{\%\ Cr\ in\ diet}{\%\ Cr\ in\ chyme} \times \frac{\%\ X\ in\ chyme}{\%\ X\ in\ diet} \times 100\%\right)$$

[3]Hoechst Holland NV, Amsterdam, The Netherlands.
[4]Microcen 13, Herolab GmbH, Laborgeräte, D-6908, Wiesloch, Germany.
[5]Brookfield Engineering Laboratories Inc., Stoughton, MA 02172.
[6]Skalar Analytical B.V., Breda, The Netherlands.
[7]Boehringer Mannheim GmbH, Tutzing, Germany.

Statistical Analysis

In a 2 × 2 factorial arrangement, the effects of heat treatment (toasting vs extrusion cooking) and enzyme addition (no addition vs addition *per se*) were studied. Analyses of variance of data were performed using the contrast statements of the General Linear Models procedure of SAS® (SAS Institute, 1985), with cage mean as the experimental unit. Moreover, effects of different shear forces as well as the use of different enzyme preparations were studied by using Tukey's multiple comparison test (SAS Institute, 1985).

RESULTS

The first objective was to study differences between toasting (tSBM) and single-screw eSBM on growth performance, apparent ileal nutrient digestibilities, and chyme characteristics in broiler chicks (Treatments 1 to 4 vs Treatments 6, 8 to 10). In addition, a possible enzyme effect was also studied in this experimental design (Treatments 1 and 6 vs Treatments 2 to 4 and 8 to 10). The results are presented in Table 2, including possible interaction terms between thermal processing and enzyme treatment. Despite the fact that there were no significant differences in BW gain and FI between chicks fed tSBM or eSBM, FCR on the extruded diets were significantly improved ($P < 0.05$). Enzyme treatment did not affect BW gain, FI, and FCR. No interaction between thermal processing and enzyme treatment was found for these trials.

Apparent ileal digestibilities of CP, starch, and NSP were significantly improved in the eSBM compared with the tSBM ($P < 0.05$). Also, enzyme treatment significantly increased apparent ileal CP and NSP digestibility compared with no enzyme treatment ($P < 0.05$). Apparent NSP digestibility also showed an interaction between thermal processing and enzyme treatment (Table 2). The WHC of the water-insoluble solids in the chyme of eSBM birds was significantly increased compared with that of birds fed tSBM ($P < 0.05$). The chyme viscosity and the concentrations of soluble NSP in the chyme did not change as a result of thermal processing or enzyme treatment. However, there was an interaction in chyme viscosity between thermal processing and enzyme treatment (Table 2).

Differences between the enzymes in animal characteristics are summarized in Table 3. No significant effects were found in growth performance. Apparent ileal digestibility of CP showed no differences between the enzyme additions. Treatment with the protease preparation gave higher apparent ileal digestibilities of starch and fatty acid compared with the addition of both the protease preparation and carbohydrase preparation, although no differences where found compared with the carbohydrase preparation treatment alone. The apparent ileal digestibility of NSP was significantly increased after the separate treatment with the carbohydrase preparation compared with combined treatment with the

TABLE 2. Growth performance, apparent ileal nutrient digestibility, and chyme characteristics (± SD) in broiler chicks fed diets with thermal processed soybean meal and diets treated with a protease, carbohydrase, or a combination of both

Item	Thermal processing		Enzyme treatment		Probabilities		
	Toasting	Extrusion	No enzyme	Enzyme	Thermal processing (T)	Enzyme Treatment (E)	T × E
Chick performance 7 to 25 d of age							
BW gain, g	1,319 ± 46	1,344 ± 48	1,321 ± 53	1,335 ± 47	NS	NS	NS
FI, g	2,145 ± 92	2,098 ± 68	2,104 ± 64	2,125 ± 88	NS	NS	NS
FCR, g:g	1.62 ± 0.03	1.56 ± 0.03	1.59 ± 0.05	1.59 ± 0.04	0.001	NS	NS
Apparent ileal nutrient digestibility							
DC_{CP}, %	82.2 ± 1.4	87.5 ± 0.08	83.7 ± 3.3	85.2 ± 3.1	0.001	0.024	NS
DC_{Starch}, %	99.22 ± 0.07	99.33 ± 0.08	99.29 ± 0.08	99.28 ± 0.09	0.002	NS	0.013
DC_{Fat}, %	77.0 ± 5.4	76.6 ± 8.0	75.0 ± 5.5	77.4 ± 7.1	NS	NS	NS
DC_{NSP}, %	11.4 ± 5.5	26.7 ± 2.8	14.5 ± 10.8	20.6 ± 7.8	0.001	0.001	0.034
Chyme characteristics							
Soluble NSP, %	9.7 ± 3.9	10.6 ± 4.9	9.1 ± 2.5	10.5 ± 4.9	NS	NS	NS
WHC, g:g	5.14 ± 0.13	6.24 ± 0.28	5.61 ± 0.58	5.72 ± 0.61	0.001	NS	NS
Chyme viscosity, cP	3.52 ± 0.48	3.41 ± 0.41	3.53 ± 0.42	3.44 ± 0.46	NS	NS	0.016

TABLE 3. Growth performance, apparent ileal nutrient digestibility, and chyme characteristics (± SD) in broiler chicks fed with diets treated with a protease, carbohydrase, or a combination of both

Parameter	Neutrase®	Energex™	Neutrase® and energex™
Weight gain, g	1,316 ± 55	1,327 ± 38	1,362 ± 37
FI, g	2,106 ± 76	2,125 ± 85	2,142 ± 109
FCR, g:g	1.60 ± 0.02	1.60 ± 0.03	1.57 ± 0.06
DC_{CP}, %	85.6 ± 3.7	85.7 ± 2.8	84.5 ± 3.0
DC_{Starch}, %	99.32 ± 0.10[a]	99.27 ± 0.08[ab]	99.24 ± 0.10[b]
DC_{Fat}, %	79.8 ± 6.6[a]	77.8 ± 6.6[ab]	74.7 ± 7.9[b]
DC_{NSP}, %	18.3 ± 9.9[a]	23.6 ± 6.1[b]	19.8 ± 7.0[a]
Soluble NSP, %	12.6 ± 4.2[a]	9.3 ± 5.1[b]	9.6 ± 5.1[b]
WHC, g:g	5.71 ± 0.71[ab]	6.86 ± 0.64[a]	5.61 ± 0.47[b]
Chyme viscosity, cP	3.35 ± 0.22	3.58 ± 0.38	3.41 ± 0.68

[a,b]Values in a row with no common superscript differ significantly ($P < 0.05$).

protease preparation or the protease preparation alone ($P < 0.05$). If the carbohydrase preparation was supplied to the diets (alone or combined with the protease), the concentration of soluble NSP in the chyme was significantly lower than that of the separate protease preparation treatment ($P < 0.05$). The WHC of the water-insoluble solids in the chyme was the lowest after treatment with both the protease preparation and carbohydrase preparation and differed significantly with the WHC obtained after treatment with the carbohydrase preparation alone (Table 3). Chyme viscosity was not altered by the addition of the different enzymes.

The results of shear forces during single-screw extrusion of uSBM on growth performance, apparent ileal nutrient digestibilities and chyme characteristics in broiler chicks are shown in Table 4. Different shear forces had no significant effect on growth performance ($P < 0.05$); however, FCR seemed to be negatively affected in birds fed a diet containing Ex-8 ($P < 0.10$). Apparent ileal nutrient digestibilities showed no significant differences between shear levels. It should be mentioned that in birds fed the diet containing Ex-4, the apparent ileal digestibility of CP had the tendency to give a maximum protein digestibility ($P < 0.10$). Extrusion at the highest shear level (Ex-8) significantly increased ($P < 0.05$) chyme viscosity, WHC, and concentration of soluble NSP compared with extrusion at lower shear levels (Table 4).

In order to study the Mw distribution of soluble NSP in the chyme, high-performance size-exclusion chromatography (HPSEC) analysis was performed. Figure 1a shows the dextran standard. In Figure 1b are the results given for chyme of chicks fed diets containing tSBM and eSBM extruded at different shear levels (Ex-0, Ex-4 or Ex-8, respectively). With increasing intensity of the process (toasting, extrusion with zero, four and eight flights, respectively), the concentration of high molecular NSP (apparent Mw > 500 kDa) sharply increased. Also, NSP with an apparent Mw of 10 kDa increased with intensity of the process. Carbohydrates with an apparent Mw of 1,000, which are oligosaccharides, had the tendency to decrease after extrusion compared with toasting. If the protease, carbohydrase, and a combination of both enzymes were supplied to diets containing tSBM (Figure 1c) or eSBM; Ex-4 (Figure 1d), the concentration of high molecular NSP (apparent Mw > 500 kDa) decreased. This result appeared especially after enzyme treatment of the eSBM diets, indicating that enzymatic breakdown of cell wall material had occurred. Mortality of the birds averaged 6.1%.

TABLE 4. Growth performance, apparent ileal nutrient digestibility, and chyme characteristics (± SD) in broiler chicks fed with diets supplied with soybean meal extruded at different shear levels (Ex-0, Ex-4, and Ex-8)

Parameter	Ex-0	Ex-4	Ex-8
Weight gain, g	1,384 ± 59	1,357 ± 49	1,330 ± 51
FI, g	2,167 ± 37	2,111 ± 79	2,138 ± 95
FCR, g:g	1.57 ± 0.04	1.56 ± 0.03	1.61 ± 0.01
DC_{CP}, %	84.9 ± 1.4	86.6 ± 1.0	85.6 ± 0.6
DC_{Starch}, %	99.30 ± 0.06	99.28 ± 0.13	99.30 ± 0.05
DC_{Fat}, %	74.0 ± 6.1	71.9 ± 4.5	76.4 ± 5.8
DC_{NSP}, %	24.2 ± 3.6	24.4 ± 1.5	21.3 ± 2.6
Soluble NSP, %	4.3 ± 3.5[a]	7.7 ± 1.9[a]	13.4 ± 1.8[b]
WHC, g:g	5.65 ± 0.20[a]	6.12 ± 0.29[ab]	6.45 ± 0.40[b]
Chyme viscosity, cP	3.20 ± 0.29[a]	3.80 ± 0.35[a]	5.16 ± 0.44[b]

[a,b]Values in a row with no common superscript differ significantly ($P < 0.05$).

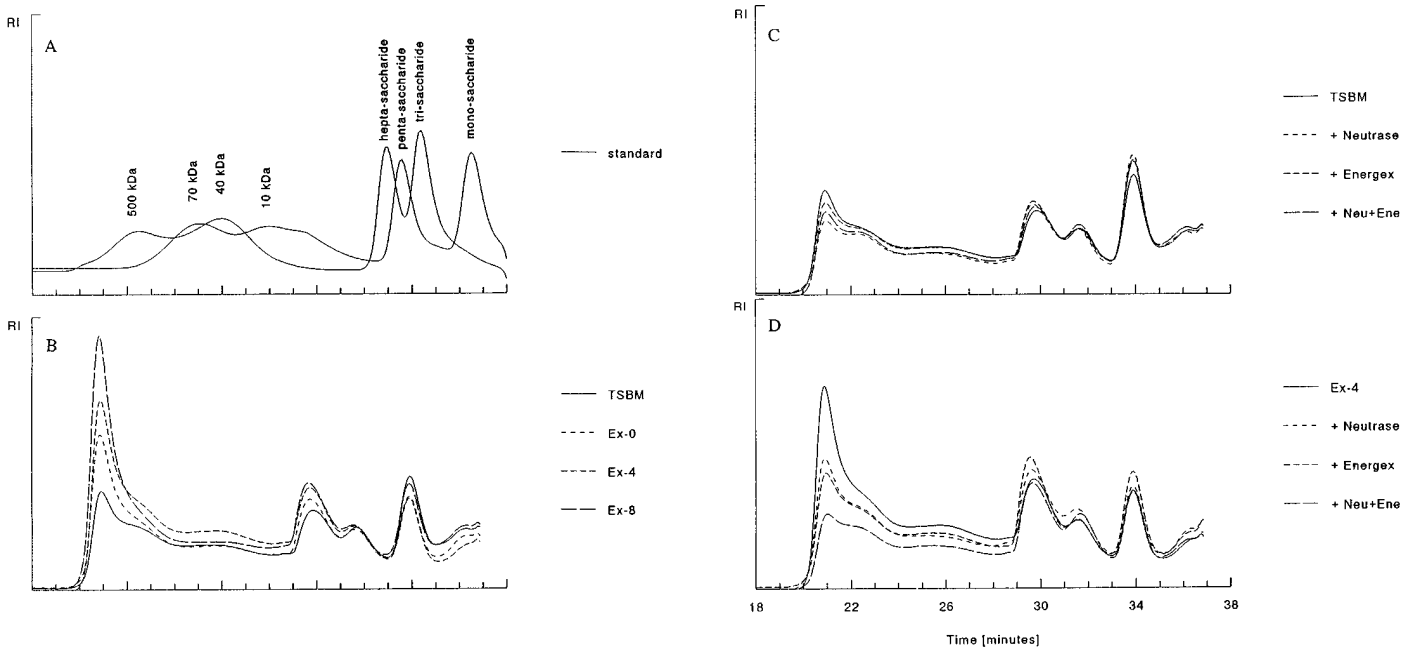

FIGURE 1. A) Elution pattern (HPSEC) of the standard. Molecular weights: 1) 500 kDa, 2) 70 kDa, 3) 40 kDa, 4) 10 kDa, 5) hepta-saccharide, 6) penta-saccharide, 7) tri-saccharide, and 8) mono-saccharide. Elution pattern (HPSEC) of the soluble NSP fractions of chyme collected after feeding broiler chicks a diet containing: B) toasted soybean meal (tSBM) and untoasted soybean meal (uSBM extruded at different shear levels (Ex-0, Ex-4 and Ex-8, respectively), C) toasted soybean meal (tSBM) and tSBM treated with a protease preparation, carbohydrase preparation, and a combination of both, and D) extruded SBM (Ex-4) and extruded soybean meal (Ex-4) treated with a protease preparation, carbohydrase preparation and a combination of both.

DISCUSSION

Growth Performance

Extrusion of SBM improved FCR of broiler chicks compared with toasting (1.56 vs 1.62). In a previous study (Marsman *et al*., 1995b) it was shown that the FCR of chicks was also improved if tSBM was extruded (1.65 vs 1.71) using a Wenger X-20 single extruder. Both experiments clearly show the positive effect of SBM extrusion on growth performance. Conflicting results are reported in literature concerning the effect of extrusion on the nutritional value of soybeans. Meyer and Froseth (1988) found an improved BW gain and FCR in broiler chicks fed a diet containing extruded vs toasted soybeans. However, FCR of broiler chicks fed extruded soybeans (138 or 154 C) were identical compared with those of birds fed a solvent-extracted SBM, but were significantly weaker after extrusion at 104 C (Zhang *et al*., 1993). Both studies do not report trypsin inhibitor contents. In the present study, the TIA were 23.9, 2.9, 3.5, 1.7, and 1.1 mg/g SBM for uSBM, tSBM, eSBM (Ex-0, Ex-4, and Ex-8), respectively (not tabulated). All thermal treatments were sufficient to decimate initial TIA levels. Therefore, the differences in growth performance between chicks fed tSBM vs eSBM diets cannot fully be explained by residual trypsin inhibitor levels.

In general, the enzyme treatments did not improve growth performance of broiler chicks. Moreover, among the enzyme treatments no differences in growth performance were noticed. However, treatment with both the protease and carbohydrase showed a possible synergistic effect ($P > 0.05$) on BW gain and FCR as compared with the separate enzyme treatments. At a relatively stable FI, BW gain increased and FCR improved, indicating that by proteolytic and cell wall degrading activity some nutrients are better absorbed in the gastrointestinal tract of the chicken. Irish *et al*. (1993) found no improvement in growth performance of 1- to 21-d-old broiler chicks fed SBM diets, treated with a mixture of different carbohydrases. Although no differences in growth performance were found, *in vitro* experiments showed that several enzyme preparates were active on SBM. Treatment with Viscozyme™ and Xylanase X-250 preparations, which are both carbohydrases, increased nitrogen solubility in SBM (Cone *et al*., 1994), caused by release of proteins by cell wall degrading activities but also by some proteolytic activity in the crude enzyme preparates. When the protease and carbohydrase preparations used in this research were studied in *in vitro* experiments with uSBM, tSBM, or eSBM (Ex-4), it appeared that the protease preparation was able to solubilize higher amounts of proteins in the extruded samples compared with tSBM. On the other hand the carbohydrase preparation was able to solubilize considerable amounts of neutral sugars in both tSBM and eSBM (Ex-4) (Marsman *et al*., unpublished data). The protease preparation was also able to solubilize considerable amounts of neutral sugars, whereas the carbohydrase preparation could release proteins. This result may explain why it is difficult to find differences in

growth performance between both enzymes. Nevertheless, the previously observed *in vitro* enzyme activities do not fully match the *in vivo* growth performance in broiler chicks in this experiment. This result must be attributed to other factors in the gastrointestinal tract of the chicken, such as pH, viscosity, or the influence of other components, which may play an important role in the effectiveness of enzyme activity on the animal.

Extrusion at different shear levels did not alter growth performance of the chicks. Data on the effect of different screw-configurations on animal performance are scarce in the literature. Shear forces induced by specially designed screw elements, such as the torpedo elements in this study or the so-called twin lead slotted screws in a previous study (Marsman *et al*., 1995b), were not powerful enough to result in significant differences in growth performance in broiler chicks. Exposure to higher shear forces with the single-screw extruder is, from a technical point of view, not feasible and economically less attractive.

Apparent Ileal Nutrient Digestibility

In eSBM, a significantly higher apparent ileal CP digestibility was noticed compared with tSBM (87.5 vs 82.2%). Several studies showed that extrusion under mild conditions may improve *in vivo* CP digestibility. Extruded soybeans had superior CP digestibility in pigs compared with other thermal processes, e.g., jet exploding and roasting (Marty and Chavez, 1993). With the presence of shear forces and a high energy input during extrusion, proteins are better unfolded and denatured compared with toasting. The susceptible bonds for enzyme hydrolysis, which are buried in the interior of the proteins, are more easily accessible for enzyme attack after extrusion (Bhattacharya and Hanna, 1988).

The amount of starch in the chyme was very low for all the treatments (12 to 22 g/kg DM, results not shown), whereas in the experimental diets the starch content was at least 435 g/kg. The apparent ileal starch digestibility appeared to be very high (> 99%) for all thermal treatments, but even so, a significant increase ($P < 0.05$) was noted after extrusion compared with toasting.

The apparent ileal digestibility of NSP increased as a result of extrusion compared with toasting (26.7 vs 11.4%). As monogastric animals lack the proper enzymes to breakdown cell wall materials, the higher NSP digestibility after extrusion may be the result of improved fermentation of cell wall components by bacteria in the gut. The effect has to be tremendous due to the fact that the major site for fermentation in birds is the ceca, which is "postileal". However, chyme refluxing may provide bacterial populations in the used part of the ileum. In experiments with wheat-based diets fed to rats, it is known that after extrusion the ratio of insoluble to soluble dietary fiber is lowered, resulting in a decrease in fecal recovery of arabinose, xylose, and glucose compared with raw wheat flour (Björck *et al*., 1984). The higher solubility is probably responsible for this increased fermentability. Lintas *et al*. (1988) reported that soluble fiber content of extruded legumes increased compared with the raw materials, but the extent of solubilization depended upon the type of legume. The HPSEC analysis of the carbohydrate fractions in the chyme also showed that more soluble NSP components were present after extrusion compared with toasting. Therefore, disruption and homogenization of fiber by intense mechanical treatment during extrusion cooking could render dietary fiber more available to fermentation.

Enzyme treatment, which did not affect growth performance of the broiler chicks, significantly increased apparent ileal CP digestibility compared with no enzyme treatment (85.2 vs 83.7%). Among the enzymes no differences occurred in CP digestibility, which means that all enzymes to some extent increased *in vivo* CP digestibility. As mentioned before, in a previous study, the carbohydrase preparation was able to solubilize considerable amounts of protein, because it also exhibited proteolytic activity (Marsman *et al*., unpublished data). This finding may explain the overall increase in apparent ileal CP digestibility and also why no differences were found among the enzyme treatments. It could also mean that proteins were abundantly available in all diets. Therefore, the increased CP digestibility did not lead to a better chick performance.

Although enzyme treatment, in general, improved apparent ileal NSP digestibility compared with no enzyme treatment (20.6 vs 14.5%), it can be seen that, in particular, the treatment with the carbohydrase preparation increased the NSP digestibility (23.6%). From *in vitro* studies it is known that this enzyme preparation is very effective in solubilizing high amounts of cell wall material (Marsman *et al*., unpublished data). One might expect that a higher degree of solubilization, thus a higher fermentation rate in the gut, may contribute to a higher amount of ME in the diet which result in an improved growth performance (Chesson, 1993). The present study did not show this improvement in growth.

With respect to apparent ileal NSP digestibility, there was an interaction between thermal processing and enzyme treatment. It appeared that addition of enzymes to tSBM increased apparent ileal NSP digestibility relatively better (13.6 vs 4.6%) than enzyme addition to eSBM (27.5 vs 24.4%). However, HPSEC analysis of chyme showed that enzyme treatment had more impact on the soluble NSP fractions in eSBM than in tSBM. These results should be interpreted with care, because two factors are largely influencing the results. First, extrusion solubilized more cell wall material than toasting, which resulted in a higher apparent ileal NSP digestibility. A certain amount of NSP was solubilized but could not be fermented by the bacteria, resulting in an accumulation of a high molecular undigestible NSP fractions (apparent MW > 500 kDa and 10 kDa, respectively). Secondly, enzyme activity was very effective in hydrolyzing those undigestible cell wall components into oligo- and monosaccharides. The HPSEC analysis shows the resultant of both phenomena, but it is clear that enzymatic breakdown of both soluble and

insoluble cell wall components may contribute to improved NSP digestibility.

Chyme Characteristics

The concentration of soluble NSP in the chyme of chicks fed tSBM or eSBM did not differ significantly (9.7 vs 10.6%); however, HPSEC analysis showed that after extrusion more high molecular weight NSP fractions and less oligosaccharides were obtained than with toasting. It was expected that high molecular weight NSP fractions may contribute more to the viscosity in the ileum than do the smaller ones (Bedford, 1995). However, no significant differences in ileal viscosities were obtained between toasting and extrusion 3.52 vs 3.41 cP). Therefore, it is difficult to conclude whether the lower growth performance and apparent ileal CP digestibility after toasting may be related to an increase in chyme viscosity, resulting in a decrease in nutrient absorption in the small intestine. A positive relationship existed between the concentration of soluble NSP and chyme viscosity in the chyme after feeding the chicks SBM extruded at different shear levels. Extrusion at the highest shear level significantly increased the concentration of soluble NSP (13.4 vs 4.3 and 7.7%) and chyme viscosity (5.16 vs 3.20 and 3.80 cP) compared with extrusion at lower shear levels (Ex-0 and Ex-4, respectively). The HPSEC analysis also showed that increasing shear forces resulted in an increasing concentration of high molecular NSP, which also explained the increase in chyme viscosity. The increase in chyme viscosity is also accompanied by an increase in WHC at the highest shear level compared with the lowest shear level. Nevertheless, this increase in viscosity did not significantly depress growth performance nor apparent ileal nutrient digestibilities. Body weight gain had the tendency to decrease with increasing chyme viscosity. Also, FCR and apparent ileal CP digestibility tended to worsen ($P < 0.10$) after extrusion with eight flights. This result may also be explained by the increase in ileal viscosity. Several studies reported an increase in viscosity in the small intestine due to high soluble molecular weight forms of, e.g., β-glucans and arabinoxylans, which resulted in decreased diffusional rates of the nutrients (Walsh *et al.*, 1993; Bedford, 1995). Polysaccharides of legumes are more complex than their counterparts in cereals (Annison and Choct, 1993). This fact makes it difficult to target these cell wall components for enzyme supplementation.

In conclusion, extrusion significantly improved FCR compared with toasting, which can be explained by higher apparent ileal digestibilities of CP, starch, and NSP. Enzyme treatment did not improve growth performance, but apparent ileal CP and NSP digestibility were significantly increased compared with no enzyme treatment. Among the enzymes, no differences were found in growth performance, despite the increasing apparent ileal NSP digestibility after treatment with the carbohydrase preparation. The use of different torpedo elements in order to increase the amount of shear forces during extrusion were not powerful enough to induce significant differences in growth performance and apparent ileal nutrient digestibilities among the different shear levels. The increasing chyme viscosity caused by a higher concentration of NSP did not affect growth performance or apparent ileal nutrient digestibilities.

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