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Stress Indicators to Pre-Slaughter Transportation of Broiler Chickens Fed Diets Supplemented with a Synbiotic

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Abstract: There is a growing interest concerning the welfare problems associated with harvesting, transportation and pre-slaughter handling of broilers. Transportation is a multifactor process associated with a variety of stressors which may covertly reduce welfare. Plasma corticosterone is elevated following a road which is consistent with the post-transport increase of heterophil: lymphocyte ratios (H/L ratios). Plasma corticosterone was compared with H/L ratio responses to various stressors and the latter was the better indicator of stress in poultry. In the present study, stress responses were evaluated in broiler chickens fed for 5 weeks a synbiotic Biomin® IMBO (a combination of *Enterococcus faecium*), a pre-biotic (derived from chicory) and immune modulating substances (derived from sea algae), with a dose of 1 kg/ ton of the starter diets and 0.5 kg/ton of the grower diets). The birds were subjected to 80 km transport journey (90 min approximately). Heterophil (H) counts, Lymphocyte (L) counts and Heterophil to Lymphocyte (H/L) ratios were determined immediately on arrival and at 24 h following bird's transport. The H/L ratios decreased after 24 h from arrival from transport journey by about 17 % for birds fed synbiotic BIOMIN IMBO compared with controls. However, a non significant increase in L counts and a non significant decrease in H counts and H/L ratios at 24 h following bird's transport for birds fed BIOMIN IMBO compared with controls. In conclusion, feeding of BIOMIN IMBO relatively modulates the stress indicator of transported birds and relatively enhances tolerance to stress after pre-slaughter handling and transportation.

Key words: Broiler, transport, stress, H/L ratio

INTRODUCTION

Stress occurs when an animal experiences changes in the environment that stimulate body responses aimed at re-establishing the homeostatic condition. The physiological indices of broiler welfare, which have been commonly used include: body temperature, heart rate, blood cell counts and corticosterone. The main haematological response is a change in the Heterophil/Lymphocyte ratio (H/L) and in number of leucocytes. The number of heterophils per unit of blood increases and the number of lymphocytes decreases in birds under stress but the ratio of these cell types is less variable and thus H/L ratio is a better measure than individual cell numbers (Gross and Siegel, 1983). A normal ratio is about 0.4 but this can rise to 8 in birds under severe stress. Changes in H/L have been observed in response to thermal stress and treatment with corticosterone.

Harvesting and transportation of broilers to the slaughterhouse causes a severe stress to the birds if not the severest in their short life. 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. For example, harvesting and transport of poultry represents a severe stressor based upon measurements of Tonic Immobilty (TI), heart rate and plasma corticosterone concentrations (Cashman *et al*., 1989; Nicol and Scott, 1990). Plasma corticosterone is elevated following a road journey (Freeman *et al*., 1984 and Satterlee *et al*., 1989). This is consistent with the post-transport increase of heterophil: lymphocyte ratios (Satterlee *et al*., 1989; Mitchell *et al*., 1992 and Maxwell, 1993). The Heterophil: Lymphocyte ratio (H/L ratio) was used as an index of stress (Zulkifli *et al*., 2000a). For example 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 latter is a better indicator of stress in poultry.

So that, attenuating the adverse physiological consequences associated with harvesting and transportation of broiler chickens is recognized within the poultry industry. Dietary supplementation of Ascorbic Acid (AA) has yielded some promising results in relation to petrurbation 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 (Sattelere *et al*., 1989).

Recently, addition of prebiotic chicory and probiotic lactobacillus sp. decrease the H/L ratios after transportation of broilers (Ghareeb *et al*., 2008). Interestingly, supplementation of diet with 0.1% probiotic (Bioplus 2B) 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). Furthermore, 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). So far, no information are available regarding the effects of a synbiotic (a combination between prebiotic and probiotic) on physiological stress response of broiler chickens after transportation. Therefore the present study was conducted to investigate the effect of dietary supplementation of synbiotic product (Biomin® IMBO) on H counts, L counts and H/L ratios after transportation of broiler chickens.

MATERIALS AND METHODS

Birds and housing: Four hundred, 1-d-old broiler chicks (males and females) were obtained from a commercial hatchery. The birds were randomly divided without regard to sex into 2 groups (200 bird/group) and housed in pens of identical size (1.75 x 6 m) in a deep litter system. Wood shavings were used as the litter material. 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 h of light were provided with a reduction to 20 h afterwards.

Diets: The control group was fed starter and grower diets based on corn, soya HP, soya oil and a premix with vitamins, minerals, amino acids (Lysine, Methionin, Threonine), salt and monocalcium phosphate. The synbiotic group was fed the basal diet plus the synbiotic product (1 kg of Biomin® IMBO/ton of the starter diets and 0.5 kg/ton of the grower diets). Biomin® IMBO is a combination of probiotic strain *Enterococcus faecium* (DSM 3530), added to the starter diet with 5 x 10^8 cfu/kg and 2.5 × 10^8 cfu/kg to the grower diet, prebiotic derived from chicory rich in inulin and immune-modulating substances derived from sea algae. The chicks were fed with the starter diets from days 1-13 and grower feed from day 14-35 (Table 1). The feed additives were delivered by Biomin® GmbH, Herzogenburg, Austria. The birds had free access to water and feed.

Table 1: Composition of the experimental diet (%)

Ingredient	Starter	Grower
Corn	57.93	59.75
Soya HP	31.25	29.60
Soya oil	2.50	2.00
Megafat	1.25	2.50
Monocalciumphosphate	0.25	
Lysine	0.38	0.15
Methionin	0.08	
Threonine	0.13	
Premix[a]	6.25	6.00
Calculated composition[b]		
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	0.30	0.28
Mg	0.32	0.28

[a]BR 5 Universal Vetmed, Biomin GmbH, Herzogenburg, Austria. Each kg contains calcium 196 g, phosphorous 64 g, sodium 30 g, magnesium 6 g, 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 1600 mg, vitamin K_3 34 mg, vitamin C 1300 mg, 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 30 g. [b]Based on a dry matter content of 88 %

Transportation stress: At 35 day old, 15 birds from each group 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 the laboratory, they were housed in battery cages (5 birds/cage) and subjected to physiological measurements.

Heterophil counts, lymphocyte counts and 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 reponse 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 lymphocte, 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 5 birds from each group were used and their blood was collected for determination of H/L ratio at 24 h after transportation journey.

RESULTS

H counts, L counts and H/L ratios immediately up on arrival for both dietary and control groups are shown in (Table 2). The H counts are numerically lower for synbiotic treated group than control group. The L counts are numerically higher synbiotic treated group compared to control. Moreover, the H/L ratios are lower by about 17 % for synbiotic group (1.09%) compared with the control group (1.18%). These results indicate that addition of synbiotic to diets of broilers could help to overcome stress due to transportation with less physiological response.

Transportation stress produced substantial increase in H counts after 24 h for control birds but this did not occur for the synbiotic treated group. The H counts are numerically lower for synbiotic treated group compared with control group (Table 3). L counts were numerically higher for synbiotic group compared with control group. Furthermore the H/L ratios were lower for synbiotic group (0.99%) by about 17% than the control group (1.16%, Table 3) indicating that these feed supplementations enable the birds to overcome the stresses with less physiological reponses.

Table 2: H counts, L counts and H/L ratio immediately up on arival from transportation journey (90 min)

	Dietary treatments		
Parameters	Control (n= 10)	Synbiotic (n= 10)	P value
Heterophils (H)%	49±3	48±1	0.281
Lymphocytes (L)%	43±2	45±4	0.564
H/L ratio	1.18±0.11	1.09±0.1	0.639

The results are presented as means±SEM (Independent samples t-test)

Table 3: H counts, L counts and H/L ratio after 24 h from transportation journey (90 min)

	Dietary treatments		
Parameters	Control (n= 5)	Synbiotic (n= 5)	P value
Heterophils (H)%	51±2	43±5	0.249
Lymphocytes (L)%	45±2	47±4	0.593
H/L ratio	1.16± 0.10	0.99±0.2	0.538

The results are presented as means ± SEM (Independent samples t-test)

DISCUSSION

Transportation is a multifactor process associated with a variaty of stressors which may covertly reduce welfare. The stressors of transportation process include thermal extremes, feed and water deprivation, noise, overcrowding, sudden movement and vibration (Mench, 1992 and Mitchell and Kettlewell, 1998). Physical injuries, pain and mortality are reported after transportation of broilers (Knowles and Broom, 1990 and Bayliss and Hinton, 1990). 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 bird's fearfulness (Cashman *et al*., 1989; Nicol and Scott, 1990). Plasma corticosterone is elevated following a road journey (Freeman *et al*., 1984 and Satterlee *et al*., 1989) which is consistent with the post-transport increase of heterophil: lymphocyte ratios (Satterlee *et al*., 1989; Mitchell *et al*., 1992 and 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. Based on all previous information the need for modulating the adverse physiological consequences following harvesting and transportation of broilers has a great concern in poultry industry.

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 and Zulkifli *et al*., 2000a). 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 (Huff *et al*., 2006). Furthermore, supplementation of diet with 0.1% probiotic (*Bioplus 2B*) 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). Recently, addition of either pre-biotic chicory rich in inulin or probiotic *Lactobacillus* sp. decreased the H/L ratios after road transportation (Ghareeb *et al*., 2008). It seems that nutritional supplementation of broilers could help in decreasing the physiological consequences of

transportation stress. The results of the present experiment revealed that, the H counts are numerically lower and L counts are higher for synbiotic treated group than control group in both blood samples collected either immediately up on arrival or after 24 h from transportation. Moreover, the H/L ratios are decreased by 17% by dietary inclusion of synbiotic. These results indicate that dietary supplementation of synbiotic relatively increased the L counts and relatively decreased the H counts and the transported broilers which could help to overcome the transportation stresses with less immune response. H/L ratios were lower for synbiotic group compared with control group by about 17% suggesting that supplementation of broiler diet with synbiotic BIOMIN IMBO can modulate the physiological stress responses.

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, Rahimi and Khaksefidi (2006) reported that supplementation of diet with 0.1 % probiotic (*Bioplus 2B*) 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. Recently, it was shown that dietary inclusion of either prebiotic or probiotic decreased the H/L ratios of the transported birds (Ghareeb *et al*., 2008).

Conclusion: In conclusion, from this study we could conclude that supplementation of broiler diet with synbiotic BIOMIN IMBO can relatively modulate the physiological response of birds after transportation stress.

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