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Energy digestibility and concentration of nitrogen-corrected apparent metabolizable energy of Azolla and Duckweed in broiler

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Abstract The most practical system for estimating the energy of an ingredient and diet is used for the apparent metabolizable energy (AME), corrected to zero nitrogen retention (AMEn) in poultry. Results revealed that soybean meal (SBM) had similar higher values on gross energy (GE) and AMEN to Azolla than Duckweed. Although Azolla was significantly higher than Duckweed in terms of GE, AME, and AMEN, it had similar values on body weight gain, N gain, and GE for N gain, suggesting that Duckweed was more digestible than Azolla despite having lower values in terms of GE, AME, and AMEN, but still inferior when compared to SBM. In conclusion, Azolla can be used as an alternative feed source in broiler.

Keywords: GE, AME, AMEN, N gain, Azolla, Duckweed, Broiler chickens

Introduction

The energy systems provide a collective basis for diet formulation by identifying and measuring the inherent energy concentration of a feed ingredient. Nutrients such as proteins, carbohydrates, and fats that are absorbed will yield energy, which is important for the proper functioning of the body. Currently, the most practical system used in estimating the energy of an ingredient or diet is to determine the apparent metabolizable energy (AME) and correct it to zero nitrogen retention (AMEn), which are presumed to be additives in the mixed diet of poultry.

As proven by various published reports, older birds are more efficient in terms of energy utilization from feedstuffs than growing broilers (Garnsworthy *et al.*, 2000; Svihus and Gullord, 2002; Cozannet *et al.*, 2010a). Physiological differences due to age and breeds have different digestion and absorption coefficients and may obtain different energy values (Begin, 1967; Pym and Farrell, 1977; Lopez and Leeson, 2005; Cozannet *et al.*, 2010b).

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Furthermore, nutrient composition, form, and type of diet being tested are the determinants of AME values (Nitsan *et al*., 1997; Noblet *et al*., 2010). Common examples in the AME system are high-fat ingredients with lower estimates of energy values, while high-protein feedstuffs have generally higher estimated energy values compared to carbohydrates (De Groote, 1974; Carré *et al*., 2014). Conventionally, to make the energy values of each feedstuff more consistent and reflective of different types of birds, it is necessary to utilize the reported AME, which will be corrected with zero retention of nitrogen in body tissues, or AMEn (Mollah *et al*., 1983; Hätel, 1986; Bourdillon *et al*., 1990; Farrell *et al*., 1997). Azolla and duckweed were both unconventional feed ingredients. However, the shortages of supply and fluctuating prices of conventional feed ingredients motivate local producers and researchers alike to find alternative feed ingredients that would replace totally or at least in part the conventional feed sources.

Most of the studies conducted on these two ingredients focused on nutritional composition, growth and reproductive performances, meat qualities, humoral immunity response, antioxidant properties, and economics of production, but less on digestibility of nutrients and energy concentration. Hence, the objectives of the study were to determine the energy value and the apparent total tract digestibility (% ATTD) of azolla and duckweed in the broiler.

Materials and methods

Animal and experimental design and treatments

In this study, a Randomized Complete Block Design (RCBD) was used. As experimental birds, 24 heads of straight-run 14-day-old broilers were employed. These birds were randomized randomly to three dietary treatments, replicated eight times. Each bird was placed in the metabolic cage at random using its initial weight as a blocking factor, and that served as a replicate. Treatment 1: corn-soy diet (basal); Treatment 2: basal + 20 azolla; and Treatment 3: basal + 20% duckweed.

Feeding and excreta collection

Birds were fed a common diet, a chick booster ration from days 1 to 13 ad libitum, with water always available. On day 14, the birds were weighed and transferred to individual metabolic cages, and the dietary treatments were given gradually from 65 g to 130 g up to the 19th day as an adaptation period. The birds were weighed again on the 20th day after fasting for 4 hours, and after weighing, feeds were given at 130 g per day divided by 3 meals to minimize feed wastage. Every morning, the collection of excreta was done for 3 consecutive days, and on the last day, the birds were fasted for 8 hours and weighed to determine the final weight of the bird.

Chemical analysis

Excreta samples were pooled per bird, and before analysis, they were oven-dried and ground to pass through a 20-mm sieve. Using bomb calorimetry (Model 6400, Parr Instruments, Moline, IL) to determine the Gross Energy (GE) and Proximate Analysis for other nutrients determination (AOAC, 2007), all samples of excreta, diet, and ingredient were analyzed in triplicate.

Energy calculations

The amount of energy lost in the excreta and the DE and ME of the diets were calculated using these equations (Adeola, 2001).

$$\boldsymbol{DE_{diet}} = \frac{\boldsymbol{GE_{intake} - Fecal\ energy_{output}}}{\boldsymbol{ADFI}}$$

$$\boldsymbol{ME_{diet}} = \frac{\boldsymbol{GE_{intake} - \left(Fecal\ energy_{output} + Urine\ energy_{output}\right)}}{\boldsymbol{ADFI}}$$

To calculate the contribution of the basal diet on the DE and ME in diets containing each feedstuff is to multiply it by 80%, while the DE and AMEn in duckweed and azolla will be calculated by difference using this equation (Widmer *et al.,* 2007):

$$\boldsymbol{DE_A} = \frac{\boldsymbol{DE_D - (S_B\ x\ DE_B)}}{\boldsymbol{S_A}}$$

where DE_A is the DE of the test ingredient (kcal/kg), DE_D is the DE of the treatment diets (kcal/kg), DE_B is digestible energy of the reference ingredient (kcal/kg), S_B is contribution level of the reference ingredient (%), and S_A is contribution level of component from test ingredient to the diet (%).

Digestibility calculations

The apparent total tract digestibility (**ATTD, %**) of DM, and GE were calculated using this equation:

$$\boldsymbol{ATTD}\ (\%) = \frac{\boldsymbol{[Nutrient_i - Nutrient_f]}}{\boldsymbol{Nutrient_i}}\ \boldsymbol{x\ 100}$$

where ATTD is the apparent total tract digestibility, $Nutrient_i$ is the total nutrient intake (g) from d 6 to d 11; and $Nutrient_f$ is the total fecal output (g) of the nutrient originating from the diet fed from d 6 to d 11 (Almeida and Stein, 2010).

Statistical analysis

The mixed procedure of SAS (SAS Institute Inc., Cary, NC; 1999) was used to analyze the data, with birds as the experimental unit, diet as the fixed effect, and replication as the random effect. Each independent variable was calculated with the least squares mean, and the PDIFF option of SAS was used to separate the means with an α-level of 0.05 for significance and 0.10 for tendencies.

Results

The daily energy balance of broiler chicks fed with a basal diet and diets with 20% duckweed and 20% azolla (Table 1) revealed that GE intake (kcal/kg) tended to decrease on diets with 20% azolla and significantly differ (P<0.05) from diets with 20% duckweed. This might be due to the higher fiber content of duckweed, which will cause an enlargement of the crop, leading to a gut-fill sensation and thus decreasing gross energy intake. The decreased energy intake of birds fed with 20% duckweed and 20% azolla resulted in a highly significant decline in body weight gain (P<0.0001). The significant decline in body weight gain also resulted in a highly significant decrease in nitrogen (N) gain and the GE of N gain (P<0.0001).

Table 1. Daily energy balance (as-fed basis) of broiler chicks fed basal diet and diets with 20% duckweed and 20% azolla

ITEM	Basal	Duckweed	Azolla	SEM	*P* - value
DM intake, g	94	87	89	3.59	0.35
GE intake, kcal	375^{a}	331^{b}	369^{ab}	14.41	0.09
Fecal output, g	34	34	35	1.99	0.81
Fecal GE loss, kcal	124	116	134	7.46	0.25
Body weight gain, g	76^{a}	45^{b}	46^{b}	3	0.0001
N gain, g	2.4^{a}	1.6^{b}	1.6^{b}	0.1	0.0001
GE of N gain, kcal/g	71^{a}	50^{b}	50^{b}	2.14	0.0001
ATTD, % of GE	67	65	64	1.22	0.17
AME of diet, kcal/kg	$2,658^{a}$	$2,493^{b}$	$2,658^{a}$	48.34	0.01
AMEn of diet, kcal/kg	$2,444^{a}$	$2,342^{b}$	$2,508^{a}$	46.69	0.02

Note: Values with different superscripts in same row differ significantly (P<0.05).

The apparent metabolizable energy (AME, kcal/kg) of the diet showed a significant decrease with 20% duckweed (P<0.01) when compared to the basal diet and with 20% azolla, and when the diets were corrected with N (P<0.02) showed the same results with the AME of the diet. When the two ingredients were compared on AME and AMEN as feed or as dry matter basis, azolla was significantly higher than the duckweed (P<0.02) (Table 2). Despite being inferior to SBM and azolla on GE, AME, and AMEN, duckweed has similar values on body weight gain, N gain, and GE for N gain compared to azolla, and this might mean that duckweed has a higher digestibility compared to azolla.

Table 2. Apparent metabolizable energy (AME) and nitrogen corrected AME concentration in duckweed (*Lemna minor*) and azolla *(Azolla sp.*) fed to broiler chickens

ITEM	Duckweed	Azolla	SEM	*P*-value
As-fed basis				
AME, kcal/kg	1,473^{b}	2,138^{a}	219.60	0.02
AMEn, kcal/kg	1,352^{b}	2,018^{a}	217.74	0.02
DM basis				
AME, kcal/kg DM	1,831^{b}	2,657^{a}	272.87	0.02
AMEn, kcal/kg DM	1,680^{b}	2,507^{b}	270.55	0.02

Note: Values with different superscripts in same row differ significantly (P<0.05).

Discussion

Feed ingredient digestibility is inherent for a specific ingredient or a mixed diet. There are also other factors that will affect the digestibility of feed ingredients, such as the age of the animal (Tarvid, 1995). However, digestibility of ingredients as affected by age also had contradictory results; it increased digestibility with age (Wallis and Balnave, 1984; Ten Doeschate *et al*., 1993; and Huang *et al*., 2005), while others found the opposite (Hakansson and Eriksson, 1974; Fonolla *et al*., 1981; Zelenka and Liska, 1986).

In this study, the birds used were 20 days old, although some reports say that birds at 14 days old already have a complete gut in terms of enzyme secretions and other factors needed by the gut during digestion and absorption. But in general, there was an increase in the digestibility coefficients of broiler chickens with advancing age (Huang *et al*., 2005), and Ravindran (2013) noted that broilers have lower enzyme capacity like most younger animals because of undeveloped enzymes and therefore need supplementation with exogenous enzymes to boost the digestibility of essential nutrients for maintenance, muscle development, and growth. However, it is noteworthy to mention the report of Obst and Diamond (1992) that the nutrient uptake capacity in chickens' peaks on the second week after hatching, abruptly reduces on the third week to the fifth week, with a similar rate on the first week, and will peak again on the sixth week.

In addition, the inherent high fiber content of duckweed and azolla and its bulkiness contributed greatly to decreased digestibility values and resulted in poorer bird growth performance because broilers have a lower capacity to digest fiber (Rojas and Stein, 2017). The water-soluble fractions of the dietary fiber content of azolla and duckweed bind an enormous volume of water from the digesta as it goes down from the anterior part to the posterior portion of the small intestine, making the intestinal lumen more viscous (Bedford and Classen, 1992). Higher digesta viscosity will result in a decreased movement of digesta in the lumen and will increase the area of unstirred water in the gut, leading to a lesser interaction of the digesta and digested products with the intestinal microvilli and decreased access of endogenous enzymes to the substrate, resulting in a reduced digestibility of carbohydrate fractions and other nutrients (Choct *et al*., 1996; Smits and Annison, 1996).

Furthermore, the birds in this study were fed with mashed diets, and the energy utilization of birds eating mash diets was higher compared to pelleted feeds (Serrano *et al*., 2012). Pelleting increases feed intake with a higher feed conversion ratio, resulting in better growth performance in broilers (Abdollahi *et al*., 2013, 2018a, b). Meanwhile, energy digestibility values in pig diets vary from 70 to 90%, or even more, from 0 to 100% for feed ingredients, and these variations are due to the existence of dietary fiber (NSP + lignin) (Noblet and van Milgen, 2004). In addition, dietary fiber is less digestible among other nutrients (<50%), and it will also affect the digestibility of other nutrients, such as crude protein and fat (Noblet and Perez, 1993; Le Goff and Noblet, 2001). However, the negative effect of dietary fiber decreased as body weight increased, as did enzyme supplementation and feed processing, including pelleting and optimum particle size (Noblet and van Milgen, 2004).

Many factors have been previously reported to affect the digestibility of nutrients, including type of animal, age, and the inherent nutritional composition of the ingredient, among others. In the current study, duckweed had an inferior digestibility coefficient on GE, AME, and AMEN, resulting in poor growth performance, thus decreasing N gain and GE for N gain compared to SBM. In addition, azolla had better GE, AME, and AMEN but similar values on body weight gain, N gain, and GE for N gain as compared to duckweed. The poor digestibility might be due to the higher fiber content of duckweed. Therefore, azolla may be used as an alternative feed ingredient for broilers at lower levels of inclusion. While duckweed results need further studies for validity.

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