The Effect of Fat Type, Carbohydrase, and Lipase Addition on Growth Performance and Nutrient Utilization of Young Broilers Fed Wheat-Based Diets

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ABSTRACT A 2 × 2 × 2 factorial experiment was conducted to evaluate the effects of fat type (beef tallow [50 g/kg diet] or canola oil [50 g/kg of diet]), carbohydrase addition (none or carbohydrases [0.4 g/kg diet]), and lipase addition (none or lipase [0.2 g/kg of diet]) on growth performance and nutrient utilization of male broilers fed a wheat-based diet from 5 to 18 d. The carbohydrase supplement contained xylanase, glucanase, cellulase, and other enzyme activities. The experimental diets were formulated to be suboptimal in major nutrients and each was fed in a mash form to 10 replicate pens of 5 broilers per pen. Body weight gain was not affected by fat type but a poorer feed / gain ratio ($P < 0.001$) was noted for tallow-containing diets. Regardless of fat type, carbohydrase enzyme supplementation improved ($P < 0.001$) BW gain and feed / gain ratio. There was no effect of lipase addition on chicken performance and nutrient utilization. When compared with canola oil, tallow-containing diets had a lower ($P < 0.001$) apparent fat digestibility and consequently a lower dietary AME_n content. Carbohydrase enzyme addition improved ($P < 0.001$) fat, starch, nitrogen, and nonstarch polysaccharide (NSP) digestibilities in the small intestine, improved AME_n, and reduced ($P < 0.001$) jejunal digesta viscosity in both fat types. Carbohydrase supplementation increased water-soluble ($P < 0.001$) and decreased water-insoluble ($P < 0.001$) NSP concentrations in the small intestine. The interaction between fat type and carbohydrase addition was only significant for fat digestibilities, with greater improvements seen for diets containing tallow. Significant interactions between carbohydrase addition and intestinal segment were noted for fat, starch, nitrogen, and NSP digestibilities, with the enzyme effects being greater in the jejunum than the ileum. It is evident from the present study that an appropriate carbohydrase preparation could eliminate the negative effects of soluble NSP on animal fat utilization in a wheat-based broiler diet.

(*Key words*: broiler, enzyme, fat type, nutrient utilization, wheat)

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INTRODUCTION

Animal fats and vegetable oils are usually added to broiler diets to increase energy concentration and to improve productivity. The digestibility of a dietary fat depends on the chemical nature of its constituent fatty acids (Garrett and Young, 1975; Ketels and De Groote, 1989; Danicke et al., 2000). Fats rich in unsaturated fatty acids are better digested and absorbed than saturated fats (Danicke, 2001). Beef tallow is characterized by a lower fat digestibility and a lower ME content than vegetable oils, and these have been attributed to its higher content of long-chain saturated fatty acids (LCSFA) (Blanch et al., 1995).

Water-soluble fractions of nonstarch polysaccharides (NSP), including arabinoxylan of rye and wheat and β-glucan of barley, are known to exert adverse effects on performance and nutrient digestibility in broilers (Bedford and Classen, 1992; Choct and Annison, 1992a). Such negative effects are thought to be caused by an increased digesta viscosity and can be largely eliminated by the addition of viscosity-reducing carbohydrase enzymes such as xylanase and β-glucanase (Choct and Annison, 1992b; Simon, 1998; Steenfeldt et al., 1998a, b). Several studies have shown that, among the nutrients, fat digestion suffers the most pronounced impairment due to high digesta viscosity (Campbell et al., 1983; Ward and Marquardt, 1983; Choct and Annison, 1992b). Furthermore, an increase in intestinal viscosity is more detrimental to the digestion and absorption of dietary fats containing high proportions of saturated fatty acids. Antoniou et al. (1980) observed a greater depression in performance and fat digestibility in rye-fed birds when tallow rather than soy oil was added to the diet. Using similar rye-based

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Abbreviation Key: LCSFA = long-chain saturated fatty acids; NSP = nonstarch polysaccharides.

broiler diets, Danicke et al. (1997, 1999, 2000) demonstrated that dietary fat type influenced the degree of the carbohydrase enzyme effects on fat digestion.

Compared with rye, wheat contains a lower level of arabinoxylan and thus produces lower viscosity (Henry, 1987). However, Preston et al. (2001) demonstrated a significant interaction between fat type and enzyme effect in a wheat-based (70%) broiler diet. Furthermore, Pasquier et al. (1996) reported reduced fat emulsification and hydrolysis with every increment in medium viscosity over a range from 0 to 20 mPa s. These results suggest that the negative effect of viscous NSP on fat digestion and absorption may not be confined to diets that induce high intestinal viscosity.

It is well known that fat digestion is facilitated by the combined action of bile acids, lipase, and colipase. It has been demonstrated that the physiological functions necessary for efficient fat digestion in young chickens are immature and continue to develop for several weeks after hatching (Jin et al., 1998). Noy and Sklan (1995) reported that in broiler chickens, secretion of lipase was low at hatching and increased 20-fold between 4 and 21 d of age. Krogdahl and Sell (1989) reported that dietary tallow and animal-vegetable fat were not efficiently used until lipase activity reached its maximum level. Because young birds have insufficient secretion of endogenous lipase, dietary supplementation of bacterial lipase may improve fat use.

In many studies, fat type × enzyme interactions were evaluated using high fat inclusion rates (e.g., 10%) and highly viscous semisynthetic diets (e.g., rye-based), but such information regarding practical wheat-based broiler diets is limited. Therefore, the goal of the present study was to investigate the growth performance and nutrient use responses of broilers to dietary fat type, carbohydrase, and lipase supplementation using a wheat-based diet.

MATERIALS AND METHODS

Experimental Design and Diets

A 2 × 2 × 2 factorial experiment was used to evaluate the effects of dietary fat type [beef tallow (50g/kg diet) or canola oil (50g/kg diet)], carbohydrase addition [none or carbohydrases (0.4 g/kg diet)], and lipase addition [none or lipase (0.2 g/kg of diet)] to a wheat (60%)-based broiler diet. The 2 basal diets (Table 1) containing beef tallow or crude canola oil were formulated to meet 95% of the NRC (1994) requirement for AME and 92% of CP, calcium, available phosphorus, methionine, and methionine + cysteine. Other nutrients met or exceeded NRC specifications. Beef tallow (melting point, 43°C; saturated fatty acids, 49.5%; monounsaturated fatty acids 42.0%; and polyunsaturated fatty acids, 3.0% of the total fat) and crude canola oil (melting point, –10°C; saturated fatty acids, 6.0%; monounsaturated fatty acids, 55.0%; and polyunsaturated fatty acids, 36.0% of the total fat) were provided by a local feed manufacturer. The carbohydrase enzyme preparation was a multicarbohydrase cocktail and supplied xylanase (1000 units/kg of diet), glucanase (400 units/kg of diet), cellulase (120 units/kg of diet), and other NSP-degrading activities that were determined in this laboratory (Meng et al., 2002). In addition to wheat, the carbohydrase preparation was effective in soybean meal, canola meal, and pea NSP hydrolysis that, under the conditions of the assay (pH 5.2, 45°C), averaged 26, 36, and 28%, respectively. The lipase enzyme supplied 100 units of activity per kilogram of diet. Both enzyme supplements were provided by Canadian Bio-Systems Inc., Calgary, Canada. Chromic oxide at 3.0 g/kg of diet was mixed with the diets and used to calculate the nutrient digestibilities and dietary AME_n content.

TABLE 1. The composition and chemical analysis of basal diets (g/kg)

Ingredient	Canola oil diet	Beef tallow diet
Wheat (13.5% CP)	600.0	600.0
Soybean meal (46% CP)	215.0	215.0
Canola meal (36% CP)	60.0	60.0
Peas (22% CP)	30.0	30.0
Canola oil	50.0	
Beef tallow		50.0
Limestone[1]	14.6	14.6
Dicalcium phosphate[2]	10.8	10.8
DL-Methionine	0.8	0.8
L-Lysine-HCl	0.8	0.8
Mineral premix[3]	5.0	5.0
Vitamin premix[4]	10.0	10.0
Chromic oxide	3.0	3.0
Total	1,000.0	1,000.0
Calculated analysis		
CP (%)[5]	21.0	21.0
Crude fat (%)	7.8	7.8
AME (kcal/kg)	3,040.0	2,990.0
Lysine (%)	1.15	1.15
Methionine (%)	0.46	0.46
Methionine + cysteine (%)	0.86	0.86
Calcium (%)	0.92	0.92
Available phosphorus (%)	0.41	0.41

[1]Contained 38% calcium.

[2]Contained 21% calcium and 18% phosphorus.

[3]Mineral premix provided per kilogram of diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.36 mg; Na, 1.6 g.

[4]Vitamin premix provided per kilogram of diet: vitamin A, 8,250 IU; vitamin D_3, 1,000 IU; vitamin E, 11 IU; vitamin B_{12}, 0.012 mg; vitamin K, 1.1 mg; niacin, 53 mg; choline, 1,020 mg; folic acid, 0.75 mg; biotin, 0.25 mg; riboflavin, 5.5 mg.

[5]Calculated based on analyzed values of ingredients.

Growth Performance

One-day-old male Arbor Acres broiler chicks were obtained from a local commercial hatchery. The birds were held in electrically heated Jamesway battery brooders[2] for a 4-d preexperimental period and fed commercial chick starter crumbles (21% CP). On d 5, birds were fasted for 4 h, individually weighed and sorted into 5 weight classes. Groups of 5 birds were then randomly assigned to pens such that the average initial BW of birds was

[2]James Mfg. Co., Mount Joy, PA.

similar across pens. Ten replicate pens of 5 birds each were randomly assigned to the 8 dietary treatments. All diets were fed in a mash form throughout the 2-wk experimental period. The birds had free access to water and feed and were provided with continuous light. Body weight and feed intake were monitored weekly with pen as the experimental unit. Before weighing, the birds were fasted for 4 h. Mean BW, feed intake, and feed / gain ratio were used to determine the performance of birds.

Nutrient Utilization

At the termination of the experiment (on d 18), excreta samples from each pen were collected over a 3 h period and subsequently frozen, freeze-dried, and finely ground. The samples were analyzed for chromic oxide, gross energy, nitrogen, NSP, and fat content. The total tract digestibility of fat and NSP and AME_n content of experimental diets were calculated. On d 19 and 20, 20 birds from each treatment were randomly selected and killed by cervical dislocation. The contents of the jejunum (from the end of the duodenum to Meckel's diverticulum) and the ileum (from Meckel's diverticulum to 1 cm above the ileo-cecal junction) were collected. The digesta samples were frozen, freeze-dried, ground, and pooled to yield 5 replicate samples per treatment. The samples were analyzed for chromic oxide, nitrogen, NSP, starch, and fat to determine their jejunal and ileal digestibilities. The water-soluble and water-insoluble NSP concentrations were measured for jejunal and ileal digesta samples.

In addition, 10 birds per treatment were randomly selected for intestinal viscosity measurement. The birds were killed by cervical dislocation and the contents of the jejunum were collected and pooled for 2 birds to yield 5 replicate samples per treatment. Fresh samples of 1.5 g each were centrifuged at 9,000 rpm for 10 min and the viscosity of the supernatant was determined at 40°C using a Brookfield digital viscometer.[3]

All animal procedures were conducted according to the guidelines of the Canadian Council on Animal Care and the protocol for this study was approved by the Local Animal Care Committee of the University of Manitoba.

Chemical Analysis

Diet, digesta, and excreta samples were analyzed in duplicate for fat content using AOAC method 920.39 (AOAC, 1990). Chromic oxide was determined using the procedure described by Williams et al. (1962). Nitrogen content was analyzed by the combustion method using the LECO model FP 2000 combustion analyzer.[4] Gross energy was determined using a Parr 1261 adiabatic bomb calorimeter.[5] Starch was determined colorimetrically using Sigma Glucose (HK) 20 kit and the procedure described by Aman and Hasselman (1984).

Total NSP were determined by gas-liquid chromatography (component neutral sugars) and by colorimetry (uronic acids) using the procedure described by Englyst and Cummings (1984, 1988) with minor modifications (Slominski and Campbell, 1990). In brief, 100-mg diet samples or 50-mg digesta or excreta samples were boiled with 2 mL of dimethylsulfoxide for 1 h and then incubated at 45°C overnight with a sodium acetate buffer solution (pH 5.2) of starch-degrading enzymes amylase, pullulanase, and amyloglucosidase.[6] Ethanol was added and the mixture was left for 1 h at room temperature before being centrifuged. The supernatant was discarded and the dried residue was dissolved in 1 mL of 12 *M* sulfuric acid and incubated for 1 h at 35°C. Six milliliters of water and 5 mL of myo-inositol (internal standard) solution were then added and the mixture was boiled for 2 h. One milliliter of the hydrolysate was taken and neutralized with 12 *M* ammonium hydroxide, reduced with sodium borohydride, and acetylated with acetate anhydride in the presence of 1-methylimidazole. Component sugars were separated using an SP-2340 column and a Varian CP 3380 gas chromatograph.[7] Water-soluble NSP content of the digesta samples was determined according to the method described by Slominski et al. (1993). Water-insoluble NSP content was calculated as the difference between total NSP and water-soluble NSP content.

Calculations and Statistical Analysis

The following equations were used for calculation of the digestibility of various dietary components (using fat calculation as an example) and AME_n content of experimental diets (Hill et al., 1960):

$$\text{digestibility (\%)} = \{1 - [(Cr_2O_{3\ \%\ \text{diet}} / Cr_2O_{3\ \%\ \text{digesta/ excreta}}) \times (\text{fat}_{\%\ \text{digesta/ excreta}} / \text{fat}_{\%\ \text{diet}})]\} \times 100,$$

$$AME_n\ (\text{kcal/kg}) = GE_{\text{kcal/kg diet}} - [GE_{\text{kcal/kg excreta}} \times (Cr_2O_{3\ \%\ \text{diet}} / Cr_2O_{3\ \%\ \text{excreta}})] - 8.22 \times \{N_{\%\ \text{diet}} - [N_{\%\ \text{excreta}} \times (Cr_2O_{3\ \%\ \text{diet}} / Cr_2O_{3\ \%\ \text{excreta}})]\}$$

where GE is gross energy, N is nitrogen, Cr_2O_3 is chromic oxide, and 8.22 is the energy equivalent of uric acid nitrogen, i.e., 8.22 kcal/kg of uric acid nitrogen.

Three-way factorial ANOVA was applied for performance parameters, jejunal viscosity, AME_n, and total tract digestibility of fat and NSP:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + (A \times B)_{ij} + (A \times C)_{ik} + (B \times C)_{jk} + (A \times B \times C)_{ijk} + e_{ijkl}$$

[3]Model DV-II+LV, Brookfield Engineering Laboratories, Stoughton, MA.
[4]LECO Corp., St. Joseph, MI.
[5]Parr Instrument Co., Moline, IL.
[6]Sigma Chemical Co., St. Louis, MO.
[7]Varian Canada Inc., Mississauga, Ontario, Canada.

where Y_{ijkl} = tested parameter of a broiler l fed a diet containing fat type i, carbohydrase level j and lipase level k; A_i = fat type (beef tallow, canola oil); B_j = carbohydrase addition (none, carbohydrase 0.4 g/kg diet); C_k = lipase addition (none, lipase 0.2 g/kg diet); $(A \times B)_{ij}$ = interactions between fat type and carbohydrase addition; $(A \times C)_{ik}$ = interactions between fat type and lipase addition; $(B \times C)_{jk}$ = interactions between carbohydrase and lipase addition; $(A \times B \times C)_{ijk}$ = interactions among fat type, carbohydrase, and lipase addition; e_{ijkl} = error term.

The digestibility of fat, starch, nitrogen, and NSP and water-soluble and water-insoluble NSP concentrations in the jejunum and ileum were analyzed by a 4-way ANOVA as a split-plot experiment with repeated measurements. Because the 3-way and 4-way interactions were tested nonsignificant, these components were removed from the complete model and the final model used was:

$$Y_{ijklm} = \mu + A_i + B_j + C_k + (A \times B)_{ij} + (A \times C)_{ik} + (B \times C)_{jk} + e_m(a \times b \times c) + D_l + (A \times D)_{il} + (B \times D)_{jl} + (C \times D)_{kl} + e_{ijklm}$$

where Y_{ijklm} = tested parameter of a broiler m fed a diet containing fat type i, carbohydrase level j, lipase level k, in intestinal segment l; A_i = fat type (beef tallow, canola oil); B_j = carbohydrase addition (none, carbohydrase 0.4 g/kg diet); C_k = lipase addition (none, lipase 0.2 g/kg diet); $(A \times B)_{ij}$ = interactions between fat type and carbohydrase addition; $(A \times C)_{ik}$ = interactions between fat type and lipase addition; $(B \times C)_{jk}$ = interactions between carbohydrase and lipase addition; these above components were tested using $e_m(a \times b \times c)$ as error term, and $e_m(a \times b \times c)$ = effect of repeated measurements (different intestinal segments) within the same bird m; D_l = effect of intestinal segment (jejunum, ileum); $(A \times D)_{il}$ = interactions between fat type and intestinal segment; $(B \times D)_{jl}$ = interactions between carbohydrase addition and intestinal segment; $(C \times D)_{kl}$ = interactions between lipase addition and intestinal segment; e_{ijklm} = error term.

All experimental data were subjected to the GLM procedure of SAS (SAS Institute, 1986) as a complete randomized design. All statements of significance are based on a probability of less than 0.05.

RESULTS

Growth Performance

The results of growth performance of the broilers during the 2-wk experimental period are presented in Table 2. Birds fed diets containing beef tallow consumed 3.4% more ($P < 0.05$) feed to achieve a similar ($P > 0.05$) BW gain to chicks fed canola oil-containing diets. As a result, a poorer ($P < 0.001$) feed / gain ratio was noted for the diets containing tallow. Carbohydrase addition improved ($P < 0.001$) BW gain and feed / gain ratio by 5.4 and 3.4%, respectively. There was no effect of lipase addition on chicken performance. No interactions were observed between fat type and enzyme supplementations.

Nutrient Utilization

The results of the apparent digestibility of fat, starch, nitrogen, and NSP in different segments of the small intestine are summarized in Table 3. An effect ($P < 0.001$) of fat type was only observed for fat digestibility, which was 5.7% lower for tallow-containing diets than for canola oil-containing diets. Carbohydrase enzyme addition improved ($P < 0.001$) the digestibility of fat, starch, nitrogen, and NSP, irrespective of fat type. However, lipase addition had no effect ($P > 0.05$) on these parameters. An interaction ($P < 0.01$) between fat type and carbohydrase addition was only noted for fat digestibility, with a greater improvement for the tallow-containing diets. Interactions ($P < 0.01$) between carbohydrase enzyme and intestinal segment were observed for fat, starch, nitrogen, and NSP digestibilities; the improvements due to carbohydrase enzyme addition were greater in the jejunum than in the ileum.

The water-soluble and water-insoluble NSP concentrations in different intestinal segments are shown in Table 4. Fat type and lipase addition had no effect ($P > 0.05$) on the concentrations of both NSP fractions, but carbohydrase addition increased ($P < 0.001$) soluble NSP and decreased ($P < 0.001$) insoluble NSP concentration. Interactions ($P < 0.05$) between carbohydrase addition and intestinal segment were noted for soluble and insoluble NSP concentration, with a greater increase in soluble NSP and a greater decrease in insoluble NSP concentration in the jejunum than in the ileum.

Total tract digestibility of fat and NSP, jejunal digesta viscosity, and AME_n content of diets are shown in Table 5. Tallow-containing diets had a lower ($P < 0.001$) total tract digestibility of fat, which was paralleled by a lower ($P < 0.001$) AME_n content when compared with canola oil-containing diets. As opposed to lipase addition, which showed no effect, the carbohydrase addition improved fat ($P < 0.001$) and NSP ($P < 0.05$) digestibilities, increased AME_n content ($P < 0.001$), and reduced ($P < 0.001$) jejunal viscosity. An interaction between fat type and carbohydrase addition was again noted for fat digestibility only ($P < 0.001$), the enzyme effect being more pronounced for tallow-containing diets.

DISCUSSION

The present study clearly demonstrated that performance of birds fed wheat-based diets was affected by fat type and that chicks fed tallow-containing diets had a poorer feed / gain ratio. Poorer feed / gain ratios were observed by Brenes et al. (1993), Langhout et al. (1997), and Preston et al. (2001) when similar wheat-based diets were supplemented with beef tallow rather than vegetable oils. Nutrient digestibility results (Tables 3 and 5) indicated that the difference observed in feed / gain ratio between beef tallow- and canola oil-containing diets was only due to the difference in fat digestibility, because digestibilities of starch, nitrogen, and NSP were not affected by fat type. It is well known that beef tallow is

TABLE 2. Effect of dietary fat type, carbohydrase, and lipase addition on growth performance of broilers (5 to 18 d) fed wheat-based diets

Effect	Feed intake (g/bird)	BW gain (g/bird)	Feed:gain (g feed/g gain)
Fat type			
Tallow	698	476	1.47
Canola oil	675	482	1.40
Pooled SEM	7.6	3.9	0.006
Carbohydrase addition			
–	682	466	1.46
+	692	491	1.41
Pooled SEM	7.6	3.9	0.006
Lipase addition			
–	678	475	1.44
+	695	482	1.43
Pooled SEM	7.6	3.9	0.006
Source of variation		Probability[1]	
Fat type	0.037	0.248	<0.001
Carbohydrases	0.362	<0.001	<0.001
Lipase	0.113	0.184	0.122
Fat type × carbohydrases	0.937	0.882	0.799
Fat type × lipase	0.387	0.633	0.219
Lipase × carbohydrases	0.542	0.563	0.624
Fat type × lipase × carbohydrases	0.894	0.985	0.729

[1]An effect with a probability of less than 0.05 is considered significant.

TABLE 3. Effect of dietary fat type, carbohydrase, and lipase addition on apparent digestibility (%) of fat, starch, nitrogen, and nonstarch polysaccharides (NSP) in the jejunum and the ileum of broilers fed wheat-based diets

Effect	Fat	Starch	Nitrogen	NSP
Fat type				
Tallow	68.8	88.7	55.0	10.1
Canola oil	72.7	89.7	56.8	12.2
Pooled SEM	0.61	0.44	0.83	0.88
Carbohydrase addition				
–	68.0	87.3	53.5	6.4
+	73.5	91.1	58.4	15.9
Pooled SEM	0.61	0.44	0.83	0.88
Lipase addition				
–	70.8	89.4	56.0	11.8
+	70.8	89.0	55.9	10.5
Pooled SEM	0.61	0.44	0.83	0.88
Fat type × carbohydrases				
Tallow				
–	64.8			
+	72.9			
Canola oil				
–	71.3			
+	74.1			
Pooled SEM	0.86			
Carbohydrases × intestinal segment				
Jejunum				
–	58.5	81.5	34.0	–0.7
+	66.3	87.1	41.2	11.8
Ileum				
–	77.6	93.0	72.9	13.6
+	80.7	95.1	75.5	20.0
Pooled SEM	0.66	0.60	0.63	0.97
Source of variation		Probability[1]		
Fat type	<0.001	0.129	0.134	0.110
Carbohydrases	<0.001	<0.001	<0.001	<0.001
Lipase	0.963	0.491	0.926	0.319
Fat type × carbohydrases	0.004	0.498	0.655	0.110
Fat type × lipase	0.855	0.610	0.659	0.474
Lipase × carbohydrases	0.861	0.795	0.502	0.826
Intestinal segment	<0.001	<0.001	<0.001	<0.001
Fat type × intestinal segment	0.727	0.959	0.095	0.094
Carbohydrases × intestinal segment	0.002	0.005	0.001	0.004
Lipase × intestinal segment	0.959	0.341	0.934	0.397

[1]An effect with a probability of less than 0.05 is considered significant.

TABLE 4. Effect of dietary fat type, carbohydrase, and lipase addition on water-soluble nonstarch polysaccharides (SNSP) and water-insoluble nonstarch polysaccharides (INSP) concentration (mg/g) in the jejunum and the ileum of broilers fed wheat-based diets

Effect	SNSP	INSP
Fat type		
Tallow	22.7	182
Canola oil	22.2	178
Pooled SEM	0.36	5.3
Carbohydrase addition		
–	24.6	197
+	20.4	164
Pooled SEM	0.36	5.3
Lipase addition		
–	22.4	180
+	22.6	180
Pooled SEM	0.36	5.3
Carbohydrases × intestinal segment		
Jejunum		
–	14.7	154
+	20.2	112
Ileum		
–	26.0	240
+	28.9	216
Pooled SEM	0.50	3.6
Source of variation	Probability[1]	
Fat type	0.327	0.608
Carbohydrases	<0.001	<0.001
Lipase	0.724	0.983
Fat type × carbohydrases	0.168	0.592
Fat type × lipase	0.283	0.704
Lipase × carbohydrases	0.390	0.770
Intestinal segment	<0.001	<0.001
Fat type × intestinal segment	0.901	0.197
Carbohydrases × intestinal segment	0.027	0.018
Lipase × intestinal segment	0.811	0.702

[1]An effect with a probability of less than 0.05 is considered significant.

TABLE 5. Effect of dietary fat type, carbohydrase, and lipase addition on total tract digestibility of fat and nonstarch polysaccharides (NSP), AME_n content, and jejunal digesta viscosity of broilers fed wheat-based diets

Effect	Fat (%)	NSP (%)	AME_n (kcal/kg)	Viscosity (mPa s)
Fat type				
Tallow	84.1	20.7	3048	3.9
Canola oil	88.0	22.1	3115	3.4
Pooled SEM	0.23	0.94	11.0	0.20
Carbohydrase addition				
–	84.3	19.5	3032	5.0
+	87.8	23.3	3132	2.4
Pooled SEM	0.23	0.94	11.0	0.20
Lipase addition				
–	86.2	21.4	3085	3.7
+	85.9	21.3	3078	3.6
Pooled SEM	0.23	0.94	11.0	0.20
Fat type × carbohydrases				
Tallow				
–	81.5			
+	86.7			
Canola oil				
–	87.1			
+	88.9			
Pooled SEM	0.33			
Source of variation	Probability[1]			
Fat type	<0.001	0.300	<0.001	0.098
Carbohydrases	<0.001	0.012	<0.001	<0.001
Lipase	0.304	0.952	0.650	0.781
Fat type × carbohydrases	<0.001	0.841	0.080	0.964
Fat type × lipase	0.465	0.812	0.860	0.957
Lipase × carbohydrases	0.464	0.864	0.775	0.950
Fat type × lipase × carbohydrases	0.175	0.356	0.787	0.332

[1]An effect with a probability of less than 0.05 is considered significant.

characterized by low digestibility, particularly in young birds (Ketels and DeGroote, 1989). Ward and Marquardt (1983) attributed such poor digestibility of tallow to the degree of saturation of its fatty acids. Beef tallow contains mainly palmitic and stearic acids (LCSFA), and unsaturated oleic acids (Danicke, 2001). Danicke (2001) suggested that the LCSFA in beef tallow are nonpolar and thus rely on an adequate presence of bile salts for efficient emulsification and micelle formation, which are essential for fat digestion and absorption. Conversely, crude canola oil is primarily composed of long-chain unsaturated oleic, linoleic, and linolenic acids (NRC, 1994), which can be easily absorbed even in the absence of bile salts (Garrett and Young, 1975). Despite the need for bile salts to digest tallow, birds younger than 3 wk have been observed to produce inadequate secretions of bile acids, particularly when tallow is provided as dietary fat (Krogdahl, 1985). Support for this was given by Polin et al. (1980) and Fengler et al. (1988), who showed that feeding exogenous bile salts to chicks increased their ability to digest tallow. Hence, in the present study, the insufficiency of bile salts may account for the observed lower fat digestibility in the chicks fed the tallow-containing diet.

The specific arrangement of the saturated and unsaturated fatty acids on the glycerol moiety of a triglyceride molecule may contribute to the observed differences between fat types. Pancreatic lipase shows specificity for the fatty acids esterified to glycerol in the 1- and 3- positions and leaves the 2-monoglycerides intact and absorbed in this form (Leeson and Summers, 2001). The 2-position LCSFA in the form of monoglycerides have greater solubility for micelle formation than the same fatty acids released from the 1- or 3- positions, which are more nonpolar and insoluble and thus less digestible. Sibbald and Kramer (1977) reported that in beef tallow, 73 to 81% of palmitic and stearic acids are bound at the 1- and 3- positions, whereas the long-chain unsaturated fatty acids (mainly oleic acid) are esterified at the 2-position. This may be an important factor contributing to the poorer fat digestibility of tallow-containing diets observed in the present study. Evidently, the lower AME_n value of tallow-containing diets, compared with canola oil-containing diets, is a consequence of a lower fat digestibility.

The lack of responses in chicken performance and fat digestibilities to the lipase addition suggests that the insufficiency of pancreatic lipase production may not contribute to the lower fat digestibility of tallow-containing diets in the current study. Examination of the published data indicates that although the daily net secretion of lipase into the duodenum increases significantly as the bird ages (Noy and Sklan, 1995), the secretion of lipase when calculated per gram of feed intake is less dramatic (Uni et al., 1996). This indicates that the lipase secretion of young birds may not be as inadequate as expected when their feed intake is considered (Sklan, 2001). Although some increase in diet AME content and fat digestibility with lipase supplementation has been reported, lipase addition caused a significant reduction in feed intake and consequently lowered BW gain (Al-Marzooqi and Leeson, 1999). This was not the case in the current study, as performance parameters were similar to the control diets and no reduction in feed intake was observed. It would appear that such an "anorexic effect" observed in the earlier study (Al-Marzooqi and Leeson, 1999) may have been a consequence of the high inclusion rates of lipase (i.e., pancreatic preparation). This was not the case in the current study because enzyme addition, when calculated per gram of feed intake, accounted for approximately 30% of the endogenous lipase secretion to the duodenum of the young chicken.

It has been shown that the antinutritional effect of wheat arabinoxylan amplifies the digestibility differences between fat types (Choct and Annison, 1992b; Choct et al., 1996). In the absence of carbohydrase addition, the difference in fat digestibility between tallow- and canola oil-containing diets (10% at the intestinal level and 7% at fecal level, Tables 3 and 5, respectively) is relatively small when compared with other studies, in which fat digestibility differences of 29% (Panicke et al., 1997; Langhout et al., 1997) and 36% (Preston et al., 2001) were noted. This may be due to the relatively lower fat inclusion rate and a less viscous dietary background [jejunal viscosity of 5.2 and 4.7 mPa s (data not shown), respectively] in the present study. Consequently, the growth rate of birds fed tallow-containing diets was depressed in these studies when compared with vegetable oil-containing diets. In the current study, however, BW gain was not affected by fat type, suggesting a less dramatic dietary situation. Under such conditions, the birds fed tallow-containing diets achieved a similar growth rate to those fed canola oil-containing diets by increasing feed intake to compensate for the compromised fat digestibility.

Significant interactions between fat type and carbohydrase addition for fat digestibility were detected in the current study at the intestinal and total tract levels. For example, at the intestinal level, carbohydrase supplementation improved fat digestibility by 12.5% (from 64.8 to 72.9%) in tallow-containing diets, but an improvement of only 3.8% (from 71.3 to 74.1%) was noted for canola oil-containing diets, suggesting that the degree of enzyme effect on fat digestibility is directly related to the type of dietary fat. Although the exact mechanism of action of dietary carbohydrases is not clear, their beneficial effects have been associated with the reduction of digesta viscosity caused by water-soluble and viscous NSP (Bedford and Classen, 1992). Water-soluble arabinoxylan of wheat has been shown to increase intestinal viscosity and exert antinutritive effects (Choct and Annison, 1992b). Smulikowska (1998) suggested that an increased intestinal viscosity might lead to reduced gut motility, which in turn may decrease the rate of diffusion and the convective transportation of emulsion droplets, fatty acids, mixed micelles, bile salts, and lipase within the small intestine. Such a situation would be particularly detrimental for the digestion of LCSFA, as they rely more on vigorous digesta mixing for optimal emulsification. In addition, Krogdahl (1985) suggested that palmitic and stearic fatty

acids are nonpolar and cannot spontaneously form mixed micelles, but can be solubilized into micelles formed from unsaturated fatty acids and conjugated bile salts. Therefore, such dependence of digestion and absorption of LCSFA upon the presence of bile salts, unsaturated fatty acids, and formed micelles would explain why tallow digestion is more sensitive to small increases in intestinal viscosity, as observed in the present study. Pasquier et al. (1996) demonstrated that triglyceride hydrolysis and the amount of emulsified lipids were reduced in vitro as the viscosity of solution containing different concentrations of soluble fiber increased from 0 to 20 mPa s. The sensitivity of tallow digestion to viscosity would explain the more pronounced carbohydrase enzyme effect on improving fat digestibility of tallow-containing diets by reducing digesta viscosity from 5.0 to 2.4 mPa s. Notably, the significant interaction between fat type and carbohydrase addition occurred at an intestinal viscosity level below 10 mPa s, a level most likely encountered with broilers fed practical Canadian wheat-based diets (Slominski et al., 2000). These results seem to indicate that the negative effect of viscous NSP on animal fat digestion is substantial in a practical wheat-based diet, and even relatively small reductions in viscosity due to enzyme action could greatly improve animal fat digestion and absorption.

Choct et al. (1996) attributed poorer digestibility of saturated, compared with unsaturated fatty acids, to increased intestinal fermentation. These authors suggested that, due to hindrance of nutrient digestion by the viscous intestinal environment, more unabsorbed material reaches the ileum, which promotes the proliferation of detrimental microflora. Intestinal bacteria can deconjugate bile acids (Christle et al., 1997; Smits et al., 1998), making the bile acids inactive in fat emulsification and micelle formation (Smulikowska, 1998), and leading to a further depression in lipid digestion. This might be the case in the present study, where antibiotic-free diets were used. Studies by Kritchevsky and Story (1974) and Kritchevsky (1978) have demonstrated that fibrous foods bind bile acids and thereby increase their excretion. The viscous wheat arabinoxylan might exert a similar effect on bile acids and increase bile acid excretions (Langhout et al., 1997). The problems described above might further exacerbate the deficiency of bile salts in the young broilers, but to what extent they contributed to the more depressed tallow digestion cannot be ascertained from the current data. The fact that the fat type × carbohydrase interactions were not detected for other nutrient digestibilities, AME_n value, or growth performance confirms previous findings that digestion of fat is more affected by the negative effects of viscosity than that of other nutrients (Campbell et al., 1983; Choct and Annison, 1992b).

The present study demonstrated that carbohydrase addition had an overall significant effect on nutrient digestibilities, AME_n, and growth performance of birds fed wheat-based diets. Similar results were observed in earlier studies when enzyme preparations were supplemented to broilers fed wheat-based diets (Annison, 1992; Friesen et al., 1992; Marquardt et al., 1994; Steenfeldt et al., 1998a,b). These authors attributed the beneficial effects of enzyme supplementation partially to depolymerization of soluble NSP into smaller polymers, and the resultant reduced digesta viscosity. This was evident in the current study, with the improvements in nutrient use and growth performance accompanied by a significantly increased NSP digestibility and a decreased digesta viscosity, both of which would be the consequence of breakdown of soluble NSP by the action of enzymes. Such effects of carbohydrase enzymes on reducing viscosity have been suggested to alleviate the constraints on diffusion of substrates, enzymes, and products (Fengler and Marquardt, 1988), leading to enhanced nutrient digestion.

Enzymatic disruption of cell wall structure may accelerate digestion by allowing the rapid access of endogenous enzymes to cell wall encapsulated nutrients, and this might contribute to the observed improvements in the present study as indicated by the intestinal NSP concentration. A significant increase in soluble NSP and a decrease in insoluble NSP concentration were noted in the small intestine, indicating a partial breakdown of the plant cell wall structure. In earlier studies from this laboratory (Meng et al., 2002), it was demonstrated that the carbohydrase cocktail used in the current study was capable of depolymerizing cell wall NSP in wheat, soybean meal, canola meal, and peas in vitro. The carbohydrase cocktail was effective in improving nutrient digestibility and growth performance of broilers fed a diet similar to that used in the present study. Because soybean meal, canola meal, and peas accounted for approximately 30% of the basal diet, enhanced digestion of these protein supplements, other than wheat, may also contribute partially to the overall enzyme effects. Therefore, it might be concluded that the positive effect of carbohydrase supplementation was a combination of released intracellular encapsulated nutrients through disruption of cell wall polysaccharides and of reduced digesta viscosity.

It is noteworthy that a significant interaction between carbohydrase addition and intestinal segment was observed for the digestibility of fat, starch, nitrogen, and NSP; the improvements in nutrient digestibilities were more pronounced in the jejunum than in the ileum when diets with enzyme were compared with diets without enzyme supplementation. This would indicate a shift of nutrient digestion and absorption toward the upper region of the small intestine in enzyme-supplemented broilers and would thus limit microbial growth in the hindgut due to substrate limitation. This is of practical importance, as such enzyme effects would be more pronounced for poorly digestible rather than readily digestible ingredients. Therefore, one of the most important benefits of enzyme use would be a reduced variation in nutrient digestion.

In conclusion, the findings of the current study suggest that the negative effects of soluble NSP on animal fat digestion is substantial even in a practical wheat-based broiler diet known to result in low intestinal viscosity.

This finding was supported by the detection of interaction between fat type and carbohydrase addition for fat digestibility at the intestinal and total tract level. The importance of such interaction would be even more apparent with increasing dietary wheat and animal fat inclusion rates during the early growing period of broilers. This leads to a recommendation of supplementation with an appropriate carbohydrase preparation when animal fat is used in diets based on viscous cereals. The multicarbohydrase cocktail may have exerted its beneficial effects on nutrient use and thus growth performance by decreasing intestinal viscosity and by eliminating the nutrient encapsulating effect of cell wall structural polysaccharides.

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