THE EFFECT OF DIETARY FAT COMPOSITION AND POST-HATCHING AGE ON MICROORGANISMS OF THE GASTRO INTESTINAL TRACT (GIT) IN BROILER CHICKS

[1]S. K. Mutayoba; [2]R. C. Noble and M. Smith., [1]Sokoine University of Agriculture, Department of Animal Science and Production, P.O. Box 3004, Morogoro and [2]Scottish Agricultural Colleges, Auchincruive, Ayr, KAH, 58W Scotland

SUMMARY

The effect of dietary fat sources differing in fatty acid composition and post-hatching age on the establishment of gut microflora in young broiler chicks was studied. The dietary treatments were Diet 1 (a commercial broiler diet), containing high levels of polyunsaturated fatty acids (PUFA) and free fatty acids (FFA); Diet 2, (a tallow oil based diet) high in saturated fatty acids (SFA) and low levels of (FFA) and Diet 3, (soyabean oil based diet) high in PUFA but with low levels of FFA. One hundred and twenty day-old broiler chicks were randomly distributed to the three dietary treatments and fed the respective diets for 4 weeks. Feed and water were given *ad libitum*. The levels of Streptococci, Clostridia, Lactobacilli and Coliform species were determined in the duodenum, ileum and caecum on days 1, 7, 14 and 26 post-hatch. The bacterial population was low in all gut segments on day 1 post-hatch but progressively increased for most of the species to the 14th day post-hatch. However decreasing levels of most species was noted thereafter. Dietary fat composition had an effect on the level and type of microorganisms present in the gut. On day 7 post-hatch the level of Streptococci and Coliform species were highest in chicks fed Diet 3 whereas Lactobacilli species were higher in chicks receiving Diet 1 and Diet 2. The number and type of bacterial species studied varied between the gut segments. Most of the bacterial species studied were highest in the caeca and lowest in the duodenum. The present study has shown that the GIT flora in chicks is low at hatching, but rapid establishment occurs soon after hatching and is to some extent influenced by nutrient composition.

INTRODUCTION

Most studies in the chicken have shown that the establishment of microorganisms begins soon after hatching and may be completed within a period of 2-3 weeks post-hatch (Shapiro and Sarles, 1949; Humbert *et al.*, 1989). The establishment, type and level of microorganisms present in the gastro intestinal tract (GIT) is significantly influenced by age, environmental conditions and diet (Muramatsu *et al.*, 1988; Furuse *et al.*, 1991). Generally it has been shown that the performance of germ free chicks in terms of nutrient utilization and body weight gain is higher than in the conventional chicks (Morishita *et al.*, 1982; Furuse and Yokota, 1984). This has led to the assumption that nutrient requirements may be higher in conventional chicks due to the extra demands arising from the presence of large numbers of microorganisms. Whereas a lot of work has been done to compare the performances between germ free and conventional chicks, few studies have been carried out to evaluate the effects of different dietary nutrients on GIT flora. Harrison and Coates (1972); Morishita *et al.* (1982) and Orban *et al.* (1997) reported that dietary proteins, carbohydrates and other nutrients have an influence on number and type of microbes in the GIT. However, the information on the effect of dietary fat sources on the establishment, type and level of GIT flora in chickens is scanty. Thus the main objective of the present study was to investigate the effect of dietary fat on GIT flora in broiler chicks and to assess the changes in the GIT with respect to age and diet.

MATERIALS AND METHODS

Diets

Three diet treatments with different fat sources were used. The treatments were designated as Diet 1 (a commercial diet high in polyunsaturated fatty acids (PUFA) and high levels of free fatty acids (FFA); Diet 2 (a tallow oil-based diet containing high levels of saturated fatty acids (SFA) and Diet 3 (a soyabean oil-based diet (containing high levels of PUFA and low FFA). Table 1 shows the ingredients composition of the experimental diets.

Experimental chicks

One hundred and twenty day old Ross Breeder chicks were randomly distributed to the three dietary treatments after weighing. The chicks were reared on floored pens (3 x 2.5 mm) covered with wood shavings to 2.5 cm thick. Feed and clean water was provided *ad libitum*.

Sampling

Collection of gut samples was carried out in a microbiological cabinet to minimize contamination. Samples were collected from three chicks per treatment on days 1, 7, 14 and 26 post-hatch. Gut contents from individual sections of the GIT (namely the duodenum, ileum and caecal pouches were individually squeezed into pre-weighed sterilized bottles. After weighing, the contents were thoroughly mixed and 10 g of the sample was taken and mixed with 90 ml of Sterile Reinforced Clostridial Medium (RCM). The mixtures were then homogenized for 1 minute. This was then followed by serial dilutions using RCM.

Isolation of microorganisms

Bacterial populations were enumerated using the established media on double layered plates. Three dilutions (i.e. 10^{-6}, 10^{-7} and 10^{-8} were used) Rogosa agar (Rogosa *et al.*, 1951) was used for Lactobacilli species isolation. MacConkey agar (Fuller, 1973) was used to isolate Coliforms and Thallous Tetrazolium Acetate Agar (TTAA) adapted by Mead (1978) was used to isolate Streptococci species. The Clostridial species were estimated using the Most Probable Number (MPN) method of Hirsch and Grinsted (1954). The presence of Clostridial species was confirmed by using the Black tube method.

RESULTS

The mean body weight of chicks during the experimental period is shown in Table 2. The average weight gain was highest and lowest in chicks fed the soyabean oil and the tallow oil-based diets, respectively.

Clostridial species

The distribution of Clostridial species within the different sections of the GIT during the experimental period is shown in Table 3. Clostridial species were not detected in the duodenum on day 1 post-hatch, but were present in the ileum and caecum. The level of Clostridial species in the ileum remained fairly constant throughout the experimental period in chicks receiving Diet 1, whereas Clostridial changes observed in this section for chicks receiving the other diets did not show any consistency. Similarly, no definite trend was observed over time in the levels of Clostridial species in the duodenum and caecum in birds given different dietary treatments.

Lactobacilli species

On day 1 post-hatch the Lactobacilli species were not detected in any of the segments. At day 7 post-hatch, the levels of Lactobacilli species in the ileum were highest and lowest in chicks receiving Diet 1 and Diet 2, respectively (Figure 2i). An increase in Lactobacilli species was observed in chicks receiving Diet 2 after day 7 post-hatch, whereas, decreases occurred in chicks receiving Diet 1 and Diet 3. The level of Lactobacilli species in the caecum was slightly higher in chicks receiving Diet 1 at day 7 and 14 post-hatch. The changes observed for the Lactobacilli species between day 7 and 14 post-hatch were inconsistent.

Streptococci species

In the duodenal contents, Streptococci species were only detected on day 26 post-hatch (Figure 1) and levels were highest and lowest in chicks receiving Diet 2 and Diet 1, respectively. Substantial numbers were present in the ileum on day 1 post-hatch. However, decreases at varying rates were observed in all dietary treatments thereafter. By day 26 post-hatch, the level of Streptococci species was highest in chicks receiving Diet 2 whereas no difference was observed in birds receiving the other two diets (Figure 2ii). The level of Streptococci species in the caecum was slightly lower than in the ileum on day 1 post-hatch (Figure 3ii). Increases were however observed between day 1 and 7 post-hatch in all dietary treatments. On day 14 post-hatch the level was highest in chicks fed Diet 2 but decreases were observed thereafter.

Coliform species

The ileum contained high levels of Coliform species on day 1 post-hatch (Figure 2iii). The Coliforms remained high in chicks fed Diet 2, whereas decreases with post-hatching age occurred in chicks fed Diet 1 and 3. On day 14 and 26 post-hatch the differences in Coliform levels were relatively small. The caeca also contained substantial levels of Coliforms on day 1 post-hatch (Figure 3iii). Between day 1 and 7 post-hatch increases in the levels of Coliform species were observed in chicks fed Diets 2 and 3. Decreases in Coliform levels occurred in all dietary treatments after day 7 post-hatch.

Table 1: Ingredient composition of the experimental diets

	Diet 1	Diet 2	Diet 3
Ingredient g/kg			
Barley	100	100	100
Wheat	250	250	250
Maize meal	245	245	245
Fishmeal	50	50	50
Soyabean meal	220	220	220
Limestone	5.3	5.3	5.3
Grass meal	50	50	50
Dicalcium	21.7	21.7	21.7
phosphate	2.5	2.5	2.5
Salt	2.5	2.5	2.5
Vitamin mix	0.5	0.5	0.5
Amprol mix	-	50	-
Tallow fat	-	-	50
Soyabean oil	50	-	-
Commercial fat	5.3	5.3	5.3
Limestone			

Table 2: The effect of different dietary fat sources on body weight gain of chicks

	Diet 1	Diet 2	Diet 3
1	37.5	35.8	34.8
12	273.0±11.7	254.4±4.1	253.3±16.7
25	964.4±22.9	905.1±23.7	1049.3±27.8
Average wt gain	925.5	870.1	1011.8

Table 3: The effect of age and dietary fat sources on the distribution of the Clostridia along the GIT of broiler chicks

Age (days)	Diet	*Duodenum*	*Ileum*	*Caecum*
1		N.D	$<10^8$	$>10^9$
7	1	$<10^8$	$<10^8$	<10
	2	N.D	$>10^9$	<10
	3	$<10^6$	$<10^6$	<10
14	1	N.D	$<10^8$	$<10^8$
	2	N.D	$<10^7$	$<10^8$
	3	N.D	N.D	$>10^6$
26	1	$<10^5$	$<10^8$	<10
	2	$<10^5$	$<10^9$	<10
	3	$<10^5$	$<10^8$	<10

N.D: Not detected

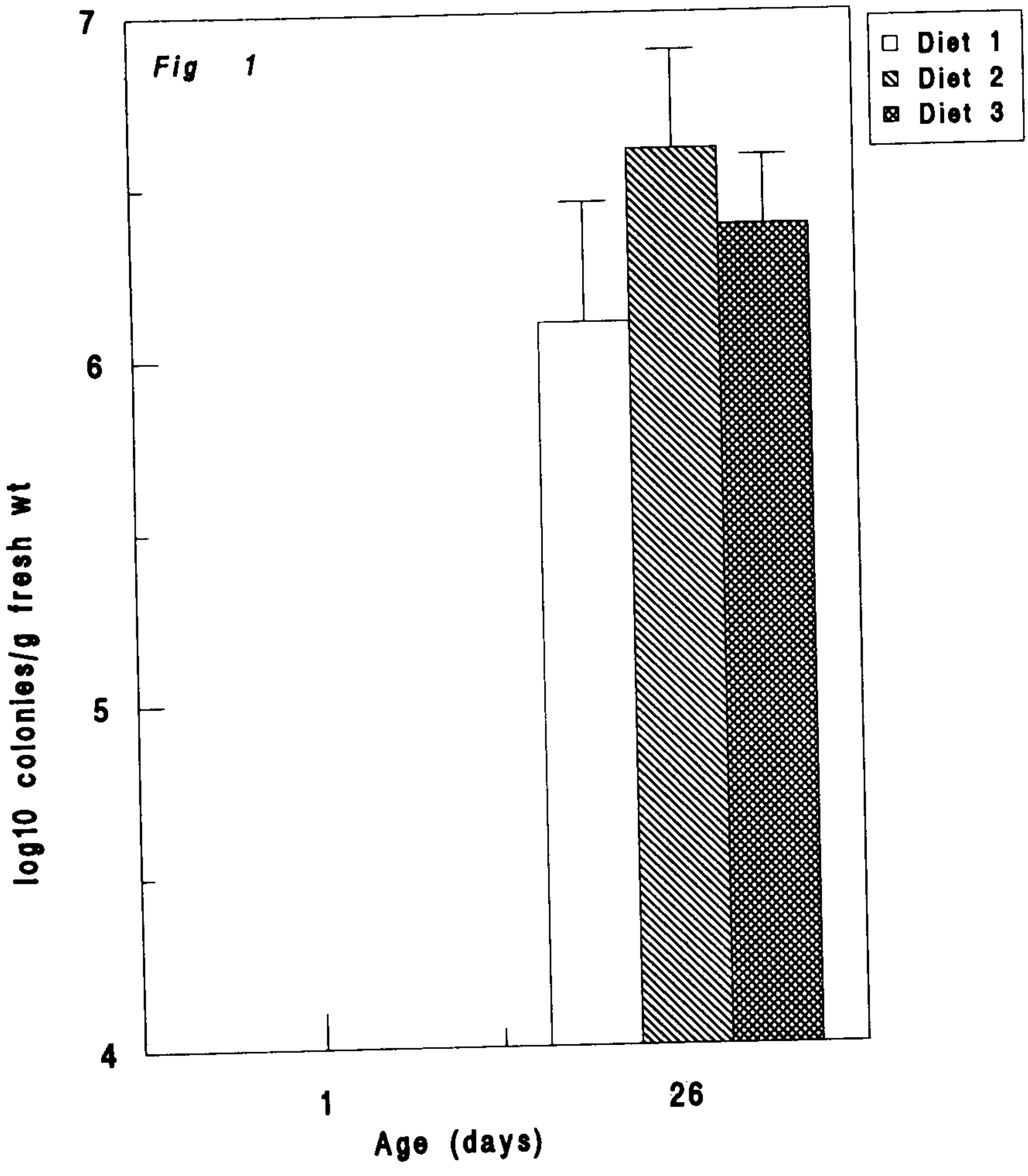

Figure 1: The distribution of Streptococci species in the duodenum

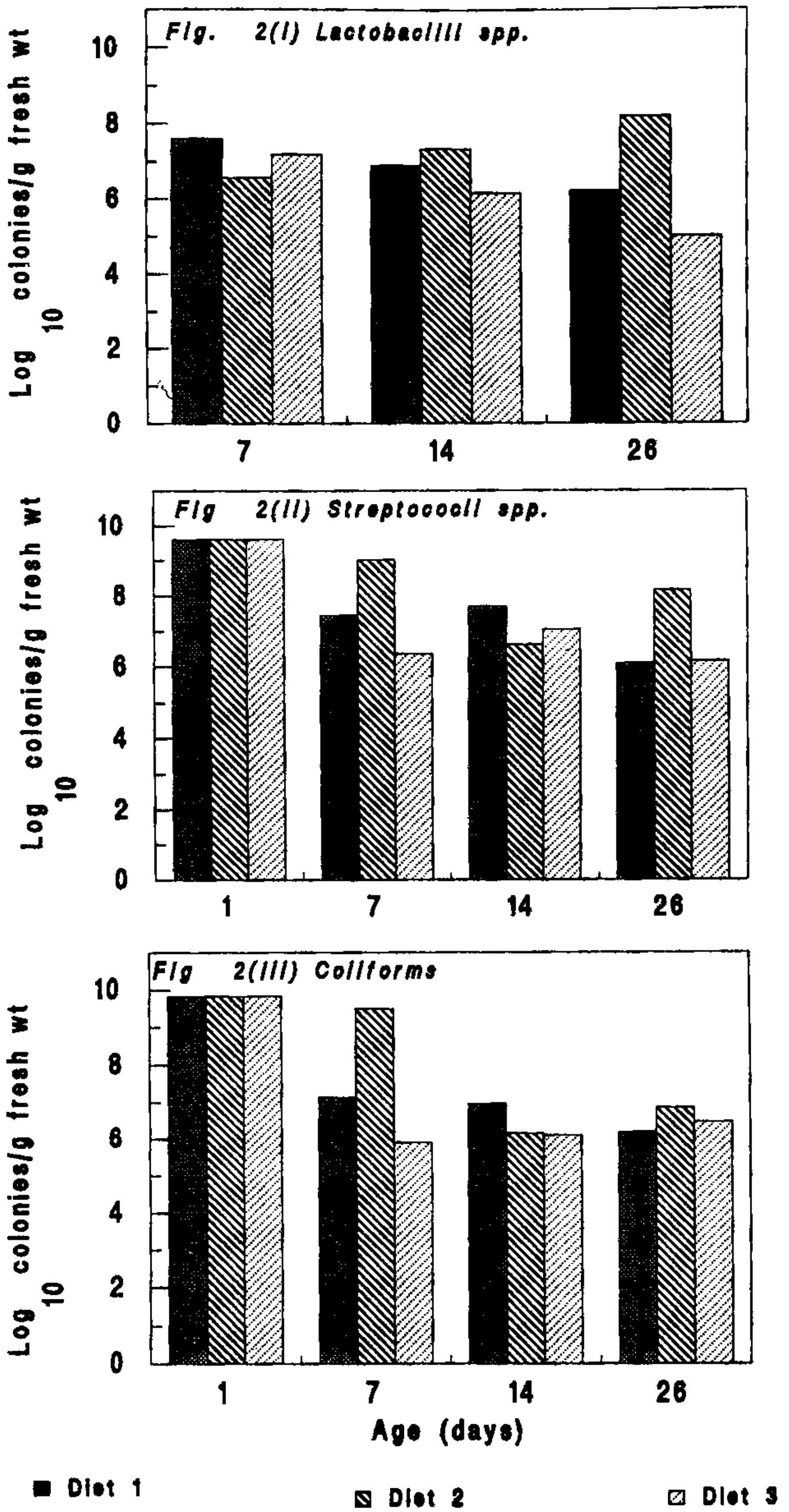

Figure 2 (i – iii): The distribution of the different micro-organisms in the ileum

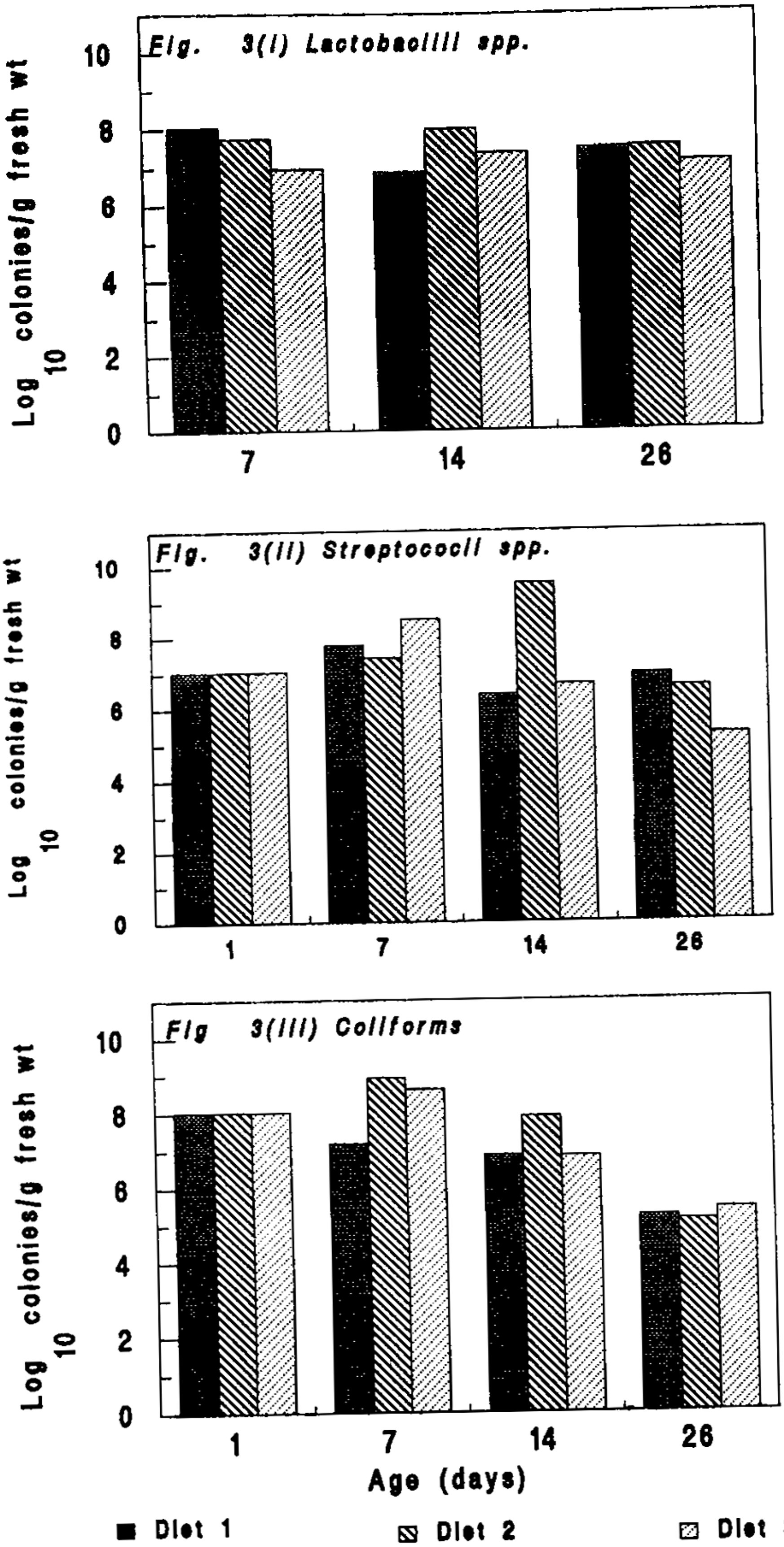

Figure 3 (i – iii): The distribution of different micro-organisms in the caecum

DISCUSSION

The results of the effect of age and dietary fat sources on the establishment, type and numbers of micro-organisms in the different parts of the gastro intestinal tract (GIT) of broiler chicks observed in the present study are discussed in relation to other findings.

The low microbial population within the GIT on day 1 post-hatch observed in the present study concurs with findings reported elsewhere (Lev and Briggs, 1956; Salanitro *et al.*, 1978; Furuse *et al.*, 1991). This accords the view of Humbert *et al.* (1989) and Furuse *et al.* (1991) that the chick harbours few microorganisms at hatching. At day 7 post-hatch substantial numbers of Clostridia and Streptococci species were present in the duodenum whilst neither Lactobacilli nor Coliforms were detected. The absence of Lactobacilli species in the present study after the second week post-hatch is in contrast with findings reported by Barnes *et al.* (1978) which showed that the duodenum at this stage was dominated by Lactobacilli species. During the present study dietary fat had little influence on the microbial population in the duodenum. This might have been due to the fact that most of the lipids in this section is of biliary origin and thus there is very little influence of dietary fat (Freeman, 1976).

The ileal microbial population was higher than that of the duodenum. This is in agreement with findings reported by Shapiro and Sarles (1949) which showed that the ileal microbial population was higher than that of the duodenum. The microbial changes in the ileum during the present study were mainly influenced by dietary fat composition whereby significant differences were observed between chicks receiving saturated and unsaturated fats. The number of the different species with the exception of Lactobacilli were higher in chicks fed the saturated fat based diet and lower in chicks fed diets containing unsaturated fats. This indicates that saturated fats have a positive effect on the growth of microorganisms in the GIT. This concurs with findings reported by Jayne-Williams and Fuller (1971) and Harrison and Coates (1972) that there is an influence of dietary fat on the establishment and level of microorganisms in the GIT of chicks. The higher numbers of Lactobacilli species present in the ileum of chicks receiving the soyabean oil based diet is in agreement with the work reported by Nath *et al.* (1948), that high levels of oleic acid in the fat had a growth stimulating effect on Lactobacilli. These findings show that probably different species of microorganisms require different types of fat and nutrients. It has also been noted that the present practice of feeding diets containing high levels of soyabean meal and fat to chicks during the early post-hatch period has a destabilising effect on the GIT flora. It reduces the activity of the sucrase and also has a negative effect on the development of the digestive system (Drasar *et al.*, 1973; Ferket, 1991).

The absence of Lactobacilli species in the caeca and high levels of Clostridia, Coliforms and Streptococci on day 1 post-hatch observed in the present study accord the findings reported by Barnes *et al.* (1978) that the caeca mainly contains Streptococci and Coliform species. The establishment of the Lactobacilli species in this section is normally slow. Variation in the caecal flora between chicks receiving the different dietary treatments in this study were in contrast to findings reported by Furuse *et al.* (1991) which showed that dietary fat composition had no influence on the type and distribution of caecal flora. The changes in the numbers of micro-organisms with age particularly up to day 14 post-hatch in this study accord findings reported in other studies which showed that the morphological and quantitative changes of the caecal microflora GIT as a whole were completed within 9 - 14 days post-hatch, (Humbert *et al.*, 1989). Decreases in most of the micro-organisms after the 14th day post-hatch regardless of the diet was a natural progression to attaining levels similar to those present in adult birds (Barnes *et al.*, 1978; Baba *et al.*, 1991).

Lower body weight gain was observed in chicks receiving the saturated fat diet compared to those receiving unsaturated-based diets. This might have been due to the large numbers of microbes present in the GIT of chicks fed diets containing saturated fats resulting into a competition of nutrients between the chick and micro-organisms (Furuse and Yokota, 1984). It has also been shown that high numbers of micro-organisms in the GIT result in lower energy retention by the chick and also increases in the weight and thickness of the intestinal wall. These physical changes to the GIT reduce its absorptive capacity of nutrients (Coates *et al.*, 1981). The variations in the GIT flora observed in the present study with post-hatching age were in accordance with findings reported elsewhere. Differences in the GIT flora between chicks receiving the different dietary treatments indicate that fat composition like other nutrients affects the establishments and growth of intestinal micro-organisms.

REFERENCES

Baba, E; Nagaishi, S; Fukata, T and Arakawa, A (1991). The Role of intestinal microflora on the prevention of Salmonella colonization of gnotobiotic chicks. Poultry Science 70: 1902 - 1907.

Barnes, E.M; Mead, G.C; Impey, C.S and Adams, B.W (1978). Analysis of the avian intestinal flora. Royal Society for Applied bacteriology, Technical Series, London Academic Press 4: 89 -105.

Coates, M.E; Cole, C.B; Fuller,R; Houghton, S.B and Yokota, H. (1981). The gut microflora and the uptake of glucose from the small intestine of the chick. British Poultry Science 22: 289-294.

Drasar, B.S; Crowther, J.S; Goddard, P; Gabrielle Hawksworth, M.J; Hill, S.P and Williams, R.E.O (1973). The relation between diet and gut microflora in man. Proceedings of Nutritional Society 32: 49-54

Ferket, P.R (1991) Effect of diet on gut microflora of poultry. Zootecnica International, July/August 44-49

Freeman, C.P (1976) Digestion, absorption and transport of fat in non-ruminants. In Fats in Animal nutrition (J. Wiseman, ed). Butterworths, London. pp 105-122

Fuller, R (1973). Differences in microfloras of the intestine and the Bursa of Fabricius as illustrated by the Coliform/Lactobacillus ratio in the two sights. British Poultry Science 14: 221-224

Furuse, M; Yang, S.I; Niwa, N and Okumura, J. (1991). Effect of short chain fatty acids on the performance and intestinal weight in germ free and conventional chicks. British Poultry Science 32: 159-165

Furuse, M and Yokota, H. (1984). The effect of gut microflora on the growth of chicks fed diets high or marginally energy. Nutrition Reports International 29: 1293-1300

Harrison, G.F and Coates, M.E. (1972). Interrelationships between the growth promoting effect of fish solubles and the gut flora of the chick. British Journal of Nutrition 28: 213-221

Hirsch, A. and Grinsted, E. (1954). Methods for growth and enumeration of anaerobic spore-formers from cheese, with observation on the effect of nisin. Journal of Diary Research 21: 101-110

Jayne-Williams, D.J and Fuller, R (1971). The influence of intestinal microflora on nutrition. In "Physiology and Biochemistry of the Domestic Fowl", Vol. 1 (Bell, D.J and Freeman, B.M eds.) London Academic Press. pp 73-92

Humbert, F; Salvat, G; Morin, M; Laheillec, C and Bennejean, G (1989). Microbial colonization of the caecal mucosa of specific-pathogen-free chicks and adherenceof an adult SPF chicken microflora to the caeca of newly hatched chicks: Study by scanning electron microscopy. Avian Pathology 18:577-589

Lev, M and Briggs, C.A.E (1956). The gut microflora of the chick: ii) The establishment of the flora. Journal of Applied Bacteriology 19: 224-230

Mead, G.C (1978). Isolation media for Streptococci: Streptococci of normal intestinal flora. In Streptococci. Society of Appplied Bacteriology Symposium Series No.7 (Skinner, F.A and Quensel, L.B. eds) London Academic Press. pp 385

Morishita, Y; Fuller, R and Coates, M.E (1982). Influence of dietary lactose on the gut flora of chicks. British Poultry Science 23: 349-359

Muramatsu, T; Takasu, O; Furuse, H and Okumura, J.I. (1988). Effect of diet on enhanced intestinal protein synthesis by the gur microflora in the chick. Journal of Nutrition 188: 1068-1074

Nath, H; Barki, V.H; Sarles, W.B and Elvehjem, C.A (1948). Microrganisms in the caecal contents of rats fed various carbohydrates and fats. Journal of Bacteriology 56: 783-793

Orban, J.l; Patterson, J.A.; Sutton, A.L and Richards, G.N (1997) Effect of Sucrose Thermal Olligosaccharide Caramel, Dietary Vitamin-Mineral Level and Brooding Temperature on Growth and Intestinal Bacterial Populations of Broiler Chickens Poultry Science 76: 482-490

Rogosa, M; Mitchell, J.A and Wiseman, R.F (1951). A selective medium for the isolation and enumeration of oral faecal lactobacilli. Journal of Bacteriology 62: 132-133

Salanitro, J.P; Blake, I.G; Muirhead, P.A; Maglio, M and Goodman, P (1978). Bacteria isolated from the duodenum, ileum and caecum of young chicks.

Applied and Environmental Microbiology 35: 782-790
Shapiro, S.K and Sarles, W.B. (1949). Micro-organisms in theintestinal tract of normal chickens. Journal of Bacteriology 58: 531-544