

Comparative biology of germ-free and conventional poultry

A. J. Cowieson*

DSM Nutritional Products, Wurmisweg 576, 4303 Kaiseraugst, Switzerland

ABSTRACT Interaction between the host and the enteric microbiome is highly complex. Microbial involvement in certain pathologies is moderately well established, but the contribution of the microbiome to animal welfare, behavior, sustainability, immune development, nutritional status, physiology, and maturation is less clear. A valuable experimental model to enable scientists to explore the role of the microbiome in various domains is to compare various phenotypes of a conventionally reared (CV) cohort with those in a germ-free (GF) state. A GF animal is one that is devoid of any detectable microbial life including bacteria, viruses, protozoa and parasites. The GF state is different from gnotobiotic animals where the microbiome is fully described, or ‘specific pathogen free’ (SPF) animals where a moderately normal microbiome is present but devoid of pathogenic microorganisms. Pioneering GF research in poultry in the late 1940s and 1950s has its origin in a need understand the mode of action of antibiotics. Early researchers

quickly established that GF chicks responded differently to antibiotics than CV counterparts. The GF experimental model has since been exploited in many divergent fields including pathology, immunology, metabolism, anatomy, physiology, and others. The absence of a microbiome presents the host with a range of advantages and disadvantages. For example, GF chicks often grow more quickly and have lower feed conversion ratio (FCR) than their CV counterparts but may be less resilient to external stress and have a compromised immunological maturation rate. This review will summarize the literature on GF animal research with a special emphasis on poultry. The objective of the review is to establish a frame of reference to understand the extent of the role of the microbiome in animal health, welfare, nutrition, and growth, to provide opportunities for targeted modulation of the microbiome to achieve desired phenotypic responses whilst simultaneously minimizing unintended collateral effects.

Key words: germ-free, poultry, microbiome, health, nutrition

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INTRODUCTION

In ruminants the enteric microbiome has co-evolved with the host and is central to the capacity of the animal to survive on feed with a relatively low energy density and high fiber concentration. In nonruminant animals such as poultry and swine, the enteric microbiome has a more nuanced role in nutrient recovery and partitioning and the net value of the microbiome to the host is difficult to fully calculate. Cohendy (1912) and Balzam (1937) were the first to successfully rear GF chicks for use in animal experimentation. However, GF chicken research was not widely used until the pioneering research of Reyniers and colleagues from the University of Notre Dame (Reyniers et al., 1950). Subsequently, the

GF chick model has been used extensively to explore the mode of action of antibiotics (Forbes and Park, 1959; Coates et al., 1963), nutrient digestion and absorption (Edwards and Boyd, 1963; Boyd and Edwards, 1967; Campbell et al., 1983), coccidiosis (Radharkrishnan and Bradley, 1973; Gaboriaud et al., 2021), gastrointestinal development (Cook and Bird, 1973; Ford, 1974; Corring et al., 1981; Philips and Fuller, 1983; Furuse and Yokota, 1984a; Furuse and Okumura, 1994), protein metabolism (Salter and Coates, 1971; Salter et al., 1974; Coates et al., 1977; Okumura et al., 1978; Furuse and Yokota, 1985; Furuse et al., 1985; Muramatsu et al., 1985; Muramatsu et al., 1987; Yokota et al. 1989; Muramatsu et al., 1993b), metabolic rate (Harrison and Hewitt, 1978; Muramatsu et al., 1988), energy metabolism (Coates et al., 1981; Hedge et al., 1982; Furuse et al., 1991b,c; Muramatsu et al., 1992) and feed additive efficacy (Furuse et al., 1991a; Muramatsu et al., 1993a; Langhout et al., 2000; Drew et al., 2003; Cheled-Shoval et al., 2014). The entire body of work from 1950 to 2022 (approximately 40 independent studies) on germ-free chickens gives considerable insight into the

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*Corresponding author: aaron.cowieson@dsm.com

role of the microbiome in development of the intestine and support organs, the ability of the chick to extract nutrients from the feed, development of immune function, retention of protein and nonprotein nitrogen (N), energy metabolism, metabolic rate, and disease resilience. Additionally, complementary germ-free work in pigs and mice exploring differential gene expression, metagenomics and biomarkers (Chowdhury et al., 2007; Tlaskalova-Hogenova et al., 2011; Sun et al., 2018; Mishima et al., 2020; Diviccaro et al., 2021; Yang et al., 2021) confirms several important observations around the central role of the microbiome in host immunity, growth, the gut-brain axis and N cycling. The present review will summarize key observations from germ-free research in poultry, supported with work in pigs and rodents, to create a foundational framework for ongoing initiatives to leverage microbiome modulatory technologies to achieve desirable end points in poultry nutrition, veterinary health, live production, and environmental sustainability.

DEFINITIONS AND EXPERIMENTAL APPROACHES

With respect to the microbiome, animals may be classified into 4 distinct groups (adapted from Furuse and Okumura, 1994).

1. A GF animal is entirely devoid of any detectable microbial life. This includes viruses, protozoa, fungi, parasites, and bacteria. In early GF research the GF state was confirmed via regular swabbing of the animal, the diet it received and the environment it was reared in. In more recent GF research, the GF state is confirmed via metagenomic sequencing of excreta or other biological matrices (Guitton et al., 2020).
2. A gnotobiotic animal differs from the GF animal in that there is detectable microbial life but this is fully characterized. For example, when the intestine of a GF animal is deliberately colonized by a single strain or specific consortia of bacteria.
3. A specific pathogen free (SPF) animal is free from certain known pathogenic bacteria, parasites or viruses but otherwise has a 'normal' microbiome.
4. A CV animal has a full, typically undefined, microbial complement.

The GF experimental model was developed in the 1950s and was initially used mainly to explore the interaction between the host animal and therapeutic antibiotics (Reyniers et al., 1950). The GF chick is hatched from sterilized eggs, reared in a sterile environment, and typically fed an irradiated diet and double distilled drinking water, sometimes treated with broad-spectrum antibiotics. The GF state is subsequently confirmed via microscopic examination of fecal smears and culture of excreta samples. A helpful summary of the procedure can be found in Langhout et al. (2000) and Guitton et al. (2020).

GROWTH AND FEED EFFICIENCY

Germ-Free and Conventional Poultry Experiments

A summary of the growth performance of GF and CV chicks is presented in Table 1. The early work by Reyniers et al. (1950) has been excluded from this summary as the authors were optimizing the GF experimental model and the GF chicks used were variably vitamin deficient and consequently had poor growth, feather development and overall health.

The growth rate and feed efficiency (FCE) of GF chicks is typically higher than of CV counterparts though there are several important exceptions. Overall, across 31 experiments from 20 independent peer-reviewed papers, GF chicks returned a 9.7% performance advantage over their CV counterparts. However, this effect was not consistent and ranged from 45.7% (Furuse and Yokota, 1984b) for chicks fed on a semisynthetic, low protein diet, to -44.3% (Furuse and Yokota, 1984b) for chicks fed a semisynthetic, high protein diet. Generally, CV chicks out-perform GF chicks under 2 dietary conditions. First, if the diet contains a high concentration of fiber or fermentable carbohydrate and/or, second, when the diet contains a high nutrient density (high crude protein [CP], or apparent metabolizable energy [AME]). Alternatively, GF chicks outperform CV chicks on highly digestible, low fiber diets and especially so when the diets are formulated to be marginal in AME. Whilst these conclusions are generally true, there may be a threshold in AME and digestible amino acid concentration below which CV chicks have an advantage over GF chicks for example, in very high fiber diets or in a fasted state. Unfortunately, many of the growth performance advantages of GF over CV chicks reported in the literature may be artefacts created by the over-reliance on semisynthetic diets based on isolated soy protein, maize starch, casein, and vegetable oil. Despite these caveats, there is clear evidence from multiple controlled experiments that GF chicks usually have more rapid growth rate and feed efficiency than CV chicks and so it can be reasonably concluded that the microbiome is involved in regulation of growth rate and metabolic efficiency. The extent to which the microbiome influences growth rate compared with feed efficiency is not clear as many studies only reported body weight. However, the presence of a microbiome appeared to suppress growth and feed efficiency indiscriminately. These data suggest that zootechnical or pharmaceutical feed additives designed to elicit beneficial modification to the enteric microbiome in poultry may have differential effects depending on diet nutrient density, nutrient bio-availability or fermentable carbohydrate concentration. For example, unintended negative consequences of microbial modulators may occur if modification of the microbiome results in a reduced capacity of the host to extract energy from fiber (Muramatsu et al., 1992), especially if the diet contains a high fiber concentration and/or a low AME density. It may therefore be important to

Table 1. Comparative growth rate (body weight gain or body weight; BWG, BW) and feed conversion efficiency (FCE) of germ-free (GF) or conventionally reared (CV) chicks.

Publication	End point ¹	Diet ²	Relative performance of GF vs. CV (%) ³
Forbes and Park (1959)	BW on d28 (males)	Semisynthetic	21.9
Forbes and Park (1959)	BW on d28 (females)	Semisynthetic	15.4
Forbes and Park (1959)	BW on d28 (males)	Semisynthetic	8.8
Forbes and Park (1959)	BW on d28 (females)	Semisynthetic	5.6
Forbes and Park (1959)	BW on d28 (males)	Corn/SBM	20.7
Forbes and Park (1959)	BW on d28 (females)	Corn/SBM	15.7
Forbes and Park (1959)	BW on d28 (males)	Corn/SBM	15.2
Forbes and Park (1959)	BW on d28 (females)	Corn/SBM	19.8
Edwards and Boyd (1963)	BW on d10	Corn/SBM	19.7
Coates et al. (1963)	BW on d28 (Exp 1)	Corn/SBM	3.7
Coates et al. (1963)	BW on d28 (Exp 1)	Corn/SBM	24.0
Coates et al. (1963)	BW on d28 (Exp 2)	Corn/SBM	0.0
Coates et al. (1963)	BW on d28 (Exp 2)	Corn/SBM	1.4
Coates et al. (1963)	BW on d28 (Exp 3)	Corn/SBM	-11.5
Coates et al. (1963)	BW on d28 (Exp 3)	Corn/SBM	20.9
Coates et al. (1963)	BW on d28 (Exp 4)	Corn/SBM	23.4
Coates et al. (1963)	BW on d28 (Exp 4)	Corn/SBM	13.3
Coates et al. (1963)	BW on d28 (Exp 4)	Corn/SBM	32.4
Coates et al. (1963)	BW on d28 (Exp 5)	Corn/SBM	9.6
Coates et al. (1963)	BW on d28 (Exp 5)	Corn/SBM	13.4
Coates et al. (1963)	BW on d28 (Exp 6)	Corn/SBM	39.1
Coates et al. (1963)	BW on d28 (Exp 6)	Corn/SBM	12.3
Coates et al. (1970)	BW on d21 (Exp 2)	Semisynthetic + raw soy	25.2
Coates et al. (1970)	BW on d21 (Exp 2)	Semisynthetic + heated soy	11.8
Coates et al. (1970)	BW on d21 (Exp 3)	Semisynthetic + raw soy	17.1
Coates et al. (1970)	BW on d21 (Exp 3)	Semisynthetic + heated soy	8.3
Coates et al. (1970)	BW on d21 (Exp 4)	Semisynthetic + raw soy	17.8
Coates et al. (1970)	BW on d21 (Exp 4)	Semisynthetic + heated soy	6.8
Coates et al. (1977)	BW on d11	Semisynthetic	3.3
Coates et al. (1977)	BW on d11	Isolated soy protein	7.0
Coates et al. (1977)	FCE on d11	Semisynthetic	9.5
Coates et al. (1977)	FCE on d11	Isolated soy protein	-7.2
Campbell et al. (1983)	BW on d21 (Exp 1)	Rye-based	11.7
Campbell et al. (1983)	BW on d21 (Exp 1)	Wheat-based	3.0
Campbell et al. (1983)	BW on d21 (Exp 2)	Rye-based	40.2
Campbell et al. (1983)	BW on d21 (Exp 2)	Wheat-based	12.2
Campbell et al. (1983)	FCE on d21 (Exp 2)	Rye-based	33.1
Campbell et al. (1983)	FCE on d21 (Exp 2)	Wheat-based	13.6
Furuse and Yokota (1984b)	BWG to d14	Semisynthetic 50 g/kg CP	45.7
Furuse and Yokota (1984b)	BWG to d14	Semisynthetic 200 g/kg CP	-4.4
Furuse and Yokota (1984b)	BWG to d14	Semisynthetic 400 g/kg CP	-44.3
Furuse and Yokota (1984b)	FCE to d14	Semisynthetic 50 g/kg CP	41.0
Furuse and Yokota (1984b)	FCE to d14	Semisynthetic 200 g/kg CP	1.0
Furuse and Yokota (1984b)	FCE to d14	Semisynthetic 400 g/kg CP	6.0
Furuse and Yokota (1984b)	BW on d14	Semisynthetic 50 g/kg CP	12.5
Furuse and Yokota (1984b)	BW on d14	Semisynthetic 200 g/kg CP	-5.1
Furuse and Yokota (1984b)	BW on d14	Semisynthetic 400 g/kg CP	1.6
Furuse and Yokota (1985)	BW on d14	Semisynthetic 227 g/kg CP	21.3
Furuse and Yokota (1985)	BW on d14	Semisynthetic 293 g/kg CP	16.0
Furuse and Yokota (1985)	FCE on d14	Semisynthetic 227 g/kg CP	22.2
Furuse and Yokota (1985)	FCE on d14	Semisynthetic 293 g/kg CP	17.1
Furuse et al. (1985)	BW on d10 (Exp 1)	Semisynthetic 11.7 MJ/kg ME	-1.1
Furuse et al. (1985)	BW on d10 (Exp 1)	Semisynthetic 14.8 MJ/kg ME	2.3
Furuse et al. (1985)	BW on d10 (Exp 2)	Semisynthetic 11.7 MJ/kg ME	6.0
Furuse et al. (1985)	BW on d10 (Exp 2)	Semisynthetic 14.8 MJ/kg ME	7.1
Furuse et al. (1985)	FCE on d10 (Exp 2)	Semisynthetic 11.7 MJ/kg ME	5.8
Furuse et al. (1985)	FCE on d10 (Exp 2)	Semisynthetic 14.8 MJ/kg ME	7.0
Muramatsu et al. (1987)	BWG to d21	Corn/SBM	9.7
Muramatsu et al. (1987)	FCE to d21	Corn/SBM	6.8
Muramatsu et al. (1988)	BWG to d13	Semisynthetic	23.3
Muramatsu et al. (1988)	FCE to d13	Semisynthetic	14.6
Furuse et al. (1991a)	BW on d10	Semisynthetic	-14.4
Furuse et al. (1991a)	BW on d10	Semisynthetic + acetic acid	11.0
Furuse et al. (1991a)	FCE on d10	Semisynthetic	-27.0
Furuse et al. (1991a)	FCE on d10	Semisynthetic + acetic acid	13.5
Furuse et al. (1991b)	BWG to d14	Semisynthetic	34.8
Furuse et al. (1991b)	BWG to d14	Semisynthetic + sorbose	13.8
Furuse et al. (1991b)	FCE to d14	Semisynthetic	14.9
Furuse et al. (1991b)	BWG to d14	Semisynthetic	34.8
Furuse et al. (1991c)	BWG to d14	Semisynthetic 220 g/kg CP	-2.3
Furuse et al. (1991c)	BWG to d14	Semisynthetic 445 g/kg CP	0.7
Furuse et al. (1991c)	BWG to d14	Semisynthetic 116 g/kg CP	7.0

(continued)

Table 1 (*Continued*)

Publication	End point ¹	Diet ²	Relative performance of GF vs. CV (%) ³
Muramatsu et al. (1992)	FCE to d14	Semisynthetic + 280 g/kg cellulose	-19.0
Muramatsu et al. (1992)	BWG to d14	Semisynthetic + 280 g/kg cellulose	-18.8
Muramatsu et al. (1993a)	FCE to d10	Semisynthetic + glucose	-4.8
Muramatsu et al. (1993a)	FCE to d10	Semisynthetic + fructose	-2.7
Muramatsu et al. (1993a)	BWG to d10	Semisynthetic + glucose	-2.6
Muramatsu et al. (1993a)	BWG to d10	Semisynthetic + fructose	6.5
Muramatsu et al. (1993b)	FCE to d17	Corn/SBM + 34 g/kg cellulose + NH ₃	2.1
Muramatsu et al. (1993b)	BWG to d17	Corn/SBM + 34 g/kg cellulose + NH ₃	7.8
Muramatsu et al. (1993b)	FCE to d17	Corn/SBM + 34 g/kg cellulose	9.5
Langhout et al. (2000)	BWG to d17	Corn/SBM + 34 g/kg cellulose	11.0
Drew et al. (2003)	BWG to d21	Corn/SBM	-4.9
Drew et al. (2003)	BWG to d21	Corn/SBM + pectin	-0.3
Grand Mean			9.8

¹When FCR was reported this was transformed to FCE to enable more convenient comparisons with BWG, that is, higher numbers regarded as positive.

²CP, crude protein; ME, AME; NH₃, ammonia; SBM, soybean meal.

³A higher number means that the GF animals grew more quickly or had improved feed efficiency relative to their CV counterparts.

monitor microbially expressed cellulases and hemicellulases in the ileum and caecum when designing a microbiome modulator to overcome an adjacent challenge to gut health. It is inadvisable to inadvertently reduce the capacity of the enteric microbiome to ferment fiber. The gut microbiome also influences the consequences of ingestion of antinutrients on host growth rate. In a series of 3 experiments, Coates et al. (1970) observed that CV chicks fed raw soy meal had a 20% lower body weight than GF chicks fed the same diet whereas the body weight differential was only 8.9% when heated soy meal was fed. This suggests that the enteric microbiome may modify the antinutritional potential of trypsin inhibitors or hemagglutinins (or potentially other antinutrients). In this specific case, raw soy had a reduced antinutritional consequence on GF relative to CV chicks. These interactions and additional putative reasons for the growth headwind associated with the microbiome will be discussed in detail later.

Antibiotic Treatment

Growth-promoting effects of antibiotics in poultry have been appreciated for decades although the mode of action is not fully understood (Dibner and Richards, 2005). Removal of antibiotic growth promoters in poultry production generates increases in mortality and FCR (typically 1%–3%; Dibner and Richards, 2005). These effects are muted relative to the delta change between GF and CV chicks, suggesting that the presence of a microbiome per se is influential at a more fundamental level than can be approximated by feeding antibiotics. Recently, via 16S and shotgun metagenomic sequencing and untargeted metabolomics, further insights into the effect of specific antibiotics on the composition and function of the microbiome in poultry has been presented (Plata et al., 2022). These authors noted that antibiotic treatment had profound effects on N cycling in the caeca, enteric bile salt and fatty acid concentrations, gut antioxidant status, carbohydrate

metabolism, mucin degradation and inflammation. Many of these areas have previously been explored using GF animal models and the importance will be discussed in subsequent sections.

INTESTINAL PHYSIOLOGY

Cook and Bird (1973) were the first authors to specifically publish a peer-reviewed paper on role of the microbiome on intestinal morphology in chickens although several groups had previously published similar work in rodents and Coates et al. (1970) presented some preliminary data on the dry weight of selected intestinal segments in GF and CV chicks. Cook and Bird (1973) demonstrated that GF chicks (reared from hatch to d7) had significantly lower villus (37.4 vs. $63.0 \text{ mm}^2 \times 10^{-3}$ at d7) and lamina propria (8.5 vs. $14.0 \text{ mm}^2 \times 10^{-3}$ at d7) area, than CV chicks. Furthermore, the crypt depth in GF chicks was lower than those in the CV cohort. These effects became significant from d4 to 5 and the differences between the CV and GF chicks grew as the chicks aged. The crypt pool size was 54% lower in GF compared with CV chicks and there was a significant decrease in epithelial cell migration. These results indicate a role of the microbiome in maturation of villi and in migration rate of epithelial cells with a profound consequence on absorptive area.

Ford (1974) explored the role of the microbiome on intestinal pH in chicks and the results are presented in Figure 1. The intestinal pH of GF chicks was significantly higher (approximately 0.2–0.3 pH points) than of CV chicks in all intestinal segments except for the proventriculus and gizzard. In addition, GF chicks had significantly lower intestinal buffering capacity than CV chicks. Interestingly, there was no difference in pH in the gastric gut between GF and CV chicks suggesting that gastric acid production may be unaffected by the enteric microbiome. Furthermore, whilst GF chicks had reduced buffering capacity compared with CV chicks, they were able to increase small intestine pH readily,

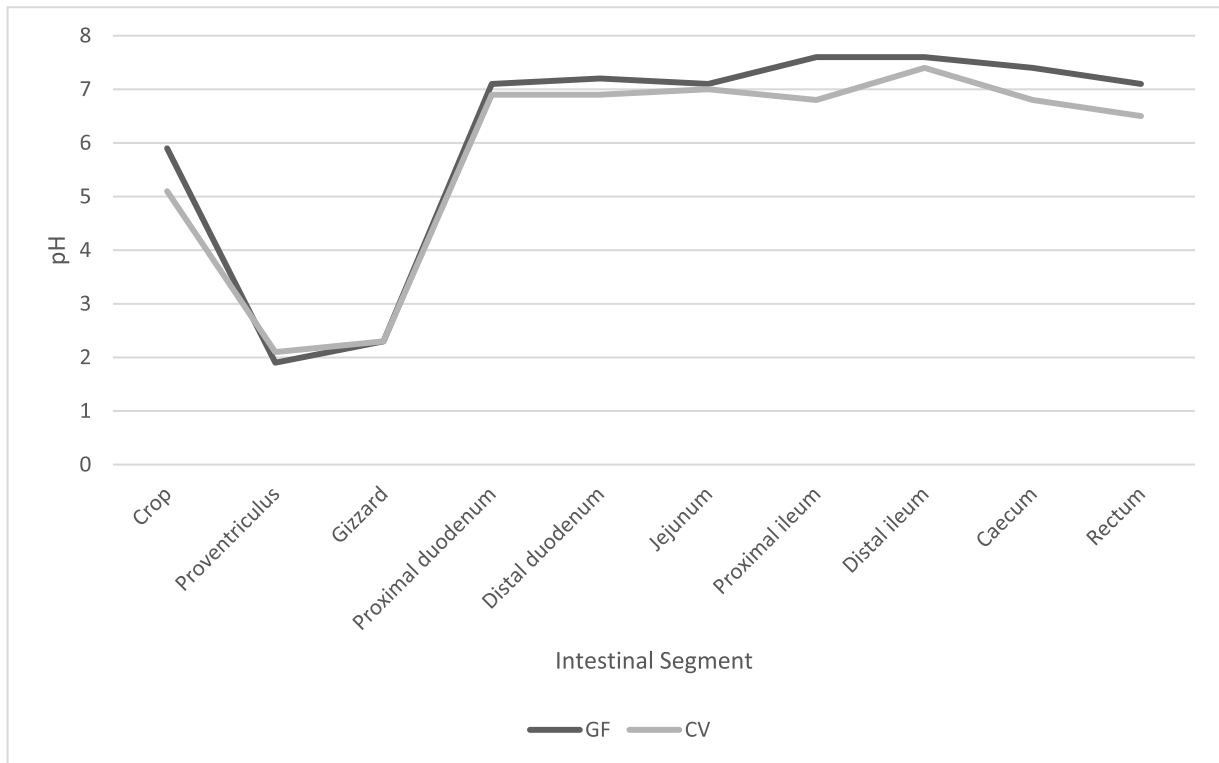


Figure 1. Comparative intestinal pH in germ-free (GF) and conventionally reared (CV) chicks fed a maize-based, high fibre, diet (adapted from Ford, 1974).

indicating that endogenous sodium bicarbonate production may also be independent of the microbiome. The consequence of the more basic intestinal environment in GF chicks on nutrient bioavailability is likely to be limited but a higher pH in the small intestine may be advantageous for absorption of bile salts and vitamin B₁₂ due to the pH dependency of binding between vitamin B₁₂ and intrinsic factor (Shum et al., 1971). Modification of the enteric microbiome via specific feed additives in order to increase or decrease the pH of specific intestinal segments is an interesting area for future research and may be expected to influence vitamin, mineral, and amino acid digestibility as well as suppression of some pathogenic species.

Coates et al. (1981) noted that GF chicks had thinner intestines than CV chicks and that this was independent of chick body weight. This observation was confirmed by Furuse and Yokota (1984a) who observed that GF chicks had lower absolute and relative weights of the duodenum, jejunum, ileum, cecum, and colon when the chicks were fed a diet with a standard (200 g/kg) crude protein concentration. Furthermore, the absolute and relative length of the duodenum, jejunum, ileum, and cecum was lower in GF chicks. The authors did not observe any effect of the microbiome on the absolute or relative weight of the kidney, heart, spleen, adrenal glands, liver or pancreas although liver fat content was higher in GF chicks which suggests a role of the microbiome in hepatic lipid export, fat metabolism and bile synthesis and absorption (these elements will be further described later). Muramatsu et al. (1987) noted that GF

chicks had lower relative weights of the liver, spleen, duodenum, jejunum + ileum, cecum, and gut + liver than CV birds. This is in partial agreement with earlier reports though the relatively smaller liver in GF chicks contrasted with the work of Furuse and Yokota (1984a). These differences are likely to be diet-related and in particular crude protein and AME concentrations. Importantly, Muramatsu et al. (1987) reported significantly higher fractional protein synthesis rate in the liver, small intestine and cecum of CV chicks compared with GF. These differences were less dramatic in other tissues with a less immediate connection to the microbiome. The stimulation of protein synthesis by the microbiome is likely to be mediated via short-chain fatty acid (SCFA) production and an effect of microbial metabolites such as cholic acid (from bile deconjugation), amines and ammonia and these effects also increase maintenance energy requirements in CV birds (Muramatsu et al., 1987; Muramatsu et al., 1988; Furuse et al., 1991a; Muramatsu et al. 1993b). Similar responses were more recently confirmed in pigs using transcriptomics (Chowdhury et al. 2007). GF piglets had several differentially expressed genes compared with CV piglets and these included genes involved in interferon signaling cascades, mucin biosynthesis, immune development, and epithelial cell turnover. It can be concluded that the presence of an enteric microbiome profoundly influences intestinal maturation rate, epithelial turnover and fractional protein synthesis in the intestine and liver. Many of these effects are likely to be conferred to the host indirectly via primary and secondary microbial metabolites

such as those generated by bacterial metabolism of nitrogen-containing compounds such as biogenic amines and ammonia and SCFA from fiber fermentation.

Chowdhury et al. (2007) noted that the enteric microbiome induces a state of chronic inflammation in the host and that this may be necessary to maintain tight junction integrity and ensure active maturation of the epithelium. More recently, Cheled-Shoval et al. (2014) explored the efficacy of mannan-oligosaccharides in GF and CV chicks. These authors observed that GF chicks have lower neutral and acidic goblet cell number and density, absence of sialylated goblet cells and reduced MUC2 mRNA expression, compared with CV chicks. These are symptoms of a poorly developed mucosa and immature mucin architecture. Interestingly, whilst there were fewer goblet cells in the small intestine of GF chicks compared with CV chicks, the opposite occurred in the cecum. This may be a result of entry of digestive enzymes, notably proteases, from the pancreas of the host into the cecum of the GF chick. Sun et al. (2018) recently explored transcriptomes of GF and CV pigs from the same litter. More than 70% of pig transcriptomes were microbially regulated and especially those involving host immunity and response to external stressors. Interferon-associated genes were downregulated in GF piglets. Additionally, the spleen and liver were underdeveloped in GF animals. Thus, while GF animals may benefit from lower maintenance energy requirements, they lack a fully functional intestinal tract, especially the lamina propria, spleen, and liver.

ENERGY METABOLISM AND METABOLIC RATE

When it comes to energy metabolism, as far as the host is concerned, the microbiome is both a blessing and a curse. On one hand the microbiome can assist the host to extract energy from fiber and other dietary nutrients with an inherently low digestibility. However, the presence of the microbiome in the intestinal tract generates a substantial increase in basal metabolic rate, energy requirement and compromises bile integrity and fat digestion.

Boyd and Edwards (1967) were the first authors to report a negative influence of the microbiome on fat digestion in chickens. In this work GF chicks had higher (85.1% vs. 75%) retention of beef tallow compared with CV chicks. Interestingly, there was little difference between GF and CV chicks (87.6% vs. 85.6%) in their capacity to retain corn oil. The microbiome disrupts micelle formation and stability, and GF chicks also have more esterase activity in the brush border. These microbiome-related differences are particularly important for retention of unsaturated fatty acids such as palmitic and stearic whereas unsaturated fatty acids such as oleic or linoleic are less affected. Corring et al. (1981) reported that bacterial hydrolases can split the bond between bile salts and taurine and glycine which is why GF animals have more primary, unmodified bile salts in the

intestinal tract. Liver bile biosynthesis in GF animals is also lower than in CV animals and GF animals also have lower rates of catabolism of hepatic cholesterol, which explains the higher liver fat concentration in GF animals (Furuse and Yokota, 1984a). Thus, microbiome modifying feed additives may be more effective in diets containing animal fat sources or mixed animal/vegetable fat blends given the disproportionately negative role of the microbiome in retention of saturated fatty acids. In addition, end point targets for next generation microbiome modulators may include mitigation of fatty liver, improvement in intestinal lipid emulsification, fat soluble vitamin absorption and choline and glycine requirement.

Despite a negative influence of the microbiome on lipid metabolism, diets fed to CV chicks typically have a higher AME than the same diet fed to GF chicks. Hedge et al. (1982) explored the effect of adding wheat straw (100, 200, or 300 g/kg) to diets of chickens on AME. Addition of 300 g/kg wheat straw reduced the AME to gross energy ratio of the diet from 0.811 to 0.532 in GF chicks and from 0.811 to 0.573 in CV chicks (Figure 2). The authors calculated that GF chicks can extract 0.09 MJ/kg of AME from wheat straw whereas CV chicks can extract 2.73 MJ/kg. Furuse and Yokota (1984b) also noted a lower AME in GF chicks (13.7 MJ/kg) compared with CV chicks (14.1 MJ/kg). However, despite the higher AME in CV chicks, the utilization of energy in CV chicks was lower than in GF chicks and GF chicks retained more body lipid. Basal metabolic rate was also calculated to be lower in GF chicks than in CV chicks and CV chicks returned a higher energy maintenance requirement (118.92 vs. 98.75 kJ/24 h² birds) than GF chicks. Furuse and Yokota (1985) also observed lower AME in GF chicks (10.6 MJ/kg) compared with CV birds (11.5 MJ/kg). However, this separation was only observed when the chicks were fed a diet with a low energy density and high fiber concentration. When a high energy density, highly digestible, diet was offered, there was no difference between GF (14.4 MJ/kg) and CV (14.9 MJ/kg) groups. Muramatsu et al. (1992) found that digestible energy was higher in CV (9.5 MJ/kg) than GF (7.6 MJ/kg) chicks. Furthermore, CV chicks had higher neutral detergent fiber digestibility than GF chicks (12.3% vs. 0.9% respectively). The contribution of fiber digestion to the enhanced digestible energy concentration in CV chicks was around 35% suggesting that the microbiome may be involved in energy extraction from other dietary nutrients, beyond fiber, or that fiber fermentation by the microbiome influences the energy digestibility of other nutrients such as starch or protein. Presumably this is associated with a general disruptive effect of fiber, protein, starch, and lipid matrices by the action of the microbiome and not that the microbiome increases nutrient absorption. Indeed, Coates et al. (1981) found that GF birds had higher glucose uptake than CV chicks (34 μg vs. 32 μg/mm of small intestine respectively).

Campbell et al. (1983) fed a rye-based diet to GF and CV chicks and observed a higher bodyweight in the GF

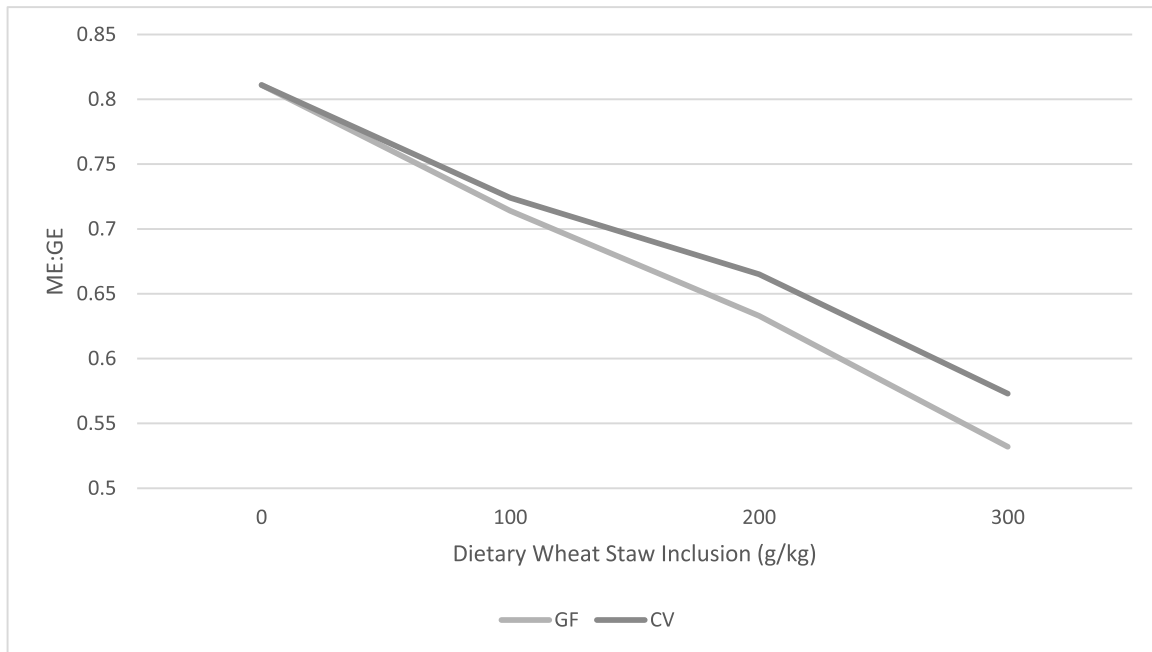


Figure 2. Effect of wheat straw inclusion on the AME to gross energy ratio (ME:GE) in germ-free (GF) and conventionally reared (CV) chicks (adapted from Hedge et al., 1982).

chicks and a lower excreta fat concentration (56 g/kg in GF vs. 97 g/kg in CV). These effects were interpreted to be due to deconjugation of bile salts by the enteric microbiome which is exacerbated by high intestinal viscosity and poor nutrient diffusion rates. Langhout et al. (2000) explored the effect of citrus pectin (to elevate intestinal viscosity) in GF and CV chicks and noted that pectin addition depressed AME in CV (13.32 MJ/kg vs. 11.81 MJ/kg) but not in GF chicks (13.76 vs. 14.26 MJ/kg). This illustrates the fine line the host animal must navigate between cost and benefit when it comes to the influence of the microbiome on nutrient availability. It can be concluded that the enteric microbiome can assist the host to extract energy from energy sources that would otherwise be poorly available to the animal but that there is a metabolic 'cost' to harboring a microbiome and net energy is similar in GF and CV states. If a microbiome modulator could be developed that optimized fiber fermentation and SCFA production but reduced metabolic processes in the microbiome responsible for increasing maintenance energy requirements in the host, for example, inflammatory cytokines, mucin biosynthesis and epithelial cell turnover, then this may significantly enhance net energy under CV feeding conditions.

Harrison and Hewitt (1978) measured the deep body temperature of GF and CV chicks. They noted that deep body temperature of chicks rose gradually from hatch to d16 in both CV and GF birds. However, the core body temperature of CV chicks was approximately 0.3°C lower than in GF chicks and rose less quickly, especially in the first week post-hatch. Core body temperature is related to metabolically active mass and the more rapid rise in body temperature in GF compared with CV chicks may be associated with more efficient

conversion of yolk to metabolically active tissue. The higher body temperature of GF chicks was confirmed by Muramatsu et al. (1988) who reported a core body temperature for GF and CV chicks of 41.5 and 40.7°C respectively without noting a difference in plasma thyroxin (3.5 vs. 3.5 $\mu\text{g}/100\text{ml}$ of plasma). These authors suggested that the higher body temperature in GF chicks may be related to higher loss of endogenous nitrogen (this will be considered in a later section). Muramatsu et al. (1993b) noted that in a fasted state, energy loss from the carcass was lower in CV chicks compared with GF chicks. The microbiome may assist the host in times of low/no nutrient intake by recycling endogenous protein and energy sources. However, as energy intake increases the microbiome exerts an increasing metabolic cost on the host, competes with the host for nutrients and negatively influences metabolic rate. Finally, the microbiome may directly influence the hypothalamus, prostaglandin production and body set-point temperature, perhaps in response to microbiome-host interactions via secondary metabolites (Huus and Ley, 2021). The potential to modify the microbiome to adjust host deep body temperature to control heat stress or to disadvantage certain pathogenic microorganisms is a rich area for future study.

PROTEIN METABOLISM AND NITROGEN CYCLING

One of the most significant roles of the enteric microbiome is N cycling. Salter and Coates (1971) explored the role of the enteric microbiome in protein digestion and N cycling using a GF experimental model and radio-labeled ^{14}C unheated or heated egg white. There were

few differences between GF and CV chicks in terms of distribution of ^{14}C or N in different intestinal segments or tissues though the heated egg white diet resulted in a substantial increase in the N concentration in the cecum of both groups. However, there was less urea N in the distal gut and cecum of CV compared with GF chicks ($14\ \mu\text{g}$ vs. $70\ \mu\text{g}$ respectively) and this was especially the case in the group that received the diet based on damaged egg white. On the other hand, uric acid N concentration was higher in the cecum of CV chicks and uric acid formed a higher proportion of total N in the excreta of CV, compared with GF, chicks. These results may be explained by the action of microbial urease and some absorption of ammonia in the CV birds. Nucleic acid N was also higher in CV compared with GF chicks. A summary of the composition of nitrogenous material in the excreta of CV and GF chicks fed the ^{14}C labeled heat damaged egg white can be found in [Table 2](#). [Salter et al. \(1974\)](#) explored the effect of feed a range of protein sources of divergent quality to GF and CV chicks. There was no difference in net protein utilization between GF and CV chicks. However, uric acid excretion tended to be higher in CV chicks compared with GF counterparts, supporting previous observations by [Salter and Coates \(1971\)](#). Endogenous N excretion was higher in GF compared with CV chicks (9.33 vs. 7.59 mg/g of feed) suggesting that the microbiome may be involved in recycling endogenous N in the distal gut. Thus, the microbiome may not be centrally involved in protein digestibility in the small intestine but could play an important role in N cycling (especially nonprotein N) in the large intestine and cecum.

[Okumura et al. \(1978\)](#) confirmed the work of [Salter and Coates \(1971\)](#) that GF chicks have higher endogenous N loss than CV chicks (223 mg/kg BW vs. 168 mg/kg BW respectively). This was especially true for uric acid (136 mg/kg BW vs. 88 mg/kg BW in GF vs. CV chicks respectively). Additionally, when fed an N-free diet, excreta from GF birds contained more Ser, Pro, Cys and Leu and less Lys, His, Ala, and Met than CV birds. However, when CV and GF birds were fed a casein-based diet, the CV chicks excreted more N and gained less weight than the GF birds. When N intake is low the microbiome may assist the host in conserving N, probably by release of ammonia and absorption of ammonia and amino acids in the distal gut. Interestingly, urinary ammonia dropped when sodium

Table 2. Composition of nitrogenous material (mg nitrogen) in excreta of germ-free (GF) or conventionally reared (CV) chicks fed a meal of heat-damaged ^{14}C egg white.

N in excreta	CV	GF
Total N	120.9	141.6
Uric acid N	45.9	35.4
Ammonia N	9.9	5.1*
Urea N	2.13	2.63
RNA N	0.83	0.58*
DNA N	0.19	0.15*
Ninhydrin-positive substances	69.8	91.8

*Significance between the CV and GF groups for a given parameter is indicated by an asterisk.

bicarbonate was included in the feed suggesting that acid:base balance may influence volatility of N in poultry waste streams. [Corring et al. \(1981\)](#) reported that pancreatic and gastric output is similar in GF and CV animals but the absence of a microbiome in GF animals increases the persistency of endogenous enzyme activity in the intestine and loss of endogenous N from the gut. In CV animals more of the host-derived N is metabolized by bacteria and then either absorbed by the host where it is used to synthesize nonessential amino acids or is converted into microbial biomass. Thus, the major difference in N cycling in GF versus CV animals may be mostly associated with nonprotein N and endogenous protein flow.

[Campbell et al. \(1983\)](#) explored the effect of feed a rye-based diet to GF and CV chicks on amino acid retention. A summary of the results is presented in [Figure 3](#). Amino acid retention was 4.6% higher in GF compared with CV chicks and this ranged from 12.9% for Ala to -0.3% for Cys. Thus, whilst GF chicks have higher endogenous N losses, this may only be relevant during periods of low protein intake and is not reflected in amino acid retention when GF chicks are fed a practical diet. The difference between GF and CV chicks in terms of amino acid retention may be associated with loss of microbial biomass from the intestine. However, [Miner-Williams et al. \(2009\)](#) reported the amino acid composition of bacterial protein from pigs fed a casein-based diet and there is a nonsignificant correlation between the delta change in amino acid retention between GF and CV chicks reported by [Campbell et al. \(1983\)](#) and the amino acid profile of bacterial protein ($P > 0.05$; [Figure 4](#)). Nevertheless, GF chicks may have some advantages in amino acid retention compared with CV chicks when protein intake is adequate, and this may be associated with a lack of bacterial protein loss from the intestine of GF animals or enhanced amino acid absorption under GF conditions. In fact, [Philips and Fuller \(1983\)](#) noted appreciable protease activity in the cecum of chicks that was not of host origin, so the possibility remains that the microbiome in CV chicks may convert dietary N into both bacterial biomass and metabolically active protein ([Yang et al., 2021](#)), some of which will be naturally lost from the intestinal tract. [Furuse and Yokota \(1984b\)](#) also observed a higher protein retention rate (g protein retained/g of protein consumed) in GF chicks compared with CV chicks when fed a diet based on soy protein isolate and this was repeated by [Furuse and Yokota \(1985\)](#) where GF chicks retained more N than CV chicks (14.2 g/kg vs. 11.34 g/kg).

[Yokota et al. \(1989\)](#) explored the requirement of GF and CV chicks for nonprotein N by supplementing a diet with diammonium citrate. Supplemental diammonium citrate improved the performance of GF chicks more than CV chicks and this was especially evident in FCR. Retention of N was higher in GF chicks and GF chicks utilized the nonprotein N to a greater extent than the CV chicks suggesting that the microbiome may competitively interfere with ammonia metabolism by the host. [Muramatsu et al. \(1993b\)](#) also fed ammonia to GF and

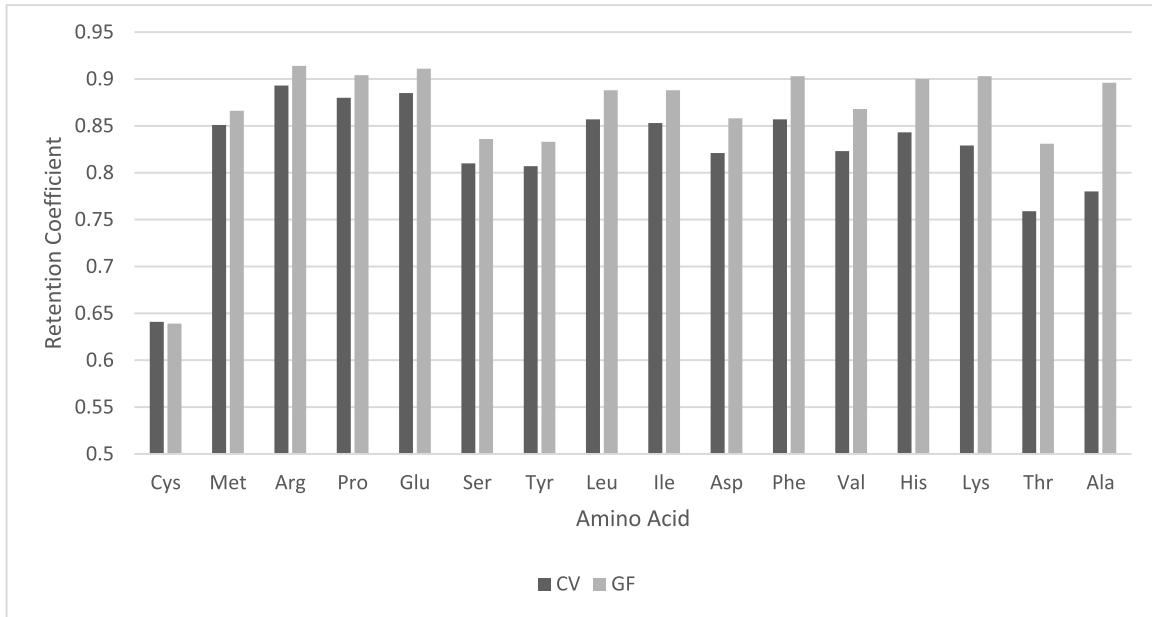


Figure 3. Amino acid retention coefficients of an irradiated rye-based diet in germ-free (GF) and conventional (CV) chicks (adapted from Campbell et al., 1983).

CV chicks and while there was no effect on growth rate, supplemental ammonium bicarbonate generated an increase in liver protein synthesis in the GF chicks. This confirms the absorption of ammonia by the host and its subsequent metabolism in the liver. It is likely that the microbiome is therefore a source of ammonia for chickens, and this may influence nonessential amino acid synthesis and requirement. Recently, Mishima et al. (2020) reported the importance of the enteric microbiome in detoxifying purines. Plasma levels of inosine, guanosine, xanthine and hypoxanthine were lower in GF than SPF mice. The gut microbiome plays a crucial role in

recycling purine metabolites via nucleosidase activity. If the microbiome is lost the host can partially compensate but not to the level of a mature, functional, microbiome. Guanosine, inosine, xanthine and urate concentrations in feces were higher in GF mice than CV mice. Finally, feeding adenine to GF and CV mice to induce renal damage resulted in more damage to renal tubules and macrophage infiltration in GF mice. These observations show how crucial the symbiotic relationship between the enteric microbiome and the host is and how carefully microbiome modulatory technologies should be deployed to avoid disrupting this balance.

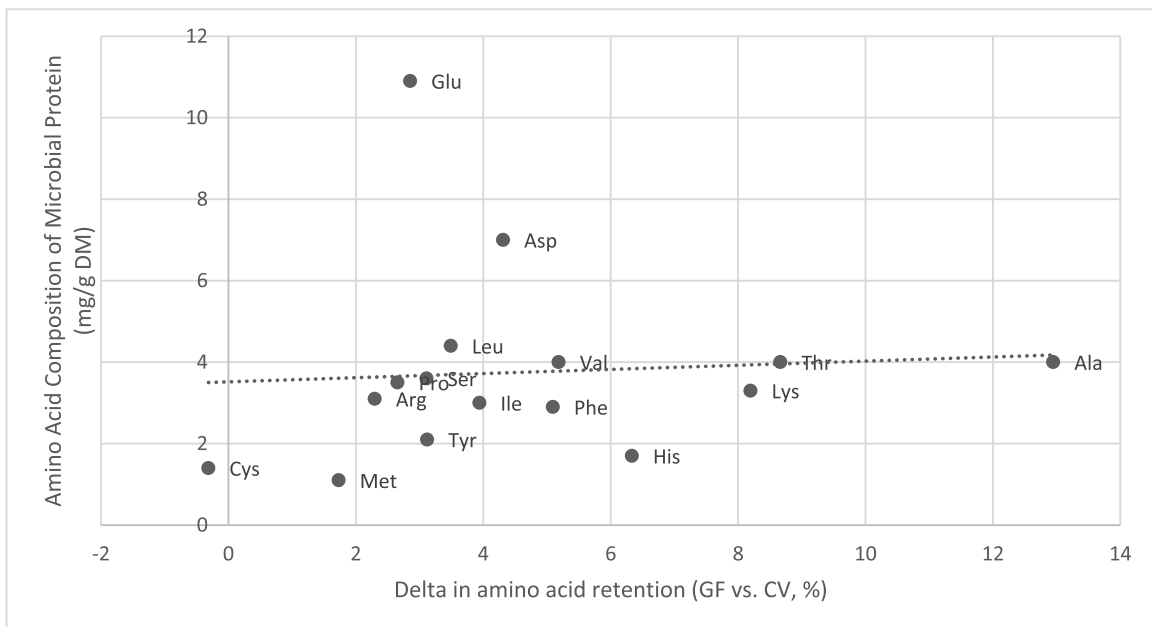


Figure 4. Correlation ($r^2 = 0.17$) between the amino acid composition of bacterial protein (Miner-Williams et al., 2009) and the delta in amino acid retention between germ-free (GF) and conventionally reared (CV) chicks fed an irradiated rye-based diet (Campbell et al., 1983).

In contrast to the previous reports, [Muramatsu et al. \(1992\)](#) found that CV chicks had higher N retention than GF chicks (272 mg/d vs. 146 mg/d), lower N excretion (368 mg/d vs. 443 mg/d) and lower urinary N excretion (270 mg/d vs. 367 mg/d). Additionally, GF chicks had higher total excreta N (186 vs. 136 mg/d), uric acid N (112 vs. 83 mg/d) and creatinine N (7.4 vs. 4.7 mg/d). Excreta ammonia was higher in CV chicks compared with GF chicks (32 vs. 13.6 mg/d). The reason for the apparent disagreement between this study and previous reports may be associated with the high fiber concentration of the diet (280 g/kg cellulose was used in the diets by [Muramatsu et al., 1992](#)) which increases the importance of the microbiome in nutrient retention. Excreta from GF birds is typically high in urea or uric acid N and low in ammonia N whereas the opposite is true in CV birds. These differences are primarily associated with microbial urease activity and suggest an important role of the enteric microbiome in recycling nonprotein N and nonessential amino acid metabolism.

[Drew et al. \(2003\)](#) fed methionine or L-2-hydroxy-4-methylthiobutanoic acid to GF and CV chicks. On d21, residual L-2-hydroxy-4-methylthiobutanoic acid in the distal ileum was lower in GF than CV chicks (4.7 vs. 10.2%) whereas there was no difference between the 2 groups for methionine. Methionine may be absorbed from the intestine more quickly than L-2-hydroxy-4-methylthiobutanoic acid, reducing the involvement of the microbiome in Met metabolism. Furthermore, not all bacteria can utilize L-2-hydroxy-4-methylthiobutanoic acid. [Hegedus et al. \(1993\)](#) observed that *Lactobacillus plantarum*, *Leuconostoc mesenteroides*, and *Lactobacillus casei* could utilize methionine but not L-2-hydroxy-4-methylthiobutanoic acid. Thus, modulation of the microbiome via feed additives may influence the capacity of the microbiome to process certain forms of dietary amino acids and this should be considered, especially if nonspecific or broad-spectrum modulators are used.

ADDITIONAL EFFECTS OF THE MICROBIOME

Most of the focus of GF work in chickens has been on growth, organ development and, protein and energy metabolism. However, a selection of additional relevant work did not fit conveniently into these categories. For example, [Edwards and Boyd \(1963\)](#) noted that following oral delivery of Ca⁴⁷, GF had higher tibia and whole blood calcium (Ca) than CV birds, but no such differences were evident when the Ca⁴⁷ was administered intraperitoneally. This work suggests that the microbiome may play a role in Ca metabolism, perhaps via changes in intestinal pH, absorption, solubility or via indirect mechanisms involving vitamin D or phosphatase and phosphorus absorption. Helpfully, [Campbell et al. \(1983\)](#) noted that GF chicks fed a rye-based diet had

increased bone ash than CV chicks (481 g/kg vs. 443 g/kg) and bone ash in CV chicks was also considerably more variable than in GF chicks. These results suggest that the enteric microbiome may interfere with Ca metabolism and so modulatory approaches to control this may be warranted.

[Furuse et al. \(1991a\)](#) explored the effect of exogenous acetic acid supplementation on GF and CV chick development. In GF chicks the addition of acetic acid increased fat retention (6.15 vs. 4.54 g/10 d), protein retention (13.9 vs. 11.1 g/10 d) and energy retention (570 vs. 439 kJ/10 d) but had no effect in CV chicks. Interestingly the retention of fat, protein and energy in GF chicks fed acetic acid was higher than CV chicks fed without or with acetic acid indicating a particular role of acetic acid in GF chicks. The control diet in this experiment contained kaolin as a diluent and it is possible that this influenced the growth of GF and CV chicks and the response to supplemental acetic acid. Nevertheless, this study shows the potential value of acetic acid in GF chicks and the potential of the microbiome to substantially alter the value of feed additives for the host.

[Radharkrishnan and Bradley \(1973\)](#) observed a significant reduction in mortality associated with *Eimeria tenella* infection in GF chicks compared with either CV or SPF chicks. No tissue damage from coccidiosis infection was noted in GF chicks and the GF chick cohort returned 0% mortality as opposed to 22% to 38% mortality in the SPF or CV groups. *Eimeria tenella* development was observed to be reduced in the GF state. This observation has recently been confirmed by [Gaboriaud et al. \(2021\)](#) where GF and CV Ross PM3 broilers were infected with *Eimeria tenella*. Oocysts were counted in cecal contents on d6, d7, and d9 postinfection and oocyst counts were much lower in GF compared with CV chicks. Importantly, sporozoite excystation was higher in GF chicks and this was also the case when oocysts were incubated with bile from the GF birds. The absence of a microbiome delays asexual phase development, and this may be responsible for the decreased oocyst load. It is possible that some microbiome metabolites are required for parasite replication. If such metabolites could be defined and specifically silenced via modulatory technologies, then the microbiome could be leveraged as an indirect coccidiosis mediation tool.

[Zhou et al. \(2021\)](#) recently assessed the influence of GF and CV pigs with a particular focus on immune development and plasma biomarkers. The GF pigs had similar BW than the CV pigs but higher FCE (0.67 vs. 0.62) although a third cohort of pigs that were specifically transplanted with fecal microbiota from healthy sows returned the highest FCE (0.77). GF pigs had a smaller liver than CV pigs and lower white blood cell count, neutrophils, lymphocytes, eosinophils, and basophilic granulocytes than CV pigs. Additionally, GF pigs had lower plasma alanine aminotransferase, aspartate aminotransferase, total protein, albumin, globulin, and higher serum urea than CV pigs. These changes in plasma biochemistry are suggestive of reduced immune maturation. The GF pigs also had lower digestibility of

Table 3. Potential targets for precision modulation of the enteric microbiome of poultry generated from work in conventionally reared and germ-free chickens.

End point	Microbiome-related plasticity ¹
<i>Nutrition & Growth</i>	
Weight gain and feed conversion efficiency	9–10%
Emulsification and digestion of saturated fat sources	10%
Apparent metabolizable energy	0.5–0.8 MJ/kg
Calcium retention	5–10%
Endogenous nitrogen loss	3–5%
Retention and utilization of nonprotein nitrogen	8–12%
Ammonia excretion	20–30%
Fiber digestion	30–40%
Apparent metabolizable energy of fiber sources	2–3 MJ/kg of fiber
Short-chain fatty acid production in the distal gut	30–45%
Fasting heat production	20–25%
Effect of antinutritional factors ²	40–50%
Amino acid digestibility	1–2%
Fractional protein synthesis rate in tissue	3–4%
Fat and fat-soluble nutrient absorption	5–10%
Efficacy of feed additives ³	Up to 100%
<i>Physiology</i>	
Pancreatic enzyme persistence in the gut	7–8%
Pancreatic size	4–5%
Liver size	8–12%
Liver fat content	8–12%
Gut mass, especially caudal gut and lamina propria	20–25%
Core body temperature	0.2–0.4 °C
Goblet cell number and mucin biosynthesis	8–10%
Mucin composition (sulfation and sialylation)	50–70%
Purine metabolism and renal function	20–30%
Intestinal (nongastric) pH	0.2–0.3 pH points
Spleen development	20–30%
Absorptive area of the small intestine	80–90%
<i>Immunity and Health</i>	
Severity of coccidiosis infection ⁴	50–100%
White blood cell, neutrophil, and lymphocyte counts	5–15%
Gut redox	20–30%
Tight junction integrity	20–30%
Immune tissue maturation rate	70–90%
Liver enzyme function and plasma protein profile	5–8%
Transcription-factor encoding gene expression	70–90%
Neuroactive steroid concentrations in plasma and brain	80–100%
Interferon inducible gene expression	50–70%

¹Possible plasticity of selected end point achievable via microbiome modulation based on summary of available literature.

²For example trypsin inhibitors or viscous nonstarch polysaccharides.

³Including acids, carbohydrates & prebiotics, salts, nonprotein nitrogen sources, amino acids, and minerals.

⁴At time of publication this has only been demonstrated for *Eimeria tenella*.

dry matter, gross energy, and crude protein than the CV pigs and also lower serum, ileum, cecum and colon SCFA concentration, especially acetate and butyrate. It is possible that swine are more dependent on the microbiome for nutrient recovery than poultry.

Finally, [Diviccaro et al. \(2021\)](#) explored the role of the microbiome on steroid hormones in mice. Higher

allopregnanolone and lower 17β -diol levels were observed in plasma of GF vs. CV mice. In the hippocampus, GF mice had higher dihydroprogesterone, isoallopregnanolone, and 17β -diol than CV mice. In the same brain region dihydrotestosterone was lower in GF versus CV mice. In the cerebellum and cerebral cortex GF mice had higher dihydroprogesterone and isoallopregnanolone than CV mice. In the hypothalamus of GF mice there was significantly higher dehydroepiandrosterone and less testosterone versus CV mice. Gut microbiome presence influenced plasma and central nervous system levels of neuroactive steroids but were influenced differently suggesting divergent impact of the microbiome on steroidogenesis and neurosteroidogenesis. This work confirms a direct link between the microbiome and neuroendocrine pathways which offers potential opportunities for microbiome modulation to improve host welfare, stress or other neurological conditions.

The enteric microbiome offers the host animal a number of competitive advantages but simultaneously creates headwind in several domains. Thus, deployment of nonspecific microbiome modulators may confer inconsistent benefits to the host if they interfere with beneficial roles of the microbiome, for example, fiber digestion or nonessential amino acid metabolism. A summary of potentially achievable targets for microbiome modulation in poultry is presented in [Table 3](#). In future, broad-spectrum microbiome modulators are likely to become obsolete as more surgical modulatory technologies are developed that can deliver desirable end points without unintended collateral effect.

CONCLUSIONS

The microbiome-host interaction is extremely complex, and cause and effect relationships are difficult to establish conclusively. The GF animal model offers an opportunity to explicitly explore the role of the microbiome in host development, immunity, nutritional state, neurology, pathology, physiology, and biochemistry. In broiler chickens, the microbiome is a net cost to the host unless diet conditions are extremely disadvantageous for the animal, and it seems likely that the microbiome has co-evolved with chickens at least partly to conserve nutrients during periods of inadequate nutrient intake. An obvious area for exploitation of plasticity in the microbiome in this regard is in broiler breeder nutrition and stress management. However, as feed cost and environmental sustainability motivates decreases in dietary CP and increases in dietary fiber, the microbiome may offer solutions to mitigate inevitable upward drift in FCR in conventionally reared broilers. The enteric microbiome is evolutionarily adapted to exist in symbiosis with the host, to alleviate nutritional stress when nutrient intake is low or nutrient quality is poor and to recycle potentially toxic metabolites associated with the N cycle. Maturation of the microbiome post-hatch is also intricately involved in liver, spleen and gastrointestinal tract development,

absorptive area, nutrient transport, and mucin biosynthesis. If negative effects on fat emulsification, bile salt deconjugation, and amino acid digestibility can be avoided, alongside obvious threats from pathogenic bacteria and viruses, then the microbiome may be leveraged to confer consistent advantages for the host. As an industry, it is critical that we gain a more complete understanding of microbial function and develop more precise intervention measures that achieve our goals without unintended collateral effect and loss of microbial biodiversity. If we can more precisely ‘nudge’ the microbiome to accomplish what the poultry industry requires without broad-spectrum, nonspecific effects, it is highly likely that animal health, welfare and sustainability will be delivered more consistently and with considerably more success.

DISCLOSURES

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