



HAL
open science

Effect of *Enterococcus faecium* M-74 strain on selected blood and production parameters of laying hens

Marcela Capcarova

► **To cite this version:**

Marcela Capcarova. Effect of *Enterococcus faecium* M-74 strain on selected blood and production parameters of laying hens. *British Poultry Science*, 2010, 51 (05), pp.614-620. 10.1080/00071668.2010.513961 . hal-00638521

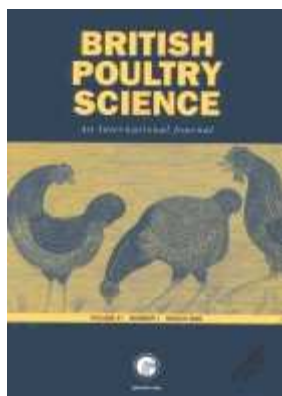
HAL Id: hal-00638521

<https://hal.science/hal-00638521>

Submitted on 5 Nov 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Effect of Enterococcus faecium M-74 strain on selected blood and production parameters of laying hens

Journal:	<i>British Poultry Science</i>
Manuscript ID:	CBPS-2009-128.R2
Manuscript Type:	Original Manuscript
Date Submitted by the Author:	11-Feb-2010
Complete List of Authors:	Capcarova, Marcela; Slovak Agricultural University, Animal Physiology
Keywords:	Biochemistry, Fats and fatty acids, Haematology, Laying hens, Probiotics

SCHOLARONE™
Manuscripts

1
2
3 1 **Effects of *Enterococcus faecium* M 74 strain on selected blood and production**
4
5 2 **parameters of laying hens**
6
7
8 3

9
10 4 M. CAPCAROVA, L. CHMELNICNA¹, A. KOLESAROVA, P. MASSANYI AND J.
11
12 5 KOVACIK

13
14
15 6 *Department of Animal Physiology, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2,*
16
17 7 *SK – 949 76, Slovak Republic* ¹*Department of Poultry Science and Small Animal Husbandry,*
18
19 8 *Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, SK – 949 76, Slovak Republic*
20
21
22 9

23
24
25 10
26 11
27 12 **Running title: Probiotics in poultry nutrition**
28 13
29 14
30 15
31 16
32 17
33 18
34 19
35 20
36 21
37 22
38 23
39 24
40 25
41 26
42 27
43 28
44 29
45 30
46 31
47
48
49
50
51
52
53
54
55
56
57
58
59
60

27 Correspondence to: M. Capcarova, Department of Animal Physiology, Slovak University of
28 Agriculture in Nitra, Tr. A. Hlinku 2, SK – 949 76, Slovak Republic.
29 E-mail: marcela.capcarova@uniag.sk
30

31
Accepted for publication: 17th March 2010

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56

Abstract: 1. The aim of the present study was to evaluate the functional efficiency of a probiotic strain, *Enterococcus faecium* M 74, in the feed on selected biochemical, haematological and production parameters in ISA Brown hens.

2. Feed in the experimental group was enriched with a probiotic preparation containing of 5×10^9 viable *Enterococcus faecium* M 74 per g. Blood samples were collected during the egg laying period at 5 (w5), 25 (w25) and 45 (w45) weeks of production. Body weight, rate of lay and egg weight were recorded every 4 weeks during the 48-week laying period.

3. Significantly lower concentrations of total cholesterol and total lipids in blood plasma were observed in the experimental group at all sampling times compared with their respective controls. Concentrations of triglycerides did not differ. Significantly lower concentrations of plasma calcium were found in the experimental group at w5 and w45. Concentrations of inorganic phosphorus in the experimental group were significantly higher at w25 but significantly lower at w45. Erythrocyte count was significantly higher in the experimental group at w25 and w45 when compared with controls. Leucocyte counts were significantly lower in the experimental group at all sampling times. Significantly lower values of haematocrit at w5 and w45 were observed in the experimental group than controls. Body weight, number of eggs and average egg weight were not significantly affected by probiotic addition.

4. In conclusion, the addition of probiotic strain *Enterococcus faecium* M 74 to the feed of ISA Brown hens reduced cholesterol, lipids, calcium, leucocyte counts and haematocrit values in blood plasma in at least two sampling times, while erythrocyte counts were increased. No significant effects of probiotic on triglyceride concentration and egg production parameters were observed.

INTRODUCTION

57
58
59
60

The European Union has banned the use of antibiotic feed additives due to the negative effect

1
2
3 57 on human health (Immerseel *et al.*, 2004); antibiotic resistance is among the greatest threats to
4
5 58 public health (Finch and Sharland, 2009). Probiotics show promising effects as alternatives to
6
7
8 59 antibiotics as pressure to eliminate growth-promoting antibiotic use increases (Awad *et al.*,
9
10 60 2009).

11
12 The use of probiotic cultures in the poultry industry has recently become more common
13
14 (Torres-Rodriguez *et al.*, 2007). Probiotics are available as single microbial strains (*e.g.*,
15 62
16 *Bacillus clausii*, *Lactobacillus*) or as a mix of multiple strains of *Lactobacillus* (*acidophilus*,
17 63
18 *sporogenes*, *lactis*, *reuteri*, *plantarum*), *Bifidobacterium* (*bifidum*, *longum*, *infantis*),
19 64
20 *Streptococcus* (*thermophilus*, *lactis*, *fecalis*), *Saccharomyces boulardii* etc. (Singhi and
21 65
22 Baranwal, 2008). They have potential health benefits for conditions such as gastrointestinal
23 66
24 infections, genitourinary infections, allergies and certain bowel disorders, all of which afflict
25 67
26 a considerable proportion of the global population (Senok *et al.*, 2005). Research over the last
27 68
28 century has shown that lactic acid bacteria and certain other micro-organisms can increase
29 69
30 resistance to disease and that lactic acid bacteria can be enriched in the intestinal tract by
31 70
32 feeding specific carbohydrates (Patterson and Burkholder, 2003).
33 71

34
35
36 72 A single dose of *Lactobacillus* administered intragastrically improved weight gain and
37
38 food conversion efficiency (Khan *et al.*, 2007; Liu *et al.*, 2007; Zulkifli *et al.*, 2000), had a
39 73
40 growth-promoting effect in broiler chickens (Cavazzoni *et al.*, 1998) and modulating effects
41 74
42 on immune system of layer- and meat-type chickens (Koenen *et al.*, 2004). The application of
43 75
44 *Rhodobacter capsulatus* into diet may be a feasible way to reduce cholesterol concentration
45 76
46 and improve the ratio of unsaturated fatty acids to saturated fatty acids in broiler meat (Salma
47 77
48 *et al.*, 2007a). After *Bacillus subtilis* treatment, egg production, feed consumption and feed
49 78
50 conversion efficiency in layers (Li *et al.*, 2006) and growth performance of goslings (Wu *et*
51 79
52 *al.*, 2008) was improved.
53 80
54
55
56
57
58
59

60 81 Probiotic beneficial action in human and animals was also confirmed by Balevi *et al.*

1
2
3 82 (2001), Juntunen *et al.* (2001), Wielen *et al.* (2002), Glück and Gebbers (2003), Weese and
4
5 83 Arroyo (2003), Gil de los Santos *et al.* (2005), Capcarova *et al.* (2008), Siggers *et al.* (2008),
6
7 84 Al-Salami *et al.* (2008) and others.

8
9
10 85 Based on the data from literature and the trends of research in the field of probiotics, the
11
12 86 aim of the present study was to evaluate the functional efficiency of a probiotic strain
13
14 87 *Enterococcus faecium* M 74 in the feed on selected biochemical, haematological and
15
16 88 production parameters of laying hens.

19 89 MATERIAL AND METHODS

20 90 **Birds, management and diets**

21
22 91 Forty ISA Brown laying hens were housed at 20 weeks of age in individual cages with a
23
24 92 space allowance of 573 cm² and given diet KKZ HYD 10 (Table 1). Feed and water were
25
26 93 provided *ad libitum*. Twenty birds acted as controls and 20 were given the experimental diet.
27
28 94 Blood collections were made at 5, 25 and 45 weeks of a 48-week laying period. The
29
30 95 experimental group of hens received a probiotic preparation with concentration of 5x10⁹
31
32 96 living organisms of *Enterococcus faecium* M 74 in 1 g. Probiotic preparation (Probiotics
33
34 97 International Ltd., UK) was in powder form for ease of incorporation into the diet, and added
35
36 98 to the feed at the rate of 0.5 g/1 kg to produce colony forming units (CFU) of 2.5x10⁹ in 1 kg
37
38 99 of finished feed.

Table 1 near here

45 100 **Measurements**

46
47 101 Blood was obtained from *vena basilica* and samples collected into EDTA treated tubes. A
48
49 102 0.2-ml blood sample was removed by micropipette to micro-capillaries for haematological
50
51 103 analyses and placed in a MPW-310 centrifuge for haematocrit measurement. The blood
52
53 104 plasma was separated from whole blood by centrifugation at 3000 g for 30 mins. Biochemical
54
55 105 parameters of mineral profile (calcium and inorganic phosphorus iP), energy profile (total
56
57 106 cholesterol, total lipids, triglycerides) and haematological parameters (erythrocytes and

1
2
3 107 leucocytes counts) were analysed using a semi-automated clinical chemistry analyser
4
5 108 Microlab 300 (Vilat Scientific, Dieren, The Netherlands) and Bürker chamber after dilution
6
7
8 109 with Natt-Herich solution. Haematocrit was determined by the micro-haematocrit centrifuge
9
10 110 method.

11 111 Body weight (BW), rate of lay and egg weight were determined. The birds were weighed
12
13 112 during whole laying period at 4-weekly intervals. The average egg weight was the mean of all
14
15 113 eggs produced during the 48-week laying period. Mortality was recorded daily.

16 114 **Statistical analysis**

17 115 SAS software was used to conduct statistical analyses. Analysis of variance and *t*-test were
18
19 116 used to calculate basic statistic characteristics and to determine significant differences
20
21 117 between experimental and control groups. The data presented concerning the biochemical and
22
23 118 haematological effects of probiotic strain are means of values obtained at three separate
24
25 119 sampling ages.

26 120 **RESULTS**

27 121 **Blood biochemical parameters**

28 122 Clinical examination showed no changes in the health status of the control and experimental
29
30 123 groups. The effects of probiotic strain *Enterococcus faecium* on selected biochemical
31
32 124 parameters are shown in Table 2. Table 2 near here

33 125 The concentration of total cholesterol in blood plasma was significantly lower in the
34
35 126 experimental group with probiotic supplementation compared with controls at all sampling
36
37 127 times (week 5, $P < 0.05$; weeks 25 and week 45, $P < 0.01$).

38 128 Significantly lower mean concentrations of total lipids were found at all sampling ages in
39
40 129 comparison with controls. The lowest lipid concentration was found at week 45 in the
41
42 130 experimental group and the highest concentration at week 5 in the control group. Mean
43
44 131 concentrations of triglycerides were not significantly different throughout the study and

1
2
3 132 ranged from 12.51 mM to 13.06 mM in both groups.
4

5 133 Mean concentrations of plasma calcium were lower in the experimental group than
6
7
8 134 controls at all three sampling times, but the difference was only significant at weeks 5 and 45.
9

10 135 Mean concentrations of inorganic phosphorus were in a narrow range from 1.81mM to 1.92
11
12 136 mM in both groups, but that of the experimental group was significantly higher than controls
13
14
15 137 after 25 weeks of production but significantly lower at week 45.
16

17 138 **Haematological parameters**

18
19 139 Erythrocyte counts at week 5 were similar for the two groups, significantly higher at week 25
20
21
22 140 in the experimental group compared with the control group, but significantly lower than
23
24 141 controls at week 45 (Table 3). Mean leucocytes counts were significantly lower at all
25
26 142 sampling ages in the experimental group compared with controls ($P < 0.001$). The haematocrit
27
28 143 values fell within narrow limits in both the experimental and control groups, but the mean
29
30 144 haematocrit values in the experimental group were significantly lower than controls at weeks
31
32
33
34 145 5 and 45.

Table 3 near here

36 146 **Production parameters**

37
38 147 Results of production parameters are shown in Table 4. Although the BW of birds given the
39
40 148 probiotic strain treatment was higher than controls at all times, the differences between groups
41
42 149 were not statistically significant. *Enterococcus faecium* strain had slight beneficial effect on
43
44 150 egg production, with the experimental group averaging 308 eggs in the 48 weeks compared
45
46 151 with 285 eggs for the control group (data not shown). The average egg weight was also higher
47
48 152 in the experimental group than the controls, but again the difference was not statistically
49
50 153 significant. Only one bird died during whole experimental period, but a post-necropsy
51
52 154 investigation confirmed that the treatment had no negative effect on the health and internal
53
54
55 155 organs of the experimental hens. No other treatment-related sickness was found.
56
57
58
59
60

Table 4 near here

DISCUSSION

1
2
3 157 In the present experiment, the probiotic strain *Enterococcus faecium* M 74 significantly
4
5 158 decreased concentrations of total cholesterol as well as total lipid in blood plasma. Since a
6
7
8 159 large proportion of the cholesterol in the body is synthesised *de novo*, mostly in the liver, the
9
10 160 search for feed additives to inhibit cholesterol biosynthesis has long been pursued as a means
11
12 161 of lowering the concentrations of plasma cholesterol (Regnstrom *et al.*, 1992). Regulation of
13
14
15 162 the serum cholesterol level is important to prevent atherosclerosis, as it has been shown that
16
17 163 atherosclerosis could be suppressed by controlling the concentration of cholesterol in the
18
19 164 serum (Kapila *et al.*, 2009).

21
22 165 Lower concentrations of cholesterol in yolk and blood in connection with probiotic strains
23
24 166 supplementation have been described by Panda *et al.* (2003), Kurtoglu *et al.* (2004) Li *et al.*
25
26 167 (2006) and Salma *et al.* (2007b). Reduced blood plasma cholesterol concentrations effected
27
28 168 by *Lactobacillus acidophilus* were shown in some studies, and cholesterol decrease was
29
30 169 detected to be transient (de Roos and Katan, 2000). The mechanism of that action is still not
31
32 170 clear, though Buck and Gilliland (1994) and Pereira and Gibson (2002) suggested that
33
34 171 *Lactobacillus* was able to assimilate the cholesterol molecules. In a similar study, de Smet *et*
35
36 172 *al.* (1994) suggested that probiotic bacteria increase the synthesis of unconjugated bile acids,
37
38 173 thus contributing to the regulation of serum cholesterol, which is the precursor of bile acids.
39
40 174 The result of increased synthesis of these acids reduces the concentration of cholesterol. After
41
42 175 adding probiotics to the diet, the cholesterol concentrations in the liver and blood serum were
43
44 176 significantly lower in cockerels (Endo *et al.*, 1999) and in rats (Hosono, 2000, 2001).

45
46 177 According to Kurtoglu *et al.* (2004), supplementation of 250, 500 and 750 mg/kg
47
48 178 probiotic increased egg production, reduced the number of cracked eggs, and decreased the
49
50 179 egg yolk and serum cholesterol concentrations. Serum triglyceride concentrations were
51
52 180 reduced by diet supplementation with 500 and 750 mg/kg probiotic. There are indications that
53
54 181 probiotic supplementation of the diet may lead to changes in serum global lipid profiles, as

1
2
3 182 reflected in increased triglyceride levels (Kekkonen *et al.*, 2008). The results of Xiao *et al.*
4
5 183 (2003) showed the potential of the probiotic *Bacterium longum* strain to improve the plasma
6
7 184 lipid panel in rats when added to diet. The hypocholesterolaemic effect of *Lactobacillus*
8
9 185 strains is attributed to its ability to reduce serum and liver total cholesterol concentrations, as
10
11 186 was demonstrated in the hamster (Chiu *et al.*, 2006). However, results from Greany *et al.*
12
13 187 (2008) do not support a beneficial effect of *Lactobacillus acidophilus* strain DDS-1 or
14
15 188 *Bifidobacterium longum* strain UABL-14 on plasma lipids in young women and men who
16
17 189 have a normal cholesterol concentration. Simon *et al.* (2006) suggested that *Lactobacillus*
18
19 190 *fermentum* did not appear to produce a major change in serum lipid fractions in humans, but a
20
21 191 small effect could not be excluded.

22
23
24
25
26
27 192 A lower mean concentration of plasma calcium was found at all weeks in the experimental
28
29 193 group in comparison with the control group. These findings are in contrast with work of
30
31 194 Panda *et al.* (2003), who reported significantly higher serum calcium levels following
32
33 195 probiotic supplementation. Mean concentrations of iP in our experiment were within narrow
34
35 196 limits in both groups of birds. No clear effect of probiotic supplementation on iP in blood
36
37 197 plasma was observed and results seem to be inconclusive. Similarly, Panda *et al.* (2003),
38
39 198 could not confirm an effect of probiotic supplementation on the concentration of plasma
40
41 199 inorganic P.

42
43
44
45
46 200 In another report, Panda *et al.* (2006) confirmed that addition of a probiotic strain
47
48 201 (*Lactobacilu sporogenes*) had a positive effect on bone breaking strength and bone ash
49
50 202 content and could be attributed to the favourable environment in intestinal tract created by the
51
52 203 feeding of a probiotic strain. This probiotic supplementation may have helped to digest and
53
54 204 absorb more calcium and subsequently support bone metabolism resulting in a higher bone
55
56 205 density and strength.

57
58
59
60 206 Tang *et al.* (2007) showed that fermenting calcium-fortified soya milk with selected

1
2
3 207 probiotics can potentially enhance the calcium bioavailability of calcium-fortified soya milk
4
5 208 due to an increase in calcium solubility and bioactive isoflavone aglycone enrichment.
6
7

8 209 Cetin *et al.* (2005) concluded that probiotic supplementation resulted in significant
9
10 210 increases in the erythrocyte count, haemoglobin concentration and haematocrit values, but
11
12 211 had no effect on total leucocytes and differential leucocytes counts. In contrast, the current
13
14 212 study only showed significantly higher erythrocytes counts in the experimental group at week
15
16 213 25. We found significantly lower leucocyte counts in all observed weeks, which is in contrast
17
18 214 to the findings of Cetin *et al.* (2005) and Zhou *et al.* (2000), who reported no significant
19
20 215 effect of probiotics on leucocyte count.
21
22
23

24 216 The current data show significantly lower mean haematocrit values in hens with probiotic
25
26 217 administration, which concurs with the report of Agawane and Lonkar (2004), who found a
27
28 218 significant decrease in the haemoglobin and packed cell volume in birds with probiotic
29
30 219 treatment.
31
32
33

34 220 Production parameters were not influenced by probiotic supplementation during our
35
36 221 experiment, which is in agreement with the findings of Balevi *et al.* (2001) that egg weight
37
38 222 was not influenced by probiotic addition. Kurtoglu *et al.* (2004) and Yörük *et al.* (2004)
39
40 223 reported increased egg production, reduced mortality, and improved feed conversion
41
42 224 efficiency with probiotic supplementation but with no improvement in egg quality. Irshad
43
44 225 (2006) found that poultry growth was promoted with gradually increasing doses of probiotics,
45
46 226 up to 1 g per 10 kg feed. However, doses of probiotics higher than that may have had reversed
47
48 227 effect on poultry growth.
49
50
51
52

53 228 In conclusion, the addition of probiotic strain *Enterococcus faecium* M 74 to the diet of
54
55 229 ISA Brown hens lowered cholesterol, lipids, calcium, leucocyte counts and haematocrit, and
56
57 230 increased erythrocyte counts, in blood plasma in at least two sampling times. The effect of
58
59 231 probiotic on iP was inconclusive and no effect was observed on triglyceride concentrations or
60

1
2
3 232 egg production parameters.
4

5 233 ACKNOWLEDGMENTS

6
7
8 234 The authors are grateful to Jenifer Poston B.S. and Ida Holaskova PhD for linguistic revision
9
10 235 of this paper.
11

12 236 REFERENCES

13
14
15 237 AGAWANE, S.B. & LONKAR, P.S. (2004) Effect of probiotic containing *Saccharomyces*
16
17 238 *boulardii* on experimental ochratoxicosis in broilers: hematobiochemical studies. *Journal*
18
19 239 *of Veterinary Science*, **4**: 359-67.
20

21
22 240 AL-SALAMI, H, BUTT, G. TUCKER, I. & MIKOV, M. (2008) Influence of the
23
24 241 semisynthetic bile acid MKC on the ileal permeation of gliclazide *in vitro* in healthy and
25
26 242 diabetic rats treated with probiotics. *Methods and Finding*, **2**: 107-13.
27

28
29 243 AWAD, W.A., GHAREEB, K., ABDEL-RAHEEM, S. & BÖHM, J. (2009) Effects of dietary
30
31 244 inclusion of probiotic and symbiotic on growth performance, organ weights, and intestinal
32
33 245 histomorphology of broiler chickens. *Poultry Science*, **88**: 49-56.
34

35
36 246 BALEVI, T., UCAN, U.S., COSKUN, B., KURTOGLU, V. & CETINGUL, I.S. (2001)
37
38 247 Effect of dietary probiotic on performance and humoral immune response in layer hens.
39
40 248 *British Poultry Science*, **4**: 456-61.
41

42
43 249 BUCK, L.M. & GILLILAND, S.E. (1994) Comparison of freshly isolated strains of
44
45 250 *Lactobacillus acidophilus* of human intestinal origin for ability to assimilate cholesterol
46
47 251 during growth. *Journal of Dairy Science*, **77**: 2925-33.
48

49
50 252 CAPCAROVA, M., KOLESAROVA, A., MASSANYI, P. & KOVACIK, J. (2008) Selected
51
52 253 blood biochemical and haematological parameters in turkeys after experimental probiotic
53
54 254 *Enterococcus faecium* M 74 strain administration. *International Journal of Poultry*
55
56 255 *Science*, **7**: 1194-1199.
57

58
59
60 256 CAVAZZONI, V., ADAMI, A. & CASTROVILLI, C. (1998) Performance of broiler

- 1
2
3 257 chickens supplemented with *Bacillus coagulans* as probiotic. *British Poultry Science*, **39**:
4
5 258 526-9.
6
7
8 259 CETIN, N., GÜÇLÜ, B.K & CETIN, E. (2005) The effects of probiotic and
9
10 260 mannanoligosaccharide on some haematological and immunological parameters in
11
12 261 turkeys. *Journal of Veterinary Medicine, A*, **6**: 263-7.
13
14
15 262 CHIU, C.H., LU, T.Y., TSENG, Y.Y. & PAN, T.M. (2006) The effects of Lactobacillus-
16
17 263 fermented milk on lipid metabolism in hamsters fed on high-cholesterol diet. *Applied*
18
19 264 *Microbiology and Biotechnology*, **2**: 238-45.
20
21
22 265 DE ROOS, N.M. & KATAN, M.B. (2000) Effects of probiotic bacteria on diarrhoea, lipid
23
24 266 metabolism, and carcinogenesis: a review of papers published between 1988 and 1998.
25
26 267 *American Journal of Clinical Nutrition*, **2**: 405-11.
27
28
29 268 DE SMET, I., VAN HOORDE, L., DE SAEYER, M., VAN de WOESLYNE, M. &
30
31 269 VERSTRAELE, W. (1994) *In vitro* study of bile salt hydrolase (BSH) activity of BSH
32
33 270 isogenic *Lactobacillus plantarum* 80 strains and estimation of cholesterol lowering
34
35 271 through enhanced BSH activity. *Microbial ecology in health and disease*, **7**: 315-29.
36
37
38 272 ENDO, T., NAKANO, M., SHIMIZU, S., FUKUSHIMA, M. & MIYOSHI, S. (1999) Effects
39
40 273 of a probiotic on the lipid metabolism of cocks fed on a cholesterol-enriched diet.
41
42 274 *Bioscience, Biotechnology and Biochemistry*, **9**: 1569-75.
43
44
45 275 FINCH, R. & SHARLAND, M. (2009) November and beyond: observations on the EU
46
47 276 Antibiotic Awareness Day. *Journal of Antimicrobial Chemotherapy*, **63**: 633-5.
48
49
50 277 FOOKS, L.J. & GIBSON, G.R. (2002) Probiotics as modulators of the gut flora. *British*
51
52 278 *Journal of Nutrition*, **1**: 39-49.
53
54
55 279 GIL DE LOS SANTOS, J.R., STORCH, O.B. & GIL-TURNES, C. (2005) *Bacillus cereus*
56
57 280 *var. toyoi* and *Saccharomyces boulardii* increased feed efficiency in broilers infected
58
59 281 with *Salmonella enteritidis*. *British Poultry Science*, **46**: 494-7.

- 1
2
3 282 GLÜCK, U. & GEBBERS, O. (2003) Ingested probiotics reduce nasal colonization with
4
5 283 pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, and β -hemolytic
6
7 284 streptococci). *American Journal of Clinical Nutrition*, **77**: 517-20.
8
9
10 285 GREANY, K.A., BONORDEN, M.J., HAMILTON-REEVES, J.M., MCMULLEN, M.H.,
11
12 286 WANGEN, K.E, PHIPPS, W.R., FEIRTAG, J., THOMAS, W. & KURZER, M.S. (2008)
13
14 287 Probiotic capsules do not lower plasma lipids in young women and men. *European*
15
16 288 *Journal of Clinical Nutrition*, **2**: 232-7.
17
18
19 289 HOSONO, U. A. (2000) Effect of administration of *Lactobacillus gasseri* on serum lipids and
20
21 290 faecal steroids in hypercholesterolemic rats. *Journal of Dairy Science*, **8**: 1705-11.
22
23
24 291 HOSONO, U. A. (2001) Hypocholesterolemic effect of *Lactobacillus gasseri* SBT0270 in
25
26 292 rats fed a cholesterol-enriched diet. *Journal of Dairy Research*, **4**: 617-24.
27
28
29 293 IMMERSEEL, F.V., DE BUCK, J., PASMANS, F., HUYGHEBAERT, G.,
30
31 294 HAESEBROUCK, F. & DUCATELLE, R. (2004) *Clostridium perfringens* in poultry: an
32
33 295 emerging threat for animal and public health. *Avian Pathology*, **33**: 537-49.
34
35
36 296 IRSHAD, A. (2006) Effect of probiotics on broilers performance. *International Journal of*
37
38 297 *Poultry Science*, **5**: 593-7.
39
40
41 298 JUNTUNEN, M., KIRJAVAINEN, P.V., OUWEHAND, A.C., SALMINEN, S.J. &
42
43 299 ISOLAURI, E. (2001) Adherence of probiotic bacteria to human intestinal mucus in
44
45 300 healthy infants and during rotavirus infection. *Clinical and Diagnostic Immunology*, **2**:
46
47 301 293-6.
48
49
50 302 KAPILA, S., VIBHA, P. & SINHA, R. (2009) Antioxidative and hypocholesterolemic effect
51
52 303 of *Lactobacillus casei* ssp. *Casei* (biodefensive properties of lactobacilli). *Indian Journal*
53
54 304 *of Medical Science*, **60**: 361-370.
55
56
57 305 KEKKONEN, R.A, SYSI-AHO, M., SEPPANEN-LAAKSO, T., JULKUNEN, I.,
58
59 306 VAPAATALO, H., ORESIC, M. & KORPELA, R. (2008) Effect of probiotic

- 1
2
3 307 *Lactobacillus rhamnosus* GG intervention on global serum lipidomic profiles in healthy
4
5 308 adults. *World Journal of Gastroenterology*, **20**: 3188-94.
6
7
8 309 KHAN, M., RAOULT, D., RICHET, H., LEPIDI, H. & LA SCOLA, B. (2007) Growth-
9
10 310 promoting effects of single-dose intragastrically administered probiotics in chickens.
11
12 311 *British Poultry Science*, **6**: 732-5.
13
14
15 312 KOENEN, M.E., KRAMER, J., VAN DER HULST, R., HERES, L., JEURISSEN, S.H. &
16
17 313 BOERSMA, W.J. (2004) Immunomodulation by probiotic lactobacilli in layer- and meat-
18
19 314 type chickens. *British Poultry Science*, **45**: 355-66.
20
21
22 315 KURTOGLU, V, KURTOGLU, F., SEKER, E., COSKUN, B., BALEVI, T. & POLAT, E.S.
23
24 316 (2004) Effect of probiotic supplementation on laying hen diets on yield performance and
25
26 317 serum and egg yolk cholesterol. *Food Additives & Contaminants*, **9**: 817-23.
27
28
29 318 LI, L., XU, C.L., JI, C., MA, Q., HAO, K., JIN, Z.Y. & LI, K. (2006) Effects of a dried
30
31 319 *Bacillus subtilis* culture on egg quality. *Poultry Science*, **2**: 364-8.
32
33
34 320 LIU, J.R., LAI, S.F., YU, B. (2007) Evaluation of an intestinal *Lactobacillus reuteri* strain
35
36 321 expressing rumen fungal xylanase as a probiotic for broiler chicken fed on a wheat-based
37
38 322 diet. *British Poultry Science*, **48**: 507-14.
39
40
41 323 PANDA, A.K., REDDY, M.R., RAMA RAO, S.V & PRAHARAJ, N.K. (2003) Production
42
43 324 performance, serum/yolk cholesterol and immune competence of white leghorn layers as
44
45 325 influenced by dietary supplementation with probiotic. *Tropical Animal Health and*
46
47 326 *Production*, **1**: 85-94.
48
49
50 327 PANDA, A.K., RAMARAO, S.V., RAJU, M.V.L.N. & SHARMA, S.R. (2006) Dietary
51
52 328 supplementation of *Lactobacillus sporogenes* on performance and serum biochemico-lipid
53
54 329 profile of broiler chickens. *Journal of Poultry Science*, **43**: 235-240.
55
56
57 330 PATTERSON, J.A. & BURKHOLDER, K.M. (2003) Application of prebiotics and probiotics
58
59 331 in poultry production. *Poultry Science*, **4**: 627-31.
60

- 1
2
3 332 PEREIRA, D.I.A. & GIBSON, G.R. (2002) Cholesterol assimilation by lactic acid bacteria
4
5 333 and Bifidobacteria isolated from the human gut. *Applied and Environmental*
6
7 334 *Microbiology*, **68**: 4689-96.
- 8
9
10 335 REGNSTROM, J., NILLSON, J., TORNVALL, P., LANDOU, C. & HAMSTEN, A. (1992)
11
12 336 Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man.
13
14 337 *Lancet*, **339**: 1183-1186.
- 15
16
17 338 SALMA, U., MIAH, A.G., MAKI, T., NISHIMURA, M. & TSUJII, H. (2007a) Effect of
18
19 339 dietary *Rhodobacter capsulatus* on cholesterol concentration and fatty acid composition in
20
21 340 broiler meat. *Poultry Science*, **9**: 1920-26.
- 22
23
24 341 SALMA, U., MIAH, A.G., TAREQ, K.M., MAKI, T. & TSUJII, H. (2007b) Effect of dietary
25
26 342 *Rhodobacter capsulatus* on egg-yolk cholesterol and laying hen performance. *Poultry*
27
28 343 *Science*, **4**: 714-9.
- 29
30
31 344 SENOK, A.C., ISMAEEL, A.Y. & BOTTA, G.A. (2005) Probiotics: facts and myths.
32
33 345 *Clinical Microbiology and Infection*, **12**: 958-66.
- 34
35
36 346 SIGGERS, R.H., SIGGERS, J., BOYE, M., THYMANN, T., MØLBAK, L., LESER, T.,
37
38 347 JENSEN, B.B. & SANGILD, P.T. (2008) Early administration of probiotics alters
39
40 348 bacterial colonization and limits diet-induced gut dysfunction and severity of necrotizing
41
42 349 enterocolitis in preterm pigs. *Journal of Nutrition*, **8**: 1437-44.
- 43
44
45 350 SIMONS, L.A., AMANSEC, S.G. & CONWAY, P. (2006) Effect of *Lactobacillus fermentum*
46
47 351 on serum lipids in subjects with elevated serum cholesterol. *Nutrition, Metabolism &*
48
49 352 *Cardiovascular Diseases*, **8**: 531-5.
- 50
51
52 353 SINGHI, S.C. & BARANWAL, A. (2008) Probiotic use in the critically ill. *Indian Journal of*
53
54 354 *Pediatrics*, **6**: 621-7.
- 55
56
57 355 TANG, A.L., SHAH, N.P., WILCOX, G., WALKER, K.Z. & STOJANOVSKA, L. (2007)
58
59 356 Fermentation of calcium-fortified soymilk with *Lactobacillus*: effects on calcium

- 1
2
3 357 solubility, isoflavone conversion, and production of organic acids. *Journal of Food*
4
5
6 358 *Sciences*, **9**: 431-6.
7
8 359 TORRES-RODRIGUEZ, A., DONOGHUE, A.M., DONOGHUE, D.J., BARTON, J.T.,
9
10 360 TELLEZ, G. & HARGIS, B.M. (2007) Performance and condemnation rate analysis of
11
12 361 commercial turkey flocks treated with a *Lactobacillus* spp.-based probiotic. *Poultry*
13
14 362 *Science*, **3**: 444-6.
15
16
17 363 WEESE, J. S. & ARROYO, L. (2003) Bacteriological evaluation of dog and cat diets that
18
19 364 claim to contain probiotics. *Canadian Veterinary*, **3**: 212-6.
20
21
22 365 WIELEN, P., LIPMAN, L.J., KNAPEN, F. & BIESTERVELD, S. (2002) Competitive
23
24 366 exclusion of *Salmonella enterica* serovar enteritidis by *Lactobacillus crispatus* and
25
26 367 *Clostridium lactatifermentans* in a sequencing fed-batch culture. *Applied and*
27
28 368 *Environmental Microbiology*, **2**: 555-9.
29
30
31 369 WU, L.Y., TAN, R.B. & SHI, K.J. (2008) Effect of a dried *Bacillus subtilis* culture on gosling
32
33 370 growth performance. *British Poultry Science*, **49**: 418-22.
34
35
36 371 XIAO, J.Z., KONDO, S., TAKAHASHI, N., MIYAJI, K., OSHIDA, K., HIRAMATSU, A.,
37
38 372 IWATSUKI, K., KOKUBO, S. & HOSONO, A. (2003) Effects of milk products
39
40 373 fermented by *Bifidobacterium longum* on blood lipids in rats and healthy adult male
41
42 374 volunteers. *Journal of Dairy Science*, **7**: 2452-61.
43
44
45 375 YÖRÜK, M.A., GÜL, M., HAYIRLI, A. & MACIT, M. (2004) The effects of
46
47 376 supplementation of humate and probiotic on egg production and quality parameters during
48
49 377 the late laying period in hens. *Poultry Science*, **1**: 84-8.
50
51
52
53 378 ZHOU, J.S., SHU. Q., RUTHERFURD, K.J. PRASAD, J., BIRTLES, M.J., GOPAL, P.K. &
54
55 379 GILL, H.S. (2000) Safety assessment of potential lactic acid bacterial strains
56
57 380 *Lactobacillus rhamnosus* HN001, *Lb. acidophilus* HN017, and *Bifidobacterium lactis*
58
59 381 HN019 in BALB/c mice. *International Journal of Food Microbiology*, **56**: 87-96.
60

1
2
3 382 ZULKIFLI, I., ABDULLAH, N., AZRIN, N.M. & HO, Y.W. (2000). Growth performance
4
5 383 and immune response of two commercial broiler strains fed diets containing *Lactobacillus*
6
7
8 384 cultures and oxytetracycline under heat stress conditions. *British Poultry Science*, **41**:
9
10 385 593-7.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review Only

386
387**Table 1.** *Diet composition of feed formulation KKZ HYD-10*

Ingredients	KKZ HYD-10
Crude protein	min. 153 g/kg
ME	min. 11.5 MJ/kg
Ash	max. 160 g/kg
Fibre	max. 60 g/kg
Lysine	min. 7 g/kg
Methionine and cystine	min. 6 g/kg
- of which methionine	min. 3.5 g/kg
Linoleic acid	min. 15 g/kg
Calcium	min. 28 - 45 g/kg
Phosphorus	min. 5 g/kg
Sodium	1.2 – 2.5 g/kg
Manganese	min. 40 mg/kg
Iron	min. 40 mg/kg
Copper	min. 4 mg/kg
Zinc	min. 60 mg/kg
Retinol	min. 5333 mg/kg
Riboflavin	min. 4 mg/kg
Cyanocobalamin	min. 0.01 mg/kg
Cholecalciferol	min. 1066 mg/kg
Vitamin E (α -tocopherol)	min. 10 mg/kg

388

Table 2. Effects of dietary treatment (*Enterococcus faecium* M 74) on some blood biochemical parameters of ISA Brown laying hens

		Control group			Experimental group		
		Sampling times (weeks of production)					
		5	25	45	5	25	45
cholesterol	mean	3.96	3.53	3.17	3.80*	2.92**	2.63**
	mM SD	0.27	0.30	0.52	0.31	0.53	0.50
total lipids	mean	11.05	10.29	10.20	10.71*	10.11*	9.80*
	g/L SD	0.32	0.45	0.37	0.24	0.28	0.26
triglycerides	mean	12.64	12.97	12.91	12.51	12.92	13.06
	mM SD	0.25	0.46	0.66	0.45	0.18	0.44
calcium	mean	8.28	7.94	8.12	7.88*	7.88	7.93*
	mM SD	0.11	0.47	0.09	0.27	0.60	0.16
phosphorus	mean	1.86	1.81	1.92	1.85	1.85*	1.88*
	mM SD	0.06	0.05	0.05	0.05	0.08	0.07

Significantly different from the control values of the same week (* $P < 0.05$; ** $P < 0.01$)

Table 3. *Effects of dietary treatment (Enterococcus faecium M 74) on some blood haematological parameters of ISA Brown laying hens*

		Control group			Experimental group		
		Sampling times (weeks of production)					
		5	25	45	5	25	45
erythrocytes	mean	3.39	3.04	3.06	3.40	3.08*	3.03*
	T/l SD	0.08	0.05	0.07	0.06	0.05	0.10
leucocytes	mean	24.49	24.38	24.43	21.25***	20.60***	20.71***
	G/l SD	0.54	0.15	0.13	0.61	0.11	0.06
hematocrit	mean	33.85	32.44	33.43	33.09*	32.48	32.72*
	% SD	0.52	0.39	0.49	0.39	0.34	0.53

Significantly different from the control values of the same week (* $P < 0.05$; ** $P < 0.01$)

Table 4. Effect of dietary treatment (*Enterococcus faecium* M 74) on body weight, rate of lay and egg weight of ISA Brown hens

	Weeks of production											
	4	8	12	16	20	24	28	32	36	40	44	48
Body weight (g)												
C	1656	1702	1773	1749	1727	1735	1771	1781	1830	1825	1889	1907
SD	135	152	151	147	164	161	169	196	178	202	201	198
E	1702	1759	1803	1816	1787	1792	1834	1870	1887	1894	1941	1958
SD	142	124	121	125	155	165	152	173	192	199	210	215
Rate of lay (eggs/100 bird.d)												
C	91	93	95	95	92	96	95	92	92	87	85	80
SD	9.8	11.2	5.8	6.6	9.0	5.6	5.9	8.8	8.1	10.1	11.1	17.7
E	93	95	94	98	93	97	95	93	90	83	85	84
SD	8.1	6.6	7.9	4.0	7.8	4.8	6.7	9.4	10.2	17.2	14.8	15.4
Egg weight (g)												
C	51.2	57.5	62.1	63.3	63.5	62.7	62.9	63.6	63.4	62.9	61.9	63.5
SD	5.4	3.6	2.8	3.1	2.9	2.5	2.5	3.8	2.8	3.0	9.5	3.6
E	52.6	58.9	62.9	65.1	64.5	63.7	63.9	64.2	63.9	62.2	64.0	63.2
SD	4.1	4.4	3.9	3.7	3.7	3.3	2.9	4.1	4.0	6.5	4.0	4.2

No significant differences between groups ($P > 0.05$)