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Research note: Comparison of total tract endogenous fat losses and fat digestibility determined by fat-free diet and regression methods in broiler chickens and laying hens

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ABSTRACT

This study aimed to compare endogenous fat losses (EFL) and fat digestibility determined using fat-free diet (FFD) and regression methods in broiler chickens and laying hens. Two metabolism trials were conducted with 28 broiler chickens (21 d of age, 0.96 ± 0.04 kg BW) and 28 laying hens (72 wk of age, 2.01 ± 0.05 kg BW). Birds within each strain were randomly allotted to 4 dietary treatments: FFD and the supplementation of 2, 4, or 6 % corn oil to the FFD. Results indicated that increasing supplementation of corn oil increased (linear, $P < 0.001$) apparent total tract digestibility (ATTD) of fat for both broiler chickens and laying hens. The basal EFL estimated using the FFD method was similar between broiler chickens (1.75 g/kg DM intake) and laying hens (1.62 g/kg DM intake). Supplemental levels of corn oil did not influence standardized total tract digestibility (STTD) of fat for both strains. The ATTD and STTD of fat at each level of corn oil supplementation did not differ between strains. Regression analysis indicated that total EFL and true total tract digestibility (TTTD) of fat were 2.024 g/kg DM intake and 97.4 % for broiler chickens, and 1.686 g/kg DM intake and 97.1 % for laying hens, respectively. However, no differences in total EFL and TTTD of fat were observed between strains based on their 95 % confidence intervals. Furthermore, comparisons between FFD and regression methods revealed no differences between basal and total EFL in both strains. Moreover, values for the STTD of fat did not differ from those for the TTTD of fat in both strains. In conclusion, the comparable values for the STTD and TTTD of fat suggested that the STTD of fat can serve as a reliable and practical measure of dietary fat utilization in poultry. For highly digestible fat sources (i.e., corn oil), fat digestibility appeared to be similar between broiler chickens and laying hens.

Introduction

Dietary fat serves as an important energy source for poultry to satisfy their energy requirements. The recent rise in feed costs has heightened the need for accurate estimations of dietary fat utilization in poultry because dietary energy constitutes the most expensive component of poultry diets (Ravindran et al., 2016). Fat digestibility in poultry diets is traditionally measured as apparent total tract digestibility (**ATTD**) with no consideration of endogenous fat losses (**EFL**). It is well-known that the ATTD of fat tends to underestimate dietary fat utilization in animals (Stein, 2017). Consequently, the standardized total tract digestibility (**STTD**) and true total tract digestibility (**TTTD**) of fat, which accounts for EFL, are regarded as more accurate assessments of dietary fat utilization (Kil et al., 2010; Stein, 2017). However, research on the STTD and TTTD of dietary fat in poultry remains largely limited.

The regression method has been used to determine total EFL as an intercept and the TTTD of fat as a slope of the regression equation (Kil et al., 2010). However, a possible limitation of this method is that it requires multiple levels of dietary fat intake and a higher fat supplementation in treatment diets may change other dietary compositions (Wang et al., 2020). On the other hand, a fat-free diet (**FFD**) method, used to measure basal EFL for estimating the STTD of fat, is more widely adopted due to its relatively simple methodology (Wang et al., 2020). While both methods have been extensively validated and compared for dietary fat utilization in swine (Kil et al., 2010; Wang et al., 2020), their application and direct comparison in poultry are currently lacking. Furthermore, advanced understanding of potential differences in dietary fat utilization among poultry strains is essential for precise feed

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formulation. For instance, broiler chickens and laying hens, as the major commercial poultry strains, exhibit substantial differences in physiological states and nutrient metabolism, which may have a considerable effect on fat digestibility. However, to our knowledge, comparative data for fat digestibility, particularly regarding the STTD and TTTD of fat, between these 2 commercial strains have not been reported. Therefore, this study aimed to assess and compare EFL and fat digestibility determined using FFD and regression methods in broiler chickens and laying hens.

Materials and methods

Experimental design

All procedures in this study were approved by the Institutional Animal Care and Use Committee of Chung-Ang University (Republic of Korea) for both broiler chickens (Approval No. 202401030147) and laying hens (Approval No. 202501020048). Two independent metabolism trials were conducted with 28 Ross 308 male broiler chickens (21 d of age, average BW = 0.96 ± 0.04 kg) and 28 Hy-Line Brown laying hens (72 wk of age, average BW = 2.01 ± 0.05 kg). Birds within each strain were assigned to 4 dietary treatments with 7 replicates per treatment (1 bird per replicate) in a completely randomized design. All birds were housed individually in metabolic cages (35 × 45 × 55 cm = width × length × height) for total excreta collection. Environmental conditions and managements were followed by individual management guidelines for each strain (Aviagen, 2022; Hy-Line International, 2024).

Four treatment diets were prepared for both strains. The FFD for each strain was formulated based on a previous study (Tancharoenrat et al., 2014; Table 1). Three oil-supplemented diets were prepared by adding 3 levels of corn oil (2, 4, and 6 %) to the FFD at the expense of cornstarch. All diets were formulated to meet or exceed nutrient recommendations suggested by the management guidelines of broiler chickens (Aviagen, 2022) and laying hens (Hy-Line International, 2024). All birds were fed 70 g per d with free access to water in both metabolism trials. All birds consumed their designated feeds with little leftover. Each metabolism trial lasted for 10 d, consisting of a 5-d adaptation period followed by a 5-d total collection periods. The diet and collected excreta samples were analyzed in duplicate for DM (method 930.15; AOAC, 2005) and acid-hydrolyzed ether extract (method 954.02; AOAC, 2005). The analyzed acid-hydrolyzed ether extract concentrations in treatment diets supplemented with 2, 4, and 6 % corn oil were 2.87 %, 4.70 %, and 7.10 % for broiler chickens and 2.24 %, 4.56 %, and 6.43 % for laying hens, respectively.

Calculations and statistical analysis

The ATTD of fat in 3 oil-supplemented diets was determined for both strains, and their STTD of fat was calculated by correcting the ATTD of fat for basal EFL derived from each FFD-fed strain (Wang et al., 2020). A linear regression analysis was conducted using 3 oil-supplemented groups by regressing apparently digested fat (g/kg DM intake) against dietary fat intake (g/kg DM intake), where the slope multiplied by 100 represented the TTTD of fat and the intercept represented total EFL (Wang et al., 2020).

All data were analyzed by ANOVA using the MIXED procedure of SAS (SAS Institute, Cary, NC). Each replicate served as the experimental unit for all analyses. The LSMEANS procedure was used to calculate treatment means. Orthogonal polynomial contrast tests were performed to determine linear and quadratic effects of increasing supplementation of corn oil on the ATTD and STTD of fat for each strain. The REG procedure was also used for the regression analysis to estimate the slope for TTTD of fat and intercept for total EFL. The slope and intercept between strains were compared using the 95 % confidence intervals derived from the SE of regression coefficients (Kil et al., 2010). Finally, t-test procedures were used to compare basal EFL as well as the ATTD and STTD of fat at each level of corn oil supplementation between strains. Statistical significance was set at $P < 0.05$.

Table 1
Ingredient composition of a fat-free diet (FFD) for each strain.

Items	Broiler chickens	Laying hens
Ingredients, %		
Casein	20.00	18.00
Cornstarch	67.00	60.00
Solka-Floc	6.50	6.60
$NaHCO_3$	2.00	2.00
Monocalcium phosphate	1.30	1.30
Limestone	1.10	10.00
DL-Met (98.5 %)	0.30	0.20
Choline (50.0 %)	0.20	0.30
K_2CO_3	0.50	0.50
MgO	0.20	0.20
Salt	0.30	0.30
Vitamin premix[1]	0.30	0.30
Mineral premix[2]	0.30	0.30
Total	100	100
Energy and nutrient contents[3]		
AME_n, kcal/kg	3,141	2,813
CP, %	17.65	15.85
Digestible Lys, %	1.36	1.21
Digestible Met, %	0.81	0.66
Digestible Met + Cys, %	0.86	0.71
Digestible Thr, %	0.71	0.64
Digestible Val, %	1.02	0.92
Digestible Ile, %	0.78	0.70
Digestible Arg, %	1.61	1.45
Digestible Trp, %	0.20	0.18
Digestible Leu, %	1.74	1.57
Ca, %	0.65	4.03
Available P, %	0.37	0.35
Acid-hydrolyzed ether extract, %	0.79	0.46

[1] Provided per kg of the complete diet: vitamin A, 12,000 IU (retinyl acetate); vitamin D_3, 4,000 IU; vitamin E, 80.0 mg; vitamin K_3, 4.0 mg (menadione dimethylpyrimidinol); vitamin B_1, 4.0 mg; vitamin B_2, 10.0 mg; vitamin B_6, 6.0 mg; vitamin B_{12}, 20.0 μg; folic acid, 2.0 mg; biotin, 200 μg; niacin, 60 mg.

[2] Provided per kg of the complete diet: iron, 60 mg ($FeSO_4$); zinc, 100 mg ($ZnSO_4$); manganese, 120 mg (MnO); copper, 16 mg ($CuSO_4$); cobalt, 1,000 μg ($CoSO_4$); selenium, 300 μg (Na_2SeO_3); iodine, 1.25 mg [$Ca(IO_3)_2$].

[3] Values were calculated from CVB feed tables (CVB, 2023), except that acid-hydrolyzed ether extract contents were analyzed (method 954.02; AOAC, 2005).

Results and discussion

Increasing supplementation of corn oil from 2 % to 6 % in FFD increased (linear, $P < 0.001$) the ATTD of fat for both broiler chickens and laying hens (Table 2). The basal EFL estimated using the FFD method was comparable between strains (1.75 and 1.62 g/kg DM intake for broiler chickens and laying hens, respectively). However, the STTD of fat for both strains was not affected by increasing supplementation of corn oil. The ATTD and STTD of fat at each level of corn oil supplementation did not differ between strains. The regression analysis of apparently digested fat against dietary fat intake was significant ($P < 0.001$) for both strains. According to the regression analysis, total EFL and TTTD of fat were estimated to be 2.024 g/kg DM intake and 97.4 % for broiler chickens, and 1.686 g/kg DM intake and 97.1 % for laying hens, respectively. Nonetheless, no differences in total EFL and TTTD of fat were observed between strains based on their 95 % confidence intervals.

The current estimated values for the ATTD of fat in corn oil (90.1 – 94.8 %) were placed within the previously reported range for the ATTD of fat in plant oils (84.0 – 96.0 %) for poultry (Baião and Lara, 2005). Despite the limited information available for poultry, the observation of a linear increase in the ATTD of fat with increasing fat intake in both strains was consistent with previous findings in pigs (Kil et al., 2010; Wang et al., 2020). This phenomenon is primarily attributed to the

Table 2
Endogenous fat losses and fat digestibility in broiler chickens and laying hens.[1,2]

Items	Broiler chickens	Laying hens	T-test SEM	T-test *P* – value
Basal EFL, g/kg DM intake	1.75	>1.62	0.132	0.339
ATTD of fat, %		>		
2 % corn oil	91.0	>90.1	0.95	0.339
4 % corn oil	93.6	>93.9	0.67	0.723
6 % corn oil	94.8	>94.7	0.88	0.839
SEM	0.56	>0.62		
P – value (Linear)	<0.001	><0.001		
P – value (Quadratic)	0.334	>0.064		
STTD of fat, %		>		
2 % corn oil	96.5	>96.7	0.95	0.504
4 % corn oil	97.0	>97.1	0.67	0.579
6 % corn oil	97.0	>96.9	0.88	0.624
SEM	0.56	>0.62		
P – value (Linear)	0.947	>0.770		
P – value (Quadratic)	0.869	>0.717		
Regression analysis[3]				
Items	Broiler chickens		Laying hens	
Regression equations	$y = 0.974x - 2.024$		$y = 0.971x - 1.686$	
Slope				
SE	0.008		0.010	
P-value	<0.001		<0.001	
Intercept				
SE	0.464		0.530	
P-value	<0.001		<0.001	
RMSE	0.708		0.941	
R^2	0.999		0.997	
TTTD of fat, %	97.4 [95.7, 99.1]		97.1 [95.0, 99.2]	
Total EFL, g/kg DM intake	2.024 [1.052, 2.996]		1.686 [0.587, 2.786]	

[1] Data are least squares means of 7 observation per treatment.
[2] EFL, endogenous fat losses; ATTD, apparent total tract digestibility; STTD, standardized total tract digestibility; TTTD, true total tract digestibility.
[3] Regression model for each strain: apparently digested fat (g/kg DM intake) = a × dietary fat intake (g/kg DM intake) - b, where slope (a) multiplied by 100 represented the TTTD of fat (%) and intercept (b) represented total EFL (g/kg DM intake). Values in brackets for the TTTD and total EFL showed their 95 % confidence intervals.

reduced relative contribution of EFL to total fat excretion with increasing fat intake (Kil et al., 2010; Wang et al., 2020). However, it is acknowledged that the STTD of fat remains unaffected by dietary fat intake (Stein, 2017), aligning with our findings for both strains. These results showing the independence of the STTD of fat from dietary fat intake clearly indicate that the STTD of fat provides a more accurate assessment of dietary fat utilization in poultry than the ATTD of fat.

For comparison of FFD and regression methods, values for basal EFL measured in each strain fell within the 95 % confidence intervals of total EFL measured for each strain, indicating no differences between basal and total EFL in both strains. Furthermore, values for the STTD of fat did not differ from those for the TTTD of fat in both strains based on the 95 % confidence intervals of the TTTD of fat.

Basal EFL is known to be lower than total EFL because the latter includes diet-specific EFL, which generally results in a higher TTTD of fat compared with the STTD of fat (Stein, 2017). Therefore, our observation for similarity between basal and total EFL is likely due to the fact that the same basal diet containing highly digestible ingredients, which induces minimal diet-specific EFL, was used for both FFD and corn oil-supplemented diets (Wang et al., 2020). Likewise, the use of corn oil, a highly digestible fat source, in this study may explain the lack of differences between the STTD and TTTD of fat for both strains (Wang et al., 2020). It has been recognized that regression methods are more complicated and costly compared with FFD methods because regression methods should establish graded levels of dietary fat intake (Wang et al., 2020). Consequently, our findings of no differences between the STTD and TTTD of fat may suggest that the STTD of fat obtained by adjusting ATTD for basal EFL is a more reliable and practical measurement than the TTTD of fat for assessing dietary fat utilization in poultry.

Various animal factors, including age, physiological state, and genotype, may influence fat digestibility in poultry (Ravindran et al., 2016). Nonetheless, data regarding the effect of poultry strains on fat digestibility, specifically focusing on the STTD and TTTD of fat, are currently lacking. In this study, we found that the STTD of fat in all diets supplemented with 3 different levels of corn oil as well as the TTTD of fat estimated from regression methods did not differ between broiler chickens and laying hens. In addition, the values for the STTD and TTTD of fat for both strains approximated 97 %, indicating that both strains have comparable and very high fat digestibility for corn oil at the STTD and TTTD levels. However, it should be noted that fat digestibility for less digestible fat sources (e.g., animal fat, oxidized fat) than corn oil and intact-form fat present in plant ingredients may differ between strains. Further research is required to compare fat digestibility of a variety of dietary fat sources, particularly less digestible ones, among poultry strains.

In conclusion, the STTD and TTTD of fat provided more accurate estimations of fat digestibility in broiler chickens and laying hens compared with the ATTD of fat. The comparable values for the STTD and TTTD of fat supported the use of the STTD of fat as a reliable and practical measure of dietary fat utilization in poultry. For highly digestible fat sources (i.e., corn oil), fat digestibility appeared to be similar between broiler chickens and laying hens.

CRediT authorship contribution statement

Min Sung Kang: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Hyun Woo Kim:** Writing – review & editing, Validation, Software, Resources, Methodology. **Ji Hye Lee:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis. **Dong Yong Kil:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Disclosures

The authors declare no conflict of interest for the data presented in this experiment.

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