

ISSN 1682-8356
ansinet.org/ijps



INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSI*net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com



Research Article

The Fatty Acid Composition of Excreta of Broiler Chickens Fed Different Dietary Fatty Acids

¹Khaled Kanakri, ¹John Carragher, ^{2,3}Robert Hughes, ¹Beverly Muhlhausler, ²Carolyn de Koning and ¹Robert Gibson

¹FOODplus Research Centre, School of Agriculture, Food and Wine, University of Adelaide, Adelaide, SA, Australia

²South Australian Research and Development Institute (SARDI), Roseworthy Campus, 5371 SA, Australia

³School of Animal and Veterinary Sciences, The University of Adelaide, 5371 SA, Australia

Abstract

Background and Objective: Excreted fatty acids represent the net result of fat digestion, absorption and bioconversion by chickens or their intestinal microbiome and thus provide information on the capacity of the birds to utilize different fat types. This study aimed to clarify the relationship between the fatty acid profile of diet and excreta in broiler chickens. **Materials and Methods:** Male Cobb 500 broilers (n = 240) were fed (*ad libitum*) one of 6 different diets supplemented with 4% (w/w) beef tallow, flaxseed, corn, macadamia, canola or coconut oils (4 replicate pens/treatment) from hatching day. At day-40 post-hatch, excreta samples were collected for fatty acids analysis. **Results:** Significant positive linear correlations ($R = 0.82-0.99$) were found in the fatty acid content of diets and excreta for all fatty acid groups in all treatments. Comparing the individual fatty acid content of diet and excreta suggested that the broilers preferentially utilized (in descending order, if present) omega-3 polyunsaturated fatty acids, omega-9 and omega-7 monounsaturated fatty acids and most saturated fatty acids (except C16:0 and C18:0), but the omega-6 polyunsaturated fatty acids were under utilized even when they were the most abundant. **Conclusion:** Fat sources which are high in the C16:0, C18:0 and omega-6 fatty acids may not be ideal for broiler feed formulations for nutritional and economical reasons.

Key words: Oils, fatty acid, diet, excreta, chicken broiler

Received: August 03, 2017

Accepted: September 15, 2017

Published: October 15, 2017

Citation: Khaled Kanakri, John Carragher, Robert Hughes, Beverly Muhlhausler, Carolyn de Koning and Robert Gibson, 2017. The fatty acid composition of excreta of broiler chickens fed different dietary fatty acids. Int. J. Poult. Sci., 16: 424-433.

Corresponding Author: Khaled Kanakri, FOODplus Research Centre, School of Agriculture, Food and Wine, University of Adelaide, Urrbrae, 5064 SA, Australia Tel: +618 8313 6857 Fax +618 8313 7135

Copyright: © 2017 Khaled Kanakri *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

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

Fats from animal and/or plant origins are added to commercial chicken feed as a source of essential fatty acids and a source of energy. Manipulation of the fat composition (e.g., the types and inclusion level) is commonly implemented for economic and nutritional purposes and results in altered dietary fatty acids composition (e.g., chain length, degree of saturation and molecular structure). Previous studies in chickens have shown different associations between the fatty acid content of diets and tissues depending on tissue type¹⁻³. There is considerable evidence that dietary fatty acid composition is related to growth performance⁴ and health status^{5,6} in chickens, but less is known about whether dietary fat composition influences the utilization of different fat types. The utilization of fatty acids as an energy source in broilers is limited in the first two weeks post-hatch but it improves as birds get older and physiological functions develop⁷. Fatty acid analysis of digesta sampled from along the gastrointestinal tract is commonly used to evaluate the digestion of nutrients and shows variation in the fat digestion coefficient between different intestinal segments^{8,9} with jejunum being the segment where the majority of fat is digested and absorbed¹⁰. To complicate matters further, variation in the microbial content between different intestinal sections, such as ileum and cecum, has been reported in broilers¹¹ which suggests intestinal microbiota could play a role in fat metabolism. In support of this, changes in gut microbiota are known to affect the performance parameters of broiler chickens¹².

Many aspects of dietary fatty acids, including inclusion level¹³, source¹⁴, re-esterification¹⁵ as well as their interactions with other dietary macronutrients⁹, micronutrients^{16,17} and enzymes¹⁸ have been reported to affect their intestinal absorption and excretion in broilers.

Number of studies have investigated different aspects of broiler excreta. These studies have led to better understanding of lipid utilization and losses¹⁹, fatty acid influence on excreta microbiota²⁰, estimating metabolizable energy^{13,21}, feed conversion efficiency²², using excreta as a dietary component itself^{23,24}, influencing manure mineral content¹⁷ and even how excreta affects the foot pad health of the birds¹⁹. However, it is important to acknowledge that the fatty acid content of excreta is a consequence of several factors, including: The lipid composition of the diet, the activities of lipase and bile salts, the efficacy of absorptive, metabolic and excretive processes throughout the length of the gut and the utilization and potential modification of fatty acids by the microbiota²⁵⁻²⁸.

To our knowledge, no published literature has compared the dietary effect of a wide variety of dietary fats which are very different in their fatty acid composition on the fatty acid composition in the excreta (as opposed to the digesta) of broilers. Therefore, the present study aimed to examine this relationship in broilers at harvest age fed diets supplemented with a range of different types of fats. A better understanding of this relationship is potentially useful to provide advice to poultry feed producers regarding the best choice of available fats to use when formulating broiler diets.

MATERIALS AND METHODS

Broilers and experimental design: The Animal Ethics Committees of the University of Adelaide and Primary Industries and Regions South Australia approved this study. A total of 240 day-old male chicks of the Cobb 500 strain were obtained from the Baiada Hatchery (Willaston, SA, Australia) and transferred to South Australian Research and Development Institute (SARDI) facility (Roseworthy, SA, Australia). A complete randomized block design (4 pens/treatment) was implemented. Birds were randomly distributed into 24 groups of 10 and allocated to 24 raised rearing floor pens (1.2×0.9 m each) in one shed. Chickens were reared on sawdust and shavings in a temperature controlled room and had free access to both feed and water at all times. Pens were heated by infrared brooder lamps (175 W) during the first 3 weeks post-hatch. Temperature in the room was 27°C for the first 4 days, gradually decreased to 20°C and then maintained for the 40-day experimental period.

Experimental diets: The study included 6 dietary treatments. In each, broilers were fed *ad libitum* 1 of 6 experimental diets by adding a different fat at 4% w/w to starter (crumble form, first 3 weeks) and finisher (pellet form, last 3 weeks) basal diets. The basal diets (containing ~3% crude fat) were obtained from a poultry feed manufacturer (Ridley Agriproducts, Australia). The added fat types were: Flaxseed oil (high omega-3 polyunsaturated fatty acids (n-3 PUFA): Four Leaf Oils, Australia), corn oil (high omega-6 polyunsaturated fatty acids (n-6 PUFA): Daisy, Malaysia), canola oil (high omega-9 monounsaturated fatty acids (n-9 MUFA): Foodland, Australia), macadamia oil (high omega-7 monounsaturated fatty acids (n-7 MUFA): Macoils, Australia), coconut oil (high saturated fatty acids (SFA): Banaban, Fiji) or beef tallow (moderate SFA and MUFA, Ridley Agriproducts, Australia). The composition and nutritional profiles of the two basal diets are shown in Table 1. Apart from the variation in the fatty acid

composition of the experimental diets (Table 2), they all were nutritionally identical and met requirements for healthy growth²⁹.

Production parameters: Total body weight (BW) of the birds in each pen was taken on the day of hatch and then on a weekly basis for the first 3 weeks. Feed intake (FI) of birds in each pen was also recorded on a weekly basis and used to calculate the feed conversion ratio (FCR, kg FI kg⁻¹ b.wt., gain). Number of deaths and culls was used to calculate mortality rate in all treatments on a weekly basis.

Sample collection: On day-40, paper drop-sheets were placed in each pen to collect excreta samples. Approximately 10-12 fresh droppings (deposited within 3 h) were randomly transferred into clean plastic containers. Samples were immediately frozen on dry ice before they were transferred to the laboratory to be stored at -18°C until subsequent determination of crude fat content and fatty acid analysis.

Fatty acid analysis: Crude lipid was extracted from homogenized feed and excreta samples³⁰. The gravimetric approach was utilized to estimate total lipid percentages. Fatty acid profiling was performed after transmethylation of the extracted crude lipids with 1% H₂SO₄ in methanol at 70°C for 3 h^{31,32}. After cooling to room temperature, the resulting fatty acid methyl esters (FAME) were extracted with n-heptane (2 mL) and transferred into gas chromatography (GC) vials containing about 30 mg of anhydrous sodium sulphate. Vials were stored at -18°C until GC analysis.

Gas chromatography analysis of FAME: The FAME were separated using a Hewlett-Packard 6890 GC (Hewlett-Packard, CA, USA) equipped with a flame ionization detector (FID), a

split injector and a BPX-70 capillary column (50 m × 0.32 mm internal diameter) with a 0.25 µm film thickness (SGE, Victoria, Australia). The operating conditions of the GC, identification of fatty acids using the GLC 463 external standard (Nu-Chek Prep Inc, MN, USA) and qualitative analysis was as described previously^{33,34}.

Statistical analysis: The effects of dietary treatment on the excreta fatty acid profile were tested by one-way analysis of

Table 1: Ingredient composition and nutrient content of the basal diet

Ingredients (%)	Basal diet ¹	
	Starter ²	Finisher ³
Wheat fine	61.42	70.77
Barley fine	5.00	5.00
Tallow mixer	0.37	1.00
Blood meal (91% CP)	1.00	0.00
Soybean meal	25.17	16.67
Limestone small	0.86	0.64
Monocalcium phosphate	0.14	0.00
Sodium chloride	0.21	0.18
Sodium bicarbonate	0.16	0.18
Choline chloride 75%	0.03	0.04
DL-methionine 58.1	0.29	0.21
L-threonine 73.7	0.13	0.25
Rovabio Excel LC	0.02	0.02
Meat meal	3.73	3.70
Ronozyme NP CT	0.02	0.02
Mineral/vitamin premix ¹	1.00	1.00
L-lysine sulphate 70	0.43	0.33

¹The starter and finisher basal diets metabolisable energy = 2899.59 and 2999.90 Kcal kg⁻¹, the nutrient contents (g kg⁻¹) were: Crude protein 225.1 and 186.7, Crude fat 23.5 and 30.0, Crude fibre 32.3 and 29.2, Ca 9.8 and 8.5, Available phosphorus 4.5 and 4.2, Na 1.8 and 1.7, K 0.8 and 6.5, Cl 0.2 and 1.8, Lysine 12.3 and 9.1, Methionine 5.7 and 4.7, Cystine 3.9 and 2.8, Threonine 7.9 and 7.6, Leucine 14.3 and 11.2, Isoleucine 7.8 and 6.4, Tryptophan 2.3 and 1.8, Arginine 12.4 and 9.8 and Valine 9.3 and 7.2, respectively, ²Used to formulate 2 experimental diets to feed broilers up to 3 weeks old, ³Used to formulate 2 experimental diets to feed broilers from 4-6 weeks old

Table 2: Crude fat content and fatty acid composition (as a percentage of total fatty acids) of the six experimental diets¹

Diet	Crude fat (%) ²	Total SFA ³	Total trans	Total n-9 ⁴	Total n-7 ⁵	Total n-3 ⁶	Total n-6 ⁷
Tallow	7.0	35.8	1.6	34.1	4.6	2.9	20.2
Flaxseed oil	7.2	18.6	0.5	25.0	2.1	29.5	24.1
Corn oil	7.2	20.3	0.5	29.3	2.0	2.7	45.0
Canola oil	7.2	16.9	0.5	44.4	3.5	6.9	27.4
Macadamia oil	7.0	20.6	0.5	45.7	14.8	2.0	16.2
Coconut oil	7.1	64.3	0.5	15.3	1.7	1.9	16.2
Excreta⁸							
Tallow	1.2±0.1	52.3±1.1 ^a	1.7 ^a ±0.1 ^a	20.5±1.0 ^{ab}	2.7±0.2 ^a	2.1±0.1 ^a	20.4±0.3 ^a
Flaxseed oil	0.9±0.1	22.4±0.4 ^b	0.3±0.1 ^{bd}	22.8±0.3 ^{ab}	2.2±0.1 ^a	16.0±0.5 ^c	36.1±0.5 ^b
Corn oil	1.0±0.1	23.3±0.3 ^b	0.2±0.1 ^d	25.7±0.6 ^b	2.4±0.1 ^a	3.2±0.1 ^b	45.1±0.4 ^c
Canola oil	1.0±0.0	21.0±0.5 ^b	0.3±0.1 ^{bd}	33.6±0.7 ^c	3.0±0.1 ^a	5.3±0.2 ^c	36.6±0.8 ^b
Macadamia oil	1.1±0.2	27.1±1.1 ^c	0.6±0.0 ^c	39.0±4.4 ^c	9.5±1.4 ^b	2.6±0.0 ^{ab}	21.0±6.6 ^a
Coconut oil	1.0±0.1	48.2±2.7 ^d	0.5±0.1 ^{ab}	17.3±0.4 ^a	2.0±0.1 ^a	2.7±0.1 ^{ab}	29.2±0.8 ^b

¹Finisher basal diet+added fat, fed to broilers between 22 and 40 days of age. ²Values are means of 4 replicates and the percentages are based on the wet weight.

³SFA = Saturated fatty acid, ⁴n-9 = Omega 9 monounsaturated fatty acid, ⁵n-7 = Omega 7 monounsaturated fatty acid, ⁶n-3 = Omega 3 polyunsaturated fatty acid, ⁷n-6 = Omega 6 polyunsaturated fatty acid, ⁸Values are mean of 4 replicates ± SEM and different superscript letters within the same column are significantly different (p<0.001)

variance (ANOVA) and Duncan's multiple comparison test was implemented when the ANOVA indicated the differences between dietary treatment effects were significant ($p < 0.05$) using SPSS version 22 for Windows (IBM Corp., NY, USA). The effects of dietary treatment on BW, FI and FCR were determined by one-way ANOVA ($p < 0.05$) using SAS 9.3 for windows.

RESULTS

Broiler productivity: There were no significant differences in any of the production parameters between different treatments. The average BW of 40-day-old broilers was 3367g, the FI was 5414 g/bird, the FCR was 1.63 g feed g^{-1} weight gain and the overall mortality rate was 5.4%.

The diets: The crude fat content of the different experimental diets ranged from 7.0-7.2% (Table 2). In the tallow diet, the two prevalent fatty acid groups were SFA and n-9 MUFA in almost equal portions and these together made up more than two thirds of total fatty acids. The major fatty acid group in the flaxseed oil diet was n-3 PUFA (at ~30% of the total fatty acids) with n-9 MUFA and n-6 PUFA also present at lower proportions. The main fatty acid group in the corn oil diet was the n-6 PUFA (45%) followed by n-9 MUFA (~29%). The n-9 MUFA was the predominant fatty acid group in the canola and macadamia oil-based diets (~44-46%). The canola oil diet also contained, in descending order, n-6 PUFA, SFA and n-3 PUFA; whereas the macadamia oil diet contained more SFA than either n-6 or n-3 PUFA and while the n-7 MUFA was only the third most abundant fatty acid group in the macadamia oil diet (at 15% total fatty acids), this level was still 3-9 times higher than in the other 5 diets. The coconut oil diet contained a higher percentage of SFA (64%) compared to all other diets. The contribution of trans fatty acids was the lowest of all the fatty acid groups and highest percentage was in the tallow diet with 1.6% (Table 2).

The excreta: The colour, shape, size or viscosity of droppings between different pens (dietary treatments) were not different; however, droppings from individual birds within the same group did differ in appearance (data not shown). The overall percentage of the crude fat in the wet excreta ranged from 0.9-1.2% with overall average approximately 1.1% (Table 2). The tallow treatment resulted in excreta with the highest SFA percentage (52%) followed by n-9 MUFA and n-6 PUFA with equal contributions (~20% each). The excreta of flaxseed oil treatment contained higher n-3 PUFA content (16%) than any other treatment. However, this excreta was

dominated by n-6 PUFA and n-3 PUFA was only the fourth main contributor. Similar to its respective dietary level (45%), n-6 PUFA was the main fatty acid group in excreta of the corn oil treatment, followed by n-9 MUFA and SFA. Likewise, but at a lower level, n-6 PUFA was the dominant (37%) fatty acid group in the excreta of the canola oil treatment followed by n-9 MUFA and SFA. Noteworthy and reflecting the dietary composition, canola oil excreta contained the second highest percentage of n-3 PUFA (5.3%). The excreta of broilers fed the macadamia oil diet was dominated by n-9 PUFA (39%) and contained the highest n-7 MUFA (~10%) content of all treatments. Excreta of birds in the coconut oil treatment group was dominated by SFA (48%), however this was 16% lower than its respective dietary level. The trans fatty acid group was the lowest contributor to the fatty acid composition of excreta of all treatment groups, reflecting the level in the diets (Table 2). The clear majority of the PUFA in the excreta were as alpha-linolenic acid (ALA, 92-99% of n-3 PUFA) and linoleic acid (LA, 98-99% of n-6 PUFA).

In general, the correlations between the content of the different groups of fatty acid in the diet and excreta were positive, strong and significant. Levels of two fatty acid groups (n-3 PUFA and n-7 MUFA) were particularly closely related between the diet and excreta, with R values ~1.00. The linear regression equations ($y = ax + b$) reflect the differences in the slopes ($a = 0.490$ for n-3 PUFA to 0.762 for n-6 PUFA) of the relationships between the diet and excreta for the different fatty acid groups (Table 3 and Fig. 1).

To provide an indication of the relative utilization of each of the fatty acid groups and individual fatty acids, the proportion of each in the diet and in the excreta was compared. It is inferred that fatty acids that were proportionally higher in the feed than in the excreta were preferentially utilized by the bird (and its microbiome). On the other hand, fatty acids that were proportionally higher in the excreta than in the diet, were preferentially under-utilized. In the light of the results of the present study it is considered that fatty acids that qualitatively differed between diet and excreta by <1% were neither preferentially utilized nor under-utilized. Comparing the relative abundance of the main fatty acid groups between diets and excreta there was a preference in utilization of n-3 PUFA, n-9 MUFA and a relative selection against the utilization of n-6 PUFA (Fig. 2a). A detailed comparison of the qualitative relative abundance of individual fatty acids in the diet and excreta showed that of the 29 fatty acids measured in the diet and excreta, 19 of them (C9:0, C10:0, C15:0, C17:0, C22:0, C24:0, C13:1, C18:1n-7, C22:1n-9, C20:3n-3, C18:3n-6, C20:2n-6, C20:3n-6, C22:2n-6, C22:4n-6, C22:5n-6, trans C18:1n-9, trans C18:1n-7 and trans C18:2n-6)

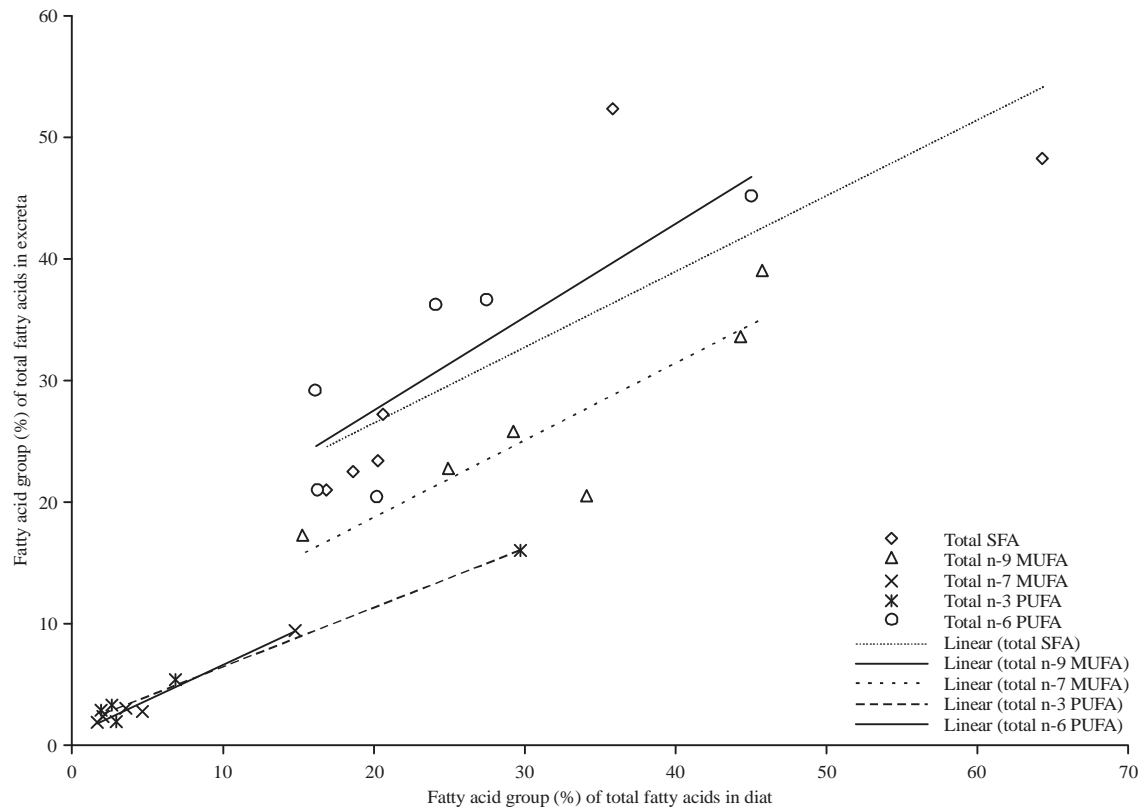


Fig. 1: Relationship between diet and excreta for different fatty acid groups (total saturates, total n-9, total n-7, total n-3 and total n-6) in broilers grown for 40 days on one of six different dietary treatments containing 4% w/w tallow, flaxseed, corn, canola, macadamia or coconut oil

Mean values for excreta are based on 4 replicates

Table 3: Relationship between the levels of the main fatty acid groups in the diets and excreta¹

Correlation term	Fatty acid group Total SFA ²	Total n-9 ³	Total n-7 ⁴	Total n-3 ⁵	Total n-6 ⁶
R value	0.82	0.89	0.99	1.00	0.85
p value	0.046	0.018	<0.001	<0.001	0.032
y ⁷	0.627x ⁸ +13.981	0.629x+6.191	0.574x+0.905	0.490x+1.552	0.762x+12.455

¹Values are the means of 4 replicates. ²SFA = Saturated fatty acid, ³n-9 = Omega 9 monounsaturated fatty acid, ⁴n-7 = Omega 7 monounsaturated fatty acid, ⁵n-3 = Omega 3 polyunsaturated fatty acid, ⁶n-6 = Omega 6 polyunsaturated fatty acid, ⁷The dependent variable (fatty acid level in excreta), ⁸The explanatory variable (dietary fatty acid level)

differed by less than 1% (data not shown) and 10 differed by more than 1% (range -13% to +16%, Fig. 2b). Seven fatty acids from 4 fatty acid groups: C18:3n-3, C16:1n-7, C18:1n-9 and the medium chain length SFA (C8:0 to C14:0) were present at relatively lower levels in the excreta compared to the diet, indicating they were preferentially utilized by the broilers. Conversely, 3 fatty acids from 2 fatty acid groups: C18:2n-6, C16:0 and C18:0 were found at relatively higher levels in the excreta, indicating these fatty acids were somewhat under-utilized by the broilers (Fig. 2b).

DISCUSSION

The productivity parameters measured in this study were similar to our previous observations^{2,3,32,34} and better than the recommended commercial values for the Cobb 500 strain³⁵. Again, in agreement with most previous studies, there were no significant differences in production parameters between treatment groups. This suggests that all the fats trialled in this study are appropriate sources of macro, micro-nutrients for broilers and their microbiome without affecting feed palatability. On the other hand, this leads us to speculate

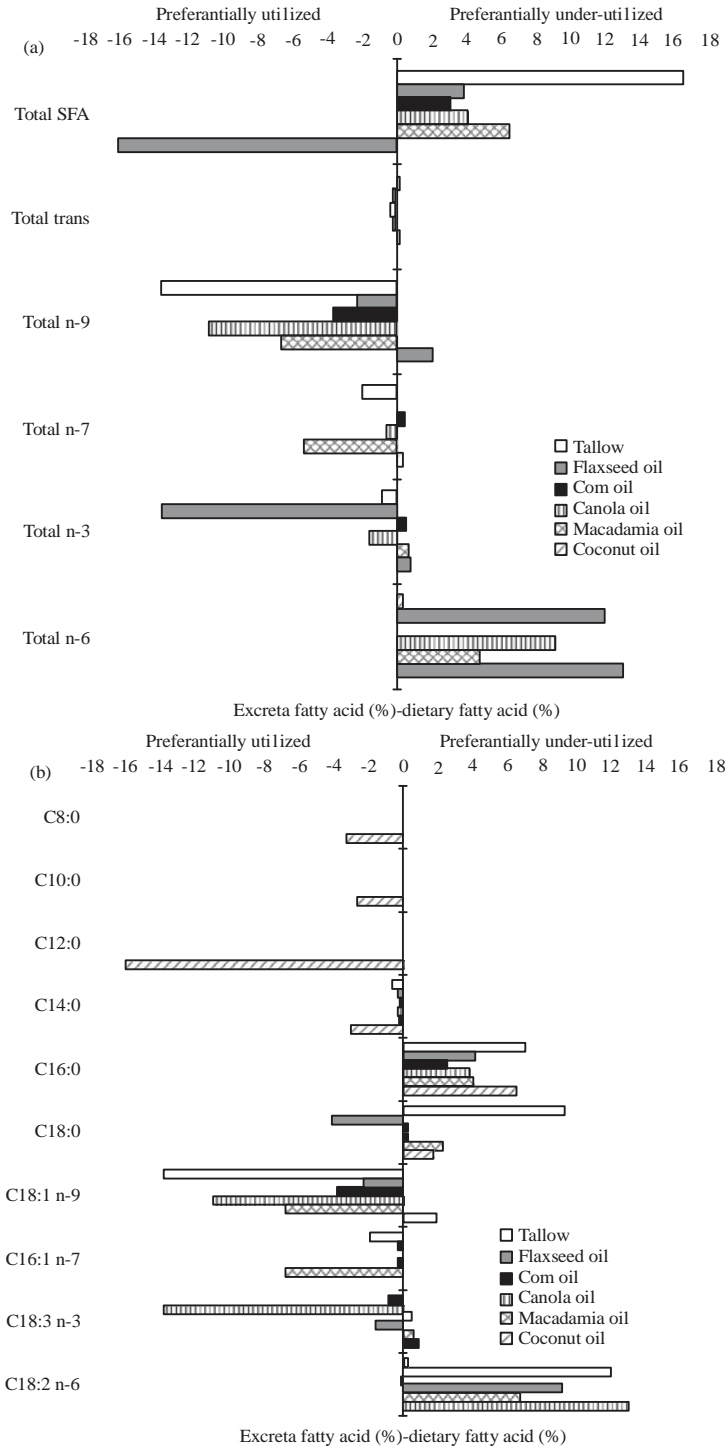


Fig. 2(a-b): Effect of six different experimental diets containing tallow, flaxseed, corn, canola, macadamia or coconut oil on altering the proportions of the (a) Fatty acid groups and (b) Individual fatty acids between diet and excreta
Presented values are for fatty acids with percentage difference >1% based on the means of 4 replicates for excreta

whether the differential effects of dietary fats on broiler performance only become evident if there is an interaction with external factors (e.g., environmental or social stress). The subjective visual observation of the excreta did not indicate

any obvious difference between different dietary treatments, however consistent with a previous report³⁶, the difference in appearance of droppings from individual birds likely reflects their caecal and faecal origins and excreta moisture content.

There is a limited number of publications which have correlated the type and percentage of excreted fatty acids to the dietary intake. Most of studies were focused on the digestion/absorption efficiency of different fatty acids along the length of the intestinal tract^{8,10,37,38}. Like the present study, those studies found that the main fatty acids detected in the digesta [(C16:0 (palmitic acid), C18:0 (stearic acid) and C18:2n-6, LA)] were also the main fatty acids present in the dietary fats (soybean oil and tallow). In addition, to the latter 3 fatty acids, C18:1n-9 (oleic acid) was also detected at high levels in the excreta in the present study.

It is acknowledged that these interpretations are general observations only, as the preferential/non-preferential utilization of most fatty acids is obviously affected by the presence or absence of other fatty acids in the diet. Thus, C18:1n-9 was preferentially utilized in all diets except when the fat was provided by coconut oil, suggesting that the medium length SFA in coconut oil were preferentially utilized instead. Similarly, C18:3n-3 (ALA) and C16:1n-7 (palmitoleic acid) were utilized when they were present at relatively high levels. At the other end of the spectrum, C18:2n-6 (LA) was preferentially under-utilized, except when it was the most abundant or at high level relative to other fatty acids (e.g., in the corn oil and tallow diets). The main exception to the rule that degree of utilization was influenced by the other types of fats in the diet was in the case of C16:0, which was always under-utilized by the broilers, irrespective of the level it was in the diet either overall or relative to other dietary fats. These observations agree with the performance data that showed that the birds grew well with good feed intake and feed conversion ratios on all diets. This suggests that whilst there may be a qualitative preference for utilization of different fatty acids, each of these fats can be used successfully to support growth.

While the correlation coefficient is beneficial in showing the type and strength of the relationship between dietary and excreta fatty acid levels, the regression analysis is also valuable. The latter create a mathematical model that allows the researcher or feed manufacturer to estimate from the level of different fatty acids in the chicken diet (independent variable) the proportion that would be excreted (dependent variable) and thereby the amount that would be retained by the birds³⁹. Although all fatty acid groups were strongly and positively correlated between the diet and excreta, there was some variation between them. Thus, the relatively low R values for SFA and n-6 PUFA compared to the other fatty acid groups was evidence of their relative under-utilization even when they were the most available fatty acids.

The flaxseed oil treatment has a particular significance as many studies have used this oil to enrich chicken products with beneficial n-3 long chain polyunsaturated fatty acids (n-3 LCPUFA)⁴⁰⁻⁴². Although the flaxseed oil diet was dominated by n-3 PUFA, the main fatty acid in the excreta of this treatment was n-6 PUFA and this resulted in increasing the n-6:n-3 ratio in the excreta to 2.25 compared to 0.82 in the diet similar to our previous findings in blood and meat^{3,32,34}. Previously it has been demonstrated that by feeding broilers a 2.5 and 5% flaxseed oil-based diet, a considerable percentage of the dietary n-3 PUFA substrate ALA can be elongated and desaturated to n-3 LCPUFA and deposited in various tissues (e.g. 30-40% of total n-3 PUFA in breast meat)^{4,33}. In the present study it is observed that 99% of excreted n-3 PUFA was in the form of ALA, indicating the bio-significance of the n-3 LCPUFA in many cellular and health mechanisms⁵.

It was interesting that all the fatty acids identified in excreta samples were also detected in the diets. Therefore, we speculate there was no modification (e.g. elongation, saturation/desaturation) of excreted fatty acids by the broiler gut and/or microbiome, despite this being reported by others^{43,44}. The only evidence of fatty acid modification was by the chicken itself³², with hepatic elongases and desaturases converting linoleic acid to arachidonic acid (n-6 PUFA) and ALA to n-3 LCPUFA (n-3 PUFA), with none of these end products being excreted. Thus, the effect of the dietary fat composition (especially the level of PUFA) on the net endogenous fat synthesis in broilers remains unclear^{45,46} and further studies will be required resolve the underlying mechanisms.

CONCLUSION

Broilers were fed 1 of 6 diets that had different sources of fat and therefore different fatty acid compositions, produced excreta that generally reflected the dietary fat composition. However, there were some subtle differences in excreta fatty acid composition which suggested variation in preferences for fatty acid utilization by the bird and/or its microbiota. The relative utilization of fatty acids was dependent on the dietary level and on the composition of other fatty acids in the feed. Several fatty acids, particularly the medium SFA (C8:0 (caprylic acid), C10:0 (capric acid), C12:0 (lauric acid) and C14:0 (myristic acid)), C18:1n-9 (oleic acid), C18:3n-3 (alpha-linolenic acid) and C16:1n-7 (palmitoleic acid) were preferentially utilized when they were present. In contrast,

C18:2n-6 (linoleic acid) and C16:0 (palmitic acid) were always under-utilized. Therefore, the non-invasive collection of excreta may provide useful data to feed manufacturers about the utilization of fatty acids in diets made using different lipid sources.

ACKNOWLEDGMENTS

Khaled Kanakri acknowledges the Australian Government Research Training Program (RTP) and the Faculty of Sciences - The University of Adelaide (full-time and supplementary) PhD scholarships. Beverly Muhlhausler is supported by a Career Development Fellowship from the National Health and Medical Research Council of Australia (NHMRC). Robert Gibson is supported by a NHMRC Senior Research Fellowship. This work was supported by the South Australian Department of Further Education, Employment, Science and Technology (DFEEST), Australia.

REFERENCES

1. Balevi, T. and B. Coskun, 2000. Effects of some oils used in broiler rations on performance and fatty acid composition in abdominal fat. *Revue Medecine Veterinaire*, 151: 937-944.
2. Kanakri, K., J. Carragher, R. Hughes, B. Muhlhausler and R. Gibson, 2017. A reduced cost strategy for enriching chicken meat with omega-3 long chain polyunsaturated fatty acids using dietary flaxseed oil. *Br. Poult. Sci.*, 58: 283-289.
3. Kanakri, K., B. Muhlhausler, J. Carragher, R. Gibson and R. Barekattain *et al.*, 2016. Relationship between the fatty acid composition of uropygial gland secretion and blood of meat chickens receiving different dietary fats. *Anim. Prod. Sci.* 10.1071/AN16268.
4. Carragher, J.F., B.S. Muhlhausler, M.S. Geier, J.D. House, R.J. Hughes and R.A. Gibson, 2016. Effect of dietary ALA on growth rate, feed conversion ratio, mortality rate and breast meat omega-3 LCPUFA content in broiler chickens. *Anim. Prod. Sci.*, 56: 815-823.
5. Cherian, G., 2007. Metabolic and cardiovascular diseases in poultry: Role of dietary lipids. *Poult. Sci.*, 86: 1012-1016.
6. Kartikasari, L.R., R. Hughes, M. Geier and R. Gibson, 2017. The effect of diet containing high alpha-linolenic acid on omega-3 fatty acids and health status of the heart in broilers. *Buletin Peternakan*, 41: 48-53.
7. Tancharoenrat, P., V. Ravindran, F. Zaefarian and G. Ravindran, 2013. Influence of age on the apparent metabolisable energy and total tract apparent fat digestibility of different fat sources for broiler chickens. *Anim. Feed Sci. Technol.*, 186: 186-192.
8. Ajuyah, A.O., D. Balnave and E.F. Annison, 1996. Determination of apparent and true dietary fatty acid digestibilities and metabolisable energy using ileal digesta and excreta from broiler chickens. *Anim. Feed Sci. Technol.*, 62: 131-139.
9. Boguśławska Tryk, M., A. Piotrowska, R. Szymeczko and K. Burlikowska, 2016. Effect of dietary lignocellulose on ileal and total tract digestibility of fat and fatty acids in broiler chickens. *J. Anim. Physiol. Anim. Nutr.*, 100: 1050-1057.
10. Tancharoenrat, P., V. Ravindran, F. Zaefarian and G. Ravindran, 2014. Digestion of fat and fatty acids along the gastrointestinal tract of broiler chickens. *Poult. Sci.*, 93: 371-379.
11. Bjerrum, L., R.M. Engberg, T.D. Leser, B.B. Jensen, K. Finster and K. Pedersen, 2006. Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and culture-based techniques. *Poult. Sci.*, 85: 1151-1164.
12. Rubio, L.A., M.J. Peinado, R. Ruiz, E. Suarez Pereira, C.O. Mellet and J.M.G. Fernandez, 2015. Correlations between changes in intestinal microbiota composition and performance parameters in broiler chickens. *J. Anim. Physiol. Anim. Nutr.*, 99: 418-423.
13. Leek, A.B.G., V.E. Beattie and J.V. O'Doherty, 2004. The effects of dietary oil inclusion and oil source on apparent digestibility, faecal volatile fatty acid concentration and manure ammonia emission. *Anim. Sci.*, 79: 155-164.
14. Tancharoenrat, P., V. Ravindran and G. Ravindran, 2015. Influence of cereal type and fat source on the performance and fat utilisation of broiler starters. *Anim. Prod. Sci.*, 55: 74-79.
15. Vilarrasa, E., R. Codony, E. Esteve-Garcia and A.C. Barroeta, 2015. Use of re-esterified oils, differing in their degree of saturation and molecular structure, in broiler chicken diets. *Poult. Sci.*, 94: 1527-1538.
16. Atteh, J.O. and S. Leeson, 1984. Effects of dietary saturated or unsaturated fatty acids and calcium levels on performance and mineral metabolism of broiler chicks. *Poult. Sci.*, 63: 2252-2260.
17. Azman, M.A. and P.T. Seven, 2005. Effects of different fat sources on nutrient and mineral retention in broiler chicken. *Indian Vet. J.*, 82: 55-58.
18. Danicke, S., O. Simon, H. Jeroch and M. Bedford, 1997. Interactions between dietary fat type and xylanase supplementation when rye based diets are fed to broiler chickens 2. Performance, nutrient digestibility and the fat soluble vitamin status of livers. *Br. Poult. Sci.*, 38: 546-556.

19. Fuhrmann, R. and J. Kamphues, 2016. Effects of fat content and source as well as of calcium and potassium content in the diet on fat excretion and saponification, litter quality and foot pad health in broilers. Eur. Poult. Sci., Vol. 80. 10.1399/eps.2016.118.
20. Lee, J.Y., S.K. Kang, Y.J. Heo, D.W. Shin and T.E. Park *et al*, 2016. Influence of flaxseed oil on fecal microbiota, egg quality and fatty acid composition of egg yolks in laying hens. Curr. Microbiol., 72: 259-266.
21. Wiseman, J., J. Powles and F. Salvador, 1998. Comparison between pigs and poultry in the prediction of the dietary energy value of fats. Anim. Feed Sci. Technol., 71: 1-9.
22. Metzler-Zebeli, B.U., A. Molnar, M. Hollmann, E. Magowan, R.J. Hawken, P.G. Lawlor and Q. Zebeli, 2016. Comparison of growth performance and excreta composition in broiler chickens when ranked according to various feed efficiency metrics. J. Anim. Sci., 94: 2890-2899.
23. Bansal, G.R., V.P. Singh and N. Sachan, 2011. Carcass quality characteristics of broilers as affected by dried poultry excreta supplementation. Asian J. Poult. Sci., 5: 116-123.
24. Nambi, J., P.N. Mbugua and B.N. Mitaru, 1992. The nutritive evaluation of dried poultry excreta as a feed ingredient for broiler chickens. Anim. Feed Sci. Technol., 37: 99-109.
25. Blanch, A., A.C. Barroeta, M.D. Baucells, X. Serrano and F. Puchal, 1996. Utilization of different fats and oils by adult chickens as a source of energy, lipid and fatty acids. Anim. Feed Sci. Technol., 61: 335-342.
26. Firman, J.D., A. Kamyab and H. Leigh, 2008. Comparison of fat sources in rations of broilers from hatch to market. Int. J. Poult. Sci., 7: 1152-1155.
27. Ravindran, V., P. Tancharoenrat, F. Zaefarian and G. Ravindran, 2016. Fats in poultry nutrition: Digestive physiology and factors influencing their utilisation. Anim. Feed Sci. Technol., 213: 1-21.
28. Zumbado, M.E., C.W. Scheele and C. Kwakernaak, 1999. Chemical composition, digestibility, and metabolizable energy content of different fat and oil by-products. J. Applied Poult. Res., 8: 263-271.
29. NRC., 1994. Nutrient Requirements of Poultry. 9th Edn., National Academy Press, Washington, DC., USA.
30. Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem., 226: 497-509.
31. Gregory, M.K., V.H.L. See, R.A. Gibson and K.A. Schuller, 2010. Cloning and functional characterisation of a fatty acyl elongase from Southern bluefin tuna (*Thunnus maccoyii*). Comp. Biochem. Physiol. Part B: Biochem. Mol. Biol., 155: 178-185.
32. Kanakri, K., J. Carragher, B. Muhlhausler, R. Hughes and R. Gibson, 2017. *In ovo* exposure to omega-3 fatty acids does not enhance omega-3 long-chain polyunsaturated fatty acid metabolism in broiler chickens. J. Dev. Origins Health Dis. 10.1017/S2040174417000216.
33. Kartikasari, L.R., R.J. Hughes, M.S. Geier, M. Makrides and R.A. Gibson, 2012. Dietary alpha-linolenic acid enhances omega-3 long chain polyunsaturated fatty acid levels in chicken tissues. Prostaglandins Leukotrienes Essential Fatty Acids, 87: 103-109.
34. Kanakri, K., J. Carragher, R. Hughes, B. Muhlhausler and R. Gibson, 2017. The effect of different dietary fats on the fatty acid composition of several tissues in broiler chickens. Eur. J. Lipid Sci. Technol. 10.1002/ejlt.201700237.
35. Cobb 500, 2015. Broiler performance and nutrition supplement. http://www.cobb-vantress.com/docs/default-source/cobb-500-guides/Cobb500_Broiler_Performance_And_Nutrition_Supplement.pdf
36. Pauwels, J., B. Taminiau, G.P.J. Janssens, M. de Beenhouwer, L. Delhalle, G. Daube and F. Coopman, 2015. Cecal drop reflects the chicken's cecal microbiome, fecal drop does not. J. Microbiol. Methods, 117: 164-170.
37. Hurwitz, S., A. Bar, M. Katz, D. Sklan and P. Budowski, 1973. Absorption and secretion of fatty acids and bile acids in the intestine of the laying fowl. J. Nutr., 103: 543-547.
38. Sklan, D., P. Budowski, I. Ascarelli and S. Hurwitz, 1973. Lipid absorption and secretion in the chick: Effect of raw soybean meal. J. Nutr., 103: 1299-1305.
39. Puvaca, N., D. Lukac, D. Ljubojevic, V. Stanacev, M. Beukovic, L. Kostadinovic and N. Plavska, 2014. Fatty acid composition and regression prediction of fatty acid concentration in edible chicken tissues. World's Poult. Sci. J., 70: 585-592.
40. Baeza, E., P. Chartrin, V. Gigaudo, S. Tauty, K. Meteau, M. Lessire and C. Berri, 2013. Effects of dietary enrichment with n-3 fatty acids on the quality of raw and processed breast meat of high and low growth rate chickens. Br. Poult. Sci., 54: 190-198.
41. Konieczka, P., M. Czauderna and S. Smulikowska, 2017. The enrichment of chicken meat with omega-3 fatty acids by dietary fish oil or its mixture with rapeseed or flaxseed-Effect of feeding duration: Dietary fish oil, flaxseed and rapeseed and n-3 enriched broiler meat. Anim. Feed Sci. Technol., 223: 42-52.
42. Lopez-Ferrer, S., M.D. Baucells, A.C. Barroeta, J. Galobart and M.A. Grashorn, 2001. n-3 enrichment of chicken meat. 2. Use of precursors of long-chain polyunsaturated fatty acids: Linseed oil. Poult. Sci., 80: 753-761.
43. Clement, J., 1980. Intestinal absorption of triglycerols. Reprod. Nutr. Dev., 20: 1285-1307.

44. Van der Hoeven-Hangoor, E., J.M.B.M. van der Vossen, F.H.J. Schuren, M.W.A. Verstegen, J.E. de Oliveira, R.C. Montijn and W.H. Hendriks, 2013. Ileal microbiota composition of broilers fed various commercial diet compositions. *Poult. Sci.*, 92: 2713-2723.
45. Crespo, N. and E. Esteve-Garcia, 2002. Nutrient and fatty acid deposition in broilers fed different dietary fatty acid profiles. *Poult. Sci.*, 81: 1533-1542.
46. Snaz, M., C.J. Lopez-Bote, D. Menoyo and J.M. Bautista, 2000. Abdominal fat deposition and fatty acid synthesis are lower and β -oxidation is higher in broiler chickens fed diets containing unsaturated rather than saturated fat. *J. Nutr.*, 130: 3034-3037.