Effect of Enzyme Supplementation of Broiler Diets Based on Corn and Soybeans

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ABSTRACT Digestibility of diets based on corn and soybean meal or soybeans treated by roasting or extrusion, with or without an enzyme supplementation, was measured by "true" (Sibbald) methods, by analysis of excreta, and by analysis of ileal digesta. Only analysis of ileal digesta was able to consistently measure differences between soybean and enzyme treatments in the digestibility of CP, starch, fat, and ME. The amino acid (AA) digestibility of the diets was measured by analysis of the ileal contents. Whereas enzyme supplementation improved overall CP digestibility by 2.9%, this improvement was not equal for all AA. Of the AA most important for broilers fed corn-soybean diets, the digestibilities of Lys, Met, and Arg were not improved or not improved significantly by the enzyme supplementation; however, that of Val was improved by 2.3% and that of Thr was improved by 3.0%. A performance trial demonstrated that enzyme supplementation with equal diet formulation improved BW and the feed conversion ratio by 1.9 and 2.2%, respectively. A second performance trial compared standard diet formulations with formulations using enzyme supplementation and energy levels that were reduced by the amount of improvement provided by the inclusion of enzyme in the first performance trial. No difference was seen between treatments, showing that the improvement of nutrient utilization brought about by enzyme supplementation completely compensated for the reduced energy content. Whereas enzyme supplementation should allow a reduction in CP formulation as well, individual AA were not improved equally by supplementation and should also be balanced.

(*Key words*: metabolizable energy, amino acids, broiler, enzyme, corn-soybean diets)

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INTRODUCTION

In much of the U.S. and in other countries, including Brazil, broiler feed is based primarily on corn and soybean meal (SBM), which supplies the majority of energy and protein in the diet. Utilization of the nutrients contained in corn by broilers is generally considered to be high. Raw soybeans contain antinutritive substances, the most important of which are trypsin inhibitors and hemagglutinins. The negative effects of these are normally eliminated by heating, although over-processing can damage amino acids (AA) and reduce the availability of protein (Araba and Dale, 1990a,b).

Various techniques have been used for the measurement of nutrient content, availability, or digestibility in feed ingredients (Härtel, 1986; Sibbald, 1986; Askbrant 1990; Scott *et al*., 1998a). The "true" values obtained by precision feeding techniques of adult White Leghorn roosters (Sibbald, 1986) have been widely used and are reported widely (National Research Council, 1994). However, "true" values have been criticized because the assay uses starved, adult birds, which are not representative of broilers for which the majority of poultry feed is used, and because it uses a correction for endogenous nutrient production. Scott *et al*. (1998b) concluded that the ME content of wheat and barley was most easily and accurately measured from excreta. Protein and AA digestibility is affected by contamination by products of the urinary system and microbial fermentation in the hindgut. Because of this contamination, determination of protein and AA digestibility is often based on analysis

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Abbreviation Key: AA = amino acid; DE = digestible energy; DE_n = nitrogen-corrected digestible energy; FCR = feed conversion ratio; SBE = extruded full-fat soybeans; SBM = soybean meal; SBR = roasted full-fat soybeans.

of the contents of the ileum (Lewis and Bayley, 1995; Kadim and Moughan, 1997; Marsman *et al*., 1997; Bedford *et al*., 1998).

The beneficial effects of some feed enzymes for improving nutrient availability and bird performance are well-established (Bedford and Morgan, 1996). Wheat and barley may contain variable levels of soluble nonstarch polysaccharides, which reduce the performance of young broilers. In regions where the poultry industry uses these cereals, dietary enzymes can be used to reduce their effects (Annison and Choct, 1991). Phytic acid is present in some feeds and can bind minerals and proteins, reducing their availability. Supplementation of diets with phytase enzyme has been shown to increase protein digestibility (Sebastian *et al*., 1997). The value of enzymes for diets based on corn and soybeans is not well established in the scientific literature.

This paper reports on a series of studies that were performed to: 1) compare three methods of measuring nutrient availability; 2) measure the effect of a commercial feed enzyme preparation on nutrient availability of diets based on corn and soybeans and the performance of broilers fed these diets; and 3) compare standard feed formulation to that with lower energy levels and supplementation with the enzyme preparation.

MATERIALS AND METHODS

Digestibility Assays

Metabolizable energy [digestible energy (DE) when measured from ileal contents], and protein, starch, and fat digestibilities of the diets described in Table 1 were determined using three methods. The diets contained SBM (45% CP), extruded full-fat soybeans (SBE, 38% CP, extruded at 120 to 130 C for 10 to 20 s at a pressure of 70 atmospheres),[4] or roasted full-fat soybeans (SBR, 37% CP, treated at 100 C for 40 min),[5] and were formulated to contain 18% protein and 3,000 kcal/kg. Urease activity and protein solubility of the three SBM were measured in duplicate following the techniques of Araba and Dale (1990a) after treatment but before incorporation into the diets in order to evaluate protein quality (Table 2). Each of these diets was tested as is and supplemented with 0.1% Avizyme®1500,[6] which contains 800 μ/g xylanase from *Trichoderma longibrachiatum*, 6,000 μ/g protease from *Bacillus subtilis*, and 2,000 μ/g amylase from *Bacillus amyloliquifaciens*. The enzyme mixture replaced an equal amount of sand, included in the diets as inert filler.

In a first series of measurements, "true" values were determined using the methods described by Sibbald (1986). Seventy-two adult (15 mo of age) White Leghorn roosters were randomly distributed two to a cage to provide six replicates of two roosters each for each diet. After a 36-h period of starvation, each rooster was force-fed 25 g of the assigned diet and excreta were collected for 48 h afterward. "Endogenous" nutrient production was calculated from excreta collected from 12 roosters that underwent the same procedure without being fed.

A second series of measures used total excreta collection. Two hundred and fifty-two male Hubbard[7] broilers were randomly divided into seven replicates of six broilers each, and fed the test diets from 30 d of age. Feed intake was measured and excreta was collected for analysis every 12 h for 4 d between 37 and 40 d of age.

A third series of digestibility measures used analysis of ileal digesta. Four hundred and eighty male Hubbard broilers were randomly distributed into four replicates of 20 broilers each for each of the six diets. At 37 d, they were killed by cervical dislocation and dissected in order to

TABLE 1. Composition of the diets used for digestibility assays[1]

Ingredients	Diets 1 and 2	Diets 3 and 4	Diets 5 and 6
	(%)		
Corn	66.28	53.04	52.37
SBM 45% CP	27.48	. . .	. . .
Extruded soybeans 38% CP	. . .	. . .	35.62
Roasted soybeans 37% CP	. . .	35.47	. . .
Soya oil	1.26	. . .	. . .
Dicalcium phosphate	1.82	1.83	1.86
Limestone	1.14	1.20	1.15
NaCl	0.4	0.4	0.4
Vitamin and mineral premix	0.5	0.5	0.5
DL-Methionine	0.12	0.12	0.14
Inert filler (sand)	1.0	7.44	7.96
Calculated analysis			
AME, kcal/kg	3,000	3,000	3,000
Crude protein	18	18	18
Lysine	0.91	0.91	0.91
Methionine	0.40	0.40	0.40
Methionine + cystine	0.70	0.70	0.70
Crude fat	3.0	8.9	8.7
Calcium	0.95	0.95	0.95
Available phosphorus	0.42	0.42	0.42

[1]Diets 2, 4, and 6 included 0.1% of an enzyme preparation containing 800 μ/g xylanase, 2,000 μ/g amylase, and 6,000 μ/g proteinase.

[2]The premix contained per kilogram of diet: vitamin A, 10,500 IU; cholecalciferol, 2,100 IU; vitamin E, 22 mg; riboflavin, 4.4 mg; niacin amide, 40 mg; pantothenic acid, 14 mg; choline chloride, 560 mg; vitamin B_{12}, 16 μg; vitamin K_1, 5 mg; folic acid, 0.80 mg; biotin, 0.1 mg; Minerals: Selenium, 0.1 mg; iodine, 2 mg; cobalt, 0.2 m; copper, 4 mg; iron, 30 mg; zinc, 40 mg; manganese, 68 mg; sulfur, 125 mg.

TABLE 2. Urease activity and protein solubility in 0.2% potassium hydroxide of soybean subjected to three treatments

Treatment	Urease activity	Protein solubility
	(pH units of change)	(%)
Soybean meal (45% CP)	0.06	87.59
Extruded soybeans (38% CP)	0.03	89.12
Roasted soybeans (37% CP)	0.08	83.22

[4]Nutremix, Monte Alto, São Paulo, Brazil.
[5]Cargill SA, Monte Alto, São Paulo, Brazil.
[6]Finnfeeds International, Box 777, Marlborough, Wiltshire, U.K. SN8 1XN.
[7]Obtained from Perdigão AS Mococa, São Paulo, Brazil.

TABLE 3. Energy and CP specifications for performance trials

		Starter (1 to 21 d)		Grower (22 to 37 d)		Finisher (38 to 45 d)	
Trial	Diet[1]	AME	CP	AME	CP	AME	CP
		(kcal/kg)	(%)	(kcal/kg)	(%)	(kcal/kg)	(%)
1	All diets	3,050	20.0	3,100	19.0	3,150	17.7
2	SBM	3,050	20.0	3,100	19.0	3,150	17.7
	SBM+enzyme	2,946	20.0	2,994	19.0	3,043	17.7
	SBE	3,050	20.0	3,100	19.0	3,150	17.7
	SBE+enzyme	2,980	20.0	3,029	19.0	3,078	17.7
	SBR	3,050	20.0	3,100	19.0	3,150	17.7
	SBR+enzyme	2,964	20.0	3,013	19.0	3,061	17.7

[1]SBM is soybean meal (45% CP), SBE is extruded soybeans (38% CP), SBR is roasted soybeans (37% CP), + enzyme indicates the addition of 0.1% enzyme. Diet definitions for Trial 1 are the same as those for Trial 2.

obtain the digesta contained between Meckel's diverticulum and the ileocecal junction. Chromic oxide was included in the diets at a level of 0.5% replacing sand, and nutrient concentrations were calculated from the difference in concentration of chromic oxide in the diets and that in the digesta.

Material collected from each of the three digestibility trials was stored at –20 C. After thawing, it was dried by lyophilization and analyzed for CP, starch, fat, and energy according to methods described by the Association of Official Analytical Chemists (1990), and these values were used to calculate digestibility coefficients using formulas from Sibbald (1976). Values for TME and AME (or DE) were corrected for N balance (N intake – N excreted × 8.22 kcal). Digestibility of specific AA in the diets was determined using HPLC[8] as described by Llames and Fontaine (1994) on samples of the six diets and digesta from the third (ileal) determination.

Performance Trials

The first performance trial used 1,440 1-d-old Hubbard males distributed randomly into eight replicates of 30 birds each for each of the six dietary treatments. Diet ingredients and formulation were similar to those used in the digestibility trials and used the same samples of corn, SBM, SBE, and SBR. Feed enzyme was included at a level of 0.1% in Diets 2, 4, and 6. Starter diets were fed from 1 to 21 d, grower diets were fed from 22 to 37 d, and finisher diets were fed from 38 to 45 d. Energy and protein contents of the diets are shown in Table 3; Zanella (1998) provides specific details.

Weight gain, feed to gain ratio (FCR), and mortality to 45 d were calculated for each pen. At 25 d, one broiler per pen was slaughtered in order to determine the viscosity of the intestinal contents using a using a Brookfield Viscometer (Model LVDVII+CP),[9] expressed in centipoids (cps). At 45 d of age, two birds per pen were slaughtered and eviscerated in order to determine carcass weight as a percentage of live weight, abdominal fat, and breast (including skin and bone) weights as a percentage of carcass weight, and pancreas weight.

The second performance trial used 1,440 male 1-d-old Cobb[7] broiler chicks distributed randomly into eight replicates of 30 birds each. Six diet formulations were tested using ingredients described in Table 1 and CP and energy specifications described in Table 3. Energy specifications in Diets 2, 4, and 6, which included 0.1% enzyme, were 3.4, 2.8, and 2.3% lower for SBM, SBE, and SBR treatments, respectively, than were corresponding diets without enzyme. These reductions corresponded to the advantage associated with enzyme supplementation found in performance Trial 1. Starter, grower, and finisher diets were fed as described for Trial 1.

Weight gain, FCR, and mortality to 45 d were determined for each pen. At 45 d of age, two birds per pen were slaughtered and eviscerated for determination of carcass, abdominal fat, breast, and pancreas weights as described for Trial 1.

Statistical Analysis

Data were analyzed using the Sistema de Análises Estatística (ESTAT) program of Universidade Estadual Paulista by ANOVA including the effects of soybean treatment, enzyme addition, and the interaction between the two. Of all statistical analyses performed, the interaction between soybean treatment and enzyme addition was significant for only Tyr digestibility ($P < 0.05$). When more than two means were compared, they were separated using the Tukey means separation procedure.

RESULTS

A comparison of the three methods of measuring nutrient availability (Table 4) shows substantial differences between "true" digestibility, digestibility based on total excreta collection, and that based on analysis of ileal digesta. The digestibility (absolute values) of CP as measured by the "true" (Sibbald, 1986) method and total

[8]Nutris Tecnologia e Sistemas e Nutricão LTDA, Quatro Barra-Paraná, Brazil.

[9]Brookfield Engineering Labs, Stoughton, MA 02072.

TABLE 4. Protein, starch, fat, and energy digestibility of a corn soybean diet fed as is or supplemented with 0.1% enzyme determined by three methods[1]

Method	Treatment[2]	n	Protein	Starch	Fat	ME
			(% DM basis)			(kcal/kg)
Sibbald (1986)	SBM	6	70.9 ± 1.4	99.7 ± 0.04	90.4 ± 1.1	3,580 ± 32
	SBM+enzyme	6	73.6 ± 4.2	99.7 ± 0.1	92.2 ± 0.3	3,658 ± 17
	SBE	6	66.2 ± 2.3	99.8 ± 0.1	90.4 ± 0.6	3,553 ± 44
	SBE+enzyme	6	72.6 ± 2.3	99.6 ± 0.1	89.7 ± 1.2	3,619 ± 30
	SBR	6	70.3 ± 2.3	99.4 ± 0.2	85.3 ± 1.0	3,575 ± 55
	SBR+enzyme	6	74.1 ± 2.4(5)	99.6 ± 0.03	87.4 ± 0.6	3,592 ± 40
	Tmt					
	SBM	12	72.2 ± 2.2	99.7 ± 0.1	91.3 ± 0.6[a]	3,619 ± 21
	SBE	12	69.4 ± 1.8	99.7 ± 0.1	90.0 ± 0.6[a]	3,586 ± 27
	SBR	12	72.0 ± 1.7(11)	99.5 ± 0.1	86.4 ± 0.6[b]	3,584 ± 33
	Enzyme					
	–	18	69.1 ± 1.2	99.6 ± 0.1	88.7 ± 0.8	3,569 ± 25
	+	18	73.4 ± 1.7(17)	99.6 ± 0.1	89.8 ± 0.6	3,623 ± 18
Excreta	SBM	7	56.5 ± 1.1	98.5 ± 0.048	85.0 ± 0.5	3,176 ± 16
	SBM+enzyme	7	60.1 ± 1.3	98.7 ± 0.1	86.8 ± 0.4	3,245 ± 27
	SBE	7	59.6 ± 1.6	97.9 ± 0.1	90.9 ± 0.2	3,197 ± 29
	SBE+enzyme	7	60.9 ± 1.1	98.2 ± 0.1	90.8 ± 0.2	3,234 ± 14
	SBR	7	57.4 ± 0.8	98.2 ± 0.1	82.6 ± 1.5	3,198 ± 17
	SBR+enzyme	7	59.2 ± 1.6	98.3 ± 0.1	81.2 ± 0.4	3,197 ± 32
	Tmt					
	SMB	14	58.3 ± 1.0	98.6 ± 0.1[b]	85.9 ± 0.4[b]	3,210 ± 18
	SBE	14	60.3 ± 1.0	98.1 ± 0.1[a]	90.8 ± 0.1[a]	3,215 ± 16
	SBR	14	58.3 ± 0.9	98.2 ± 0.1[b]	81.9 ± 0.7[c]	3,198 ± 17
	Enzyme					
	–	21	57.8 ± 0.7[b]	98.2 ± 0.1[b]	86.2 ± 0.9	3,190 ± 12
	+	21	60.1 ± 0.8[a]	98.5 ± 0.1[a]	86.3 ± 0.9	3,225 ± 15
Ileal digesta	SBM	4	79.1 ± 0.5	89.3 ± 0.4	83.8 ± 0.5	3,071 ± 37
	SBM+enzyme	4	83.2 ± 0.9	91.5 ± 0.4	86.5 ± 0.3	3,160 ± 23
	SBE	4	82.0 ± 1.7	92.2 ± 0.8	90.0 ± 0.6	3,144 ± 37(3)
	SBE+enzyme	4	84.9 ± 0.8	94.0 ± 0.4	90.2 ± 0.1	3,224 ± 39
	SBR	4	79.0 ± 0.6	92.1 ± 0.4	81.4 ± 0.9	3,030 ± 42
	SBR+enzyme	4	80.6 ± 1.0	93.5 ± 0.6	83.5 ± 1.3	3,077 ± 29
	Tmt					
	SBM	8	81.2 ± 0.9[ab]	90.4 ± 0.5[b]	85.1 ± 0.6[b]	3,115 ± 26[ab]
	SBE	8	83.4 ± 1.0[a]	93.1 ± 0.5[a]	90.1 ± 0.3[a]	3,189 ± 30[a](7)
	SBR	8	79.8 ± 0.6[b]	92.8 ± 0.4[a]	82.4 ± 0.8[c]	3,054 ± 25[b]
	Enzyme					
	–	12	80.0 ± 0.7[b]	91.2 ± 0.5[b]	85.1 ± 1.1[b]	3,076 ± 25[b](11)
	+	12	82.9 ± 0.7[a]	93.0 ± 0.4[a]	86.7 ± 0.9[a]	3,153 ± 24[a]

[a–c]Main effect means within a column for each method with no common superscript differ significantly ($P < 0.05$).

[1]Mean ± SEM, when n differs from that given it is provided in parentheses after the number.

[2]SBM is soybean meal (45% CP), SBE is extruded soybeans (38% CP), and SBR is roasted soybeans (37% CP).

excreta collection was 10.2 and 22.5% lower, respectively, than that measured by analysis of ileal digesta. On the other hand, starch digestion was very high for the two methods based on excreta analysis. Fat digestibility was only slightly higher when measured by the "true" method than the other methods. The energy in the feed was substantially higher when measured by TME than when measured by total excreta collection (11.4% higher) or by analysis of ileal digesta (14.4% higher).

The "true" assay (Sibbald, 1986) demonstrated very little difference between dietary treatments. "True" fat digestion was significantly lower for the roasted soybeans, and the difference between enzyme treatments for CP digestion was nearly ($P = 0.057$) significant. Other differences between soybean and enzyme treatments were not significant. Total collection of excreta showed differences between soybean treatments in starch and fat digestibility and between enzyme treatments in CP and starch digestibility. In contrast, measuring ileal digesta demonstrated significant differences among soybean and enzyme treatment main effects for all four measures. The soybean treatment by enzyme interaction was not significant for any of the variables.

The six diets used for the digestibility trials were the same as those used for the first performance trial, permitting a regression analysis of the performance on energy content (Table 5). The regression coefficients of

TABLE 5. Regression of BW and feed conversion ratio (FCR) on nitrogen-corrected digestible energy (DE_n) and TME_n values

Regression equation	R^2	*P*
$BW = 0.00046 \times DE_n + 1.24$	0.69*	<0.05
$BW = 0.00051 \times TME_n + 0.84$	0.23	NS
$FCR = -0.00042 \times DE_n + 3.14$	0.90**	<0.01
$FCR = -0.00038 \times TME_n + 3.20$	0.20	NS

BW and FCR on N-corrected DE (DE_n) measured from ileal digesta were both significant, whereas those of BW and FCR on TME_n were not.

Measurement of ileal digesta suggested that digestion of CP in the diet using SBE was slightly better than that of the diet using SBM and significantly better than that in the diet using SBR. Analysis of the digestibility of individual AA (Table 6) suggests that extrusion improved AA digestibility; digestibility of 14 of the 16 AA measured was higher in the diets containing SBE than in one or both of the other diets.

The increase in overall CP digestibility (ileal) brought about by enzyme supplementation (Table 4) was 2.9% (absolute value, 3.6% relative increase) but was not the same for all AA (Table 6). Although the digestibility of 15 of the 16 AA was higher with enzyme supplementation, the difference was significant for only 5. The greatest difference was 4.7% for Tyr digestibility. Digestibilities of Cys, Val, and Thr were 2.1 ($P < 0.10$), 2.3, and 3.0% higher, respectively, with enzyme supplementation than without. The advantage of enzyme supplementation for the digestibility of Lys and Met, which are the most limiting AA, was not significant (1.2% for Lys) or was absent (for Met). There was a significant interaction for Tyr digestibility between soybean treatment and enzyme supplementation, with enzyme having the greatest effect on SBR. The importance of this effect is unknown.

The performance of broilers fed these six diets is shown in Table 7. Diets using SBE produced birds with higher BW and lower FCR than those based on SBR, with diets based on SBM producing intermediate results. Enzyme supplementation produced a 1.9% improvement in BW and a 2.2% reduction in the FCR. There were no interactions between soybean treatment and enzyme supplementation. Neither soybean treatment nor enzyme supplementation affected mortality, viscosity of intestinal contents, carcass weight, abdominal fat, breast weight, or pancreas weight.

Reducing the energy specifications in the diet formulation to account for the advantage of enzyme supplementation (Table 8) produced similar results to Performance Trial 1 for the soybean treatments (slightly higher BW and lower FCR for SBE). However, performance characteristics were not different between the higher energy treatment and the lower energy treatment that included enzyme supplementation. The soybean treatment by enzyme interaction was not significant for any variable.

TABLE 6. Amino acid digestibility measured from the ileal digesta[1]

Treatment[2]	n	Arg	Asp	Thr	Ser	Glu	Gly	Ala	Cys
		(%)							
SBM	4	88.0 ± 0.6	78.5 ± 1.0	77.4 ± 1.0	79.3 ± 0.4	85.5 ± 0.6	74.4 ± 0.6	83.2 ± 0.3	73.9 ± 0.6
SBM+enzyme	4	89.7 ± 0.5	82.3 ± 0.6	80.1 ± 0.5	84.4 ± 0.6	87.0 ± 0.6	78.1 ± 0.5	86.5 ± 0.5	78.2 ± 1.1
SBE	4	92.6 ± 0.6	85.5 ± 0.9	80.3 ± 1.4	84.4 ± 1.1	90.1 ± 0.6	79.7 ± 1.5	89.3 ± 0.8	76.6 ± 1.6
SBE+enzyme	4	93.5 ± 0.3	87.0 ± 0.3	84.1 ± 0.6	85.5 ± 0.2	81.5 ± 0.2	80.5 ± 0.4	89.8 ± 0.3	77.4 ± 0.7
SBR	4	87.8 ± 1.0	77.8 ± 1.5	74.1 ± 1.9	80.1 ± 1.0	84.0 ± 1.3	75.2 ± 1.9	84.6 ± 1.5	73.3 ± 0.9
SBR+enzyme	4	88.2 ± 2.2	79.7 ± 2.8	76.8 ± 2.3	81.0 ± 2.1	85.1 ± 2.5	76.7 ± 0.8	85.9 ± 2.0	74.4 ± 2.7
Treatment									
SBM	8	88.8 ± 0.5^{b}	80.4 ± 0.9^{b}	78.8 ± 0.7^{ab}	81.8 ± 1.0^{ab}	86.2 ± 0.5^{b}	76.3 ± 0.8^{b}	84.8 ± 0.7^{b}	76.0 ± 1.0
SBE	8	93.1 ± 0.3^{a}	86.2 ± 0.5^{a}	82.2 ± 1.0^{a}	85.0 ± 0.5^{a}	91.1 ± 0.3^{a}	80.1 ± 0.8^{a}	89.5 ± 0.4^{a}	77.0 ± 0.8
SBR	8	88.0 ± 1.1^{b}	78.8 ± 1.5^{b}	75.4 ± 1.4^{b}	80.6 ± 1.1^{b}	84.5 ± 1.3^{b}	76.0 ± 1.0^{b}	85.3 ± 1.2^{b}	73.9 ± 1.3
Enzyme									
–	12	89.4 ± 0.8	80.6 ± 1.2	77.3 ± 1.1^{b}	81.3 ± 0.8^{b}	86.7 ± 1.0	76.4 ± 1.0^{b}	85.7 ± 0.9	74.6 ± 0.7
+	12	90.5 ± 1.0	83.0 ± 1.3	80.3 ± 1.1^{a}	83.6 ± 0.9^{a}	87.9 ± 1.1	78.4 ± 0.6^{a}	87.4 ± 0.8	76.7 ± 1.0
Treatment	n	Val	Met	Ile	Leu	Tyr	Phe	Lys	His
		(%)							
SBM	4	78.8 ± 0.6	96.2 ± 1.8	81.1 ± 0.8	84.8 ± 0.4	81.9 ± 1.7	84.2 ± 0.5	86.3 ± 0.6	85.1 ± 0.2
SBM+enzyme	4	81.9 ± 0.5	97.4 ± 0.2	84.5 ± 0.7	87.7 ± 0.6	84.8 ± 1.3	86.3 ± 0.4	89.0 ± 0.5	88.1 ± 0.5
FSE	4	84.9 ± 1.1	98.8 ± 0.1	80.8 ± 1.5	88.9 ± 0.7	88.0 ± 1.1	89.9 ± 0.8	90.4 ± 0.7	90.1 ± 0.7
FSE+enzyme	4	86.6 ± 0.2	97.8 ± 0.05	81.9 ± 0.8	90.1 ± 0.3	88.2 ± 0.9	91.7 ± 0.2	91.4 ± 0.5	91.0 ± 0.3
FSR	4	78.9 ± 1.6	97.0 ± 0.2	79.5 ± 1.8	84.9 ± 1.2	76.5 ± 2.1	85.3 ± 1.0	85.1 ± 1.3	86.1 ± 0.9
FSR+enzyme	4	80.7 ± 2.3	96.7 ± 0.3	77.2 ± 2.2	84.8 ± 2.1	87.2 ± 2.3	83.1 ± 2.6	85.3 ± 2.5	85.3 ± 2.0
Treatment									
SBM	8	80.3 ± 0.7^{b}	96.8 ± 0.9	82.8 ± 0.8^{a}	86.3 ± 0.7^{b}	83.4 ± 1.1^{b}	85.2 ± 0.5^{b}	87.6 ± 0.6^{b}	86.6 ± 0.6^{b}
FSE	8	85.7 ± 0.6^{a}	98.3 ± 0.2	81.4 ± 0.8^{ab}	89.5 ± 0.4^{a}	88.1 ± 0.7^{a}	90.8 ± 0.5^{a}	90.9 ± 0.4^{a}	90.6 ± 0.4^{a}
FSR	8	79.8 ± 1.3^{b}	96.9 ± 0.2	78.4 ± 1.4^{b}	84.9 ± 1.1^{b}	81.9 ± 2.5^{b}	84.2 ± 1.4^{b}	85.2 ± 1.3^{b}	85.7 ± 1.0^{b}
Enzyme									
–	12	80.8 ± 1.1^{b}	97.3 ± 0.6	80.5 ± 0.8	86.2 ± 0.7	82.1 ± 1.7^{b}	86.5 ± 0.9	87.3 ± 0.8	87.1 ± 0.7
+	12	83.1 ± 1.1^{a}	97.3 ± 0.2	81.2 ± 1.2	87.5 ± 0.9	86.8 ± 1.0^{a}	87.0 ± 1.3	88.5 ± 1.1	88.1 ± 0.9

$^{a–c}$Main effect means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Mean ± SEM.

[2]SBM is soybean meal (45% CP), SBE is extruded soybeans (38% CP), SBR is roasted soybeans (37% CP), and + enzyme indicated the additon of 0.1% enzyme.

TABLE 7. Performance, digesta viscosity, and carcass characteristics of chicks fed six diets, Trial 1[1]

Treatment[2]	n	BW gain	Feed:gain	Mortality	Viscosity	Abdominal fat	Breast weight	Carcass weight	Pancreas weight
		(kg)	(g:g)	(%)	(cps)	—— (% of carcass) ——		(% of BW)	(g)
SBM	8	2.62 ± 0.02	1.87 ± 0.01	6.05 ± 1.28	2.3 ± 0.2	3.33 ± 0.20	27.8 ± 0.5	72.2 ± 0.2	4.61 ± 0.28
SBM+enzyme	8	2.69 ± 0.02	1.82 ± 0.01	4.03 ± 1.46	2.5 ± 0.2	3.26 ± 0.15	27.6 ± 0.3	73.0 ± 0.2	4.27 ± 0.21
FSE	8	2.68 ± 0.01	1.82 ± 0.01	3.63 ± 1.13	2.7 ± 0.02	3.57 ± 0.22	27.7 ± 0.5	72.3 ± 0.3	4.83 ± 0.42
FSE+enzyme	8	2.73 ± 0.01	1.80 ± 0.01	6.45 ± 1.22(7)	2.8 ± 0.1	3.09 ± 0.18	27.3 ± 0.4	72.5 ± 0.1	4.71 ± 0.20
FSR	8	2.64 ± 0.02	1.88 ± 0.02	6.86 ± 1.55	2.5 ± 0.1	3.21 ± 0.09	27.0 ± 0.3	72.5 ± 0.2	4.58 ± 0.17
FSR+enzyme	8	2.69 ± 0.02(7)	1.84 ± 0.02(7)	4.61 ± 1.38(7)	2.4 ± 0.1	3.05 ± 0.18	27.3 ± 0.2	72.6 ± 0.3	4.13 ± 0.23
Treatment									
SBM	16	2.65 ± 0.02^{b}	1.84 ± 0.01^{ab}	5.04 ± 0.98	2.4 ± 0.1	3.29 ± 0.12	27.7 ± 0.3	72.6 ± 0.2	4.44 ± 0.17
FSE	16	2.70 ± 0.01^{a}	1.81 ± 0.01^{b}	4.95 ± 0.88(15)	2.7 ± 0.1	3.33 ± 0.15	27.5 ± 0.3	72.4 ± 0.2	4.77 ± 0.23
FSR	16	2.66 ± 0.01^{ab}(15)	1.86 ± 0.01^{a}(15)	5.81 ± 1.05(15)	2.4 ± 0.1	3.13 ± 0.10	27.2 ± 0.2	72.5 ± 0.2	4.35 ± 0.15
Enzyme									
–	24	2.65 ± 0.01^{b}	1.86 ± 0.01^{a}	5.51 ± 0.79	2.5 ± 0.1	3.37 ± 0.10	27.5 ± 0.2	72.3 ± 0.1	4.67 ± 0.17
+	24	2.70 ± 0.01^{a}(23)	1.82 ± 0.01^{b}(23)	4.99 ± 0.79(22)	2.6 ± 0.1	3.13 ± 0.10	27.4 ± 0.2	72.7 ± 0.1	4.37 ± 0.13

$^{a–c}$Main effect means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Mean ± SEM, when n differs from that given it is provided in parentheses after the number.

[2]SBM is soybean meal (45% CP), SBE is extruded soybeans (38% CP), SBR is roasted soybeans (37% CP), and + enzyme indicated the additon of 0.1% enzyme.

DISCUSSION

Methods of measuring the availability of nutrients for chickens have been a subject of considerable debate over the last three decades (Achinewhu and Hewitt 1979; Farrell, 1981; Härtel, 1986; Sibbald, 1986; Askbrant, 1990; MacNab, 1996; Zelenka, 1997).

The "true" values for starch, fat, and energy digestion, obtained using starved, adult, White Leghorn roosters, force fed 25 g of diet, found here were higher than the other measures because they were "corrected" by subtraction of nutrients contained in the excreta of unfed birds. The "true" CP digestion was lower than that determined from ileal digesta because measures based on excreta include the effects of hindgut fermentation and contamination of the excreta by N or AA contained in the urinary system (ten Doeschate *et al.*, 1993; Lewis and Bayley, 1995), which results in CP digestibility determined from excreta (without correction factors) being lower than that based on ileal digesta.

Starch digestibility, as measured by Sibbald's (1986) method and by total excreta collection was very high, as has been found by other authors (Härtel, 1986; Noy and Sklan, 1995), but was lower when measured by analysis of ileal digesta. This result suggests that digestion of starch in the small intestine is incomplete and is finished by microorganisms in the ceca or hindgut, thereby eliminating variation between feeds. The similarity between values for starch and fat digestibility obtained by Sibbald's method and those found by other techniques indicates that the "correction" for endogenous production of starch and fat is very small.

With the exception of a difference in fat digestibility due to processing, the "true" assay found no differences

TABLE 8. Performance and carcass characteristics of chicks fed six diets, Trial 2[1]

Treatment[2]	n	BW gain	Feed:gain	Mortality	Abdominal fat	Breast weight	Carcass weight	Pancreas weight
		(kg)	(g:g)	(%)	—— (% of carcass) ——		(% of BW)	(g)
SBM	8	2.61 ± 0.02	1.76 ± 0.01	4.30 ± 1.01	2.83 ± 0.28	29.0 ± 0.3	72.3 ± 0.3	3.94 ± 0.24
SBM+enzyme	8	2.61 ± 0.02	1.77 ± 0.01	2.74 ± 0.92	3.14 ± 0.11	29.3 ± 0.5	72.1 ± 0.4	3.75 ± 0.21
FSE	8	2.65 ± 0.01	1.76 ± 0.003	2.35 ± 1.14	3.32 ± 0.27	27.8 ± 0.3	72.1 ± 0.4	3.94 ± 0.12
FSE+enzyme	8	2.66 ± 0.01	1.76 ± 0.01	2.74 ± 0.92	3.20 ± 0.12	28.6 ± 0.3	71.9 ± 0.3	4.14 ± 0.12
FSR	8	2.56 ± 0.01	1.81 ± 0.01	3.91 ± 1.54	3.25 ± 0.21	28.6 ± 0.4	72.0 ± 0.4	4.04 ± 0.18
FSR+enzyme	8	2.61 ± 0.02	1.81 ± 0.02	3.52 ± 0.92	2.77 ± 0.18	28.3 ± 0.3	72.9 ± 0.1	3.67 ± 0.09
Treatment								
SBM	16	2.61 ± 0.01^{b}	1.77 ± 0.01^{b}	3.52 ± 0.69	3.00 ± 0.15	29.1 ± 0.3^{a}	72.2 ± 0.2	3.84 ± 0.15
FSE	16	2.66 ± 0.01^{a}	1.76 ± 0.01^{b}	2.54 ± 0.71	3.26 ± 0.14	28.2 ± 0.2^{b}	72.0 ± 0.3	4.04 ± 0.09
FSR	16	2.58 ± 0.01^{b}	1.81 ± 0.01^{a}	3.71 ± 0.87	3.01 ± 0.15	28.5 ± 0.3^{b}	72.5 ± 0.2	3.86 ± 0.11
Enzyme								
–	24	2.60 ± 0.01	1.78 ± 0.01	3.52 ± 0.71	3.13 ± 0.15	28.5 ± 0.2	72.1 ± 0.2	3.97 ± 0.10
+	24	2.62 ± 0.01	1.78 ± 0.01	3.00 ± 0.51	3.04 ± 0.09	28.7 ± 0.2	72.3 ± 0.2	3.85 ± 0.09

$^{a–c}$Main effect means within a column with no common superscript differ significantly ($P < 0.05$).

[1]Mean ± SEM.

[2]SBM is soybean meal (45% CP), SBE is extruded soybeans (38% CP), SBR is roasted soybeans (37% CP), and + enzyme indicated the additon of 0.1% enzyme.

between treatments to be significant, in spite of differences as large as 4.3% (absolute value) between enzyme treatments. Measurement of excreta found some differences in CP, starch, and fat digestibility between treatments. Only the measurement based on ileal digesta was able to demonstrate differences between both soybean and enzyme treatments, and these differences were supported by feeding trial results. Regression analysis of the energy in the six feeds on BW and FCR showed that performance was closely related to DE_n determined from ileal digesta, but it had little relation to TME_n.

In the final analysis, the choice of an assay depends on the validity, the accuracy, and the ability of the assay to differentiate between materials having different nutrient content or digestibility. Protein and starch that survive the small intestine are altered in the hindgut, likely invalidating any method based on excreta collection. Endogenous nutrient losses are influenced by the amount and type of feed (Farrell, 1981; Angkanaporn *et al*., 1997) and digestibility is dependent on the age and type of bird (ten Doeschate *et al*., 1993; Zelenka, 1997), likely invalidating measures of TME for broilers. Accuracy and ability to differentiate between materials are related, and the analysis of ileal contents is the only one of three methods that differentiated clearly between dietary treatments. Methods based on the analysis of ileal digesta are used with increasing frequency and would appear to be the clear choice for the analysis of AA digestibility.

Traditional thought would suggest that these diets, based on corn and soybeans, would not be improved by enzyme supplementation. However, preliminary reports of trials using this commercial enzyme have demonstrated similar improvements to those shown here in digestibility (Pack *et al*., 1997) and broiler performance (Wyatt *et al*., 1997).

The starch component of corn is considered to be highly digestible, based primarily on measures obtained using Sibbald's (1986) technique. However, Brown (1996) summarized findings on starch that is resistant to digestion. Incomplete starch digestion at the ileum was completed in the hindgut, suggesting that some of the starch was indeed resistant. The enzyme mixture may have improved digestion of this fraction.

Urease activity in the soybean samples was well below the maximum acceptable level (National Research Council, 1994), protein solubility was well above that indicative of heat-damaged protein (Araba and Dale, 1990a), and trypsin inhibitors in the soybean samples did not cause an increase in pancreas weights. According to these standards, these soybean samples did not contain antinutritive substances; however, both digestibility and performance trials showed clearly that enzyme treatment improved nutrient utilization, growth, and FCR.

Studies on the effects of soybean processing (Sakomura, 1996) have found that the digestibility of energy, fat and AA is better in SBE than in SBR because the extrusion process ruptures the cell walls. This improvement in digestibility is reflected in the comparisons between SBE and SBR.

Marsmann *et al*. (1997) found that soybean processing altered nutrient digestibility, possibly by differential alteration of noncovalent interactions and disulfide bonds, an explanation that may be applicable here as well. Marsmann *et al*. (1997) also found that supplementation of soybeans with protease and carbohydrase enzymes, individually and in combination, improved the CP digestion, and that the carbohydrase improved the nonstarch polysaccharide digestibility of soybean meal. They suggested that the carbohydrase that they used also exhibited protease activity, supporting the observation by Pack *et al*. (1998) that improvements in a diet brought about by enzyme supplementation may depend on the specific enzyme preparation.

Whereas total protein digestion was improved by 2.9% by the use of the enzyme mixture, the effect was not equal for the individual AA. This difference may relate to the origin of the AA, because endogenous secretions, such as enzymes and sloughed cells, are rich in some AA (Bielorai *et al*., 1991; Angkanaporn *et al*., 1996). Diet is known to affect digestive function (Bedford, 1996) and the action of the enzyme supplementation may have been to improve overall digestion and reduce endogenous AA losses. This improvement in digestibility, in turn, would improve the energy efficiency of digestion, leaving more energy available for growth.

According to Baker *et al*. (1996) the limiting AA for boilers fed corn-soybean diets are Met, Lys, Thr, Val, and possibly Arg. Of these, the digestibility of only Thr (3.0%) and Val (2.3%) approached the overall level of improvement by enzyme. The addition of this enzyme mixture allowed a reduction of energy levels corresponding to the enzyme advantage without affecting performance. However, if the same rational is used to reduce protein formulation, care must be taken to ensure adequate levels of some specific AA.

In conclusion, the SBE diets had the highest digestibility coefficients, and broilers fed this ration had the highest weights and the best FCR. Supplementation of the diets with an enzyme mixture containing amylase, protease, and xylanase improved the digestibility of the nutrients and broiler performance and use of this mixture allowed a reduction in the energy formulation of the diets.

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