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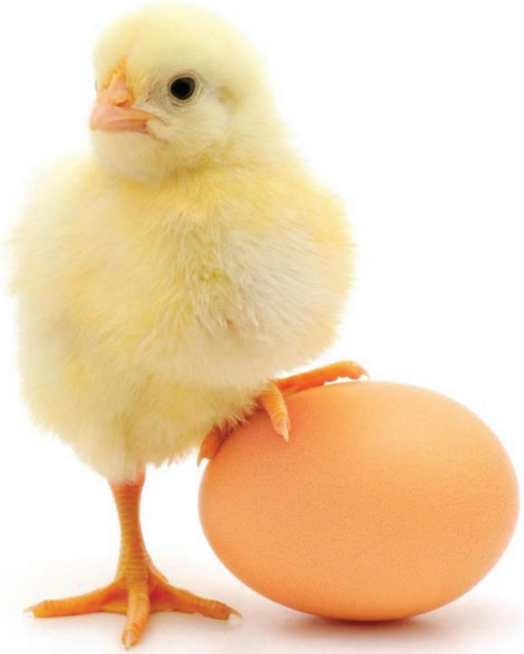

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Research Article
Maintenance Energy Requirements in Modern Broilers Fed Exogenous Enzymes

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Abstract

Objective: The objective of this study was to determine if exogenous enzymes reduce the metabolizable energy requirements for maintenance in broilers. **Materials and Methods:** Two feeds were tested, a negative control and negative control plus enzyme composite. The composite was a proprietary blend of glucanase+xylanase +cellulase+arabinofuranosidase+protease+phytase. Feed allowances were 30-100% of the *ad libitum* feed intake from 16-27 days. The retained energy in the carcass was evaluated as protein gain, $g\times5.45$ kcal g^{-1}+fat gain$\times8.95$ kcal g^{-1}. A linear regression of Y = Retention energy kcal/kg$^{0.70}$ was regressed by X = Metabolizable energy intake kcal/kg$^{0.70}$ where the metabolizable energy intake at zero carcass retention energy was the metabolizable energy of maintenance. **Results:** Body weight gain was +6.39 g day^{-1} with the enzyme treatment at *ad libitum* intake. The metabolizable energy for maintenance was 168 ± 4.2 kcal/kg$^{0.70}$ (R^2 = 0.98) for the enzyme treatment and 160 ± 4.5 kcal/kg$^{0.70}$ (R^2 = 0.98) for the control ($p<0.01$). The efficiency of energy utilization for maintenance and tissue gain was improved by 4 and 3%, respectively with the enzymes. The enzyme had -7.6 kcal/kg$^{0.70}$ metabolizable energy of maintenance which represents 4.5% lower ($p<0.01$) than the control. Energy savings from the enzyme composite ranged from 67 kcal kg^{-1} at *ad libitum* intake to 238 kcal kg^{-1} at 30% intake. **Conclusion:** The present study showed that the enzyme composite reduced the broiler energy requirement for maintenance and improved the efficiency for protein gain. To the authors' knowledge, this is the first research reporting that an enzyme composite decreases the maintenance energy and changes the tissue efficiency gain. Further investigation is required.

Key words: Maintenance, enzyme composite, modern broilers, poultry production, poultry feed, metabolizable energy, retained energy, heat production

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The poultry industry keeps improving the performance response and nutrient utilization of the broiler even when meat quality remains an issue[1]. Current and future studies will support the continuous improvement in the sustainability of poultry production. Energy remains the most expensive component of broiler feeds and maintenance energy requirements is a large component, being 42-44% of the energy intake[2]. Maintenance energy requirements include basal metabolism, thermogenesis and physical activity. Maintenance energy requirements need to be satisfied before tissue gain occurs[3], therefore, a reduction in the maintenance requirement will enable the animal to derive more nutrients for tissue gain or production. The addition of exogenous enzymes such as phytases, proteases, carbohydrases individually or in combination to poultry diets has been reported to improve performance and nutrient digestibility[4-9]. The partitioning of additional feed energy from enzymes into maintenance and production as well as the specific mechanisms involved in the energy savings is unclear. A better understanding of partitioning and energy saving mechanisms could explain the inability to measure a consistent response of exogenous enzymes in corn-soybean meal based diets[10]. Exogenous enzymes in poultry diets have been shown to decrease heat production (HP) using an indirect calorimetry system[11]. Based on maintenance energy being the largest component of HP, it is possible that enzymes could decrease this component. Metabolizable energy (ME) is the most common energy system for formulating poultry diets and normally feed intake is subject to ME concentration in the feed[12]. Maintenance energy requirement is defined as the requirement at zero tissue gain at normal physiological processes and health[13,14]. The ME maintenance requirement is determined from the intersection of the regression line for ME intake with the zero energy retention line[15], the linear regression of energy balance is fit between retained energy (RE) (RE = Y) and ME intake (MEI = X). The hypothesis of this study was to determine if adding this specific enzyme composite changes the metabolizable energy of maintenance and energy efficiencies for tissue gain in modern broilers.

MATERIALS AND METHODS

The University of Arkansas, Institutional Animal Care and Use Committee (IACUC) No. 12041 approved all management practices and procedures.

Birds, management and diets: Five hundred 1 day-old Cobb male broilers (Cobb Fayetteville, Arkansas hatchery) were reared in floor pens until day 15. On day 16, three hundred and eighty four broilers with initial BW of 449 g±27 sd (6.0% CV) were allocated to 96 wire metabolic cages (dimensions of each cage was 91×30 cm) with four broilers per cage. Temperature was reduced from 33°C for day 1 of age to 22°C at day 27. The lighting program was 23 hrs light: 1 hr dark. Two dietary treatments were tested. Diet 1: Negative control (NC) and Diet 2: NC+enzyme composite (NC+Enz). This proprietary enzyme composite (Victus®) included six different exogenous enzymes: (1) Glucanase produced from fermentation of *Aspergillus aculeatus*, (2) Xylanase produced from *Trichoderma longibrachiatum*, (3) Cellulase produced from *Trichoderma longibrachiatum*, (4) Arabinofuranosidase produced from *Trichoderma longibrachiatum* and *Aspergillus aculeatus*, (5) Serine protease with a chymotrypsin specificity from *Nocardiopsis prasina* (donor microorganism) expressed in *Bacillus licheniformis* (host or production microorganism), (6) Phytase from *Aspergillus oryzae* (Table 1). To make the NC+Enz treatment, the enzyme composite was added on-top at the rate of 350 g MT^{-1} to the basal Grower diet formulated with corn and soybean meal-based diet to have –100 kcal kg^{-1} and same amino acids and minerals as the 2018 Cobb 500 recommendations (Table 2). The negative control (NC) feed was mixed again to avoid differences on mixing between the two treatments. The basal diet contained 500 FYT kg^{-1} phytase activity with contributions of 0.10% Ca and 0.10% avP, so the added 1400 FYT kg^{-1} of phytase in the enzyme composite treatment was on-top and it did not have matrix contributions for minerals nor for amino acids. Prior to the experimental period, the NC+Enz birds were adapted to the enzyme composite by being fed the same enzyme composite during the starter period (1-15 day) at the

Table 1: Enzyme composition

[1]Enzyme composite	Description	Units	Per kg	Units kg^{-1} at 350 g MT^{-1} inclusion
β-glucanase	Endo-1,3(4)-β-Glucanase (*A. aculeatus*)	FBG	6,667	2.3
	Endo-1,3(4)-β-Glucanase (*T. longibrachiatum*)	U	186,667	65
Xylanase	Endo-1,4-β-Xylanase (*T. longibrachiatum*)	U	720,000	252
[2]Cellulase	Endo-1,4-β-Glucanase (*T. longibrachiatum*)	U	213,333	75
Arabin of uranosidase	L-arabinofuranosidase (*T. longibrachiatum* and *A. aculeatus*)	U	122,615	43
Protease	Protease (*Bacillus licheniformis*)	PROT	10,000,000	3,500
Phytase	Phytase (*Aspergillus oryzae*)	FYT	4,000,000	1,400

[1]Commercial name: Victus®, Ward[9], [2]A common terminology for cellulase glucanase

Table 2: Composition and nutrient calculations (g/100 g as fed) of the diet

	Basal	
Ingredient (%)	Starter 1-15 day	Grower 16-27 day
Yellow corn (8.3% CP)	48.78	59.62
Soybean meal (47.5% CP)	33.38	25.09
Wheat middlings (16.7%CP)	5.00	5.00
Corn DDGS (29.4% CP)	4.00	4.00
Poultry fat	4.51	2.26
DL-methionine	0.34	0.24
L-lysine HCl	0.36	0.28
L-threonine	0.16	0.11
Calcium carbonate	1.02	0.93
Dicalcium phosphate	1.60	1.17
Sodium chloride	0.35	0.35
Vitamin and mineral premix[1]	0.54	0.54
Propionic acid	0.050	0.050
Phytase[2]	0.005	0.005
Calculated composition		
ME (kcal kg^{-1})	2,975	3,008
Crude protein	21.5	20.1
Calcium[3]	0.90	0.81
Non-phytate phosphorus	0.45	0.41
Digestible lysine	1.22	1.02
Digestible methionine+cysteine	0.91	0.77
Digestible threonine	0.83	0.67
Digestible arginine	1.28	1.06
Analyzed composition		
AMEn (kcal kg^{-1})		2,966
Crude protein (%)	23.2	21.1
Calcium (%)	0.96	0.85
Total phosphorus (%)	0.74	0.68
Phytase (FYT kg^{-1})	487	374

[1]Vitamin premix, supplied per kilogram of diet: Antioxidant: 200 mg. Retinyl acetate: 21 mg, Cholecalciferol: 110 µg, D-α-tocopherol acetate: 132 mg, Menadione: 6 mg, Riboflavin: 15.6 mg, D-calcium pantothenate: 23.8 mg, Niacin: 92.6 mg, Folic acid: 7.1 mg, Cyanocobalamin: 0.032 mg, Pyridoxine: 22 mg, Biotin: 0.66 mg, Thiamine: 3.7 mg, Choline chlorine: 1200 mg, Mn: 100 mg, Mg: 27 mg, Zn: 100 mg, Fe: 50 mg, Cu: 10 mg, I: 1 mg, Se: 200 µg, [2]Ronozyme HiPhos, DSM, Nutritional Products LLC, Parsippany, NJ. The enzyme was included at a rate of 50 g MT^{-1} to the basal diet to supply a guaranteed minimum of 500 FYT kg^{-1} phytase activity, [3]Includes contribution from phytase of 0.10% Ca and 0.10% digestible P

Table 3: Enzyme activity analysis in feed

Enzyme composite	Treatments	Diet	Enzyme analyzed (U kg^{-1})	Target (U kg^{-1})	Guarantee (%)
β-Glucanase (U kg^{-1})	NC+Enz	Starter	112	70	160
		Grower	83	65	128
Xylanase (U kg^{-1})	NC+Enz	Starter	449	270	166
		Grower	257	252	102
Cellulase (U kg^{-1})	NC+Enz	Starter	133	80	166
		Grower	97	75	129
Protease (PROT kg^{-1})	NC+Enz	Starter	5711	5625	102
		Grower	3436	3500	98
Phytase (FYT kg^{-1})	NC	Starter	487	500	97
		Grower	374	500	75
	NC+Enz	Starter	1881	2000	94
		Grower	2084	1900	110

Dose in the feed: Victus starter: 375 g MT^{-1}, Victus grower: 350 g MT^{-1}, Arabinofuranosidase was analyzed in the enzyme composite but not in the feed due to difficulty on the assay

rate of 375 g MT^{-1}, however broilers were selected to have the same initial body weight between treatments on day 16. From 16-27 day of age, each diet was fed at eight controlled feeding levels: 30, 40, 50, 60, 70, 80, 90 and 100% of *ad libitum* consumption with 6replications per treatment. The amount of feed was increased daily for 11 days (16-27 day) based on feed intake of the *ad libitum* group. Samples of each diet were sent for enzyme analysis verification to a commercial enzyme laboratory (TMAS, DSM Nutritional Products, Belvidere, NJ) (Table 3).

Chemical analysis: The analysis of AMEn was evaluated for the *ad libitum* group of the control treatment. The AMEn involved analysis of gross energy, dry matter and nitrogen in feed and excreta. Gross energy (GE) was determined with a bomb calorimeter (Parr 6200 bomb calorimeter, Parr Instruments Co., Moline, IL.). Dry matter was analyzed by method 934.01[16] and nitrogen determined by the method 990.03[17]. The AMEn assay was determined by the classical total excreta collection method. The broilers underwent an adaptation to the experimental diets for 4 days (16-20 day) before excreta collection for 3 days. On the third day of collection, the excreta collections were pooled within a metabolic cage, mixed and a representative sample (120 g) was lyophilized in a freeze dryer. The lyophilized excreta samples were ground with a commercial grinder to pass through a 0.5 mm sieve. Samples were sent to the Central Laboratory at the University of Arkansas for analysis (dry matter, gross energy and nitrogen).

Body composition analysis: Broilers were analyzed for whole body composition at 16 and 27 day using the dual energy X-ray absorptiometry (DEXA). The body of 20 broilers of the same initial weight were scanned on day 16 to have the initial body protein, body fat and body mineral composition. At 27 day, broilers were humanely sacrificed by CO_2 inhalation. All 360 broilers were scanned individually by DEXA for body composition analysis. The DEXA values were adjusted using the feed restricted broiler equations as described by Caldas[11]. Briefly, the equations are:

Body protein:

$$\text{g} = 0.149\times\text{DEXA Lean g}^{1.02}$$

Body fat:

$$\text{g} = -15.9+0.095\times\text{DEXA tissue, g}+0.28\times\text{DEXA fat, g}-0.468\times\text{DEXA area, cm}^2$$

Body mineral:

$$\text{g} = e^{[1.73+0.51\times\text{Ln(Dexa BMC, g)}]}$$

Calculations: Body weight gain and feed conversion ratio (FCR) were calculated from 16-27 day, taking initial and final body weights. The FCR was accounted as 1 point for every 0.01 g g^{-1} value. The AMEn in the feed was calculated according to the equation by Hill and Anderson[18]:

$$\text{AME}_\text{n} = \frac{(\text{GE}_\text{d}\times\text{FI})\text{-}[(\text{GE}_\text{exc}.\times\text{Exc.})+(\text{N}_\text{d}\times\text{FI, g-N}_\text{exc}.\ \text{g g}^{-1}\times\text{exc. g})\times(8.22\ \text{kcal g}^{-1})]}{\text{FI}}$$

where:

AME_n = Apparent metabolizable energy, nitrogen corrected
Ge_d = Gross energy in the diet (kcal kg^{-1})
FI = Feed intake (kg)
Exc = Excreta output (kg)
N_d = Nitrogen in the diet (g g^{-1})
$\text{N}_\text{exc.}$ = Nitrogen in the excreta (g g^{-1})

MEI (metabolizable energy intake, kcal/$kg^{0.70}$) was calculated as:

$$\text{MEI} = \text{FI (kg)}\times 2966\ \text{kcal/kg/av. BW, kg}^{0.70}$$

AMEn = 2966 was the result of the energy evaluation in the feed.

The body compositions for protein, fat and bone mineral content (BMC) were reported as dry matter (DM) g kg^{-1} of body weight.

RE (retained energy, kcal/$kg^{0.70}$) was calculated as described by Caldas *et al.*[19]:

$$\text{RE} = \frac{\text{Protein gain (g)}\times 5.45\ \text{kcal g}^{-1}+\text{Fat gain (g)}\times 8.95\ \text{kcal g}^{-1})}{\text{av. BW, kg}^{0.70}}$$

Retained fat (RF) and protein (RP) (g day^{-1}) were calculated as the fat and protein at 27 day minus the tissue composition at 16 day. When fitting the linear regression of Y = Retained fat or protein vs X = Feed intake (FI) g day^{-1}, tissue retention for each treatment were calculated with the equation RF = -5.35+0.0934×FI -0.107 when calculating for the NC and +0.107 for the NC+Enz treatment (Fig. 2). {NC»-0.107, NC+Enz. »+0.107, else»} means that when no enzyme composite is added to the diet the equation subtracts 0.107 and when enzyme is added, the equation adds 0.107.

In similar manner, retained protein was calculated as RP = -0.29+0.107×FI -0.103 for NC and+0.103 for NC+Enz (Fig. 3). The slope of both equations in Fig. 2 and 3 is the tissue retention (fat or protein) g g^{-1} feed allowance and the intercept (first value of the equation) it is the value of fat or protein retention when feed intake is zero.

HP (Heat production) kcal/$kg^{0.70}$ was calculated to be = MEI-RE.

$\text{Av.BW}^{0.70}$ was the average metabolic body weight from the initial and final body weight of the feeding study elevated to the power of 0.70[20].

The energy value of the enzyme composite (kcal kg^{-1}) or matrix in the formulation was calculated by MEm (NC+Enz) minus MEm (NC) divided by the actual FI in each phase of feed restriction.

Statistical analysis: For all parameters, the experimental unit was one cage, only clarifying that for body composition the average of four broilers within each cage was pooled to make one replicate. The body weight gain (BWG), FCR and body composition data means were analyzed by ANOVA within each feed allowance level and initial body weight was included as a covariate, the means were separated by t-student and p-value was considered significant when $p \leq 0.05$. Body composition (g kg^{-1}) was also analyzed by a 2×8 factorial design (diet×feed allowance) with initial body weight as covariate. The means of feed allowance means were separated by Tukey HSD (honestly significant difference) test and p -value was considered significant when $p \leq 0.05$. Fat and protein retention (g day^{-1}) was fitted against g day^{-1} FI as a multiple linear regression (MLR), having FI and diet in the X-axis and fat or protein retention in the Y-axis. For the determination of MEm, a MLR analysis was performed including the diet effect to obtain the difference of MEm between diets. Retained energy (RE) as the dependent variable was regressed on metabolizable energy intake (MEI) adding the diet effect in the X-axis, as part of the equation according to Farrell[15]. The MEm was calculated by inverse prediction when RE = zero (0) for NC and NC+Enz. Another linear regression was fitted separately by diet and the slope of the equation was used for determining efficiency of energy utilization for gain (k_g). A logarithmic curve was fitted between HP by MEI building parameters for:

$$a \times \exp^{b \times MEI}$$

Where:

a = NEm (net energy of maintenance)

The efficiency of energy utilization of maintenance (k_m) was calculated with the ratio NEm/MEm[13]. All data were analyzed using JMP15.2[21].

RESULTS

The enzyme analysis in the feed from the experimental period resulted in 75-129% of the calculated inclusion levels showing the expected units of enzymes were in the experimental feed NC+Enz (Table 3).

Body weight gain (BWG), FCR and body composition: Body weight gain from 16-27 day was +2.02 g day^{-1} and +6.39 g day^{-1} for NC+Enz was significantly improved ($p<0.05$) for NC at 60% and 100% feed allowance, respectively. There was a tendency of higher BWG with NC+Enz at 40% feed allowance ($p = 0.065$) and 80% feed allowance ($p = 0.063$) (Table 4). The FCR was significantly better for NC+Enz at 40% (-22 points), 60% (-10 points) and 100% (-11 points) of feed allowance ($p<0.05$) and tendency to be better at 70% (-8 points) and 80% (-8 points) feed allowance ($p = 0.089$ and 0.078, respectively). The body fat composition (g kg^{-1}) was higher for the NC+Enz treatment (NC+Enz: 182 vs NC: 162 g kg^{-1} DM) only at 50% feed allowance ($p = 0.044$) (Table 5) and the difference across all feed allowances was not significant ($p>0.05$) (Table 6). As feed allowance increased from 30-100%, the fat component in the body of the broilers increased from 140-304 g kg^{-1} ($p<0.01$), the fat for broilers fed *ad libitum* was more than twice the fat amount for broilers fed at 30% feed allowance (Table 6). The body protein composition (g kg^{-1}) was lower for the NC+Enz treatment (NC+Enz: 613 vs NC:616 g kg^{-1}) at 60% feed allowance ($p = 0.025$) and a tendency of lower body protein at 70% feed allowance ($p = 0.079$) (Table 5). Protein between diets across all feed allowances was not different ($p>0.05$) (Table 6). As feed allowance increased from 30-100%, body protein decreased from 643-589 g kg^{-1}($p<0.01$) but the difference was not as large as the change in body fat composition (Table 6). The body mineral composition between diets was not significant ($p>0.05$) but as feed allowance increased, the mineral composition decreased from 101-80 g kg^{-1} ($p<0.01$) (Table 6). Body composition changes from 30-100% feed allowance are depicted in graphs per each dietary treatment (Fig. 1). At the lowest feed allowance (30%), the broiler prioritized protein synthesis and as more feed was provided, the amount of fat deposition increased for both dietary treatments. Broiler body protein and mineral composition both decreased as more feed was allowed.

The fat and protein gain (g day^{-1}) reported in Fig. 2 and 3, respectively, were different between diets ($p \leq 0.05$). Fat retention in broilers fed NC+Enz was 0.214 g day^{-1} (0.107×2) more than broilers fed NC (Fig. 2). Fat retention increased at a rate of 0.093 g day^{-1} for each gram increased FI ($p \leq 0.05$). The equation predicts that at zero feed intake, the broiler would be losing 5.35 g day^{-1} of fat during 16-27 day. Protein retention in broilers fed NC+Enz was 0.206 g day^{-1} (0.103×2) more than broilers fed NC ($p<0.05$) (Fig. 3). Protein increased at a rate of 0.107 g day^{-1} for each g of increased FI ($p \leq 0.05$). The equation predicts that at zero feed intake, the broiler would be losing 0.29 g day^{-1} of protein during 16-27 day ($p \leq 0.05$).

Maintenance energy (MEm, NEm), k_m, k_g, ERf, ERp: The MEm was 168.1 ± 4.2 kcal/$kg^{0.70}$/day for negative control (NC) broilers and 160.5 ± 4.5 kcal/$kg^{0.70}$/day for broilers from

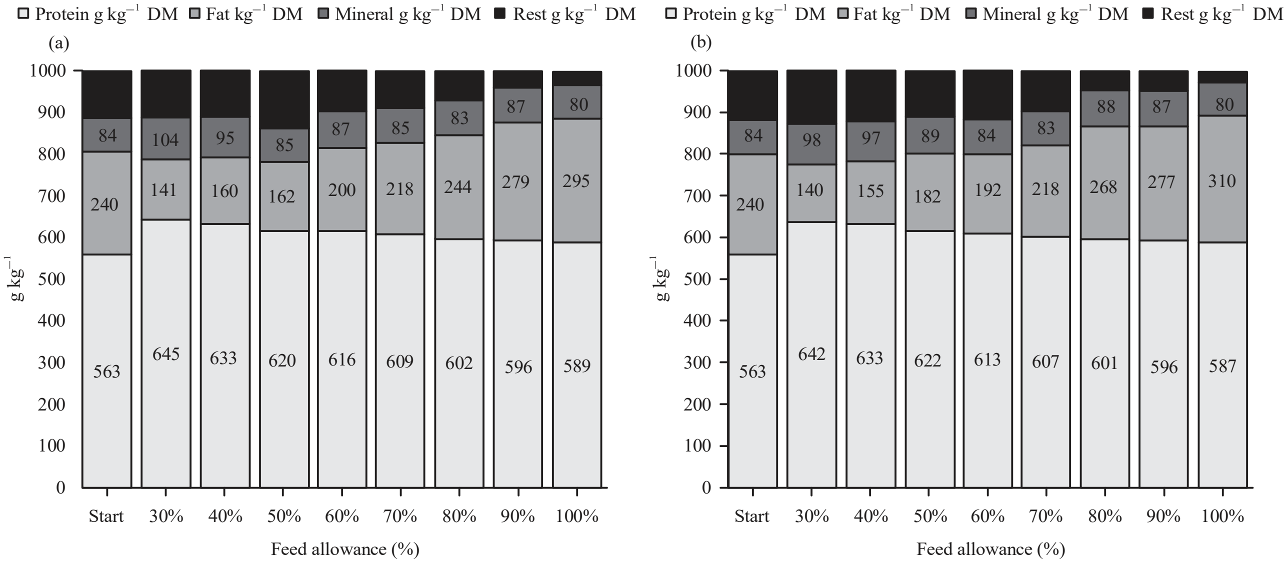

Fig. 1(a-b): Body composition of broilers fed 30-100% of *ad libitum* intake at 27 day, Start: Body composition at 16 day

NC: Negative control diet, NC+Enz: Negative control plus enzyme composite, DM: Dry matter

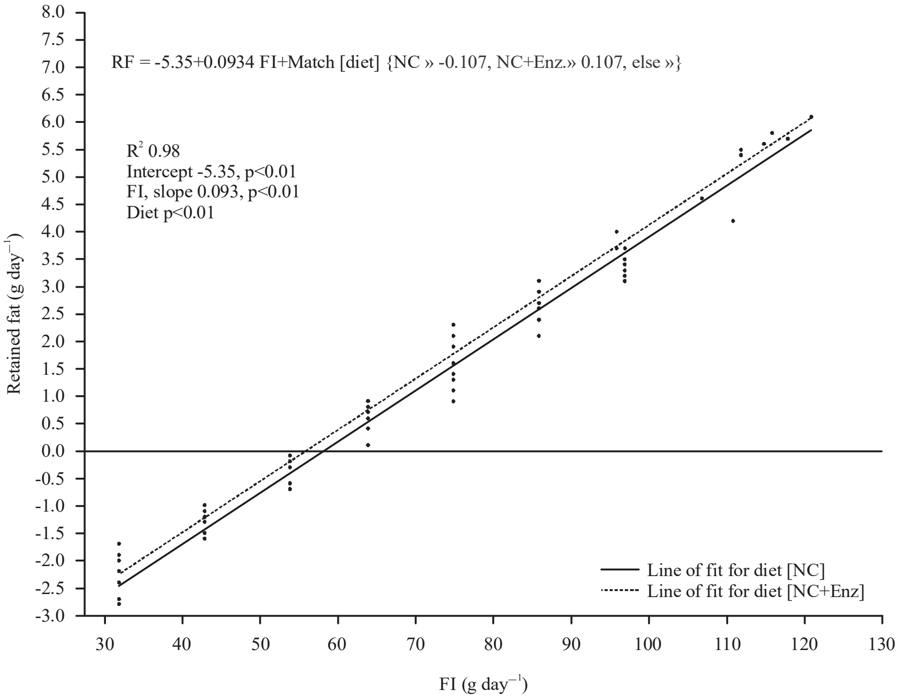

Fig. 2: Retained fat g day^{-1} for broilers 16-27 day fed increasing feed allowance

RF: Retained fat g day^{-1}, FI: Feed intake g day^{-1}, NC: Negative control, NC+Enz: Negative control plus enzyme composite, Match [diet] {NC »-0.107, NC+Enz. »0.107, else»} means that when no enzyme is added to the diet the equation subtracts -0.107 and when enzyme is added, the equation adds +0.107

the NC+Enz group ($p<0.05$). The equation coefficients of the multiple linear equation are presented in Fig. 4 and the values in Table 7. The calorie differences between treatments were 7.6 kcal/kg$^{0.70}$/day which represents +4.5% energy improvement, inferring that broilers fed the composite enzymes need less energy for maintenance and start partitioning energy toward the growing process faster than the broilers fed the diet without the composite enzyme.

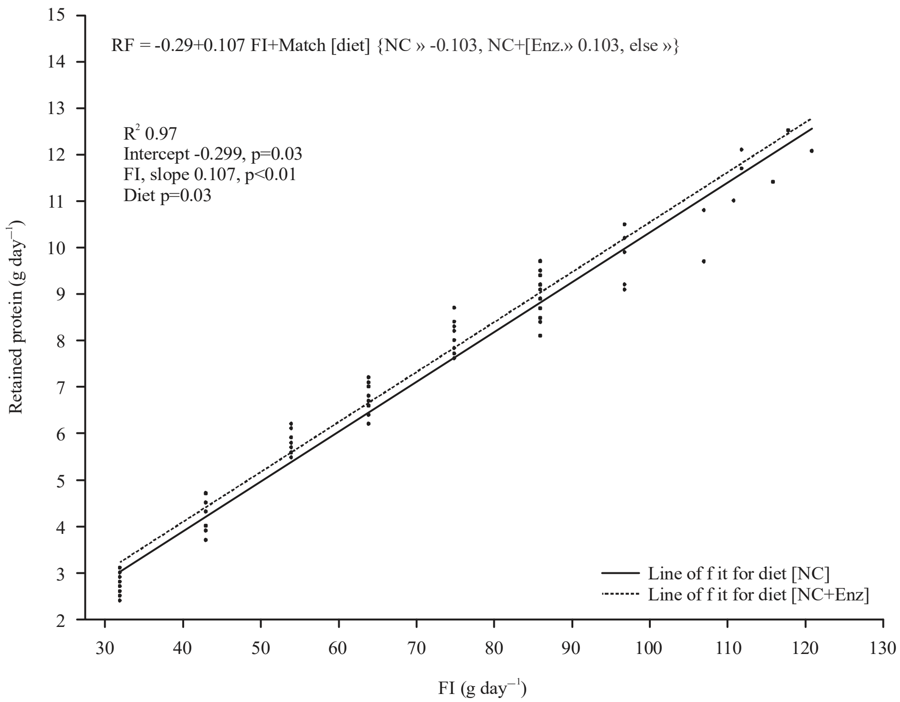

Fig. 3: Retained protein g day^{-1} for broilers 16-27 day fed increasing feed allowance

RP: Retained protein g day^{-1}, FI: Feed intake g day^{-1}, NC: Negative control, NC+Enz: Negative control plus enzyme composite. Match [Diet] {NC »-0.103, NC+Enz. »0.103, else»} means that when no enzyme composite is added to the diet the equation subtracts -0.103 and when enzyme is added, the equation adds +0.103

Table 4: Body weight gain and FCR of broilers between 16-27 day

		Body weight gain, g day^{-1} (16-27 day^{-1})				FCR, g g^{-1} (16-27 day)			
Feed allowance	Initial BW, g (16 day)	NC	NC+Enz	p-value initial BW	p-value diet	NC	NC+Enz	p-value initial BW	p-value diet
30%	445	9.71	11.34	0.061	0.410	3.43	3.28	0.010	0.732
40%	454	17.72	19.34	0.269	0.065	2.44	2.22	0.181	0.039
50%	446	28.29	29.26	0.357	0.210	1.91	1.83	0.295	0.139
60%	439	35.49	37.51	0.913	0.043	1.82	1.72	0.942	0.035
70%	436	44.68	46.49	0.641	0.153	1.69	1.61	0.656	0.089
80%	453	52.39	55.01	0.406	0.063	1.64	1.56	0.456	0.078
90%	467	61.47	62.67	0.136	0.394	1.56	1.54	0.104	0.433
100%	459	71.18	77.57	0.153	0.045	1.57	1.48	0.438	0.041

Means within a row are different at p-value diet <0.05, p-value initial BW: Initial body weight was a covariate, p-value diet: compares the difference between NC and NC+Enz, NC: Negative control, NC+Enz: Negative control plus enzyme composite

Table 5: Body composition: fat, protein and mineral g kg^{-1} dry matter of broilers at 27 day

	Fat g kg^{-1} DM				Protein g kg^{-1} DM				Mineral g kg^{-1} DM			
Feed allowance	NC	NC+Enz	p-value initial BW	p-value diet	NC	NC+Enz	p-value initial BW	p-value diet	NC	NC+Enz	p-value initial BW	p-value diet
30%	141	140	0.016	0.905	645	642	0.166	0.344	104	98	0.217	0.108
40%	160	155	0.054	0.536	633	633	0.073	0.948	95	97	0.238	0.251
50%	162	182	0.217	0.044	620	622	0.013	0.282	85	89	0.068	0.123
60%	200	192	0.050	0.305	616	613	0.001	0.025	87	84	0.209	0.271
70%	218	218	0.946	0.986	609	607	0.038	0.079	85	83	0.446	0.601
80%	244	268	0.627	0.244	602	601	0.066	0.790	83	88	0.913	0.272
90%	279	277	0.740	0.829	596	596	0.792	0.838	87	87	0.657	0.884
100%	295	310	0.743	0.378	589	587	0.068	0.368	80	80	0.786	0.715

Means within a row are different at p<0.05, p-value initial BW: Initial body weight was a covariate, p-value diet: Compares the difference between NC and NC+Enz, BW: Body weight, NC: Negative control, NC+Enz: Negative control plus enzyme composite, DM: Dry matter, SE: Standard error

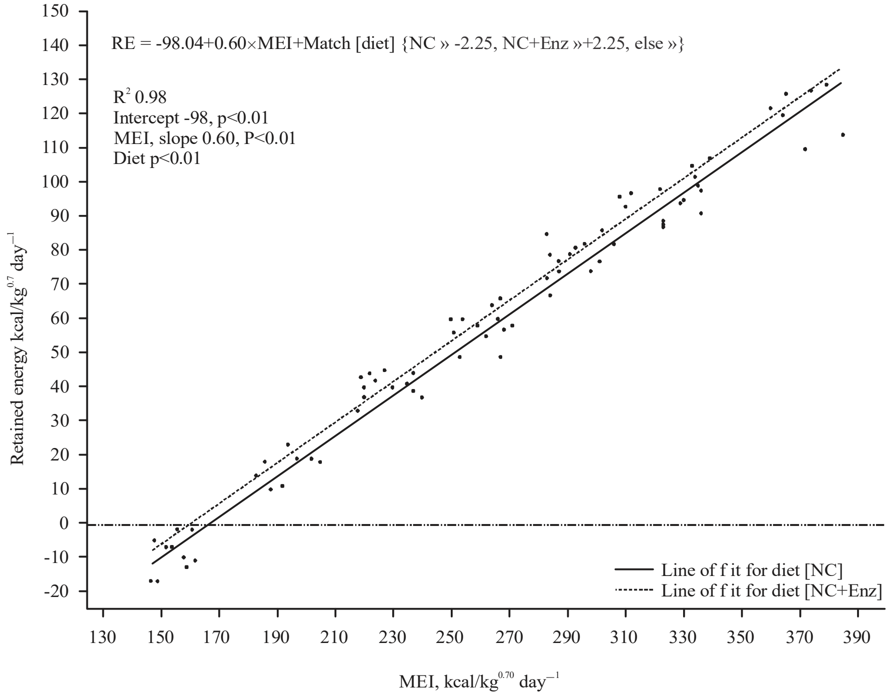

Fig. 4: Linear regression: Retained energy (RE) regressed on MEI (metabolizable energy intake) kcal/kg$^{0.70}$/day

RP: Retained protein g day^{-1}, MEI: Metabolizable energy intake, NC: Negative control, NC+Enz: Negative control plus enzyme composite. Match [Diet] {NC »-2.25, NC+Enz. »2.25, else »} means that when no enzyme composite is added to the diet the equation subtracts 2.25 and when enzyme is added, the equation adds 2.25

Table 6: Body composition (factorial design): fat, protein and mineral g kg^{-1} dry matter of broilers at 27 day

Feed allowance	Fat g kg^{-1} DM	Protein g kg^{-1} DM	Mineral g kg^{-1} DM
NC	139	645	104
NC+Enz	140	642	98
30%	140^{f}	643^{a}	101^{a}
40%	158^{ef}	633^{b}	96^{a}
50%	172de	620^{c}	87^{bc}
60%	194^{cd}	614^{d}	85^{bc}
70%	215^{c}	606^{e}	83^{bc}
80%	257^{b}	602^{f}	86^{bc}
90%	282^{ab}	597^{g}	88^{b}
100%	304^{a}	589^{h}	80^{c}
RMSE	21.31	3.56	5.67
	------------------------------p-values	--------------------------	
Diet	0.998	0.107	0.222
Feed allowance	<0.001	<0.001	<0.001
Diet×allowance	0.421	0.659	0.207
Initial body weight	0.012	<0.001	0.093

$^{a-h}$Means with no common superscripts within a column are different at $p<0.05$, p-value initial BW: Initial body weight was a covariate; p value diet: Compares the difference between NC and NC+Enz, NC: Negative control, NC+Enz: Negative control plus enzyme composite, DM: Dry matter, RMSE: Root mean square error

The energy value of the enzyme composite in the formulation was 67 kcal kg^{-1} for *ad libitum* consumption and 236 kcal kg^{-1} at 30% of the *ad libitum* feed intake which was close to the maintenance feed intake (33% of the *ad libitum* intake) (Table 8). The net energy of maintenance (NEm) was 121.21 kcal/kg$^{0.70}$/day for NC and 121.67 kcal/kg$^{0.70}$/day for NC+Enz. The net energy of maintenance values are very similar between treatments but valuable to evaluate the energy efficiency for maintenance, k_m (Nem MEm^{-1}). The k_m was 4% units higher for the NC+Enz, 75% vs 71%NC (Table 9). The energy efficiency for gain (k_g) was 3% units higher for NC+Enz, 61% vs 58% NC (Table 9).

DISCUSSION

The analyzed AMEn of the experimental basal diet was 2,966 kcal kg^{-1} and very close to the formulated energy of 3,008 kcal kg^{-1} for this study. The total collection method used for the energy evaluation may have reduced the variability compared to when markers are used. The higher body weight gain when broilers were fed the diet with the enzyme composite is indicating there is a better nutrient utilization when adding exogenous enzymes to broiler feeds. It is interesting that BWG and FCR were significantly different or close to being significantly different for 40, 60, 80 and 100% of feed allowance and not in the others. Since the enzyme composite was added extra to 100% of the basal diet, the amino acid and mineral matrixes for the enzyme composite

Table 7: Metabolizable energy intake (MEI), retained energy (RE), heat production (HP) in broilers from 16-27 day

	MEI (kcal/kg$^{0.70}$)		RE (kcal/kg$^{0.70}$/day)				HP (kcal l/kg$^{0.70}$/day)		
Feed allowance	NC, NC+Enz	NC	NC+Enz	p-value initial BW	p-value diet	NC	NC+Enz	p-value initial BW	p-value diet
30%	155	-8.7	-7.6	0.032	0.732	164	163	0.535	0.812
40%	193	15.1	17.4	0.026	0.258	179	174	0.028	0.161
50%	227	39.9	40.9	0.253	0.664	188	186	0.006	0.633
60%	261	54.9	60.5	0.131	0.033	208	199	0.014	0.034
70%	290	73.9	78.7	0.076	0.089	218	210	0.284	0.088
80%	312	87.8	91.0	0.219	0.293	226	219	0.083	0.186
90%	333	99.3	99.1	0.003	0.945	234	234	0.834	0.957
Ad lib	371	117.9	125.4	0.555	0.142	253	246	0.073	0.295

Means within a row are different at $p<0.05$. P-value initial BW: Initial body weight was a covariate, p value diet: compares the difference between NC and NC+Enz, MEI: Metabolizable energy intake, RE: Retained energy, HP: Heat production, NC: Negative control, NC+Enz: Negative control plus enzyme composite

Table 8: Metabolizable energy value of the multi-enzyme (NC+Enz) at different feed allowance levels in broilers from 16-27 day

Feed allowance	Feed intake (g day^{-1})	[1]ME kcal kg^{-1} of the enzyme composite
30%	32	236
40%	43	176
50%	54	140
60%	64	118
70%	75	101
80%	86	88
90%	97	78
100%	113	67

[1]ME kcal kg^{-1} = (7.6 kcal/feed intake) × 1000, NC: Negative control, NC+Enz: Negative control plus enzyme composite

Table 9: Retained energy (RE), heat production (HP) and energy efficiency (k_g) for gain and maintenance (k_m) in broilers from 16-27 day

Treatments	Model expression	R^2	RMSE	Energy requirements kcal kg^{-1} BW$^{0.70}$/day	Efficiencies
NC	[1]RE = -132+0.58xMEI	0.98	5.66	MEm = 168 (163-172)	$k_g = 0.58$
NC+Enz	RE = -99.49+0.61xMEI	0.98	5.79	MEm = 160 (155-164)	$k_g = 0.61$
NC	[2]HP = 121.21 e$^{(0.0020xMEI)}$	0.95	5.89	NEm = 121.21	$k_m = 0.71$
NC+Enz	HP = 121.67 e$^{(0.0019xMEI)}$	0.95	5.70	NEm = 121.67	$k_m = 0.75$

[1,2]RE, HP are in kcal/kg$^{0.70}$, MEI: Metabolizable energy intake, kcal/kg$^{0.70}$, NC: Negative control, NC+Enz: Negative control plus enzyme composite, RMSE: Root mean square error, MEm: Metabolizable energy for maintenance, NEm: Net energy for maintenance, k_g: Energy efficiency for gain, it's the slope of the RE equation, k_m: Energy efficiency for maintenance, it's the ratio Nem Mem^{-1}

were not accounted for in the formulations. There may have been an excess or deficiency of nutrients at different levels of feed restriction. Studies with super dose phytase show a better performance response of broilers fed with higher levels of phytase[7,22,23]. Broilers are also very responsive to higher levels of amino acids[24,25] but the responses to higher phytase and amino acids were performed with full fed broilers and the partitioning of nutrients and metabolism is probably very different with feed restricted broilers. A lack of a significant response for BWG and FCR with added enzyme composite for several restriction levels may have been caused by imbalances in nutrients because of priority differences in nutrient partitioning but statistical power analysis shows that nine replications instead of six would have made a significant difference between treatments. The explanation may be that not enough replications were used for the ANOVA analysis for all levels of restriction.

The body composition of the young broiler changed according to the feed intake. Water represents the highest proportion of the BW, being 72% at 27 day[19]. When adjusted to a DM basis, protein represents the highest portion of BW, followed by fat, minerals and remaining components to achieve 100%. The other components may be glycogen in the body or variability of the calculations. The body composition in g kg^{-1} or percentage showed small differences between dietary treatments, which may have added to the complexity of obtaining statistical differences from small changes using regression analysis. The body composition differences, however, clearly changed between feed allowances. With increasing feed intake, the body fat content increased and protein decreased, so fat gain increased at a higher rate and represented a larger percentage of the body. Protein may have also increased butat a slower rate. In addition, at zero fat retention only protein was retained which confirms the research from Boekholt and Schreurs[3]. Broilers fed the NC+Enz diet showed an effect on fat and protein metabolism because more fat and protein were deposited (g day^{-1}) for all feed allowance levels leading to +6.39 g day^{-1} for 100% *ad libitum* intake.

Broilers fed the enzyme composite had lower MEm and greater km allowing more energy to be available for partitioning to production which was shown with more fat and protein gain. It is highly unlikely that the diet with composite enzyme created an imbalance of amino acids and phosphorus because the enzyme treatment produced positive gains for protein and fat when fed *ad libitum*.

To the author's knowledge, the present study is the first feeding study with an enzyme composite, such as Victus® (glucanase+xylanase+cellulase+arabinofuranosidase+protease+phytase) that shows a reduction in the MEm requirement. There are limited research publications on the topic of exogenous enzymes helping partition energy for maintenance, however, there is research showing that addition of exogenous enzymes changes the energy and protein metabolism. Musigwa *et al.*[26] reported that broilers fed a corn soybean based diet with low soluble NSPs, similar to the diet utilized in present study, with an added multi-enzyme (glucanase, xylanase and arabin of uranosidase) improved broiler nitrogen efficiency which was similar to the higher efficiency for tissue protein retention found in the present study. The enzyme composite used in present study consisted of cellulase, phytase and protease in addition to the enzyme blend reported for the Musigwa *et al.*[26] study. The enzyme compositeutilized in present study contained carbohydrases for NSP in both corn and soybean meal, along with several debranching enzymes. The findings from present study indicate that different enzyme combinations produced different effects on broiler body composition making the combinations more efficient for either fat or protein gain.

The MEm of the present study was168 and 160 kcal/$kg^{0.70}$ BW for NC and NC+Enz respectively, showing a reduction of the maintenance energy needs of the broiler during the 16-27 day period. The reduction in energy utilized for maintenance caused by the composite enzyme allows extra energy to be used for tissue gain. The reduction in MEm for broilers fed composite enzyme in present study may be caused by less energy being used for maintaining protein synthesis and GI tract tissue utilized in digestion process. Broilers fed supplemental enzymes have been reported to have a reduced pancreas size as a percent body weight[27]and a lower concentration of pancreatic enzymes in the intestinal contents[28]. The gut accounts for about 20% of the body energy expenditure and nearly 12% of newly synthesized protein is devoted to the GI tract[29]. Exogenous enzyme supplementation has been shown to decrease the weight of the duodenum (by about 22%) and jejunum+ileum (by about 16%) in 14-day old broilers[28] which has obvious implications on energy utilization and maintenance requirements. Cowieson and Ravindran[30] reported a highly significant correlation between the changes in endogenous amino acid flow and mucin with supplemental phytase. The endogenous losses of nutrients during digestion would have implications for the net energy gained from feed ingredients indicating the importance of enzymes beyond phytase on energy dynamics.

The overall values of MEm 168 and 160 kcal/$kg^{0.70}$ BW for a 16-27 day broiler compared to similar studies in the past show higher maintenance levels in present study. Sakomura[13] reported MEm to be112 kcal/$kg^{0.75}$ BW for broilers from 1-8 week. Several factors can affect the MEm such as animal age, body weight, body composition, size of organs. The present experiment focused on evaluating the MEm of young broilers (16-27 day) and the maintenance based on metabolic body weight is higher compared to older broilers utilized of Sakomura[13] study (1-8 week evaluation). In addition, the NEm in Sakomura[13] study was 90 kcal/$kg^{0.75}$ at 23°C, whereas thepresent research indicates the broilers had a NEm of 121 kcal/$kg^{0.70}$ at the same temperature. The younger age of the broilers(16-27 day) in present study played a role in the higher MEm values but Sakomura[13] reported the broiler MEm based on kg metabolic body weight with exponent of 0.75 compared to metabolic body weight with exponent of 0.70 used in the present study. The metabolic body weight is used in comparative physiology, it permits an expression of the metabolic level of an animal independent of its body weight[31] that in turn is better correlated to energy efficiency than usingonly body weight. Different authoras[13,20,32] used different coefficient numbers to calculate metabolic weight based on their experiments ($BW^{0.653}$, $BW^{0.75}$ and $BW^{0.70}$, respectively) to mention a few. The present broiler studies utilized an exponent of 0.70 to express metabolic weight because the studies reported by Noblet *et al.*[20] utilized similar fast-growing broilers. Noblet *et al.*[20] reported MEm values as FHP (fasting heat production) plus physical activity. The author reported the FHP of 3-6 week old broilers in metabolic chambers was 104±6 kcal/$kg^{0.70}$, so if activity is 20% of MEm[13], then energy expenditure for activity would be 21 kcal/$kg^{0.70}$. Noblet *et al.*[20] reported that if a calculated physical activity value of 21 kcal/$kg^{0.70}$ is added to FHP for broilers the MEm would be 125 kcal/$kg^{0.70}$ compared to MEm of 168 and 160 kcal/$kg^{0.70}$ for broilers fed NC and NC+Enz, respectively, in present study. The MEm in the present study is higher than that reported by Noblet *et al.*[20]. The methodology differences for the two studies may be an important part causing the different MEm values.

The MEm requirement of the control in the present study was 33% of ME intake for broilers during 16-27day and is lower than 42-44% for MEm reported by Lopez and Leeson[2]. The difference could be due to the broiler body weight gain of 71 and 78 g day^{-1} from 16-27 day in the present study (Table 4) compared to 56 g day^{-1} from 23-28 day for the Lopez and Leeson[2] study. Genetic improvements for feed efficiency continue to increase[33] and part of this efficiency may be from less percent MEm forthe modern broiler compared to percent energy utilized for gain.

On the prediction of net energy (NE), there was nodifference between dietary treatments when compared at the NEm level. The authors do not believe the exogenous enzymes are playing a role only at the MEm level. Different methodologies are needed to properly evaluate heat production differences at the NEm level. In the present study, HPmeasurements needed for determining NE were predicted from equations and research with NE and enzymes needs to be determined withindirect or direct calorimetry.

The efficiency for ME maintenance (k_m) of 71 and 75% in present study for broilers from NC and NC+Enz, respectively, are lower than80% which was reported by De Groote[34]. The differences in k_m reported by De Groote[34] compared to present study are probably more related to genetics, age and methodology than true efficiency differences.The addition of the enzyme composite facilitated a greater efficiency to retain protein and a decreased efficiency for fat retention, while decreasing the overall amount of MEm required.

CONCLUSION

The enzyme composite used in the present experiment reduced the broiler energy requirement for maintenance and improved the efficiency for protein gain. The present research provides insight for future enzyme research for evaluating maintenance energy requirements.

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