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A Study of the Circulating Leucocytes in Swine

INCLUDING THE LEUCOCYTIC RESPONSE TO ADRENALIN
AND ADRENOCORTICOTROPHIC HORMONE

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A Study of the Circulating Leucocytes in Swine

INCLUDING THE LEUCOCYTIC RESPONSE TO ADRENALIN AND ADRENOCORTICOTROPHIC HORMONE

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INTRODUCTION

The endocrine system is an integral part of the physiological mechanisms involved in the response of animals to stress. Hormones can be administered to induce stress and correlated physiological changes. A measurement of these changes provides a means of determining the ability of the animal to compensate for unfavorable conditions. Variations in responsiveness may be related to differences in levels of performance and, consequently, may provide a good means of predicting the merit of breeding and slaughter animals.

This study was conducted to determine the response of swine to stress-inducing hormones as measured by changes in the number and kind of circulating leucocytes. Differences observed among animals in the leucocytic reaction were associated with variations in performance and carcass characteristics.

A similar study was conducted on untreated swine to ascertain the normal circulating level of leucocytes and to determine the relationship of leucocyte counts to variations in the performance of these animals.

REVIEW OF LITERATURE

Leucocytes of Swine.

Three major types of leucocytes are normally found in the blood of swine; lymphocytes, neutrophils, and eosinophils. Other types of leucocytes account for only a small percentage of the total number. Total white cell counts and the percentages of the major cell types have been determined by a number of workers. The results of these studies are summarized as follows:

| <u>Author</u> | <u>Age</u> | <u>TWC (mm³)</u> | <u>LYM (%)</u> | <u>NEUT (%)</u> | <u>EOSIN (%)</u> |
|--------------------|------------|-----------------------------|----------------|-----------------|------------------|
| Scarborough (1931) | ----- | 15800 | 52.1 | 39.0 | 4.5 |
| Frazer (1938) | 2-7 mos | 21000 | 48.0 | 39.9 | 5.5 |
| Venn (1944) | 84 days | 15980 | 52.8 | 42.5 | 2.9 |
| Dukes (1947) | ----- | 17110 | 55.0 | 42.0 | 3.0 |
| Luke (1953) | ----- | 19866 | 66.4 | 32.0 | 1.6 |
| Hennessy (1959) | ----- | 17500 | 66.0 | 30.0 | 4.0 |

Romic (1948) and Addleman (1957) reported sex differences in the number of leucocytes found in swine. Other studies have shown a decrease in the percentage of lymphocytes and an increase in the percentage of neutrophils and eosinophils with age. Palmer (1917) made differential counts on blood samples taken from 2 to 42-day old pigs. These counts showed lymphocytes, 63 percent; neutrophils, 32 percent; and eosinophils, 1.0 percent. The results of differential counts made on the blood of 100 pound pigs were: lymphocytes, 55 percent; neutrophils, 39 percent; and eosinophils, 3.0 percent. Milicevic (1959) reported a significant difference between breeds in total number of leucocytes per mm^3 of blood.

Effect of Adrenocorticotrophic Hormone (ACTH) on the Level of Circulating Leucocytes.

Leucocytes have been studied rather extensively in man and laboratory animals, and the experimental results have indicated certain characteristic responses of their cells to different stress-producing agents.

Adrenocorticotrophic hormone is produced by the pituitary gland, but it affects the concentration of leucocytes indirectly through the stimulation of the adrenal cortex (Dougherty and White 1944; Hills *et al.* 1949; Dury 1950 and Thorn *et al.* 1953). The administration of exogenous adrenocorticotrophic hormone has been found to cause a reduction in the number of lymphocytes (lymphopenia) and eosinophils (eosinopenia) and to initiate an increase in the number of neutrophils (neutrophilia) by Dougherty and White (1944), Thorn *et al.* (1948), Hills *et al.* (1948), Polak and Nemeč (1959).

Effects of Adrenalin (Epinephrine) on the Level of Leucocytes of Swine.

Previous experimental work has shown that adrenalin injections in intact animals induces lymphopenia, eosinopenia and a marked neutrophilia (Speirs and Meyer, 1949; Almy and Laragh, 1949; White *et al.*, 1950; and Samuels, 1951). White *et al.* (1950) stated that adrenalin induces changes similar to, but not identical with those produced by ACTH.

MATERIALS AND METHODS

Poland China pigs, weighing at least 175 pounds, were used in this study. They were self-fed a complete mixed ration prior to and during the experimental period.

Leucocyte Concentration in Growing-Finishing Pigs.

The experimental animals were selected at random within litters and sexes. Initial blood samples were collected and counted from two boars and two gilts per litter when possible. A total of 280 animals were studied, representing three different seasons; Spring 1959, Fall 1959, and Spring 1960.

Blood collections were obtained from a mid-ventral incision made in the distal one-third of the tail. Four to 5 ml. of blood were collected in a 10 ml. tube in which potassium oxalate had been added as an anti-coagulant. Duplicate dry smears were made immediately after collection for differential white blood cell counts. The tubes were sealed with rubber stoppers and total white cell counts were made as soon as possible, which was usually within 4-6 hours after the time of collection.

The total white cell counts were made in a 1:19 dilution of the whole blood. The diluting fluid was prepared by mixing and filtering 9.6 cc HCl, 300 cc distilled H₂O and 500 mg. of neutral red dye. Cell counts were made at 100 magnifications using the improved Neubauer hemacytometer.

The dry smears were stained with a modified Wright's Stain which is prepared as follows; for one liter of stain, 3.0 gms. powdered Wright's Stain and 0.3 gms. powdered Giemsa's Stain are mixed with 30.0 ml. glycerine and made into a light paste. A buffer solution is prepared by dissolving 1.6 grams monobasic potassium phosphate and 3.2 grams diacidic sodium phosphate in 970 ml. of methyl alcohol. The buffer solution is mixed with the stain-glycerine paste and the combination is agitated for 20 minutes, allowed to stand for 24 hours, and filtered. The dry smears are flooded with this stain for 15 seconds, diluted with distilled water for 15 seconds, then washed with distilled water and allowed to dry. Two hundred white cells (lymphocytes, neutrophils and eosinophils) were counted on each of the smears to determine the percentages of each cell type in the sample. The counts were made at 430 magnifications.

Adrenocorticotrophic Hormone (ACTH) Studies.

This study involved 53 pigs. Three littermates from nine different litters were used in the first study (Spring pigs) and 26 barrows from 22 different litters were used in the second study (Fall pigs). Initial blood samples were taken and treated as previously described.

Crystalline adrenocorticotrophic hormone (ACTH) was dissolved in physiological saline and injected intramuscularly at the rate of 0.06 U.S.P. unit per pound of body weight. The pigs were released and left undisturbed for a four-hour period after the initial collection. A second sample of blood was then collected and examined as described previously.

Adrenalin Studies.

Twenty-six Poland China barrows were used in this study. Each pig was injected intramuscularly with 0.75 cc of a 1:2000 epinephrine solution (0.5 mg. epinephrine per cc) for each 100 pounds of body weight. Initial and four-hour post-treatment blood samples were collected and counted.

RESULTS AND DISCUSSION

Leucocytes of Normal Swine.

Studies were made of the number and kinds of circulating leucocytes of 280 untreated Poland China pigs weighing an average of 175 pounds. It was felt that the number of animals in this study was adequate to determine standard leucocyte counts for swine maintained under the conditions of the experiment. Also, the large sample size gave a rather conclusive evaluation of the correlations between the level of circulating leucocytes and performance traits.

Table 1 gives the mean values for the total number of leucocytes per mm^3 of blood and the numbers and percentages of lymphocytes, neutrophils and eosinophils. Approximately two-thirds of the total number of leucocytes were

TABLE 1—NUMBER AND PER CENT OF CIRCULATING LEUCOCYTES PER MM^3 OF BLOOD IN 280 NON-TREATED PIGS REPRESENTING THREE FARROWINGS

| | <u>Mean + Standard Error</u> |
|-------------------|------------------------------|
| Total White Cells | 21, 789. 2 + 263. 9 |
| Percent: | |
| Lymphocytes | 64. 9 + 3. 9 |
| Neutrophils | 29. 7 + 1. 8 |
| Eosinophils | 5. 3 + 0. 3 |
| Number: | |
| Lymphocytes | 14, 022. 8 + 179. 3 |
| Neutrophils | 6, 501. 4 + 168. 4 |
| Eosinophils | 1, 203. 9 + 56. 5 |

lymphocytes, 30 percent neutrophils and 5 percent eosinophils. These results indicate that the normal level of circulating leucocytes in growing-finishing swine is slightly higher than mean values reported previously. Differences in age, breeding, and management of experimental animals would be expected to be at least a partial cause for this lack of agreement in results.

Statistically significant differences were found among seasons in the total number of leucocytes per mm^3 of blood, number of neutrophils and eosinophils, and the percentages of lymphocytes and neutrophils. The total number of leucocytes, the number of neutrophils, and the number of eosinophils were lower for 1960 spring-pigs than for the previous fall or spring. The percentage of lymphocytes was lower and the percentage of neutrophils higher in pigs farrowed during the fall and tested during the winter months. Figure 1 shows the seasonal differences in percentages of leucocytes.

Within-season analyses revealed that a significant litter influence existed for the total number of white cells and the number of lymphocytes per mm^3 of blood. These results indicate that part of the variation observed in these cellular components of the blood was due to differences in the genotype of the animal.

A significant sex effect was found in the number of neutrophils and percentage of eosinophils. Boars had a higher number of neutrophils than gilts and

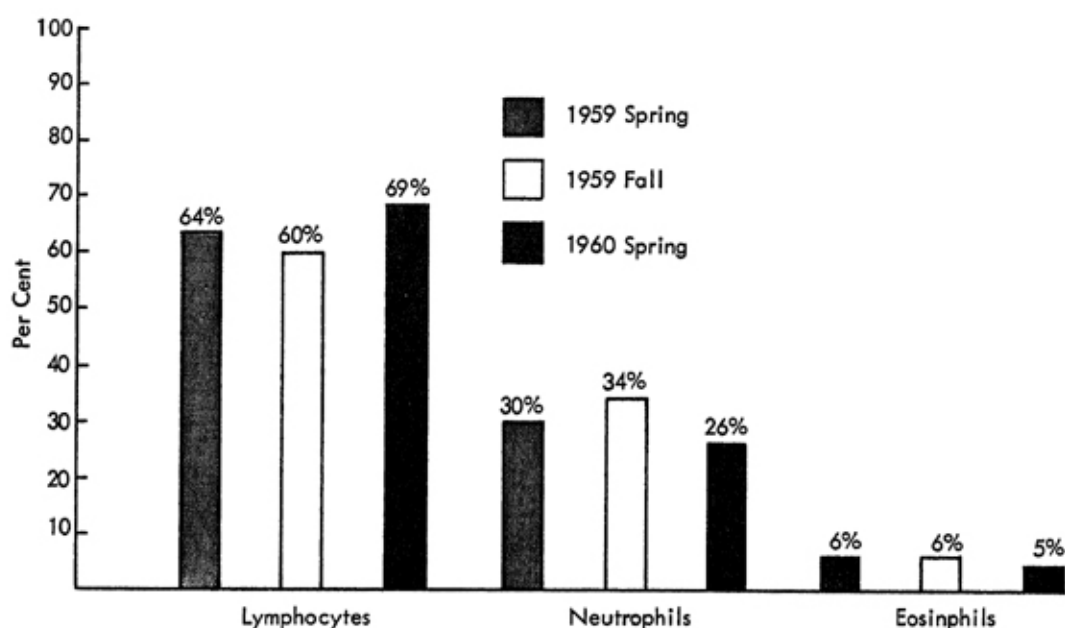


Fig. 1—The percentages of leucocytes in pigs representing three different farrowings (N = 280).

gilts had a higher proportion of eosinophils than boars. No other sex differences were noted.

Correlation coefficients were computed to determine the associations of the leucocyte enumerations with rate of gain, feed efficiency, live animal backfat probe and age at the time the blood sample was collected. Although statistically significant correlations were found between some of the variables, as shown in Table 2, the magnitude of these correlations was relatively small and, in turn, it is felt that these leucocytic measurements would be of limited value to the swine producer for the purpose of predicting the performance of individual animals.

TABLE 2—CORRELATIONS OF RATE OF GAIN, BACKFAT THICKNESS, LITTER FEED EFFICIENCY, AND AGE WITH TOTAL WHITE CELLS, PERCENTAGE AND NUMBER OF LYMPHOCYTES, NEUTROPHILS, AND EOSINOPHILS IN SAMPLES FROM 280 NON-TREATED PIGS

| (N = 280) | Rate of Gain | Backfat Thickness | Litter Feed Efficiency | Age |
|-------------------|--------------|-------------------|------------------------|--------|
| Total White Cells | -.038 | -.050 | -.001 | +.149* |
| Percent: | | | | |
| Lymphocytes | +.171** | +.027 | -.119 | -.019 |
| Neutrophils | -.149* | -.028 | +.085 | -.024 |
| Eosinophils | -.067 | +.002 | +.090 | +.126 |
| Number: | | | | |
| Lymphocytes | +.122* | -.010 | -.085 | +.156* |
| Neutrophils | -.155** | -.060 | +.031 | +.012 |
| Eosinophils | -.097 | -.021 | +.082 | +.109 |

* Statistically significant at the 5% level.

** Statistically significant at the 1% level.

Adrenocorticotrophic Hormone (ACTH) Studies.

Fifty-three animals were used in this phase of the investigation. The general leucocytic response of the experimental animals to a single injection of ACTH was an increase in the total number of leucocytes per mm^3 of blood, a decrease in the number and percentage of lymphocytes and eosinophils, and a marked increase in the number and percentage of neutrophils, as shown in Tables 3 and 4.

TABLE 3—LEUCOCYTIC RESPONSE IN SWINE TO THE ADMINISTRATION OF ADRENOCORTICOTROPHIC HORMONE (SPRING PIGS)

| | Pre-Treatment Mean \pm Standard Error | | 4-Hour Post-Treatment Mean \pm Standard Error | |
|-------------------|--|-------|--|-------|
| Total White Cells | 21,083.0 \pm 1,008.8 | | 21,226.0 \pm 915.6 | |
| Percent: | | | | |
| Lymphocytes | 62.6 \pm | 1.9 | 46.6 \pm | 2.2 |
| Neutrophils | 32.1 \pm | 1.9 | 53.3 \pm | 2.2 |
| Eosinophils | 5.4 \pm | 0.6 | 0.2 \pm | 0.1 |
| Number: | | | | |
| Lymphocytes | 12,928.2 \pm | 537.4 | 9,601.8 \pm | 405.5 |
| Neutrophils | 6,977.3 \pm | 663.4 | 11,592.5 \pm | 863.9 |
| Eosinophils | 1,177.9 \pm | 183.1 | 31.6 \pm | 11.6 |

N = 27

TABLE 4—LEUCOCYTIC RESPONSE IN SWINE TO THE ADMINISTRATION OF ADRENOCORTICOTROPHIC HORMONE (FALL PIGS)

| | Pre-Treatment Mean \pm Standard Error | | 4-Hour Post-Treatment Mean \pm Standard Error | |
|-------------------|--|-------|--|-------|
| Total White Cells | 17,481.0 \pm 742.8 | | 22,594.0 \pm 950.2 | |
| Percent: | | | | |
| Lymphocytes | 60.1 \pm | 1.7 | 37.7 \pm | 1.8 |
| Neutrophils | 31.8 \pm | 1.8 | 61.8 \pm | 1.9 |
| Eosinophils | 8.1 \pm | 0.7 | 0.5 \pm | 0.1 |
| Number: | | | | |
| Lymphocytes | 10,451.3 \pm | 464.4 | 8,294.9 \pm | 351.4 |
| Neutrophils | 5,678.3 \pm | 477.8 | 14,205.3 \pm | 895.5 |
| Eosinophils | 1,389.6 \pm | 111.7 | 94.0 \pm | 21.1 |

Differences between animals, within litters, in rate of gain did not have a significant influence on total leucocyte count or the number and percentage of eosinophils following the injection of ACTH. Significant interactions were found between the rate of gain groups and litters for the remaining leucocyte determinations. The presence of interactions for these traits indicates that the influence of exogenous ACTH on the level of circulating neutrophils and lymphocytes of pigs with different gaining ability is partially controlled by the genotype of the injected animal.

Correlation coefficients were computed between the leucocyte counts and various economic traits as shown in Tables 5 and 6. Rate of gain was not significantly correlated with the total number of leucocytes per mm^3 of blood or the differential counts of leucocytes in the initial or four-hour blood samples. Backfat thickness was significantly associated with percentage of lymphocytes, and the percentage and number of neutrophils in the initial blood sample. Although these results suggest that the numbers of lymphocytes and neutrophils in an initial blood sample are correlated with percentage of lean cuts, other more extensive studies on untreated barrows failed to confirm this association. The percentage of lean cuts in the carcasses obtained from these animals was significantly correlated with the percentage of lymphocytes and number of neutrophils in the initial blood sample.

TABLE 5—CORRELATION COEFFICIENTS OF LEUCOCYTE ENUMERATIONS BEFORE AND 4-HOURS AFTER THE ADMINISTRATION OF ACTH WITH RATE OF GAIN (SPRING PIGS)

| | Pre-Treatment | 4-Hour Post Treatment |
|-------------------|---------------|-----------------------|
| Total White Cells | + .25 | + .15 |
| Lymphocytes, % | + .14 | -.07 |
| Lymphocytes, No. | -.14 | + .13 |
| Neutrophils, % | -.14 | + .08 |
| Neutrophils, No. | -.24 | + .11 |
| Eosinophils, % | + .01 | + .28 |
| Eosinophils, No. | -.11 | -.28 |

N = 26

TABLE 6—CORRELATIONS OF LEUCOCYTE DETERMINATIONS WITH RATE OF GAIN, BACKFAT THICKNESS, AND PERCENTAGE OF LEAN CUTS BEFORE AND 4-HOURS AFTER THE ADMINISTRATION OF ACTH (FALL PIGS)

| | Pre-Treatment | | | Post-Treatment | | |
|-------------------|---------------|-------------------|-------------------|----------------|-------------------|-------------------|
| | Rate of Gain | Backfat Thickness | Percent Lean Cuts | Rate of Gain | Backfat Thickness | Percent Lean Cuts |
| Total White Cells | -.17 | -.28 | + .33 | -.32 | -.40* | + .30 |
| Lymphocytes, % | -.03 | + .54** | -.41* | + .15 | + .06 | + .01 |
| Lymphocytes, No. | -.20 | + .12 | + .05 | -.17 | -.24 | + .28 |
| Neutrophils, % | -.01 | -.54** | + .36 | -.17 | -.03 | -.03 |
| Neutrophils, No. | -.09 | -.53** | + .41* | -.28 | -.31 | + .19 |
| Eosinophils, % | + .10 | + .05 | + .09 | + .30 | -.36 | + .40 |
| Eosinophils, No. | + .02 | -.04 | + .21 | + .26 | -.40* | + .45* |

N = 26

* Statistically Significant at the 5% level.

** Statistically Significant at the 1% level.

No significant correlations were found when the total white cell counts and differential counts of lymphocytes and neutrophils in the post-treatment blood samples were associated with the yield of lean cuts. However, the number and percentage of circulating eosinophils following stress were significantly

correlated with percentage of lean cuts in the carcass. The significant correlations found between the level of circulating eosinophils in the post-treatment blood sample and both backfat thickness and percentage of lean cuts suggest that this measurement may provide a means of predicting carcass merit. However, extreme variation is encountered in these counts. The development of additional techniques in determining the number of eosinophils may reduce this variation and contribute significantly to the accuracy of the method.

Adrenalin Studies.

Twenty-six Poland China barrows were administered 0.75 cc of 1:2000 solution (0.5 mg. epinephrine per cc) of epinephrine per 100 lbs. of body weight. The leucocytic response of swine to exogenous adrenalin was similar to that observed following the injection of ACTH. A comparison of the initial and four-hour blood samples revealed an increase in the total number of leucocytes per mm³ of blood, a slight decrease in the percentage of lymphocytes and eosinophils, and an increase in the percentage of neutrophils (Table 7).

TABLE 7—LEUCOCYTIC RESPONSE IN SWINE TO ADRENALIN ADMINISTRATION

| | Pre-Treatment Mean \pm Standard Error | 4 Hour Post Treatment Mean \pm Standard Error |
|-------------------|--|--|
| Total White Cells | 15, 255.5 \pm 772.6 | 17, 025.0 \pm 806.0 |
| Percent: | | |
| Lymphocytes | 63.2 \pm 1.8 | 59.9 \pm 1.8 |
| Neutrophils | 28.6 \pm 2.0 | 35.4 \pm 1.9 |
| Eosinophils | 8.2 \pm 0.7 | 5.0 \pm 0.5 |
| Number: | | |
| Lymphocytes | 9, 678.9 \pm 577.0 | 10, 211.8 \pm 584.7 |
| Neutrophils | 4, 350.4 \pm 359.6 | 5, 977.6 \pm 416.4 |
| Eosinophils | 1, 226.5 \pm 108.9 | 835.7 \pm 96.1 |

N = 26

TABLE 8—CORRELATIONS OF LEUCOCYTE COUNTS WITH RATE OF GAIN, BACKFAT THICKNESS AND PERCENTAGE OF LEAN CUTS BEFORE AND 4-HOURS AFTER THE ADMINISTRATION OF ADRENALIN

| | Pre-Treatment | | | Four-Hour Post-Treatment | | |
|-------------------|---------------|-------------------|-------------------|--------------------------|-------------------|-------------------|
| | Rate of Gain | Backfat Thickness | Percent Lean Cuts | Rate of Gain | Backfat Thickness | Percent Lean Cuts |
| Total White Cells | -.24 | -.11 | +.04 | -.21 | -.22 | +.12 |
| Percent: | | | | | | |
| Lymphocytes | +.39* | +.03 | +.08 | +.40* | +.07 | -.01 |
| Neutrophils | -.50** | -.08 | -.05 | -.45* | -.07 | -.07 |
| Eosinophils | +.45* | +.16 | -.08 | +.31 | +.01 | +.02 |
| Number: | | | | | | |
| Lymphocytes | -.02 | -.06 | +.06 | +.01 | -.13 | +.08 |
| Neutrophils | -.57** | -.19 | -.00 | -.47* | -.23 | +.09 |
| Eosinophils | +.31 | +.12 | -.01 | +.22 | -.09 | +.09 |

N = 26

* Statistically significant at the 5% level.

** Statistically significant at the 1% level.

Rate of gain was significantly correlated with the percentage of lymphocytes and eosinophils in the initial and four-hour blood samples. (Table 8) Also, statistically significant correlations were found between average daily gain and the number of neutrophils in both the initial and four-hour determinations. Results obtained from subsequent studies were not in agreement with the significant correlations between rate of gain and the level of leucocytes in the initial blood samples.

In contrast to the results obtained in the ACTH studies, the percentage of lean cuts in the carcasses of barrows used in the adrenalin experiment was not significantly correlated with any of the leucocyte determinations made before or after treatment. A trend did exist for the leucocyte enumeration determined four hours following the injection of adrenalin to be associated with gaining ability.

SUMMARY

The circulating leucocytes of 280 Poland China pigs representing three farrowing seasons were studied to determine the normal circulating level of leucocytes in swine. The average total white cell count per mm^3 of swine blood was found to be 21,790. Differential white cell counts revealed the following average values; lymphocytes, 14,023 or 64.9 percent; neutrophils, 6,501 or 29.7 percent; and eosinophils, 1,204 or 5.3 percent. The mean differences between seasons in total number of white cells and the number and percent of lymphocytes, neutrophils, and eosinophils were statistically significant. Age of the animal was correlated with the total number of leucocytes and the number of lymphocytes. A significant difference was found between sexes in the number of neutrophils and the percentage of eosinophils with boars having the largest number of neutrophils and gilts the largest proportion of eosinophils.

Adrenocorticotrophic hormone (ACTH) was used to induce leucocytic changes in 53 pigs. The general leucocytic response elicited was an increase in the total number of leucocytes per mm^3 of blood, a decrease in the number and percentage of lymphocytes and eosinophils, and a marked increase in the number and proportion of neutrophils. Rate of gain was not significantly correlated with the total number of leucocytes per mm^3 of blood or the differential counts of leucocytes in the initial or four-hour blood samples. Some correlations between white blood cell enumerations and backfat thicknesses were statistically significant. Post-treatment counts were significantly correlated with the percentage of lean cuts in the carcass. However, it is felt that improved techniques for measuring the circulating levels of the blood cells must be developed before it will be possible to obtain a satisfactory degree of dependability in making a prediction on the merits of an individual animal.

The leucocytic response induced by the administration of adrenalin was similar to the response obtained from ACTH administration. The lymphocytes and eosinophils in the four-hour post-treatment blood samples showed a decline in both number and percent while the concentration of neutrophils increased. The percentage of lymphocytes and eosinophils and the number of neutrophils in blood samples collected after the administration of adrenalin were significantly

correlated with rate of gain but not with backfat thickness or proportion of lean cuts in the carcass. However, as indicated previously, the extreme variation encountered in these counts limits the effectiveness of the procedure in predicting the merits of individual animals.

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