Research Note: Impact of *Eimeria* on apparent retention of components and metabolizable energy in broiler chickens fed single or mixture of feed ingredients-based diets[1]

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ABSTRACT The effect of *Eimeria* on apparent retention (**AR**) of components and metabolizable energy corrected for nitrogen (**AMEn**) content in corn, wheat, soybean meal (**SBM**), and pork meal (**PM**) was investigated in broiler chickens. A total of 840 male d-old Ross 708 chicks were placed in 84 cages (10 birds/cage) and allocated either a nitrogen-free diet (**NFD**), or 1 of 6 test cornstarch-based semipurified diets: 1) corn, 2) wheat, 3) SBM, 4) PM, 5) corn, SBM, and PM (**CSP**) mixture, and 6) wheat, SBM, and PM (**WSP**) mixture ($n = 12$). Diets contained 0.3% titanium dioxide and nutrient digestibility was determined by difference method using NFD. On d 10, birds in half of replicates per diet were orally challenge with 1 mL of *E. acervulina* and *E. maxima* culture and the other half equal volume of saline. Excreta samples were collected from d 12 to 14. With exception of AR of Ca and P, there was no interaction ($P > 0.05$) between *Eimeria* and diet on AR of dry matter, crude fat (**CF**), crude protein and gross energy and AMEn of ingredients. *Eimeria* reduced AR of CF ($P = 0.01$) and had a tendency to reduce AR of DM ($P = 0.09$) and AMEn ($P = 0.063$) of ingredients. The data demonstrated exposure to *Eimeria* impacted nutrient retention and energy utilization irrespective to diet composition.

Key words: broiler chicken, digestibility, *Eimeria*, metabolizable energy

2023 Poultry Science 102:102526
https://doi.org/10.1016/j.psj.2023.102526

INTRODUCTION

Feed costs account for more than half of broiler production, with a significant proportion of this cost allotted toward the provision of energy and amino acids (**AA**) for growth, maintenance, and production (Adedokun et al., 2016). One of the leading threats to the gastrointestinal health of broilers is *Eimeria*-induced coccidiosis, a major parasitic disease with considerable economic burden (Dalloul and Lillehoj, 2006; Rochell et al., 2016). Much of the economic losses incurred from coccidiosis are due to the damage of the intestinal epithelium and reduced feed intake, resulting in energy and nutrient malabsorption, poor feed efficiency, and increased mortality (Allen and Fetterer, 2002; Adedokun and Adeola, 2016; Kim et al., 2017).

While adequate levels of nutrients in the diet play an important role in the maintenance of the immune system, deficiencies, particularly in protein and energy, can negatively influence the immune status of the bird and the susceptibility to various pathogens (Adedokun and Olojede, 2019). With increasing trends toward partial and/or complete removal of antibiotics in poultry production, the challenge is understanding how energy and nutrient utilization are affected in consideration of the renewed insults by bacteria and intestinal parasites (Adedokun and Olojede, 2019). Moreover, feed ingredients are inherently unique in terms of antinutritional factors and indigestible components, making nutrient digestibility vary between feedstuffs (Adedokun et al., 2011). Understanding the undigested dietary fractions in excreta can provide insight in developing strategies to maximize feed efficiency, particularly during an *Eimeria* infection. For this reason, it would be necessary to characterize the effect of an *Eimeria* challenge on the apparent retention (**AR**) of components and apparent metabolizable energy (**AME**) content in different feed ingredients fed to broiler chickens. Therefore, the objective of this study was to determine the AR of components and AME content in poultry feed fed as a single

Received October 24, 2022.
Accepted January 17, 2023.
[1]Presented in part at the 26th World Poultry Congress, France, Paris, August 7 to 11, 2022.
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ingredient or mixture of ingredients to broiler chicks subjected to an *Eimeria* challenge.

MATERIALS AND METHODS

The experimental protocol (#3521) was reviewed and approved by the University of Guelph Animal Care Committee and birds were cared for in accordance with the Canadian Council on Animal Care guidelines (CCAC, 2009). The birds, diet composition, and experimental procedures were reported in a companion paper that focused on aspects of amino acids digestibility (Kim et al., 2022). Briefly, 840 male d-old Ross 708 chicks were placed in 84 cages (10 birds/cage), reared on basal starter diet and transitioned to experimental diets on d 9 (Kim et al., 2022). The diets were: nitrogen-free diet (**NFD**), or 1 of 6 test diets based on a single or mixture of feed ingredients as the sole source of AA: 1) corn, 2) wheat, 3) soybean meal (**SBM**), 4) pork meal (**PM**), 5) corn + soybean meal + pork meal (**CSP**) mixture, 6) wheat + soybean meal + pork meal (**WSP**) mixture. The ratio of cornstarch to sucrose to soy oil (sole source of energy in NFD) was similar as in test diets for determination of apparent metabolizable energy (**AME** and **AMEn**) in feedstuffs using difference method (Woyengo et al., 2010). The mash diets had 0.3% TiO_2 as indigestible marker. Due to the differences in composition of ingredients, crude protein and energy contents varied among diets, however, all diets were fortified with minerals and vitamins to meet or exceed Ross 708 requirements (Aviagen, 2019). On d 10, birds in half the replicates per diet were challenged with 1 mL of an *Eimeria* culture (100,000 oocysts of *E. acervulina* and 25,000 oocysts of *E. maxima*) while the other half were given equal volumes of 0.9% saline in distilled water. *Eimeria* infection was confirmed with intestinal lesion scores reported by Kim et al. (2022) and the challenge protocol has been validated though oocyst count as we previously reported (Kim et al., 2017; Akbari Moghaddam Kakhki et al., 2019; Leung et al., 2019). Excreta samples were collected from d 12 to 14 and stored at −20°C. The excreta samples were freeze-dried and along with diets, finely ground for chemical analyses using standard procedures (AOAC, 2005).

The AR of components and AMEn were calculated according to Adeola et al. (2016). The AME contents for the test ingredients were calculated using measured AME value of the test diets (**AMEtd**) and the proportions of other (cornstarch, sucrose, and soy oil) energy yielding ingredients (**poeyi**) and the proportions of the test ingredients (**poti**) as previously described in

Table 1. Apparent retention of nutrient and energy in broiler chickens fed single or mixture-based feed ingredients with or without an *Eimeria* challenge.

Diet	*Eimeria*[1]	Dry matter	Crude fat	Crude protein	Gross energy	Calcium	Phosphorus
Nitrogen-free diet	-	80.7	76.4	−38.4	82.5	53.2^{abc}	35.4^{bcde}
Corn	-	75.6	69.6	38.3	78.0	23.2^{ef}	20.5^{de}
Wheat	-	72.8	81.0	37.0	76.3	41.4^{bcd}	32.6^{cde}
Soybean meal	-	66.3	83.6	46.6	72.5	58.9^{ab}	43.4^{abc}
Pork meal	-	73.2	76.6	62.4	76.0	38.4^{cde}	40.6^{abcd}
CSP[2]	-	72.5	79.3	60.2	75.7	70.2^{a}	56.7^{a}
WSP[3]	-	68.2	80.3	55.4	72.8	61.4^{a}	48.7^{abc}
Nitrogen-free diet	+	78.2	66.2	−57.6	80.9	36.9^{cdef}	14.3^{e}
Corn	+	71.8	66.0	38.7	75.3	17.2^{f}	13.9^{e}
Wheat	+	70.2	71.9	28.2	74.1	52.0^{abc}	38.9^{abcd}
Soybean meal	+	65.5	78.7	22.8	62.6	57.6^{ab}	42.7^{abc}
Pork meal	+	68.0	69.1	55.4	71.5	29.8^{def}	32.4^{bcde}
CSP	+	65.0	76.7	65.5	70.0	72.3^{a}	62.0^{a}
WSP	+	70.3	77.3	60.2	74.9	66.2^{a}	55.6^{ab}
SEM		1.25	1.51	9.97	1.29	4.69	4.01
Main effect of diet							
Nitrogen-free diet		79.5^{a}	71.3^{bc}	−48.0^{c}	81.7^{a}	45.1	24.8
Corn		73.7^{ab}	67.8^{c}	38.5^{ab}	76.7^{ab}	20.2	17.2
Wheat		71.5^{ab}	76.5^{ab}	32.6^{b}	75.2^{ab}	46.7	35.8
Soybean meal		65.9^{b}	81.2^{a}	34.7^{ab}	67.6^{b}	58.2	43.1
Pork meal		70.6^{ab}	72.8^{abc}	58.9^{ab}	73.8^{ab}	34.1	36.5
CSP		68.8^{b}	78.0^{ab}	62.8^{a}	72.9^{ab}	71.2	59.3
WSP		69.3^{b}	78.8^{ab}	57.8^{ab}	73.8^{ab}	63.8	52.2
SEM		1.63	1.79	14.44	1.61	6.67	5.55
Main effect of challenge							
	-	72.8	78.1^{a}	37.4	76.3	49.5	39.7
	+	69.8	72.3^{b}	30.5	72.8	47.4	37.1
	SEM	1.46	2.93	3.46	1.76	1.06	1.29
Probabilities							
Diet		0.004	<0.001	<0.001	0.043	<0.001	<0.001
Challenge		0.092	0.001	0.185	0.304	0.314	0.288
Diet × challenge		0.829	0.741	0.637	0.739	0.024	0.034

$N = 6$.
[1]"-" unchallenged, "+" challenged.
[2]Corn, soybean meal, and pork meal mixture.
[3]Wheat, soybean meal, and pork meal mixture.
[abcdef]Within a column, LSmeans assigned different letters within a response criterion are significantly different, $P < 0.05$.

Table 2. Apparent metabolizable energy content (kcal/kg DM) in feedstuffs fed to broiler chickens with or without *Eimeria* challenge.

Diet	*Eimeria*[1]	AME	AMEn
Corn	-	3,481	3,451
Wheat	-	3,371	3,336
Soybean meal	-	3,455	3,215
Pork meal	-	3,863	3,431
Corn	+	3,355	3,327
Wheat	+	3,252	3,221
Soybean meal	+	3,250	3,013
Pork meal	+	3,408	2,997
SEM		66.95	62.13
Probabilities			
Diet		0.283	0.410
Challenge		0.083	0.063
Diet × challenge		0.751	0.722

$N = 6$.
[1]"-" unchallenged, "+" challenged.

Thanabalan et al. (2021) using the following equation:

$$AMEti = [AMEtd - (AMEnfd \times poeyi)]/poti,$$

where AMEti is AME of the test ingredient (corn, wheat, SBM, or PM) and AMEnfd is AME of the NFD diet. The AMEtd and AMEnfd were calculated according to Woyengo et al. (2010).

The data were subjected to a 6 × 2 for AR of components or 4 × 2 for AMEn factorial arrangement using mixed procedures of SAS version 9.4 (SAS Institute Inc., Cary, NC). The model included diet, challenge, and associated interaction as fixed factors. An alpha level of 0.05 was used as the criterion for statistical significance and treatments were compared using Tukey test.

RESULTS AND DISCUSSION

The analyzed chemical composition of the feed ingredient samples and diets were previously reported in Kim et al. (2022). Briefly, the analyzed content (DM basis) of GE was 4,024, 4,179, 4,103, 4,409, 4,277, 4,406, and 4,322 kcal/kg for NFD, corn, wheat, SBM, PM, CSP, and WSP diets, respectively. The corresponding concentrations of crude protein and crude fat were 0.9 and 2.3, 7.5 and 3.4, 9.7 and 2.5, 22.9 and 3.0, 21.4 and 6.6, 22.6 and 3.7, and 23.2 and 3.2%, respectively. There was a diet and challenge interaction effect ($P < 0.05$) on AR of Ca and P such that *Eimeria* reduced AR in NFD, corn, and PM diets (Table 1). This aligns with the results from a study by Teng et al. (2020), where *Eimeria* challenge decreased apparent ileal digestibility (**AID**) of Ca and P linked to *Eimeria* damage on intestinal cell (Wasserman, 1981). However, it is unknown as to why decrease in AR of Ca and P retention was not seen across all diets. Diet effects ($P \leq 0.04$) were observed for AR of dry matter (**DM**), crude fat (**CF**), crude protein (**CP**), and gross energy (**GE**). Birds fed NFD retained more DM than birds fed SBM, CSP, and WSP diets, whereas SBM birds retained more CF than birds fed corn or NFD. The AR of CP was lowest and negative for NFD. However, the AR of GE was higher for NFD (81.7%) relative to SBM diet (67.6%) whereas the rest of the diets were intermediate. These differences in responses may be attributed to the differences in composition of individual ingredient, the diet type (single ingredient compared to mixed ingredients), and utilization of energy in different feed ingredients. *Eimeria* reduced AR of CF ($P = 0.001$) and tended to reduce AR of DM ($P = 0.09$). Amerah and Ravindran (2015) found a linear reduction in AID of fat with increasing intestinal lesion scores. Indeed, the severity of jejunal lesion scores was similar across diets as we previously reported in our companion paper (Kim et al., 2022). There was no interaction or main effects of diet and challenge ($P > 0.05$) on AME and AMEn in feedstuffs (Table 2). However, there was tendency for decrease in AME ($P = 0.08$) and AMEn ($P = 0.06$) with *Eimeria*. This is in line with expected poor nutrient absorption and utilization from damaged intestinal epithelium (Adedokun et al., 2016).

In conclusion, the data demonstrated that diet, *Eimeria*, and their interaction can influence nutrient and energy retention in broiler chickens. However, exposure to *Eimeria* tended to have independent effects on measured AMEn suggesting effects of *Eimeria* on energy utilization is not influenced by diet composition.

ACKNOWLEDGMENTS

The research was funded by Ontario Agri-Food Innovation Alliance (#030463), METEX NØØVISTAGO (#054288), and Wallenstein Feed & Supply Ltd. (#054297). Technical assistance by I. Wilson, D. Vandenberg, and C. Zhu appreciated.

DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. W. Lambert is an employee of METEX NØØVISTAGO.

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