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EFFECTS OF SUCROSE THERMAL OLIGOSACCHARIDE CARAMEL AND FEED RESTRICTION ON THE PERFORMANCE, HEMATOLOGICAL VALUES AND CECAL BACTERIOLOGICAL COUNTS OF BROILER CHICKENS

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Inclusion of fructooligosaccharide in poultry diets and feed restriction are some of the strategies that have received increased attention in efforts to improve production efficiency and reduce mortality and Salmonella colonization in broiler chickens. Forty-eight 3-week- old Peterson X Arbor Acres males were used in an experiment to evaluate the influence of the novel sugar sucrose thermal oligosaccharide caramel (STOC) on hematological values and cecal total gram-negative counts in growing broilers fed ad libitum or restricted diets. Broilers fed STOC consumed more feed, gained more weight ($P<0.001$) and had a similar feed conversion compared with birds on the control diet. Birds fed ad libitum consumed more feed and gained more ($P<0.001$) weight compared with birds fed the restricted diet. Chickens fed STOC had slightly but not significantly higher ($P>0.05$) MCV, heterophil counts, H/L ratio and basophils than chickens fed the control diet.

Feed-restricted birds had slightly higher PCV, MCV, WBC, heterophils, lymphocytes and monocytes than birds fed ad libitum. Six-week-old birds had slightly but non-significantly higher values for PCV, RBC, MCV, WBC, lymphocytes and basophils. Packed cell volume (PCV) was lower for 4-week -old birds and tended to increase up to 6-weeks of age. In 8-week-old-birds fed STOC ad libitum there were lower lymphocyte counts and a higher H/L ratio compared with the control group. Although diet did not influence cecal gram-negative bacterial counts (Log_{10}), the results indicate a significant decrease in Log_{10} bacterial counts in birds fed the restricted diets. Birds fed STOC had slightly less cecal gram-negative bacteria compared with the control group. Cecal gram-negative bacteria were influenced by age in broilers. Eight-week old birds had lower mean log_{10} bacterial counts in their ceca than 6-week-old birds. The results of this study showed only slight alterations in hematological profile in broilers due to the

influence of STOC and feed restriction. The observation of reduced numbers of Gram negative bacteria in birds fed STOC with feed restriction needs further investigation.

Key words: Broiler chicken, Sucrose Thermal Oligosaccharide Caramel (STOC), feed restriction, body weight hematological values, cecal gram-negative bacterial counts.

INTRODUCTION

Oligosaccharides possess several functional and nutritional properties that make them useful ingredients in human and animal foods. Among these properties, they increase calcium and iron absorption and prevent anemia in rats (Ohta et al, 1998_{ab}), enhance calcium absorption in humans (van den Heuvel, et al.,1999), increase bifidobacteria population in humans (Bouhnik et al ,1999), and pet animals (Hussien et al., 1998). In poultry they have been shown to increase the bifidobacterial population (Terada et al.,1994), improve performance of broiler chickens (Sims et al.,1998) and inhibit growth of Salmonella (Bailey et al., 1999). Recent studies reporting the use of fructooligosaccharides (FOS) in poultry diets have indicated improvements in weight gain, feed efficiency, and reduction in mortality and in Salmonella colonization (Ammerman,1988,1989;Bailey et al., 1991; Waldroup et al., 1993; Oyarzabal and Conner, 1996; Orban et al., 1997_b, Sims et al.,1998). Mannose and lactose have also been reported to reduce Salmonella colonization in poultry (Oyofo et al., 1989_a; Corrier et al., 1990, 1991, 1992; Tellez et al., 1993). Lactose was found to be more effective in reducing Salmonella colonization when it was used in combination with anaerobic cultures of cecal microflora. Terada et al. (1994) observed that lactosucrose consumption increased bifidobacteria and decreased the counts of Clostridia, Bacteriodacae, Staphylococci, anaerobic bacteria and Pseudomonas, cecal concentration of ammonia , phenol and cresol. Consequently, environmental ammonia and odor of chicken ceca were greatly reduced by lactose consumption.

Thermally produced kestose oligosaccharides increased the numbers of bifidobacteria and lactobacillus cecal contents of growing broilers (Patterson et al.,1997). FOS increased the concentrations of total lactic acid-producing bacteria and Lactobacillus sp without modifying total anaerobes (Le Blay et al.,1999). Weight gain, feed consumption and mortality of broilers fed diets containing oligosaccharides were similar to the data of those receiving a standard growth promoter (Sims et al.,1998). A novel sucrose thermal oligosaccharide caramel (STOC) has also been evaluated as a promoter of intestinal proliferation of beneficial bacteria. The studies conducted at Purdue using oligosaccharides, kestoses and STOC showed an improvement in growth performance of poultry and the growth of beneficial cecal bifidobacteria (Orban et al. 1997_b ; Patterson et al.,1997). The physiological mechanism by which growth enhancement occurs remains to be elucidated.

Nutritional deprivation was found to promote intestinal inflammation and shedding of Salmonella enteritidis in the feces of laying hens (Macri et al., 1994). Therefore, feed restriction coupled with feeding STOC may affect intestinal colonization of bacteria and the immune system, as indicated by total and differential white blood cells counts and heterophil/ lymphocyte (H/L) ratios

(Gross and Seigel, 1986). Feed restriction in broilers significantly reduced hemoglobin content (Hb), packed cell volume (PCV), mean corpuscular hemoglobin (MCH) and mean cell volume (MCV) (Maxwell et al., 1990a), while the only white blood cells to increase in number following a period of feed restriction were basophils, which might have occurred as a result of physiological tension imposed by the stress of feed restriction (Maxwell et al., 1990b). Maxwell et al. (1992) concluded that some poultry may respond to feed restriction, depending on the severity of the stress, by producing heterophilia or basophilia. The heterophil:lymphocyte ratio, which is indicative of a reactive leukocytosis occurring as in response to disease, and the proportion of basophils were raised in restricted compared to ad libitum fed birds (Hocking et al,1993).However, in newly hatched chicks, feed deprivation resulted in stressful behavior and mortality (Al-Rawashdeh et al ,1995).

In view of the expanding interest in health and improved performance and in relation to nutrition and physiologic conditions, alterations in blood values were considered essential for investigation in this study. On the other hand, feed restriction is a well known commercial practice for improving biological and economic performance of chickens. It is evident that health and well-being of the bird and its productive performance depend on normal quantities of formed elements and serum constituents in the blood. However, the physiological responses to feeding oligosaccharides, with or without restriction, and their possible protective properties have not been sufficiently studied. It is not known whether feeding oligosaccharide would affect any basic physiological data including hematological values in broiler chickens

The present paper reports the effect of restricted and ad libitum feeding of the novel sugar, STOC, on performance, hematological values, and cecal total Gram-negative bacterial counts in growing broiler chickens.

MATERIALS AND METHODS

Three week-old Peterson X Arbor Acres male broiler chicks (n= 48) were randomly assigned to one of two diets, a control diet or one containing 3.5 % STOC to provide 1.5 (Wt/Vol) of active ingredient (Table 1). All rations contained monensin (Coban) and bacitracin. Within the two diet subgroups, birds were weighed and randomly divided into four replicate groups of six birds each. Within diets, two groups were fed ad *libitum* and two groups were restricted (70% of the amount fed to the ad *libitum* group). Birds were allowed free access to feed and water during the growout period. All birds were fed the control diet or STOC diet ad *libitum* until 4 week of age. Restriction, at 70% of the amount fed ad *libitum,* was imposed at 4 week of age (day 0) until the 8th week (period 2). Birds were compared at 6 weeks of age (period 1) and 8 weeks of age (period 2). Birds were reared in wire pens (6 birds per pen) hanging over a waste pit in a room with automatic heating and ventilation systems. Feed intake and body weight were measured weekly.

Sampling Procedures:

Two birds from each treatment group at 4, 6, and 8 weeks of age were randomly selected for hematological and microbiological analyses. Sampling was done early in the morning. Chickens were individually weighed and blood samples were collected from each bird via cardiac puncture into a collecting tube

Table 1. Composition of the diet fed to growing broiler chickens fed *ad libidum* and restricted (70% of ad *libidum*)

Ingredients[ab]	%
Corn (8.2%)	61.64
Soybean Meal (48.5% CP)	32.24
Fat	2.00
Dicalcium Phosphate (18.5% P)	2.00
Calcium Carbonate	1.15
Premium Broiler Premix	0.32
Salt	0.35
Alimet (Liquid MHA)	0.25
Vitamin E 20,000 IU/lb.	0.05
Calculated analysis	
Protein %	20.70
Fat %	4.60
Fiber %	2.69
Calcium %	0.95
Phosphorus (Total) %	0.73
Phosphorus (Available) %	0.49
Sodium %	0.16
Methionine %	0.57
Methionine + Cystine %	0.93
Lysine %	1.17
M Energy (Kcal/kg)	3089

[a]STOC was added at the rate of 3.5%, replacing the same amount of the premix.
[b]Medication - monensin 90g/1000 Kg
Medication - bacitracin 25g/1000 Kg

containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant. The birds were killed by cervical dislocation. Ceca were aseptically removed and stored on ice for determination of gram-negative bacteria and for enumeration of Salmonellae as described below.

Hematological Examinations:

The hematological values obtained from the EDTA blood sample included: Packed cell volume (PCV), hemoglobin (Hb), red blood cells (RBC), white blood cells (WBC) and differential leukocyte count. Heterophils to lymphocytes (H/L) ratio, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were then calculated. The microhematocrit method (Harrison and Harrison,1986) was used for determining the hematocrit. Red blood cells were counted using a Coulter counter (Model ZBI) and Hb was read on a Coulter hemoglobinometer (Coulter Electronics, Inc., Haileah, FL). Following red blood cell lysis and prior to measuring Hb, samples were centrifuged for 10 min. (x1000 rpm) to remove nuclear material and cytoplasmic debris. Total WBC were

counted following the Eosinophil Unopette Test B-D No. 5877 (Becton-Dickson, Rutherfod, NJ). The total heterophils and eosinophils counted by the Unopette technique were used to calculate the total WBC from the percentage of heterophils and eosinophils determined by the differential leukocyte count. Differential leukocyte counts were determined on blood smears stained with Wright-Giemsa Stain.

Bacteriological culture of chicken ceca:

The pair of ceca from each bird, aseptically removed, were placed in a sterile Stomacher bag with 10 ml sterile saline. The bag was blended for 60 sec using a Stomacher Lab-Blender(Tekman Co., Cincinnati, OH) to produce a homogeneous suspension. One ml of the suspension was removed, serially diluted in sterile saline up to 10-8 dilution in sterile test tubes. One-tenth of a ml from each dilution was spread-plated on McConkey plates in duplicate. The plates were incubated for 24 h at 37^{0}C and the number of Gram-negative bacterial colonies (lactose fermenters as well as lactose nonfermenters) was counted. The final calculation used the mean $\log_{10}$ number of bacteria per pair of ceca.

To detect cecal carriage of Salmonella, 100 ml of freshly prepared tetrathionate broth was added to the cecal suspension in the Stomacher bag. The bags were incubated at 41^{0}C for 24 h. Contents were then plated on XLT4 plates and incubated for 48 h at 37 ^{0}C. The plates were examined for Salmonella suspect colonies. Salmonella suspect colonies were confirmed using recommended biochemical and serological procedures (Edward and Ewing, 1986). When Salmonella organisms were detected in a cecal sample, their count in a cecal pair was determined from a 1 ml cecal suspension sample removed from the Stomacher bag kept at 4^{0}C.

Statistical Analysis:

Statistical analyses of test treatment means were performed using the GLM procedures of SAS for analysis of variance (SAS, 1989).The performance and hematological profile data were analyzed by ANOVA as a randomized block design. Means were separated using Student-Newman-Keul's test (SAS, 1989). Pooled standard errors of the mean (SEM) were calculated and reported with least square means and probability values. Colony plate counts were transformed to logarithmic values and expressed as $\log_{10}$ Gram-negative bacteria per bird's cecal content. In addition to diet, the model included ad *libidum* vs restricted feeding, age of the bird, and their interactions.

RESULTS

Broilers fed the STOC diet consumed more ($P<0.001$) feed than the control group (Table 2). Although STOC -fed birds had a slightly higher gain in weight as a reflection of their higher feed intake, than the control group, the differences were not significant ($P>0.05$). As expected, restricted birds consumed less feed and gained less weight ($P<0.001$) and were less efficient in feed conversion than birds fed ad libitum .

Red Blood Cell Characteristics and WBC Counts:

Table 3 presents least square means and pooled SEM for the hematologic profile of broiler chickens. Broilers fed STOC had slightly, but non - significantly higher MCV, heterophil counts, H/L ratio and basophils in their blood, and lower monocyte counts than broilers fed the control diet. PCV, HB, and RBC count were

Table 2. Cumulative feed intake, weight gain and feed conversion and log10 Gram-negative bacteria/cecal pair in broiler chickens.

Item	Diet		Treatment		Period (age)		SEM	Pvalues		
	Control	STOC	Restricted	*ad libitum*	6 weeks	8 weeks		Diet	Treat.	Period
Broilers										
Feed intake. g	3342.00	3569.50	3073.00	3838.50	–	–	25.00	0.001	0.001	–
Weight gain. g	1834.20	1961.00	551.50	244.50	–	–	66.50	NS	0.001	–
Feed conversion	1.89	1.83	2.00	1.73	–	–	0.08	NS	0.02	–
Gram-negative bacteria (Log10)	7.24	6.50	6.10	7.65	7.60	6.15	0.43	NS	0.02	0..02

[a]A significant (P <0.02) Period X treatment interaction was due to the fact that restricted 8-week old birds fed the STOC diet had the lowest (3.97) log_{10} Gram-negative bacterial count in their ceca.

Table 3. Body weight and hematological profile (blood picture) in restricted and *ad libitum* fed chickens.

	Diet			Treatment			Period			P value		
Item	Control	STOC	SEM	Restricted	*ad libitum*	SEM	Period 1	Period 2	SEM	Diet	Treat.	Period
Body Weight	2374.36	2446.87	57.24	2249.75	2571.47	57.24	2023.36	2797.86	57.24	0.03	0.001	0.001
PCV, %	27.87	27.87	1.31	28.62	27.12	1.31	29.75	26.00	1.31	NS	NS	NS
Hb, g/dl	8.71	8.49	0.36	8.55	8.65	0.36	8.97	8.22	0.36	NS	NS	NS
RBCs, $10^6 \mu l^{-1}$	2.56	2.40	0.17	2.49	2.47	0.17	2.57	2.39	0.17	NS	NS	NS
MCV, fl	109.50	116.25	3.54	115.87	109.87	3.54	115.62	110.12	3.54	NS	NS	NS
MCHC, g/dl	31.62	30.87	0.86	30.37	32.12	0.86	30.37	32.12	0.86	NS	NS	NS
WBCs, $10^3 \mu l^{-1}$	22.95	23.42	3.31	25.11	21.26	3.31	3.75	22.62	3.31	NS	NS	NS
Heterophils, $10^3 \mu l^{-1}$	7.22	9.83	2.05	9.69	7.37	2.05	7.05	10.01	2.05	NS	NS	NS
Lymphocytes, $10^3 \mu l^{-1}$	12.91	10.78	2.41	12.45	11.25	2.41	14.12	9.57	2.41	NS	NS	NS
Heterophils:Lymphocytes	0.56	0.91	0.49	0.78	0.65	0.49	0.50	1.05	0.49	NS	NS	NS
Monocytes, $10^3 \mu l^{-1}$	1.62	1.24	0.32	1.65	1.20	0.32	1.07	1.79	0.32	NS	NS	NS
Basophils, $10^3 \mu l^{-1}$	1.27	1.56	0.31	1.32	1.51	0.31	1.58	1.26	0.31	NS	NS	NS

Least-square means (±SEM)

Table 4. Hematological profile (blood picture) in *ad libitum* fed chickens.

Age	4 Weeks				6 Weeks				8 Weeks			
	Diet				Diet				Diet			
Item	Control	STOC	SEM	P value	Controls	STOC	SEM	P value	Control	STOC	SEM	P value
Body Weight, g	1375.90	1344.37	57.24	NS	1962.22	2141.45	57.24	NS	3075.80	3106.4	57.24	NS
PCV, %	20.75	26.50	1.13	0.1	32.50	34.00	1.13	NS	22.00	26.00	1.13	NS
Hb, g/dl	7.05	8.17	0.36	NS	9.60	9.40	0.36	NS	7.15	7.85	0.36	NS
RBCs, $10^6 \mu l^{-1}$	1.80	2.10	0.17	0.1	2.77	2.67	0.17	NS	2.32	2.19	0.17	NS
MCV, fl	114.00	126.00	3.54	NS	117.50	127.50	3.54	NS	99.50	119.00	3.54	NS
MCHC, g/dl	34.50	31.25	0.86	NS	30.50	27.00	0.86	0.05	33.00	30.50	0.86	NS
WBCs, $10^3 \mu l^{-1}$	21.62	24.70	3.31	NS	31.57	22.30	3.31	NS	19.80	26.60	3.31	NS
Heterophils, $10^3 \mu l^{-1}$	8.05	7.69	2.05	NS	7.11	6.94	2.05	NS	6.77	17.91	2.05	NS
Lymphocytes, $10^3 \mu l^{-1}$	12.19	15.12	2.41	NS	21.57	12.45	2.41	NS	9.53	6.23	2.41	NS
Heterophils:Lymphocytes	0.66	0.51	0.49	NS	0.33	0.56	0.49	NS	0.71	2.87	0.49	NS
Monocytes, $10^3 \mu l^{-1}$	1.25	1.65	0.32	NS	2.41	0.67	0.32	NS	1.75	1.79	0.32	NS
Basophils, $10^3 \mu l^{-1}$	0.29	0.37	0.31	NS	0.60	2.23	0.31	NS	1.74	0.66	0.31	NS

NS = Not Significant (P > 0.05)
Least-square means (±SEM)

Table 5. Hematological profile in feed-restricted broiler chickens.

Age	6 Weeks			8 Weeks		
	Diet			Diet		
Item	Control (without STOC)	STOC	P value	Control (without STOC)	STOC	P value
Body Weight, g	1908.25	2081.50	NS	2551.10	2458.15	NS
Hematocrit, %	26.50	26.00	NS	30.50	25.50	NS
Hemoglobin, g/dl	8.60	8.10	NS	9.30	8.60	NS
RBCs, $10^6 \mu l^{-1}$	2.52	2.31	NS	2.63	2.40	0.04
MCV, fl	105.00	112.50	0.05	116.00	106.00	NS
MCHC, g/dl	32.50	31.00	NS	30.50	34.50	NS
WBCs, $10^3 \mu l^{-1}$	22.65	18.40	NS	17.70	26.40	NS
Heterophils, $10^3 \mu l^{-1}$	8.21	5.93	NS	6.80	8.53	NS
Lymphocytes, $10^3 \mu l^{-1}$	12.59	9.87	NS	7.94	14.58	0.06
Heterophils:Lymphocytes	0.65	0.60	NS	0.86	0.58	NS
Basophils, $10^3 \mu l^{-1}$	1.46	1.96	NS	1.21	1.41	NS

NS = Not Significant ($P < 0.10$)
Least-square means (±SEM)

similar across diets. Restricted birds had lower ($P<0.001$) body weights, slightly higher percentage PCV, MCV, WBC, heterophils, lymphocytes and monocytes than ad libitum fed birds (Table 3). Broilers at 6-weeks of age (period 1) had lower ($P<0.001$) body weights, slightly higher PCV, RBC, MCV, WBC, lymphocytes and basophils than 8-week-old (period 2) broilers, irrespective of diet or treatment. However, older birds had a slightly higher heterophil count and higher H/L ratio.

When analysis of variance was performed using orthogonal contrasts to compare the diet, treatments and periods (not shown) it was found that diet did not influence any of the hematologic values while restriction affected PCV and RBC count ($P<0.001$) and influenced basophil count and H/L ratio. PCV, RBC and Hb values were influenced by age . They were lower for 4-week -old birds and tended to increase with age up to 6 weeks of age (Table 4). In ad *libitum* fed birds (Table 4) PCV and RBC were slightly higher for STOC fed birds, while MCHC was slightly lower for 6-week - old and slightly lower for 8-week - old birds fed the STOC diet, respectively. Packed Cell Volume (PCV) and RBC tended to increase with age in the restricted birds fed the control diet (Table 5). Although H/L ratio tended to increase with age in ad *libitum* fed birds, especially in those fed the STOC diets, restricted birds fed STOC in period 2 (8- weeks of age) had a higher lymphocyte count and lower H/L ratio (Table 3).

Bacteriological Culture of cecal content:

Table (2) presents the effect of dietary carbohydrates (STOC) with and without restriction on Gram-negative bacterial in cecal contents of control and treated broiler chickens. Cecal Gram-negative bacterial counts (log_{10}) were compared after 4 weeks of incorporation of STOC into the diet of one group (treated birds) with control birds. Although diet did not significantly influence cecal Gram-negative bacterial counts, feed restriction resulted in a significant decrease in log_{10} bacterial counts in the birds. Chickens fed the STOC diet had fewer Gram-negative bacteria compared with the control group (Table 2). Ceca from restricted birds, irrespective of the diet fed, had lower mean log10 bacterial counts than ad libitum fed birds (mean log_{10} count = 7.65). Cecal gram-negative bacterial counts were influenced by age (Table 2); eight-week old broiler chicks had lower mean log10 bacterial count in their ceca than 6-week old birds. In addition, STOC-fed birds with restriction of diet for four weeks had the lowest (mean log10 count = 3.97) bacterial counts compared with the control and other treatment groups. It is worth mentioning here that the only Salmonella detected ($3x10^3$) were found in the ceca of one of the birds fed the restricted control diet.

DISCUSSION

Feed restriction in poultry has been advocated as a management tool that improves biological and economic performance (Person and Shannon, 1979; Whitehead et al., 1987; Maxwell et al., 1990a). However, Maxwell et al. (1990a) found that feed restriction from 4 up to 20 weeks of age in broiler breeders and layer strains tended to reduce Hb, PCV, but increase RBC's , while MCHC and MCV were lower than the control diet. The results of this investigation are in agreement and within the ranges reported by Maxwell et al.(1990a) for broilers. Packed Cell Volume (PCV), Hb, RBC, and WBC's in this study tended to increase with age up to 6 weeks. This is also consistent with the findings of Ross et al.,(1978). The H/L ratio, as a measure of stress, tended to increase as the

environmental stress increases (Gross and Siegel, 1983). Although H/L ratio has been used as an indicator of stress in poultry, studies have failed to show significant changes in H/L ratios in restricted broilers (Maxwell et al., 1992), probably because birds adjust to feed restriction (Gross and Siegel, 1986; Katanbaf et al., 1988). The numbers of both lymphocytes and heterophils in chicken blood tended to increase in response to stress, especially during the first period of feed deprivation (Gross and Seigel, 1986). The results of this study revealed an increase in lymphocytes up to 6 weeks of age for birds fed the control diet ad libitum and an increase in heterophils in the 8-week old group fed ad libitum or the restricted STOC diet. Similar inconsistency was reported by Gross and Siegel (1983,1986). It may be due to the fact that in the present experiment feed restriction caused less stress and that the STOC diet might have influenced the results. STOC influence on the results could have been possible since feeding oligosaccharides has been shown to increase mineral absorption (Delzenne,et al.,1995) and improve broiler performance (Orban et al. 1997b, Sims et al.,1998). This latter observation may, however point out the importance of including FOS when feed restriction is used as a practice in poultry management operations. On the other hand, STOC fed chickens have apparently higher PCV, Hb and MCV values which is in agreement with the findings of Ohta et al (1998a) who postulated that dietary fructooligosaccharides prevented anemia by increasing the levels of these blood values in rats.

Maxwell et al. (1992) have shown an increase in heterophil numbers with a concomitant increase in H/L ratio in restricted broiler chickens. Our results are generally consistent with these findings in that restriction tended to increase the numbers of heterophils and the H/L ratio when comparisons were made independent of diet and age. When comparisons were made within the 6 - week -age group, those fed the control diet had more WBC than the STOC fed group. The difference was not as conspicuous within STOC diets. Feed restriction only resulted in an increase in heterophil counts in 6-week-old chickens fed the control diet. Maxwell et al. (1990b, 1992) indicated that the failure in some experiments to show significantly higher H/L ratios following prolonged feed restriction in broilers may be because birds adjust with time to the nutritional stress.

Chickens fed ad libitum STOC diets had increased heterophil counts and a higher H/L ratio than other treatment groups. This might suggest that after an adaptation period to the STOC diet, the numbers of beneficial bacteria in the gut, tend to increase while heterophils and H/L ratios were enhanced only in STOC ad libitum fed chickens and in restricted birds fed the STOC diet. This protection apparently needed time and unrestricted feeding to develop and might have been confounded by the antibiotics contained in the premix fed to these chickens Whether STOC results in better protection against stress in birds that are not protected with sub-therapeutic doses of antibiotics or fed unrestricted diets for a longer period of time needs further investigation.

Basophils, increased in number in restricted fed birds in this study. This may be related to a stress response in these birds as suggested by Maxwell et al. (1990b).

It is not really known whether feeding STOC in an ad libitum diet for 4 weeks improves the immunocompetence or protects the birds against stress, as indicated by the heterophilia and H/L ratio, or the increased heterophil counts and H/L ratio could be the result of other stressors in the environment as suggested by Maxwell et al. (1992).

The use of sugars and complex carbohydrates in poultry diets was initiated early in this century (Barnes et al., 1979; Beach, 1925, Beach and Davis, 1925; and Beach and Card, 1925). Dietary sugars, such as lactose, were reported to increase the acidity of the cecal contents and helped in controlling coccidiosis (Beach and Davis, 1925; Beach and Card, 1925) and Salmonella infections (Barnes et al., 1979; Freter, 1974 and Oyofo et al., 1989b). This effect has been attributed to the influence of the carbohydrates on the growth, character, and fermentation of normal flora (Rettger, 1915; Rantal and Nurmi, 1973 and Meynell, 1963). The role of intestinal flora in the control of invading pathogens has been documented in animals (Bohnhoff et al., 1964, and Freter, 1974). In broiler chickens, oligosaccharides were shown to enhance bifidobacteria and lactobacilli, and prevented the growth of E. coli and C. perfringens in the intestine (Patterson et al.,1997). Salmonella did not grow when fructo-oligosaccharide (FOS) was the sole carbon source (Bailey, 1991) which also reduces Salmonella colonization (Fukata et al ,1999) and chicken cecal pH (Chambers,1997), without a consistent effect of the reduced pH on the organism. Similarly, Hinton,Jr. et al. (1990) concluded that increased lactic acid concentrations were directly correlated to decreased cecal pH values and caused a reduction in the total VFA's and an increase in the undissociated form of some VFA's. These effects which are possibly found with FOS are of paramount economic importance and need more investigation. However,it is not clear by what mechanism normal flora decrease intestinal colonization by enteropathogens, such as Salmonella, Listeria, Yersinia or E-coli. Production of bacteriostatic short chain volatile fatty acids particularly acetic, butyric and propionic acids by normal anaerobic flora present in the cecum and colon is one suggested mechanism (Barnes et al., 1979; Bohnhoff et. al., 1964; Rantala and Nurmi, 1973 and Meynell, 1963).

The results of the present study have shown only slight alterations in hematological profile in broiler chickens due to the influence of STOC and feed restriction. The observation of reduced numbers of Gram negative bacteria in birds fed the restricted diets needs further investigation. Also, whether STOC, with or without feed restriction, enhances immunocompetence or provides better protection against stress in birds that are not protected with sub-therapeutic doses of antibiotics needs to be elucidated.

REFERENCES

1. *Al-Rawashdeh, O.F., Lafi, S.Q., Abdul-Aziz,T.A., Ereifij, K. and Nour A.Y. M.*,1995. Effect of feed deprivation on the blood levels of glucose, gamma-hydroxybutyrate, triglycerides, cholesetrol and on body weight and yolk sac weight in one-day old broiler chicks. *Acta Veterinaria (Beograd) 45* No. 4: 175-186.
2. *Ammerman, E., C. Quarles and P.V. Twining, Jr.*, 1988. Broiler response to the addition of dietary fructo-oligosaccharides. *Poultry Sci., 67* (Suppl. 1): 46. (Abstr.).
3. *Ammerman, E., C. Quarles and P.V. Twining, Jr.*, 1989. Evaluation of fructo- oligosaccharides on performance and carcass yield of male broilers. *Poult. Sci. 68* (Suppl. 1):167. (Abstr.).
4. *Bailey,J.S., Blankenship L.C. and Cox N.A.*, 1991. Effect of fructo-oligosaccharides on Salmonella colonization of the chicken intestine. *Poult. Sci.* 70:2433-2438.
5. *Barnes, E.M., C.S. Impey, and Stevens, B.J.H.* 1979. Factors affecting the incidence and anti - Salmonella activity of the anaerobic cecal flora of the young chick. *J. Hyg. Camb.* 82:263-283.
6. *Beach, J.R.*, 1925. The effect of feeding Bacillus acidophilus, lactose, dry skim milk or whole milk on the hydrogen ion concentration of contents of the ceca of chickens. *Hilgardia* 1:145-166.
7. *Beach, J.R. and Card J.C.*, 1925. Studies on the control of avian coccidiosis. *Poult. Sci.* 4:83-93.
8. *Beach, J.R. and Davis D.E.*, 1925. The influence of feeding lactose or dry skim milk on artificial infection of chicks with Emeria anlum. *Hilgardia 1*:167-181.

9. *Bohnhoff, M., Miller C.P. and Martin W.R.*, 1964. Resistance of the mouse's intestinal tract to experimental Salmonella infection. I. Factors which interfere with the initiation of infection by oral inoculation. *J. Exp. Med.* 120:805-816.
10. *Chambers J. R.,. Spencer, J. L, and Modler, H. W.*, 1997. The influence of complex carbohydrates on Salmonella typhimurium colonization, pH, and density of broiler ceca. *Poult. Sci. 76* No 3:445-451.
11. *Campbell,J.M., Fahey, G. C. Jr. , Lichtensteiger, C.A., Demichele S. J. and Garleb, K.A.*. 1997a An enternal formula containing fish oil, indigestible oligosaccharides, gum Arabic and antioxidants affects plasma and colonic phospholipid fatty acid and prostaglandin profiles in pigs. *J. Nutr. 127* (No.1):137-145.
12. *Campbell,J.M., Fahey G.C. Jr. and Wolf B. W.* 1997b Selected indigestible oligosaccharides affect large bowel mass, cecal, and fecal short- chain fatty acids, pH and microflora in rats. *J. Nutr.*127 (No.1): 130-136.
13. *Corrier, D.E., Hinton, A. Jr., Kubena, L.F., Ziprin R.L. and DeLoach, J.R.* 1991. Decreased Salmonella colonization in turkey poults inoculated with anaerobic cecal microflora and provided dietary lactose. *Poultry Sci.* 70:1345-1350
14. *Corrier, D.E., Hinton, A. Jr., Ziprin R.L and DeLoach, J.R.* 1990. Effect of dietary lactose on Salmonella colonization of market-age broilers. *Avian Dis.* 34:668-676.
15. *Corrier, D.E., Snodgrass J.D., Hinton, A. Jr. and DeLoach, J.R.* 1992. Effect of anaerobic cecal microflora and dietary lactose on Salmonella colonization in Bob White Quail (Colinus virginiansus). *Poultry Sci.* 71:2022-2026.
16. *Coussment P.A.A.*1999.Inulin and oligofructose: Safe intakes and legal status. *J. Nutr.* 129: 1412s-1417s.
17. *Delzenne, N., Verplaeise J., Roccaro A., and Roberfroid, M.* 1995. Effect of fermentable fructo-oligosacchharides on mineral , nitrogen and energy digestive balance in rat. *Life Sci.* 57: 1579-1587.
18. *Edwards, P.R. and Ewing, W.H.* 1986. Identification of Enterobacteriacae. *4th Ed. Elsevier Science Publishing Co., Inc., New York, NY.*
19. *Freter, R.*, 1974. Interactions between mechanisms controlling the intestinal microflora. *Am. J. Clin. Nut.* 27:1409-1416.
20. *Fukata, T., Sasai K., Miyamoto, T.,Baba, E.* 1999. Inhibitory effects of competitive exclusion and fructooligosaccharides, singly and in combination on Salmonella colonization of chicks. *J. food Prot. 62* No. 3: 229-233.
21. *Gibson,G.R.* 1999.Dietary modulation of the human gut microflora using the prebiotics oligofructose and inulin. *J.Nutr.*129:1438s-1441s.
22. *Gross, W. B. and Siegel P.B.*. 1986. Effects of initial and second periods of fasting on heterophil / lymphocyte ratios and body weight. *Avian Diseases*, 30:345-346.
23. *Gross W. B. and Siegel H. S.*1983. Evaluation of the heterophil / lymphocyte ratio as a measure of stress in chickens. *Avian Diseases. 27:* 4, 972-979.
24 *Harrison, G.J. and Harrison L.R.*, 1986. Clinical Avian Medicine and Surgery. *W. B. Saunders and Co. Philadelphia, p.* 186-189.
25. *Hidaka, H., Eida T., Takizawa T., Tokunaga T. and Tashiro Y.*, 1986. Effects of fructo-oligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora* 5:37-50.
26. *Hocking P.M., Maxwell M.H., and. Mitchell M.A.* 1993.Welfare assessment of broiler and layer females subjected to food restriction and limited access to water during rearing. *Brit. J.Poult. Sci.* 34:443-458.
27. *Hussein S.S., Campbell J.M., Bauer L.L., Fahey G.C. Jr. , Lewis Hogarth A .J. C., Wolf B.W. and Hunter D.E.* 1998.Selected fructooligosaccharides composition of pet-food ingredients. J. *Nutr. 128* (No. 12s): 2803s-2805s.
28. *Hussein S.S. , Flickinger E.A. and Fahey G.C. Jr.* 1999. Pet food applications of inulin and oligofructose. *J. Nutr. 129:* 1454s-1456s.
27. *Katanbaf, M. N., Jones, D. E., Dunnington, E. A., Gross. and Siegel W. B.*. 1988. Anatomical and physiological responses of early and late feathering broiler chickens to various feeding regimes. *Archiv. Fur Geflugelkunde* 3:119-126.
28. *Le Blay, G., Michel, C., Blottiere, H. M., and Cherbut, C.*1999. Prolonged intake of fructooligosaccharides induces a short- term elevation of lactic acid producing bacteria and persistent increase in cecal butyrate in rats. *J. Nutr.* 129:2231-2235.
29. *Macri, N. P., Porter, R. E. Jr. and Holt, P. S.*. 1994. The effect of molting on early Salmonella enteritidis (SE) infection in laying hens. *The 7th Annual Phi Zeta Research Day of Omecron Chapter, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana.*

30. *Maxwell, M.H., Robertson, G.W., Spence S. and McCorquodale, C.C.* 1990a. Comparison of hematological values in restricted- and ad libitum-fed domestic fowls: Red blood cell characteristics. *Brit. Poult. Sci.* 31:407-413.
31. *Maxwell, M.H., Robertson, G.W., Spence S. and McCorquodale, C.C.* 1990b. Comparison of hematological values in restricted- and ad libitum-fed domestic fowls: White blood cells and Ttrombocytes. *Brit. Poult. Sci.* 31:399-405.
32. *Maxwell, M.H., Hocking, P. M. and Robertson, G. W.,* 1992. Differential leukocyte responses to various degrees of food restriction in broilers, turkeys and ducks. *Brit. Poult. Sci.*33:177-187.
33. *Mench J. A.* 1991. Feed restriction in broiler breeders causes a persistent elevation in corticosterone secretion that is modulated by dietary tryptophan. *Poult. Sci.* 70:12, 1547-2550.
34. *Meynell, G. G.,* 1963. Antibacterial mechanisms of the mouse gut. II. The role of EH and volatile fatty acids in the normal gut. *Brit. J. Exp. Pathol.* 44:209-211.
35. *Mitsuoka, T., Hidaka, H. and Eida, T.* 1987. Effect of fructooligosaccharides on intestinal microflora. *Die Nahrung* 31:427-436.
36. *Morohashi T., Sano, T., Ohta, A. and Yamada, S.*1998 True calcium absorption in the intestine is enhanced by fructooligosaccharide feeding in rats. *J. Nutr. 128* (No.10): 1815-1818.
37. *Moshfegh A.J., Friday, J.E., Goldman, J.P. and Ahuja, J.K.* 1999. Presence of inulin and oligofructose in the diets of Americans. *J. Nutr. 129:* 1407s-1411s.
38. *Ohta,A., M.Ohtsuki, M. Uehara, A.. Hosono, M. Hiryama, T. Adachi, and H. Hara.* 1998a. Dietary fructooligosaccharides prevent postgastroectomy anemia and osteopenia in rats .*J. Nutr.*(No.3): 485-490.
39. *Ohta, A., Motohashi, Y., Ohtsuki, M., Hirayama, M., Adachi, T. and. Sakuma, K.C.* 1998b. Dietary fructooligosaccharides change the concentration of cabindin- D9K differently in the mucosa of the small and large intestine of rats. *J. Nutr. 128* (No. 6):934-939.
40. *Orban J.I., Patterson, J.A., Adeola, O., Sutton, A.L. and Richards, G.N.* 1997a.Growth performance and intestinal microbial populations of growing pigs fed diets containing sucrose thermal oligosaccharidee caramel. *J. Anim. Sci.* 75: 170-175.
41. *Orban, J.I., Patterson, J. A., Sutton, A.L. and Richards, G.N.* 1997b. Effect of sucrose thermal oligosaccharide caramel, dietary vitamin- mineral level, and brooding temperature on growth and intestinal bacterial population of broiler chickens. *Poult. Sci.* 76:482-490.
42. *Oyarzabal O.A. and Conner, D.E.* 1996. Application of direct - fed microbial bacteria and fructooligosaccharides for Salmonella control in broilers during feed withdrawal. *Poult.Sci.* 75:186-190.
43. *Oyofo, B.A., DeLoach, J.R., Corrier, D.E. Normal, J.O,. Ziprin, R.L. and Mollenhauer, H.H.* 1989a. Prevention of Salmonella typhimurium colonization of broilers with D-mannose. *Poult. Sci.* 68:1357-1360.
44. *Oyofo, B.A., Deloach, J.R., Corrier, D.E. Norman, J.O., Tipoin, R.L. and Mollenbauer, H.H.* 1989b.Effect of carbohydrates on Salmonella typhimurium colonization in broiler chickens. *Avian Dis.* 33:531-534.
45. *Patterson, J.A., Orban, J.I., Sutton, A.I. and Richards, G.N.* 1997. Selective enrichment of bifidobacteria in the intestinal tract of broilers by thermally produced kestoses and effect on broiler performance. *Poult.Sci.* 76:497-500.
46. *Person, A. R. and Shannon, D.W.F.* 1979. Controlled feeding systems. In: K. N. Boorman and B.M. Freeman (eds) Food Intake Regulation in Poultry, PP 365-390. *Edinburgh British Poultry Science Ltd.*
47. *Rantal, M. and Nurmi, E.*1973. Prevention of the growth of Salmonella infantis in chicks by the flora of the allmentary tract of chickens. *Brit. Poult. Sci.* 14:627- 632.
48. *Rao A. V.* 1999. Dose- response effects of inulin and oligosaccharide on intestinal bifidogenesis effects. *J. Nutr. 129:* 1442s-1445s.
49. *Rettger, L.F.,* 1915. The influence of milk feeding on mortality and growth and on the character of intestinal flora. *J. Exp. Med.* 21:365-388.
50. *Ross, J.G., Christie, G. Halliday, W.G. and Morley Jones, R.* 1978. Hematological and blood chemistry "Comparison values" for clinical pathology in poultry. *Veterinary Record,* 102:29-31.
51. *SAS Institute,* 1989. SASR User's Guide: Statistics. SAS Institute, Inc., Cary, NC.
52. *Schneeman B.O.* 1999. Fiber, inulin and oligofructose; similarities and oligosaccharides. *J.Nutr.*129:1424s-1427s.
53. *Sims M.D, Spring, P.S. and Sefton, A.S.* 1998. Effect of mannan oligosaccharide on performance of commercial broiler chickens. *Poult. Sci.* 77 (suppl.1) Abstr. 337:89.
54. *Taper H.S. and Roberfroid, M. 1999* Influence of inulin and oligofructose on breast cancer and tumor growth. J. Nutr. 129: *1488s-1491s.*
55. *Terada A.H., Hara, T., Oishi, S., Matsui, T., Mitsuoka, S., Nakajo I., Fujimori, I. and Hara, K.*1992. Effect of dietary lactosucrose on fecal flora and fecal metabolites of dogs. *Microbial Ecol. Healt Dis.* 5: 87-92.

56. *Terada, A. , Hara, H., Kato, S., Kimura, T. Fujimori, I., Hara K., Maruyama, T. and Mitsuoka, T.*1993.Effect of lactosucrose (4G-beta-D- galactosyl-sucrose) on fecal flora and fecal putrefactive products of cats. *J. Vet. Med. Sci.* 55: 291-295.
57. *Terada, A., Hara, H., Sakamoto, J. Sato, N., Takagi, S., Mitsuoka, T., Mino, R., Hara, K, Fujimori, I. and Yamada T.*1994.Effects of dietary supplementation with lactosucrose (4G- beta - D-Galactosylsucrose) on cecal flora, cecal metabolites and performance in broiler chickens. *Poult. Sci.* 73:1663-1672
58. *Tellez, G., Dean, C.E., Corrier, D.E., DeLoach, J.R., Jaeger L. and Hargis, B.M.* 1993. Effect of dietary lactose on cecal morphology, pH, organic acids and Salmonella enteritidis organ invasion in Leghorn hens. *Poult. Sci.* 72:636-642.
59. *Van den Heuvel, E. G., Muys, T., van Dokkum, W. and G. Schaafma.* 1999. Oligofructose stimulates calcium absorption in adolescents. *Amer. J. Clin. Nutr. 69* No. 3: 544-548.
60. *Waldroup, A.L., J.T. Skinner, R.E. Hierholzer and P.W. Waldroup,* 1993. An evaluation of fructooligosaccharide in diets for broiler chickens and effects on Salmonelae contamination of carcasses. *Poult. Sci.* 72:643-650.
61. *Whitehead, C.C., Herron, K. M. and Waddington, D,* 1987. Reproductive performance of dwarf broiler breeds given different allowances of food during the rearing and breeding periods and two lighting patterns. *Brit. Poult.* Sci. 28:415-427
62. *Zou,Y., Fahey, G.C., Merchen, N.R. and Bajjalieh, N.L.* 1996.Digestion Zuo, responses to low oligosaccharide soybeanmeal by ileally cannulated dogs. *J. Anim.* Scl. 74:2441-2449.

UTICAJ SAHAROZNOG TERMALNOG OLIGOSAHARIDNOG KARAMELA I USKRAĆIVANJA HRANE NA PROIZVODNE PARAMETRE, HEMATOLOŠKE PARAMETRE I BROJ BAKTERIJA U CEKUMU BROJLERA

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SADRŽAJ

Dodavanje fruktoznih oligosaharida u hranu za živinu kao i delimično uskraćivanje hrane, privlače u poslednje vreme pažnju istraživača kao moguće strategije za povećanje proizvodnih performansi i smanjenje mortaliteta izazvanog Salmonelama. U ovom ogledu je ispitivan uticaj novog saharoznog, termički obrađenog, oligosaharidnog karamela na proizvodne i hematološke parametre, kao i na ukupan broj Gram-negativnih bakterija u cekumu pilića ukrštenog hibrida Peterson x Arbor Acres. Ogledi su izvedeni na 48 petlića koji su bili hranjeni ad libidum ili im je delimično uskraćivana hrana. Rezultati ove studije ukazuju da delimično uskraćivanje hrane i ishrana karamelom samo minimalno menjaju ispitivane proizvodne i hematološke parametre dok smanjenje broja Gram-negativnih bakterija kod brojlera hranjenih karamelom i uz delimično uskraćivanje hrane zahteva dodatna ispitivanja.