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The Interaction of Dominance Status and Supplemental Tryptophan on Aggression in *Gallus domesticus* Males[1]

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SHEA, M. M., L. W. DOUGLASS AND J. A. MENCH. *The interaction of dominance status and supplemental tryptophan on aggression in* Gallus domesticus *males.* PHARMACOL BIOCHEM BEHAV 38(3) 587–591, 1991.—In previous studies, we have found that supplemental dietary tryptophan (TRP) decreases aggression in feed-restricted male chickens (*Gallus domesticus*). The objective of this study was to determine if social status influences the effect which TRP has on aggression. In both experiments, *Gallus* males were placed on a commercial feed restriction program in which measured amounts of feed are delivered on alternate days beginning at 4 weeks of age. In the first study, birds were fed either 0.19 (control), 0.75 and 1.5% dietary TRP. In the second study, birds were fed either 0.19, 0.38, 0.75 or 1.5% dietary TRP. Dominance hierarchies were linear ($p<0.05$) in all treatment groups as indicated by Landau's indices, which ranged from 0.87 to 0.98. A significant interaction was found between dominance status and supplemental TRP for aggression, with TRP decreasing pecking more in dominant than subordinate birds. In Experiment 1, there were positive correlations ($p<0.05$) between body weight and dominance status in both the control and 0.75% TRP group ($p<0.05$) and no correlation in the 1.5% TRP group. The decrease in aggression by dominant males may have allowed subordinate birds to gain greater access to the feed. Our results indicate that dominant and subordinate males show a differential sensitivity to the effects of dietary TRP, with TRP decreasing pecking more in dominant birds.

Tryptophan Dominance Aggression Feed restriction Domestic fowl

THE primary precursor of the neurotransmitter serotonin (5-HT) is tryptophan (TRP), which is one of the least plentiful of the dietary amino acids. As an essential amino acid, TRP cannot be synthesized in animal cells. Thus diets low in TRP can have a profound effect on neural levels of 5-HT (6,22). Eating foods rich in 5-HT, however, has no appreciable effect on brain levels of the amine as 5-HT does not cross the blood-brain barrier (7).

Because of a lack of anatomical specificity in the central serotonergic system, 5-HT acts as a modulator which has broad tonic effects on behavior and physiology (12). Serotonin has been reported to influence sleep-wake mechanisms, temperature regulation, pain sensitivity and sexual behavior, with a general tendency to be associated with a tonic suppression of these functions (22, 23, 26). Serotonin also seems to have an important role as a modulator of behavioral inhibition in the control of aggression, but other factors must be present to elevate aggression before alterations in 5-HT have any effect (27).

Feed restriction, which produces a limitation of an essential resource, results in markedly elevated levels of aggression in domestic fowl males as compared to those fed ad lib (14,21). In previous studies, we have found that addition of supplemental TRP to the diet at 2, 4 and 8 times the requirement (0.19% TRP) results in a decrease in the frequency of aggressive activity among feed-restricted birds maintained on an alternate-day feeding program (21).

In nonhuman primates, dominant and subordinate animals appear to differ in the drug sensitivity of their serotonergic system. Dominant social status enhances an animal's behavioral sensitivity to drugs that augment 5-HT function (2,18). The objective of the present study was, therefore, to determine if dominance status influences the effect of supplemental dietary TRP on aggressive behavior in feed-restricted male chickens.

METHOD

Experiment 1

Subjects and housing. Nine pens of male Rock-Cornish breeder chicks (n = 14 birds/pen) purchased from a commercial hatchery were raised on a commercial starter diet (20% crude protein, 0.24% TRP) fed ad lib until four weeks of age. Birds were raised in pens that provided 0.4 m^2 of floor space and 13.80 cm of

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feeder space per bird. This experiment was initiated in March and was concluded the following July. Birds were raised in natural light with low illumination throughout the night. At 4 weeks of age, the nine pens of birds, three pens per treatment, were placed on an alternate-day feed restriction program of the type commonly used in commercial poultry production. In this program, feed intake is restricted to less than 80% of an ad lib diet. This regimen follows a 2-day cycle with 2 days of feed given on 1 day followed by a day with no feed. This schedule is maintained throughout development, but the amount of feed provided is adjusted continually to maintain a standard growth curve as specified by guidelines (Ross Poultry Breeders Inc., 1986). The restriction regime consisted of a commercial developer diet (13% crude protein, 0.19% TRP) supplied in a single batch by Penfield Corporation (Lancaster, PA), fed either as a control or supplemented to levels of 0.75 or 1.5% TRP. Alkaline hydrolysis analyses of these diets indicated actual levels of 0.20, 0.82 and 1.58% TRP (Degussa Corporation, Allendale, NJ). Birds were fed between 0700 and 0730 every other day.

Behavioral observations. Previous research in this laboratory has shown that aggressive activity in developing birds is highest on feed-off days (14). For this reason, each pen of birds was observed for three 20-min periods per week between 0730 and 0930 on feed-off days. All occurrences of aggressive pecking and threatening behavior as defined by Kruijt (10) were recorded using the methods described by Altmann (1). The observer sat approximately 1 meter in front of the pen, fully visible to the birds. The 20-min observation period began once the birds settled into basic maintenance behaviors such as grooming, eating and drinking. All chicks were marked every 5 weeks with either water-based markers or food coloring on the wing-tips and back to allow accurate identification of individual birds by the observer. For each aggressive act, initiator, recipient and outcome were recorded. If different aggressive acts occurred in the same bout, only the most severe acts were recorded. Behavioral observations were collected from 4 through 20 weeks of age. Individual body weights were recorded at the end of each week from 4 to 19 weeks of age.

Experiment 2

Subjects, housing and behavioral observations. This study was similar to Experiment 1 and was initiated in November and concluded the following February. An additional level of TRP (0.38%) was used and birds were observed from 4 to 15 weeks of age. Instead of using commercially prepared diets as in Experiment 1, isonitrogenous, isocaloric diets for Experiment 2 were mixed on-site (21). This not only ensured closer control of the nutrient composition of the diet, but enabled us to eliminate excess calories or protein as the source of any behavioral differences found. All occurrences of pecking and threatening on feed-off days were recorded during 20-min observation periods per pen as previously described. Body weights were recorded weekly on a group (per-pen) basis rather than individually as in the first experiment.

Statistical analysis. Spearman's rank correlations between body weight and dominance rank in feed restricted birds were evaluated at five different time intervals, approximately 4 weeks apart (4, 8, 12, 16, 19) for each treatment.

Once dominance hierarchies had been determined, the dominance rank (z) of each male was calculated as described by Lee et al. (13). Individual dominance ranks were converted to "relative" dominance values (RD) [RD = (minimum z − observed z)/n − 1, where z = dominance rank] to account for mortality and allow comparisons on a standardized scale among treatments. Landau's indices (9,11) were calculated to ascertain the linearity of these hierarchies across all treatments.

TABLE 1

BODY WEIGHT CORRELATIONS WITH DOMINANCE STATUS IN DEVELOPING MALE CHICKENS GIVEN ONE OF THREE LEVELS OF DIETARY TRYPTOPHAN (TRP) ON AN ALTERNATE-DAY FEEDING PROGRAM IN EXPERIMENT 1

Treatment % TRP		Age (week) 4	8	12	16	19
0.19	r	.29	.35*	.35*	.39*	.37*
	n	39	39	39	37	35
0.75	r	.37†	.33*	.35*	.42†	.43†
	n	42	42	42	41	40
1.50	r	−.13	−.20	−.32	−.23	−.13
	n	42	42	42	42	42

*$p<0.05$.
†$p<0.01$.
Correlation coefficients (r) and number of birds (n) are listed.

The effects of relative dominance and percent dietary TRP on aggression was analyzed using the SAS General Linear Models procedure (19). Because the first experiment was a three-level (TRP) study, both experiments are presented using a regression model including linear and quadratic terms with respect to TRP. The regression model used to determine the equations of the line for the predicted behavior (pecks or threats, PB), where b_0 is the intercept and b_1 to b_5 are the regression coefficients, was: $PB = b_0 + b_1TRP + b_2RD + b_3TRP^2 + b_4(TRP)RD + b_5(TRP^2)RD$.

RESULTS

Dominance Hierarchies

In both experiments, all hierarchies across treatments were calculated to be linear ($p<0.05$) as determined by Landau's index, h (11). In Experiment 1, the control birds (0.19% TRP), the 0.75% TRP birds and the 1.5% TRP birds had h values of 0.95, 0.98 and 0.98 respectively on a normalized scale of zero to one (where a value of one indicates greatest linearity of the hierarchy). Similarly, in Experiment 2, controls, 0.38, 0.75 and 1.5% TRP birds had h values of 0.88, 0.87, 0.89 and 0.89, respectively.

Body Weight Correlations With Dominance Status

Body weight correlations with dominance status are shown in Table 1. Significant positive correlations were found between body weight and dominance status in control birds and 0.75% TRP-treated birds. A correlation between body weight and dominance status was not observed, however, in the 1.5% TRP-treated birds. Body weights and coefficients of variation for body weights were similar for all treatment groups (Table 2).

Interaction Effects of Dominance Status and TRP on Aggression

The slopes of the regression lines for aggressive pecking and the standard errors of the slopes for all treatment groups for Experiments 1 and 2 are presented in Table 3. A regression estimate represents the amount of change in the dependent variable given a 1 unit increase in the independent variable. In this case, RD has been standardized on a scale of 0 to 1. Therefore, these slope es-

TABLE 2

BODY WEIGHTS AND COEFFICIENTS OF VARIATION AT THREE DIFFERENT LEVELS OF DIETARY TRYPTOPHAN (TRP) IN DEVELOPING MALE CHICKENS MAINTAINED ON A SKIP-A-DAY FEED RESTRICTION PROGRAM (0.19% TRP = CONTROLS)

Treatment		Age (week)				
% TRP		4	8	12	16	19
0.19	BW	1.01	1.21	1.75	2.64	3.10
	CV	9.26	10.47	12.68	14.00	15.17
0.75	BW	0.91	1.31	1.76	2.69	3.08
	CV	12.10	15.15	14.19	14.46	15.21
1.50	BW	0.91	1.23	1.74	2.65	3.05
	CV	10.58	13.02	15.24	15.80	15.63

Body weights (BW) and Coefficients of Variation (CV) are listed. Body weights are given in kilograms.

timates represent the actual difference in the number of aggressive pecks between the most subordinate (RD = 0) and the most dominant (RD = 1) individuals. In both studies, there was approximately a 2-fold difference in the change in pecking behavior between the control group from the least to greatest RD and each TRP-treated group ($p<0.05$). In the second study, there was a 3-fold difference between the controls and the 0.38% TRP-treated birds. The response surface plots for both Experiment 1 and 2 corresponding to the estimates of the slope are shown in Fig. 1. Relative dominance and percent TRP in the diet were found to have significant ($p<0.05$) influence on the numbers of aggressive pecks.

To further illustrate this differential response, the regression estimates of the average number of pecks for the duration of the study of the most dominant and most subordinate bird in each pen were determined. There was a greater unit decrease in aggression among TRP-treated dominant birds than TRP-treated subordinate birds. Dominant control birds (0.19% TRP) displayed an average of 18.36 pecks and 0.38, 0.75 and 1.5% TRP-treated birds showed a 12.41, 9.29 and 9.3 unit decrease in aggression, respectively, compared to dominant controls. The TRP-treated subordinate birds, however, showed only a 0.27, 0.34 and 0.75 unit decrease in aggression, respectively, as compared to subordinate control birds (pecks = 1.56). Evaluating these same numbers using percentages would be misleading because the base number of pecks among subordinate birds is markedly lower (1.56) than that

TABLE 3

REGRESSION ESTIMATES FOR DOMINANCE STATUS AND AGGRESSIVE PECKING AT DIFFERENT LEVELS OF TRYPTOPHAN (TRP)

	Experiment 1	Experiment 2
% TRP	b ± SE_b	b ± SE_b
0.19	28.0[a] ± 3.9	29.0[a] ± 4.3
0.38	—	8.6[b] ± 5.6
0.75	11.4[b] ± 5.1	15.6[b] ± 4.9
1.50	12.4[b] ± 5.3	14.5[b] ± 5.8

[a,b]Estimates with the same letter are not significantly different at $p<0.05$. b, SE_b are the estimated slope and the standard errors, respectively.

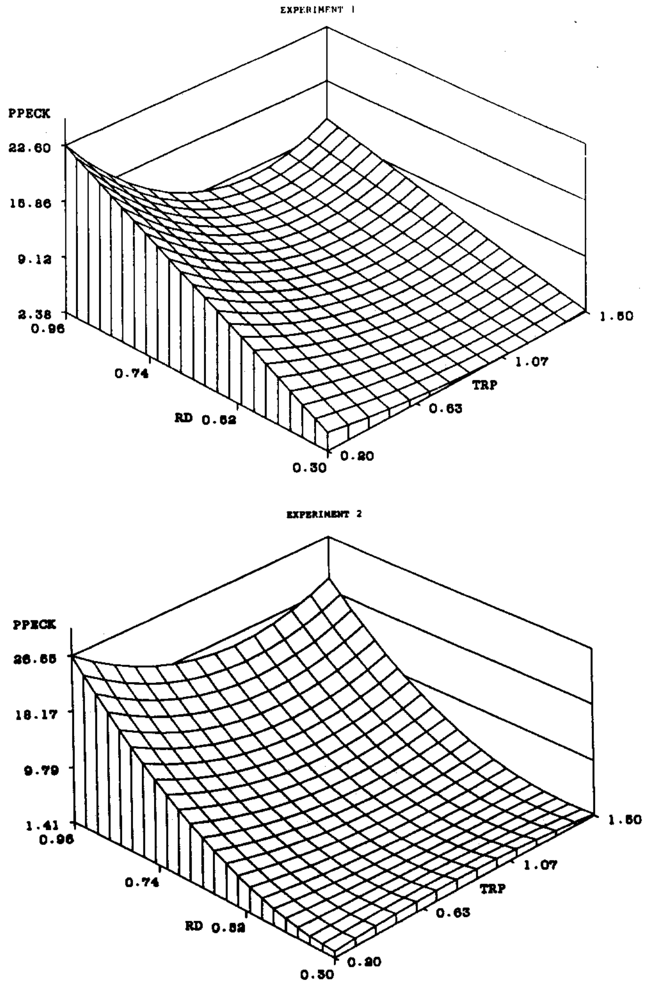

FIG. 1. Predicted pecks (PPECKS) as a function of relative dominance (RD) and percent dietary tryptophan (TRP) in developing feed-restricted male domestic fowl fed either three (Experiment 1) or four (Experiment 2) levels of tryptophan. In both experiments, the most dominant bird (RD = 0.96) showed a significantly ($p<0.05$) greater decrease in the number of PPECKS than the most subordinate bird (RD = 0.30) with increasing levels of TRP (0.20 to 1.5% TRP). Experiment 1, $R^2 = 0.50$, SD = 4.4. Experiment 2, $R^2 = 0.40$, SD = 3.2.

of dominant birds (18.36). A comparatively small change in aggression would thus represent a very large percentage of the base level in the subordinate birds. It is inappropriate to evaluate relative or percentage values when the bases for comparison differ so markedly.

There were no significant differences among predicted threats as a function of relative dominance and percent dietary TRP in either experiment.

DISCUSSION

The formation of a dominance hierarchy increases the social stability of a group by functioning as an ordering mechanism. In dominance behavior, the aggressive-submissive relationship reduces the number and/or intensity of aggressive interactions as each group member 'respects' the status of the other members (15). However, the stability provided by this hierarchy may become threatened when resources are limited.

Duncan and Wood-Gush (4) reported an increase in agonistic acts by dominant male fowl in a food frustration situation. Feed restriction programs are used extensively in commercial poultry production. *Gallus domesticus* has been genetically selected to produce an egg-laying strain (layers) and a meat-producing strain (broilers). Broilers are selected to maximize weight gain in a minimum amount of time, and breeding stock must therefore be feed restricted early in development to prevent this rapid weight gain from decreasing reproductive efficiency. Levels of aggression in such feed-restricted birds are elevated compared to the minimal levels of aggressive activity seen in birds fed ad lib (14). Not only is aggression elevated but formation of the dominance hierarchy is delayed, from seven weeks of age in birds fed ad lib to ten weeks of age in restricted fed birds. Once the hierarchy is established, supplemental dietary TRP will depress aggression in feed-restricted males. The level of TRP producing this decrease in aggression does not appear to be dose-dependent, although since all three supplemental levels investigated (0.38, 0.75 and 1.5%) significantly decrease aggression as compared to controls (0.19%) (21).

Priority of access to the limited feed source can be affected by dominance position. Greater body weight gain by dominant animals would suggest greater consumption of feed by dominant than subordinate birds. The positive correlation between body weight and dominance rank in control birds and 0.75% TRP birds found in the present study implies that dominant birds were eating more. The elimination of this correlation when birds received 1.5% TRP indicates that there is no priority of access related to social status in this group. Dominant birds did not gain weight more readily than subordinate birds, also suggesting a differential response of dominant and subordinate animals. A change in priority of access was not confirmed, however, by the coefficients of variation for body weights, which were similar among treatment groups. This discrepancy was probably due to the relatively small sample size.

The status-dependent differential sensitivity to the effects of TRP was illustrated by the change in regression of relative dominance on aggressive behavior for different treatment groups. These results concur with those of Raleigh et al. (17), who reported that dominant and subordinate adult male vervet monkeys differed in the drug sensitivity of their serotonergic systems. When treated with either a serotonin reuptake inhibitor, a receptor agonist or TRP itself, dominant male vervet monkeys were significantly more receptive behaviorally than subordinate males. Dominant males with activated serotonergic systems become more quiescent and less aggressive in their social behavior than subordinates.

It is possible that dominance is associated with higher levels of arousal. In the cat, a higher level of arousal is associated with an increased firing rate of 5-HT neurons (25). Therefore, if an elevated state of arousal exists in a food-competitive environment, altering TRP levels becomes more likely to modify aggression through increased neuronal firing. Addition of supplemental dietary TRP may, therefore, have marked effects on the more dominant animals potentiated by their highly aroused state. However, the mechanism underlying this action is unclear. Whole blood serotonin in vervet monkeys (17) and squirrel monkeys (24) has also been shown to be positively related to dominance rank. There appears to be a much higher level of conversion of peripheral TRP to 5-HT in the dominant animals than in the subordinates. Although there is a much higher level of systemic 5-HT, the lack of ability of 5-HT to cross the blood-brain barrier may result in an overall depletion of central 5-HT since most of the dietary TRP is being converted peripherally. As a result, a more aggressive behavioral state may evolve.

Although our data are not conclusive with respect to the effect of TRP in changing the priority of access to a limited resource, they do clearly demonstrate a differential sensitivity to dietary tryptophan in dominant and subordinate *Gallus domesticus*. In future studies, we will compare both peripheral and central serotonin and its metabolites in dominant and subordinate birds in an attempt to determine the physiological and neurochemical mechanisms underlying this differential responsiveness.

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