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Effects of Transportation on Stress and Fear Responses of Growing Broilers Supplemented with Prebiotic or Probiotic

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Abstract: Physiological stress response and fear levels were evaluated in broiler chickens fed control diets, chicory (1 kg/ton) and *Lactobacillus* sp. (1 x 10^8 cfu/kg) supplemented diets for 5 wk. At the end of the experiment, the birds were subjected to 80 km transport journey (90 min approximately). The fear levels of birds were measured using tonic immobility reaction (TI). The duration of TI and the susceptibility of the birds to the test were measured immediately after the birds had arrived to the laboratory and repeated after one week following their transport. Heterophil (H) counts, Lymphocyte (L) counts and Heterophil to Lymphocyte (H/L) ratios were determined immediately on arrival and at 24 h following birds transport. There was no significant differences were recorded in the fear levels of broilers supplemented with either chicory or *Lactobacillus sp.* compared with their control counterparts. However, in a comparison with fear levels after one week (without transport), a significant difference was observed (P=0.001) irrespective to dietary supplementation. Handling and transport on this relatively short journey was apparently equally frightening for broilers independent of dietary supplementation. H/L ratios either immediately on arrival or at 24 h following birds transport showed a significant difference between the dietary treatments. Chicory and *Lactobacillus sp.* supplemented groups decreased H/L ratios compared with control group which is important in reduction of stress effects on birds. In conclusion, supplementation of broiler diet with either chicory or *Lactobacillus sp.* can modulate the physiological stress response of transported birds and consequently enhance tolerance to stress without change in their behavioural index of fear.

Key words: Broiler, transport, tonic immobility, fear, stress, H/L ratio

Introduction

The welfare of poultry during transportation from sites of production to slaughter at processing places is a matter of concern. Immediately prior and during transportation birds may exposed to a wide range of potential stressors. These include catching, handling, loading, motion, acceleration, impact, thermal demands imposed by the transport microclimate, fasting, and withdrawal of water, restriction of behaviour, social disruption and noise. The adverse effect of these factors up on the bird may range from mild distress and aversion to injury and death. It has been reported that 40 per cent of mortalities in "dead on arrival" broilers are a consequence of stress (Bayliss and Hinton, 1990) and that mortality increases with the transport length (Warris *et al.*, 1990). Several studies have been attempted to characterize the behavioural and physiological responses of birds to transportation. Harvesting and transport of poultry represents a severe stressor based upon measurements of tonic immobility (TI), heart rate and plasma corticosterone concentrations. These findings are supported by studies involving measurements of TI following commercial transportation of broilers and they indicated that transport may greatly increase bird's fearfulness (Cashmann *et al.*, 1989; Nicol and Scott, 1990).

The induction of physiological stress by transportation has been suggested from a number of studies. Plasma corticosterone is elevated following a road journey (Freemann *et al*, 1984; Satterlee *et al.*, 1989). This is consistent with the post-transport increase of heterophil: lymphocyte ratios (Satterlee *et al.*, 1989; Mitchell *et al.*, 1992; Maxwell, 1993). The heterophil: lymphocyte ratio (H/L ratio) was used as an index of stress (Zulkifli *et al.*, 2000a). It was shown that both heat stress and feed restriction increase the H/L ratio in broilers (Khajavi *et al.*, 2003). In addition Gross and Siegel (1983) compared plasma corticosterone concentration and H/L ratio responses to various stressors and concluded that the H/L ratio is a better indicator of stress in poultry. While tonic immobility (TI) reaction of chickens provides a useful behavioural method of estimating their fearfulness (Jones, 1986). Cashman *et al.* (1989) mentioned that both journey duration and waiting time before transport increased the duration of tonic immobility.

Therefore, the need for attenuating the adverse physiological and behavioural consequences associated with harvesting and transportation of broiler chickens is recognized within the poultry industry. Kannan and Mench (1997) attempted to habituate broilers to preslaughter handling through repeated handling during the growing period but they failed. Alternatively, dietary supplementation of ascorbic acid (AA) has yielded some promising results in relation to perturbation of homeostasis in poultry and increase the survivability of poultry under stressful conditions (Zulkifli *et al.*, 1996) and addition of AA in water dampened adrenocortical response to the traumatic experience of harvesting and transport of broiler chickens (Sattelee *et al.*, 1989). In addition it was shown that addition of AA to the drinking water before handling of broiler decreases the duration of TI (Satterlee *et al.*, 1994; Zulkifli *et al.*, 2000a) and produced lower H/L ratios in response to preslaughter handling (Zulkifli *et al.*, 2000a). Therefore, supplementation of broiler diets with some feed additives may aid in overcome any deficiency and enhance tolerance to stresses concomitantly. Prebiotics and probiotics were generally utilized for broilers to enhance performance and productivity and to increase the immune status of broilers and consequently increase the resistance against diseases.

Probiotic have displayed a growth promoting effect when added to broiler feed and water (Fuller, 1989; Jernigan *et al.*, 1985; Mountzouris *et al.*, 2007). Preboitic chicory was shown also to increase the body weight gain, feed conversion and carcass weight in broilers (Ammermann *et al.*, 1989; Yusrizal and Chen, 2003). Morever supplementation of diet with *L. acidophilus* and mannan oligosaccharide increased the weight gain and the relative lymphoid organ weights in broilers experimentally infected with *S. Gallinarium* (Kumar *et al.*, 2003). Interestingly, supplementation of diet with probiotics increased the L counts and decreased the H counts and H/L ratio after experimental infection of broilers with *S. Gallinarium*. Furthermore, supplementation of diet with 0.1 % probiotic (*Bacillus subtilis and Bacillus licheniformis* containing 3 x 10^9 cfu/g) for broilers under heat stress increased the differential leucocytic counts and decreased the H/L ratio which is important in reducing the harmful effect of heat stress (Rahimi and Khaksefidi, 2006). In addition, supplementation of broiler diets with *Lactobacillus* improved growth performance under heat stress conditions (Zulkifli *et al.*, 2000b).

Recently, dietary supplementation of poultry with *E. coli* Nissle (1917) prevented the decrease of L counts in cold stressed birds and prevented the increase in H/L ratio in both cold stressed and transport stressed birds (Huff *et al.*, 2006). But still little informations are available regarding the effects of both prebiotic and probiotic on physiological and behavioural stress responses of broiler chickens after transportation. Therefore the present study was conducted to investigate the effects of dietary supplementation of prebiotic (derived from chicory and rich in inulin) and *Lactobacillus sp.* on the H/L ratio and level of fearfulness as indicated by TI after transportation of broiler chickens at age of marketing.

Materials and Methods

Experimental design: Six hundred 1 day-old broiler chicks (Ross 308) were obtained from a commercial hatchery. The birds were randomly divided into three groups (200 bird/group) and housed in pens of identical size in a deep litter system with wood shaving floor. Each group has 8 replicates (25 bird / pen). The control group was fed a basal diet, the chicory and the Lactobacillus sp. groups were fed the basal diet supplemented with 1kg/ ton added to the starter and grower feed. The chicks were fed with the starter diets from days 1 to 13 and grower feed from day 14-35 (Table 1). The feed additives were delivered by Biomin® GTI GmbH, Herzogenburg, Austria. The birds had free access to water and feed. The climatic conditions and lighting program were computer-operated and followed the commercial recommendations. Environmental temperature in the first week of life was 35°C, and decreased to 25°C till the end of experiment. During the first week 22 hours of light were provided with a reduction to 20 hours afterwards. At 35 day old, 30 birds were subjected to catching, handling, crating in plastic boxes, loading and transported for a journey of 80 km (90 min approximately). After the birds had arrived to our laboratory, they were housed in battery cages (5 birds/cage) and subjected to physiological and behavioural measurements.

Heterophil/lymphocyte (H/L) ratio: Immediately up on arrival, 10 birds from each group were used for determination of H/L ratio and their blood (0.3 mL) was obtained via wing vein in tubes containing EDTA as anticoagulant. Rapid H/L ratio response was demonstrated in broiler chickens after 3 h of road transportation (Mitchell *et al.*, 1992). Blood films were air dried (unfixed) and stained in concentrated May-Grunwald stain for 6 min, 1:1 May-Grunwald stain-distilled water for 1.5 min and 1:9 Geisma stain for 15 min (Robertson and Maxwell, 1990). To determine the counts of heterophil and lymphocyte, a minimum of 100 cells per film were examined by light microscopy. All blood counts were examined by the same investigator. The results are presented as the percentage of each cell occurring in each film. The H/L ratio was examined by dividing the number of heterophils by the number of lymphocytes (Gross and Siegel, 1983). H/L ratio was shown also to increase within 24-48 h in fowl in response to stresses (Gross and Siegel, 1983) and peaked after 20 h (Gross, 1990). Therefore, another 10

Table 1: Composition of the experimental diet (%)

Ingredient	Starter	Grower
Corn	57.93	59.75
Soya HP	31.25	29.6
Soya oil	2.5	2
Mega fat	1.25	2.5
Monocalciumphosphate	0.25	
Lysine	0.38	0.15
Methionin	0.08	
Threonine	0.13	
Premixa	6.25	6
Probiotic (Lactobacillus sp.)[b]		
Prebiotic (derived from chicory and rich in inulin)[c]		
Calculated composition[d]		
Dry matter	88.7	89.1
Crude protein	22.1	21.5
ME (MJ/kg)	14.28	14.66
Crude fat	7.6	8.4
Ca	1.56	1.41
P	0.97	0.83
Na	0.3	0.28
Mg	0.32	0.28

[a]BR 5 Universal Vetmed, Biomin GmbH, Herzogenburg, Austria. Each kg contains calcium 196g, phosphorous 64g, sodium 30g, magnesium 6g, copper 400 mg, zinc 1200 mg, iron 2000 mg, manganese 1200 mg, cobalt 20 mg, iodine 40 mg, selenium 8 mg, vitamin A 200,000 IU, vitamin D_3 80,000 IU., vitamin E 1600mg, vitamin K_3 34 mg, vitamin C 1300mg, vitamin B_1 35 mg, vitamin B_2 135 mg, vitamin B_6 100 mg, vitamin B_{12} 670 mcg, nicotinic acid 1340 mg, calcium pantothenic acid 235 mg, choline chloride 8400 mg, folic acid 34 mg, biotin 3350 µg, methionine 30g. [b]1 x 10^8 CFU/kg *Lactobacillus sp.* (Biomin®GTI GmbH, Herzogenburg, Austria) were added to constitute the probiotic group. [c]1 kg/ton were added to starter and grower diets to constitute the prebiotic group. [d]Based on a dry matter content of 88 %.

birds from each group were used and their blood was collected for determination of H/L ratio at 24 h after transportation journey.

Tonic immobility (TI) reaction: A total of 30 birds (10 birds per group) were tested individually for the duration of tonic immobility at day of arrival. TI reaction was carried out in a separate room having the same conditions as the bird room. Birds were out of auditory and visual contact with the other birds. TI is induced by manual restraint. The bird was placed on its back in a U-shaped cradle covered with cloth. The bird was then restrained with one hand on it's sternum for 45 seconds while holding the head and neck by the other hand. Towards the end of the induction period, hand pressure was gradually lifted so that if the chick still moved, another induction period was started immediately, until the movement ceased. After removal of the hands, a stop watch was started. The experimenter then retreated, moving out of sight of the bird and observed the behaviour of the bird. The number of induction trials to attain tonic immobility lasting at least 20 seconds and the duration of tonic immobility reaction, that is, the latency until self righting. If the bird righted in less than 20 seconds, it was considered that tonic immobility had not been induced and the restraint procedure was repeated. Conversely, if a bird did not show a righting response over the 10 min test period, a maximum score of 600 seconds was given for duration.

One week later another 10 birds per group were subjected to tonic immobility test to measure the level of fearfulness of birds after the adaptation period of birds to stress had finished. On the assumption that plasma corticosterone and related behavioural response would return to normal after 2 days following stress and that carryover effects associated with previous handling and transportation would be minimized (Kannan and Mench, 1996).

Results

The means of H counts, L counts and H/L ratios immediately up on arrival in control and dietary treated groups are presented in Table 2. The H counts decreased numerically for both prebiotic chicory and *Lactobacillus sp.* probiotic treated groups than the control group. The L counts increased ($P<0.1$) for both prebiotic and probiotic treated group compared with the control. Moreover, the H/L ratios decreased significantly for both prebiotic and probiotic groups compared with the control group ($P<0.1$). These results indicate that addition of prebiotic or probiotic to diets of broilers could help to overcome stress due to transportation with less physiological response.

Twenty four hours after transportation stress produced substantial increase in H counts and a decrease in L counts of control birds but this did not occur in the prebiotic and probiotic treated groups. The H counts are significantly lower ($P<0.05$) for prebiotic and probiotic treated groups compared with control group (Table 3). While L counts were significantly higher for both prebiotic and probiotic groups compared with control group. Furthermore the H/L ratios were significantly lower for either prebiotic or probiotic group compared with control group (Table 3) indicating that these feed supplementation enable the birds to overcome the stresses with less physiological responses.

There was no significant effect of either prebiotic or probiotic ($P>0.05$) on fearfulness of broilers as measured by tonic immobility reactions (TI) after transportation stress as well as in non-transported broilers (Table 4). However, TI duration was longer ($P<0.05$) after transportation of broilers for 90 min irrespective to their dietary supplementation with prebiotic and probiotic (Table 5) suggesting that transportation increases the level of fearfulness of broilers. Furthermore, the number of induction to achieve

Table 2: Effect of chicory and *Lactobacillus* sp. supplementation on H counts, L counts and H/L ratio immediately up on arrival from transportation journey (90 min)

	Dietary Treatment			
Parameters	Control (n= 10)	Chicory (n= 10)	*Lactobacillus* sp. (n= 10)	Probability (P)
Heterophils (H)%	49±3	43±2	43±2	0.178
Lymphocytes (L)%	43^b±2	51^a±3	52^a±2	0.058
H/L ratio	1.18^a±0.11	0.89^b±0.09	0.87^b±0.11	0.069

The results are presented as means ± SEM (ANOVA followed by Duncan' test. Means within the same row with no common superscripts differ significantly (P= 0.05)

Table 3: Effect of chicory and *Lactobacillus* sp. supplementation on H counts, L counts and H/L ratio after 24 h from transportation journey (90 min)

	Dietary treatments			
Parameters	Control (n= 10)	Chicory (n= 10)	*Lactobacillus* sp. (n= 10)	Probability (P)
Heterophils (H)%	51^a±2	35^b±5	33^b±3	0.016
Lymphocytes (L)%	45^b±2	57^b±7	61^a±4	0.059
H/L ratio	1.16^a±0.10	0.71^{ab}±0.20	0.58^b±0.12	0.047

The results are presented as means ± SEM (ANOVA followed by Duncan' test. Means within the same row with no common superscripts differ significantly ($P \leq 0.05$)

TI reaction was lower (P<0.05) for the transported birds irrespective to their dietary treatments (Table 5) suggesting that transportation increases the susceptibility of broilers to TI (to be frightened by external stimulation).

Discussion

There is a growing interest concerning the welfare problems associated with harvesting, transportation and preslaughter handling of broilers. Transportation is a multifactor process associated with a variety of stressors which may covertly reduce welfare. Catching, loading, unloading and testing in a noise environment may evoke stress and increase the fearfulness of broiler chickens (Freeman, 1984; Cashman *et al.*, 1989; Nicol and Scott, 1990; Knowels and Broom, 1990; Mitchell *et al.*, 1992; Mitchell and Kettlewell, 1998). The stressors of transportation process include thermal extremes, feed and water deprivation, noise, overcrowding, sudden movement and vibration (Mench, 1992; Mitchell and Kettlewell, 1998). Physical injuries, pain and mortality are reported after transportation of broilers (Knowels and Broom 1990; Bayliss and Hinton, 1990).

Physiological stress responses and level of fearfulness are used to assess welfare of broiler chickens after their transport in addition to other assessments as dead-on-arrivals, physical injuries, carcass characteristics and aversion (Nicol and Scott, 1990). Previous studies have characterized the behavioural and physiological responses of birds to transportation in either field or laboratory trials. Duncan (1989) suggested that transport on a vehicle represents a sever stressor based upon measurements of tonic immobility (TI), heart rate, and plasma corticosterone concentrations. These findings are supported by studies involving measurements of TI following commercial transportation of broilers that transport *per se* may greatly increase birds fearfulness (Cashmann *et al.*, 1989; Nicol and Scott, 1990). Plasma corticosterone is elevated following a road journey (Freemann *et al.*, 1984; Satterlee *et al.*, 1989) which is consistent with the post-transport increase of heterophil: lymphocyte ratios (Satterlee *et al.*, 1989; Mitchell *et al.*, 1992; Maxwell, 1993). Gross and Siegel (1983) compared plasma corticosterone concentrations and H/L ratio responses to various stressors and concluded that the latter is the better indicator of stress in poultry. Therefore, based on all previous informations the need for modulating the adverse physiological and behavioural consequences following harvesting and transportation of broilers has a great concern in poultry industry.

In the present study, the L counts are higher and H/L ratios are lower for both the chicory and *Lactobacillus sp.* treated birds compared with the control group after 90 min of road transportation. These results indicate that dietary supplementation of chicory or *Lactobacillus sp.* increase the L counts and decrease the H/L ratios of the transported broilers which could help to overcome the transportation stresses with less immune response. Twenty four hours after transportation, there was a substantial decrease in H counts and H/L ratios and an increase in L counts of both chicory and *Lactobacillus sp.* supplemented birds but this did not occur in their control counterparts. H/L ratios were lower for both chicory and *Lactobacillus sp.* treated birds compared with controls suggesting that either chicory or *Lactobacillus sp.* supplementation can modulate the physiological stress responses. In addition H counts are lower for both chicory and *Lactobacillus sp.* treated birds compared with controls. While L counts were higher for

Table 4: Effects of chicory and *Lactobacillus* sp. supplementation on fearfulness of broilers as indicated by tonic immobility reaction

	Dietary treatments			
Parameters	Control (n= 10)	Chicory (n= 10)	*Lactobacillus* sp. (n= 10)	Probability (P)
Transported birds				
TI inductions	1.1±0.10	1.1±0.10	1±0.00	0.612
TI durations	382±68	333±60	313±70	0.511
Non-transported birds				
TI inductions	1.3±0.25	1.5±0.50	1.4±0.21	0.913
TI durations	121±50	126±58	132±73	0.993

The results are presented as means ± SEM (ANOVA)

both chicory and *Lactobacillus sp.* treated birds compared with control group. To our knowledge this is the first report concerning the effects of feeding chicory and *Lactobacillus sp.* on H/L ratios of transported broiler chickens.

Blood samples either on arrival or after 24 h after stress of transportation had finished revealed that dietary supplementation of broiler chickens with chicory or *Lactobacillus sp.* can modulate the physiological stress response of transported birds and consequently enhance tolerance to stresses and overcoming it with less physiological response. These results are in agreement with the work of Huff *et al.* (2006) who suggested that dietary supplementation of poultry with *E. coli* (1917) probiotic prevented the increase in H/L ratio in both cold stressed and transported stressed birds and prevented the decrease of L counts in cold stressed birds. Similarly, Kumar *et al.* (2003) found that supplementation of diet with probiotics increased the L counts and decrease the H counts and H/L ratio after experimental infection of broilers with *S. Gallinarium*. Furthermore, supplementation of diet with 0.1 % probiotic (*Bacillus subtilis and Bacillus licheniformis*) for broilers under heat stress increased the differential leucocytic counts and decreased the H/L ratio which is important in reducing the harmful effect of heat stress (Rahimi and Khaksefidi, 2006).

Fear is an important component of stress and it has even proposed that physical stressors may fail to activate the hypothalamus-pituitary-adreno-cortical (HPA) axis if the variable of emotional arousal is avoided (Havery *et al.*, 1984). The common displays of physical stress in broilers are mainly from harvesting and transportation which elevate the plasma corticosterone concentrations (Freeman *et al.*, 1984). Although fear and stress are not synonymous (Jones, 1987), fear may also contribute to overall stress, particularly if the freighting stimulation is intense, prolonged or inescapable (Hill, 1983; Jones, 1987).

The most robust measures of fearfulness in poultry are the variables of tonic immobility (TI) reaction (Jones, 1986) which is defined as an unlearned state of reduced responsiveness to external stimulation and induced by gentle physical restraint. Both the duration of tonic immobility and the ease of being induced represent a useful behavioural index of fear (Faure and Mills, 1998). The duration of TI was considered to be a measure of the fear level of a bird immediately preceding its induction into TI and of the underlying fearfulness of a bird (Jones, 1990) under the assumption that the bird is more frightened and more fearful when TI is easily induced and remained immobile for longer duration (Jones, 1992; Scott and Moran, 1993).

In the present study transportation of broiler chickens affects both their susceptibility to TI reaction (the ease of being induced) and their durations of TI irrespective to dietary treatment. The duration of tonic immobility was appreciably prolonged by transportation in the present study. Indeed, relatively high overall fear levels were indicated as the number of inductions required to achieve TI reaction was reduced by transportation. This prolongation of TI duration and the ease of its induction represented the higher level of bird underlying fearfulness after transportation. These results are in agreement with the observations of Cashman *et al.* (1989) in broiler chickens and Scott et al. (1998) in laying hens. The catching, crating and transport of broilers was a novel stimulus because the birds are not routinely treated in this manner. Novelty is the stimulus characterized most often with fear (Murphy, 1978).

Our results also revealed that neither dietary prebiotic chicory nor probiotic *Lactobacillus sp.* could not modulate the fear levels as measured by TI reaction. Based on these results, it may assume that the trend of H/L ratios did not correlate well with the behavioural index of fear (TI reaction). Although duration of TI reaction and number of its induction were similar for different dietary treatments, H/L ratios were lower for either dietary prebiotic chicory or prebiotic Lactobacillus sp. birds compared to controls. Evidence of fear is considered as an adaptive pyschophysiological response to perceived danger as a vital component of stress response (Jones, 1996). Moreover, it was shown that H/L ratios were higher for hens showing long tonic immobility reaction than those of short tonic immobility. However, no positive relationship between H/L ratios and TI reaction in laying hens (Campo and Redondo, 1996). As well as it was shown that the relationship

Table 5: Effect of transportation on the level of fearfulness of broilers as measured by TI reactions irrespective to dietary treatments

Measurements	Transport	Without transport	P
TI inductions	1.07±0.04	1.39±0.10	0.047
TI durations	379±38	127±34	0.001

The results are presented as means ± SEM (t test of independent samples).

between physiological indictors of stress and behavioural measures of fear is not necessarily straightforward (Cashman *et al.*, 1989). As fasting and frustration of feeding elevated plasma corticosterone (Scanes *et al.*, 1980) and did not affect TI reaction (Gallup, 1987).

It was reported that supplementation of ascorbic acid in the drinking water modulated both physiological stress response (H/L ratios) and underlying fearfulness (TI duration) after preslaughter handling of broilers suggesting that supplemental ascorbic acid may offer a feasible method for alleviating fear and stress responses and enhancing poultry welfare (Satterlee *et al.*, 1994; Zulkifli *et al.*, 2000a). But prebiotic chicory and probiotic *Lactobacillus sp.* supplementation in our study can alleviate stress response without affecting the underlying fearfulness of birds.

It was shown that both prebiotic and probiotic improved the immune response of birds (Spring *et al.*, 2000; Huang *et al.*, 2004) and consequently their resistance to diseases. Whereas, broilers fed with mann-oligosaccharides (MOS) had a lower incidence of infections with *Salmonella typhimurium* (Newman, 1996), *Campylobacter jejuni* (Schoeni and Wong, 1994), and *E. coli* (Spring, 1996). Additionally, Savage *et al.* (1996) found that MOS enhanced the Ig G and Ig A production in turkeys. Mannan-oligosaccharide increases the maternal antibody titers in broiler breeders (Shashidhara and Devegowda, 2003) and titers to sheep red blood cells (Cotter *et al.*, 2000). In addition Mannan-oligosaccharide changed the lymphocyte proliferative responses in broilers (Cotter and Weiner, 1997). As well as *Lactobacillus* sp. increased the macrophages and lymphocyte activities in mice (Perdigon *et al.*, 1986), elevated the white blood cell counts and total serum protein and globulin in pigs (Pollmann *et al.*, 1980) and increases the secreting immunoglobulin IgA from the stimulated mucosal immune system in the ileum of layers (Nahshon *et al.*, 1994).

The expression of physiological functions such as growth, immune competence and disease resistance in an individual is interplay of genetics, nutrition and environment (Gross and Siegel, 1988; Thim *et al.*, 1997). These immunomodulatory effects of prebiotic and probiotic could explain how chicory prebiotic and *Lactobacillus sp.* probiotic in the present study modulated the physiological response of the transported bird and overcome the stress with less response (lower H/L ratio) compared with controls. However, their stimulation to the immune system of the bird is not necessary accompanied by decreasing the birds underlying level of fearfulness. It was shown also that improvement of housing conditions of laying hens did not affect fear levels as measures by TI after handling and transportation (Scott *et al.*, 1998).

In conclusion, supplementation of broiler diet with either prebiotic chicory or probiotic *Lactobacillus* sp. can modulate the physiological response of the transported birds but not their levels of fearfulness.

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Abbreviations: H== Heterophil, L== Lymphocyte, H/L== Heterophil to Lymphocyte, TI== tonic immobility