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The Effects of Enterococcus faecium Cernelle 68 (SF 68) on Output Properties and Some Haematological Parameters in Broilers*)

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Summary

To investigate the effects of Enterococcus faecium Cernelle 68 on output properties and some haematological and biochemical properties in broiler chicks, 130 male Ross-308 broiler chicks were used. The animals were divided into two groups as control and experimental. 35 mg/kg Enterococcus faecium Cernelle 68 was supplemented to diet of the experimental group. Body weight gain, food consumption and feed efficiency ratio were determined on day 14, 28, 42 and 49. Also on the same days, red blood cell (RBC), white blood cell (WBC), thrombocyte counts, packed cell volume (PCV), haemoglobin (Hb) amount and serum creatine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, triglyceride, cholesterol and glucose levels were determined from the blood samples of randomly selected 15 animals from both control and experimental groups. After blood samples were taken, carcass weight, small intestine weight and ileum pH of the animals were determined during autopsy. It has been determined that, food consumption was less in probiotic group than in control, and feed efficiency ratio of the probiotic group was higher than that of the control group, but these differences were not statistically significant. The carcass weight of the probiotic group was higher on day 49 ($P<0,05$). It has been determined that, the probiotic had no effect on small intestine weight and ileum pH. No statistically significant change was observed in RBC, WBC, thrombocyte, PCV, and Hb values. AST and ALT levels of the probiotic group decreased statistically on day 49 ($P<0,05$). The cholesterol level of the probiotic group was statistically lower than that of the control group on day 14 ($P<0,05$). These results show that Enterococcus faecium Cernelle 68 will bring economic advantage to the breeders by improving feed efficiency ratio and carcass weight, and it is safe for the host animal and it is well tolerated by the organism.

Keywords: Broiler, Chicken, Probiotics, Enterococcus faecium, Performance, AST, ALT, Cholesterol

Probiotics are culture of specific living micro-organisms that are used for regulating micro-flora in gastrointestinal tract, preventing pathogen micro-organisms from being harmful and contributing to host's healthiness.

Probiotics that are thought to be an alternative to antibiotics in broiler breeding and used commonly in nowadays has been investigated by many researches with the idea that probiotics are effective on body weight gain, feed efficiency ratio and development of intestines (1, 9, 11, 25). It has been reported that coccidiostats which are used in treatment of coccidiozis, increase productivity characteristics of poultry as probiotics (26) and it has been suggested that these coccidiostats do not effect probiotics negatively (22).

The effects of the probiotics used in poultry, on body weight gain, food consumption or feed efficiency ratio is in contradiction. It has been suggested that, these differences might be related with the growth phase of the animal, the species of the probiotic and its dose, or the hygiene conditions of the shelter (12). Furthermore the microflora of the shelter cause probiotics to show different effects. However, it has been reported that, the probiotics manifest their beneficial effects by competing with the pathogen and the other microorganisms of the gastrointestinal tract (20).

It has been stated that, haematological parameters are important in determining the reliability of the probiotic, the changes or insufficiency in nutrient me-

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tabolism (2) and in investigating the sub-clinical adverse effects that might develop in the host against the micro organism (23). However effects of probiotics on effects on haematological parameters are not well known.

Although the effects of *Lactobacillus* species have been investigated widely (1, 8, 13, 19), a little work has done about the effects of *Enterococcus faecium Cernelle* 68 (SF 68) which has been started to use in broiler breeding in recent years (2). However, it has been reported that, beside different species of probiotics, even different strains may cause different metabolic activities, which might affect several parameters, principally the performance of the animal (12). Therefore, in this study we aimed to investigate the effects of *Enterococcus faecium Cernelle* 68 on productivity characteristics and some physiological and biochemical parameters in broilers.

Material and Methods

A total of 130 newly hatched Ross 308 male broiler chicks were used in this study. The chicks were divided into two groups as control and experimental. These groups were also divided into subgroups, with 13 chicks in each. All animals were fed with commercial starter diet for the first three weeks and they were fed with commercial growing diet until the end of seventh week. Food and water were supplied ad *libitum*. 35 mg/kg probiotic (*Enterococcus faecium Cernelle* 68) was supplemented to diet of experimental group. Multiplying technique was used for mixing the daily prepared diet of the experimental group with probiotics (1).

Body weight of each animal and food consumption were determined daily. Body weight gain, food consumption and feed efficiency ratio were calculated from the daily records for the periods of the days 1-14, 15-28, 29-42 and 43-49. Feed efficiency ratio was calculated as weight gain / food consumption (14). In addition, 3 chicks were selected randomly from subgroups of control and experimental groups on day 14, 28, 42, 49. Blood samples of these animals were taken with and without anticoagulants from V. subcutenea ulnaris. Carcass and small intestine weights of animals that were slaughtered by bleeding after blood samplings were measured. RBC, WBC, thrombocyte numbers, PCV values and Hb amounts were determined (7). CK, LDH, AST, ALT, total protein, albumin, triglyceride, cholesterol and glucose levels of obtained serums were measured by autoanalyzer. pH of the ileum content, obtained right after the autopsy, was measured with a glass-electrode pH meter.

Means of all data and standard errors of means were determined. The significance control of the differences between control and experimental groups were determined by "student t" test. The changes in mean food consumption and mean feed efficiency ratio related with age were identified by variance analysis. The significance controls of the differences among weeks were determined by 'Duncan' test. Statistical analyses were made on SPSS 9.0 statistic program.

Findings

Data about body weight, food consumption and feed efficiency ratio are shown in Table I. It is seen that body weight and food consumption increased related with age and reached to maximal level at the end of the experiment ($P < 0.05$) in both groups. Contrary to food consumption, feed efficiency ratio decreased related with age, and in comparison with the days before the 7th week a statistically significant decrease was determined at the 7th week in both two groups.

When comparing control and probiotic groups it is noticed that food consumption was less in probiotic group than in control, although it wasn't statistically important, and feed efficiency ratio of the probiotic group was generally higher than that of the control group during the experimental period.

Data about carcass weight, small intestine weight and ileum pH are presented in Table II. It was determined that carcass weights of probiotic group were statistically higher than control on day 49. No significant difference was found between the small intestine weights of the control and probiotic group.

Data about haematological parameters are shown in Table III. There was not any statistically significant difference between haematological parameters of control and probiotic groups.

Data about CK, LDH, AST, ALT, total protein, albumin, triglyceride, cholesterol, glucose and urea levels of control and experimental groups are presented in Table IV. It is seen that AST and ALT levels of the probiotic group were statistically lower than those of the control at the 49th day of the experiment ($P < 0.05$) (Table IV). Also it was determined that serum cholesterol level were low in probiotic group compared to control on day 14 ($P < 0.05$). There was not any statistically significant alterations in other serum parameters that are presented in Table IV.

Discussion

Our findings about body weight gain shows that the probiotic (*Enterococcus faecium Cernelle* 68) which was supplemented into the diet, did not cause any statistically significant change during 49-day experimental period (Table I). Although it has been stated that contribution of *Lactobacillus sporegenes* culture, which was supplemented to the diet of broilers, to body weight gain appears after the 4th week (17), has been suggested that it is effective only during the first 3 weeks (29). However Jin et al (15) reported that the same probiotic contributed to body weight gain during every stage of 6-week breeding period. Although our results about the effects of *Enterococcus faecium Cernelle* 68 on body weight gain are in contradiction with these 3 literatures, they are in accordance with the reports de-

Table I. Body weight, feed intake and feed efficiency ratio in broilers

Parameters		Age (day) 1-14 (n = 65)		15-28 (n = 50)		29-42 (n = 35)		43-49 (n = 20)	
		$\bar{x}$	± sx	$\bar{x}$	± sx	$\bar{x}$	± sx	$\bar{x}$	± sx
Body Weight (g/day)	C	134,02^{d}	18,36	635,50^{c}	66,99	1658,07^{b}	87,62	2481,46^{a}	53,49
	P	132,50^{d}	17,47	583,68^{c}	59,86	1563,03^{b}	87,13	2427,46^{a}	56,07
Feed intake (g/day)	C	35,50^{d}	5,22	95,75^{c}	4,57	140,86^{b}	3,39	168,76^{a}	8,49
	P	31,44^{d}	4,54	89,14^{c}	4,05	135,46^{b}	4,62	164,70^{a}	6,55
Feed Efficiency Ratio Gain (g)/ Feed (g)	C	0,546^{a}	0,04	0,636^{a}	0,03	0,560^{a}	0,05	0,382^{b}	0,05
	P	0,590^{a}	0,04	0,618^{a}	0,03	0,595^{a}	0,06	0,412^{b}	0,04

a, b, c, d: Variations of means indicated by different letters at the same line are significant (P < 0,05). C = Control, P = Probiotic

Table II. Carcass and small Intestine weight and pH of ileum in broilers

Parameters		Age (day) 14 (n = 15)		28 (n = 15)		42 (n = 15)		49 (n = 20)	
		$\bar{x}$	± sx	$\bar{x}$	± sx	$\bar{x}$	± sx	$\bar{x}$	± sx
Carcass Weight (g)	C	126,63	7,96	560,40	31,87	1273,87	49,30	1658,00	37,64
	P	106,37	6,93	554,80	34,00	1199,87	57,37	1751,35*	24,65
Small Intestinal Weight (g)	C	8,43	0,42	25,78	1,60	33,76	1,49	43,13	1,84
	P	8,76	0,49	27,35	2,17	32,23	1,71	46,19	1,58
Ileum pH	C	6,13	0,22	6,67	0,24	6,32	0,30	7,78	0,22
	P	6,60	0,24	6,93	0,20	6,59	0,29	7,29	0,18

*Variations between means on the same column are significant (P < 0,05), C = Control, P = Probiotic

clare that probiotics do not affect body weight gain (3,8,27,28). Although it has been determined that dose of the probiotic (35 mg/kg) that was used in this study, was not effective on body weight gain, it should be take into account that different results would be obtained by applying different doses of the probiotic. As a matter of fact, Jin et al (15) has revealed that some *Lactobacillus* species which are effective in low doses, are not effective in high doses.

It is seen that food consumption increased related with age in both groups, but feed efficiency ratio decreased during the finishing period, which includes 43-49 days, compared with the previous weeks (P<0.05) (Table I). Although there was not any statistically significant difference between control and probiotic groups in respect of food consumption and feed efficiency ratio, it can be said that applied probiotic had positive effects on these parameters. Because, it was noticed that although the food consumption was less, feed efficiency ratio was higher in probiotic group than control. It has been reported that probiotic that was used in chicks by Crawford (5). Contributed to food consumption and feed efficiency ratio positively though this was not statistically significant. The highest difference in feed efficiency ratio between control and probiotic groups was 0.044 and determined between the 1st and 14th days (Table I). The best performance of feed efficiency ratio occurred during first 15-day period, which is the rapid growth phase of broilers, and this was considered as a positive effect of *Enterococcus faecium Cernelle* 68. Kahraman et al (16) reported that they obtain similar results by applying probiotic, which includes *S. faecium, L. acidophilus, S. cerevisiae, A. oryzae* and *B. subtilis* mixture, to chicks.

It is obvious that statistically higher carcass weight determined in probiotic group than control at the 7th week, which is slaughtering period, is going to provide economic benefits to breeders. However Kahraman et al (16) suggested that probiotic, which they used, did not affect the carcass weight. It has been thought that the contradiction appeared between these two studies may be due to the difference of probiotics.

Data about the effects of *Enterococcus faecium Cernelle* 68 on the development of small intestines and ileum pH (Table II) demonstrate that applied probiotic had no statistically significant change neither on intestine weight, nor on pH. Our results about intestine weight are in accordance with the findings of Fethiere and Miles (11) and Jin et al (15) who suggested that probiotics do not cause an alteration on intestine weight, whereas they are in contradiction with the results of Tortuero (25) who suggested that probiotics decrease intestine weight. Alp et al (1) reported that, the thickness of the intestinal wall of the chicks reared in poultry-house in which the pathogen microorganisms are effective, might increase. If a probiotic is used in such kind of a poultry-house, it is possible that the weight of intestines might decrease due to the depression of pathogen microorganisms. The contradiction between our and Tortuero's (25) results can be explained by the declaration of Alp et al (1). Although it has been reported that probiotics decrease the pH of ileum (16), our results are in accordance with the results of Toit et al (24) and Endo et al (10) who stated that probiotic supplementation does not affect pH.

It has been reported that medium levels of alterations or insufficiencies in nutrient metabolism may be exist without causing any clinical symptom or a decrease in body weight (23) In addition, it has been stated that micro-organisms that are used as probiotics may have sub-clinical adverse effects. Our results shows that RBC, WBC and thrombocyte numbers, haematocrit values and haemoglobin amounts did not change

statistically in any stage of our research and this probiotic did not have sub clinical adverse effects. It has been reported that, especially the change in WBC number is an important criterion for determining the infectivity or pathogenicity of the probiotic (30). It can be said that, as we did not observe a change in WBC number, the probiotic that we used is reliable.

Findings about biochemical parameters indicate that *Enterococcus faecium Cernelle* 68 did not change serum CK and LDH levels significantly (Table IV). Although any significant difference was determined in serum AST and ALT levels between control and probiotic groups up to 42nd day, enzyme activity of the probiotic group was lower than that of the control group on day 49 ($P<0.05$). It was thought that the decrease observed in AST and ALT levels may be due to depression of micro-organisms that are in chicks' gastrointestinal tract and have effects on serum enzyme level, by probiotic. Curtis et al (6) have reported that, *Escherichia coli* endo-toxines in the gastrointestinal tract increase serum AST level in poultries, whereas Cheng et al (4) reported that aflatoxines cause an increase in AST and ALT levels. Pedersen et al (21), reported that *Enterococcus faecium Cernelle* 68 multiplies by adhering to intestinal wall and colonising there, and decrease the number of *Escherichia coli* and other micro-organisms in the intestinal flora, and this confirms our idea.

Any statistically significant change was observed in serum total protein, albumin and glucose levels of control and probiotic groups at any stage of the experiment. These results demonstrated that applied probiotic was not effective on protein and glucose metabolisms, and are completely in accordance with Zhou et al.'s (30) data that were obtained in mice.

Table III. Haematological parameters in broilers

Parameters		Age (day) 14 (n = 15) $\bar{x}$	± sx	28 (n = 15) $\bar{x}$	± sx	42 (n = 15) $\bar{x}$	± sx	49 (n = 20) $\bar{x}$	± sx
RBC (× 10^4/ mm^3)	C	190,62	9,35	226,47	11,08	243,64	8,97	235,35	11,85
	P	203,17	9,60	228,13	8,86	247,73	9,75	219,50	9,32
PCV (%)	C	29,87	1,12	33,13	0,97	35,21	1,44	29,95	1,39
	P	31,15	1,46	32,93	0,71	35,00	1,14	29,44	0,73
Haemoglobin (g/dl)	C	6,91	0,16	7,67	0,11	7,25	0,19	7,45	0,35
	P	7,21	0,15	7,62	0,11	7,45	0,18	7,89	0,29
WBC (× 10^3/ mm^3)	C	11,92	1,58	12,00	1,66	15,79	1,80	8,90	1,13
	P	13,25	2,35	10,53	1,06	14,93	1,83	12,89	1,81
Thrombocyte (× 10^3/ mm^3)	C	11,54	1,42	22,67	3,41	16,79	4,68	13,90	1,75
	P	9,75	1,45	17,93	4,20	18,27	4,83	10,78	1,28

C = Control, P = Probiotic

Tablo IV. Serum Biochemical Parameters in Broilers

Parameters		Age (day) 14 (n = 15) $\bar{x}$	± sx	28 (n = 15) $\bar{x}$	± sx	42 (n = 15) $\bar{x}$	± sx	49 (n = 20) $\bar{x}$	± sx
CK (U/ l)	C	3659,60	761,05	2111,64	330,33	8105,43	1502,65	20102,63	5413,97
	P	3535,31	923,77	2414,80	195,21	9353,62	1916,59	15834,53	3685,15
LDH (U/ l)	C	3226,87	873,87	1061,36	134,32	1468,79	175,78	1855,05	157,55
	P	2785,50	675,93	1037,80	155,02	1912,69	369,52	1518,39	227,53
AST (U/ l)	C	291,64	38,94	179,25	7,23	241,71	9,63	315,13	22,56
	P	237,56	17,20	188,82	23,91	250,00	13,86	224,54*	36,34
ALT (U/ l)	C	3,71	0,52	3,29	0,72	3,79	0,53	4,65	0,51
	P	4,52	0,54	2,52	0,43	3,89	0,46	2,86*	0,58
T. Protein (g/dl)	C	3,27	0,25	2,76	0,08	3,40	0,25	3,93	0,14
	P	3,45	0,26	2,69	0,13	3,58	0,31	3,38	0,27
Albumin (g/dl)	C	1,51	0,08	1,31	0,08	1,76	0,15	1,76	0,06
	P	1,71	0,15	1,37	0,09	1,63	0,07	1,54	0,14
Triglyceride (mg/dl)	C	78,93	10,00	65,43	4,18	50,43	5,71	46,11	7,75
	P	83,15	8,96	61,47	6,36	63,08	10,66	38,00	4,26
Cholesterol (mg/dl)	C	162,20	14,14	113,79	4,36	124,07	10,87	144,68	7,61
	P	122,15*	9,50	116,38	10,84	121,38	6,82	150,44	7,62
Glucose (mg/dl)	C	250,73	16,28	237,71	8,71	234,00	5,19	229,95	4,07
	P	239,15	8,26	248,50	21,13	214,38	11,07	223,56	4,38

*Variations between means on the same column are significant ($P < 0,05$) C = Control, P = Probiotic

The findings about the lipid metabolism demonstrate that, although there was not a statistically significant change in serum triglyceride level between control and probiotic groups throughout the study, the cholesterol level was low on day 14 ($P < 0,05$). These findings which are in accordance with Mohan et al (18), indicate that *Enterococcus faecium Cernelle* 68 did not lead to a change in lipid digestion, but it affected the cholesterol metabolism especially during the rapid growth period. However, it has been reported that, *Lactobacillus* species implanted to the gastrointestinal tract decrease serum cholesterol level both by facili-

tating the formation of bile salts and also by increasing the fragmentation of free cholesterol in the gastrointestinal tract by bacterial enzymes (15).

In conclusion, *Enterococcus faecium Cernelle* 68 that was used as probiotic in this study, in addition to its non statistical benefit to feed efficiency ratio, it might bring a beneficial advantage to the breeder by increasing the carcass weight on days 43-49, the finishing period. The haematological and biochemical parameters also indicates that the probiotic supplemented into the diet is reliable for the host, does not cause sub-clinical adverse effects and is well tolerated by the organism.

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BIGGADIKE H. J., OHNSTAD I., LAVEN R. A., HILLERTON J. E.: Ocena przydatności pomiaru przewodnictwa elektrycznego próbek mleka z ćwiartek gruczołu mlekowego w rozpoznaniu wczesnego zapalenia gruczołu mlekowego. (Evaluation of measurement of the conductivity of quarter milk samples for the early diagnosis of mastitis). Vet. Res. 150, 655-658, 2002 (21)

Uszkodzenie nabłonka wydzielniczego gruczołu mlekowego powoduje zachwianie równowagi w mleku pomiędzy jonami sodu, potasu i chloru, czego następstwem jest zmiana przewodnictwa elektrycznego mleka. Wykonano pomiary przewodnictwa elektrycznego mleka pochodzącego z poszczególnych ćwiartek gruczołu mlekowego 31 krów ze stada liczącego 70 zwierząt w okresie 15 tyg. W tym czasie u 42% krów/tydzień i w 20% ćwiartek/tydzień zwiększyło się przewodnictwo elektryczne mleka w odniesieniu do 14 udojów. W 14% ćwiartek/tydzień przewodnictwo elektryczne wzrosło o 15% lub więcej. Średnia geometryczna komórek somatycznych była wyższa w mleku z ćwiartek o wyższym przewodnictwie elektrycznym. Jednak samo badanie przewodnictwa elektrycznego mleka pochodzącego z ćwiartek wymienia bez określenia liczby komórek somatycznych w badanej próbce nie może stanowić kryterium do wczesnej terapii antybiotykowej gruczołu mlekowego.

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