THE EFFECT OF PROTEIN ON GROWTH RATE AND ONSET OF PUBERTY IN BROWN SWISS AND EARLY WEANED ZEBU HEIFERS UNDER TROPICAL CONDITIONS

By

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ABSTRACT

THE EFFECT OF PROTEIN ON GROWTH RATE AND ONSET OF PUBERTY IN BROWN SWISS AND EARLY WEANED ZEBU HEIFERS UNDER TROPICAL CONDITIONS

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In the humid tropics of Mexico 12 Brown Swiss and 12 Zebu heifer calves were weaned at three months of age. They were then confined in individual pens and fed either an adequate protein (initially 12.8% CP) or a high protein (initially 16.4% CP) diet ad libitum until puberty. The isocaloric diets consisted of sorghum silage, sunflower meal, rice polishings, molasses and minerals. Jugular blood samples were taken and body weights were recorded bi-weekly.

Onset of puberty was determined by rectal palpation of the first corpus luteum. A plasma progesterone concentration of more than 1 ng/ml in the corresponding plasma sample confirmed the presence of a functional corpus luteum.

Growth rates of Brown Swiss and Zebu heifers tripled, to 882 and 611 g/day, and age at onset of puberty was

decreased by three to six months, to 9.4 and 12.3 months respectively, compared to what is common in the study area.

Increasing dietary crude protein from 12.8%, recommended by NRC for dairy heifers expected to gain 500 g/day, to 16.4 % had no effect on growth rate or onset of puberty.

Only five of the Brown Swiss heifers and none of the Zebus had a functional corpus luteum during their first or second estrous cycle. It is suggested that onset of puberty can occur without the development of a functional corpus luteum and a heifer may go through at least one estrous cycle before the first functional corpus luteum is developed.

In the tropics Bos Taurus and Bos Indicus heifers provided with adequate nutrition and management can grow to reach puberty at an age comparable to dairy and beef heifers in temperate climates.

I dedicate this dissertation to my Mother, Father and to Sylvia and to the memory of my aunt Greta for their unending encouragement, support, understanding and love.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBRIEVIATIONS

ACTH	adrenocorticotropic hormone
ADG	average daily gain
ANOVA	one way analysis of variance
A.O.A.C.	Association of Analytical Chemists
BHM	Brahman
BW	body weight
CL	corpus luteum
CP	crude protein
CuFe	copper iron (-solution)
CV	coefficient of variation
DM	dry matter
EDTA	ethylendiaminetriacetic acid
FR	fast (growth) rate
FSH	follicle stimulating hormone
GMP	Guanosine 3': 5'-monophosphate (cyclic)
GnRH	gonadotropin-releasing hormone
IB	IndoBrazil
ICAP	Inductively coupled argon plasma emission spectroscopy
INIFAP	Instituto Nacional de Investigaciones Forestales y AgroPecuarias
INIP	Instituto Nacional de Investigaciones Pecuarias
HQC	high quality control
LH	luteinizing hormone
LQC	low quality control
LS	least square
MR	moderate (growth) rate
NPE	non puberal estrus
NRC	National Research Council
P (PR)	probability
PBI	plasma protein-bound iodine
PGF2a	prostaglandin F2alfa
QC	quality control
SAS	Statisitcal Analysis Systems
SP	Brown Swiss (Suizo Pardo)
STD	standard
STD ERR	standard error
T3	triiodothyronine
T4	thyroxine
TRH	thyroid releasing hormone
TSH	thyroid stimulating hormone

INTRODUCTION

Productivity of cattle, as determined by calf mortality, growth rate, age at puberty, conception rate, age at first calving, milk production, calving interval and longevity, in developing countries with tropical climates is very low. Poor nutrition, low genetic potential, endemic disease, poor management and lack of trained manpower are important factors contributing to this low level of productivity (Rendel, 1973). Each of these can, in turn, be linked to the reproductive problems of cattle in the tropics.

Poor nutrition is one of the main causes of lowered fertility in tropical cattle (Vandeplassche, 1982; Williamson and Payne, 1978; Kestevan, 1973; Mahadevan 1966). Inadequately fed heifers grow slowly and, as a result, their sexual maturity is delayed. The majority of heifers in the tropics subsists on poor pastures and suffers from protein, energy and carbohydrate deficiencies during a large part of the year. Consequently, they may not attain puberty until two years of age or even older (Williamson and Payne, 1978).

Higher growth rates and earlier maturity of tropical cattle can be achieved by energy and/or protein supplementation. Oyedipe et al. (1982) showed that Zebu heifers with a high protein consumption exhibited first estrus significantly earlier than those with a low protein intake. In the trial Zebu heifers on a diet with 19% crude protein reached puberty at 570 days. In heifers on a diet with 8% crude protein presence of the first corpus luteum was detected at 704 days of age.

Mexico is a country with 25% of its area in the tropics. The tropical part of Mexico contains 60% of the national cattle population or 22.5 million bovines. It is, therefore, of considerable interest to study and improve the productivity of cattle in the Mexican tropics. Daily gains of these animals are adequate during the wet season, but loss of weight during the dry months may even exceed that gained during the wet season. For the majority of animals the final result is a net gain of 50 to 100 kg per year and poor reproductive performance. First calving when the heifers are three to four years old is a common feature (Barradas, 1980; Monroy, 1982).

In Mexico, studies of estrous cycles of Brown Swiss and Zebus have been conducted under tropical conditions, but only with mature cows (Galina, 1986; Galina et al., 1985; Jimenez et al., 1984). Very little information exists about onset of puberty of these two breeds in the tropics. There is a need for much more research on factors that affect

onset of puberty in cattle in Mexico.

REVIEW OF LITERATURE

INTRODUCTION

The literature review consists of three major subjects, whereof the last two have a number of subtopics. The first subject is effect of protein on both growth rate and onset of puberty and conception rates in heifers in the tropics. The second area is plasma thyroid hormone concentrations as related to growth rate and heat stress in cattle and to diet composition in both cattle and humans. The third subject is puberty in heifers. It begins with the proposed endocrine mechanism for onset of puberty, continues with plasma progesterone concentrations and then centers around onset of puberty and estrous cycles reported for heifers in the tropics. The last subsection focuses on the new concept of nonpuberal estrus and also the opposite event of ovulation without estrus and how the two are related to nutrition of the prepuberal heifer.

Materials and methods used in the trials reviewed are described in some detail, because these parts of the studies are essential to the subsequent discussion.

THE EFFECT OF PROTEIN ON GROWTH RATE, ONSET OF PUBERTY AND CONCEPTION RATE IN HEIFERS IN THE TROPICS

Few studies of the effect of protein on both puberty and growth rate in heifers in the tropics have been reported. An early study was conducted by Anderson (1933) and two recent studies have been reported (Oyedipe et al., 1982; Trong Trung and Escano, 1980). The trials continued past puberty through breeding of the heifers. Two of the trials were conducted in Africa and one in Asia.

Anderson (1933) reported that 27 of 32 Zebu (White Fulani) heifers receiving isocaloric supplements containing either 7.9% or 21.5% crude protein reached sexual maturity and became pregnant compared to 3 of 19 grazing poor pastures in northern Nigeria. Supplementary feeding was done individually. All heifers began the experiment at ages between 12 to 18 months and body weights of approximately 167 kg. The trial lasted for a year and final body weights were 217 kg for non-supplemented heifers and 268 kg for all others. First service by a bull kept with the heifers was used as the criterion for their of attainment of sexual maturity. It was further reported that increasing the dietary crude protein level did not influence sexual maturity.

Over the whole trial growth rate increased 100% in the supplemented heifers, regardless of composition of the supplement, compared to the those on pasture only. During

the rainy season no significant effect of supplementation on growth rate was observed. Over a four month period when amount of pasture was scarce but of good quality, supplementation improved growth rate but dietary protein level did not have an effect. It was only during a four month period of scarce grazing and low quality of the pasture (50% decrease in nitrogen content compared to previously) that increasing the dietary crude protein level significantly improved growth rate.

In this experiment it was demonstrated that age at sexual maturity and growth rate of Zebu heifers fed supplemental concentrate was improved compared to heifers subsisting on pasture only. No effect of dietary crude protein level was observed.

Oyedipe et al. (1982) in Nigeria began a trial with 60 Zebu heifers when they were approximately a year old and averaged 100 kg body weight. The heifers were divided into three equal groups and fed diets with different levels of crude protein. Dietary crude protein levels were 8.3%, 13.37% (NRC requirement for 0.5 kg gain/day) and 19.17% . The rations were based on corn silage. They were isocaloric and contained between 2.61 and 2.79 Mcal/kg of metabolizable energy. Feeding was done individually and the heifers were kept in single pens. The average daily dry matter consumption was 3.5 kg/head.

A significant effect of dietary crude protein level on growth rate was observed. Growth rate was 0.12 kg/day for

heifers on the 8.3% CP diet and increased to 0.41 kg/day on the 13.37% CP. When dietary crude protein level was raised to 19.17%, growth rate of the heifers reached 0.58 kg/day.

The heifers were palpated weekly and puberty was determined by detection of a mature corpus luteum. Age at puberty differed significantly among protein levels. The high protein group reached puberty at 570.4 days, the medium group at 640.8 days and the low protein group at 704.2 days. The respective body weights at puberty were 207.1 kg, 187.0 kg and 161.7 kg. Bulls were introduced after the heifers reached puberty and once they exceeded 200 kg live weight. Observation of estrus began at the same time and was carried out four times daily.

Nutrition affected conception rates in the heifers. On the high protein diet 58.8% became pregnant by the 90-day post-breeding period while only 27.8% became pregnant on the medium protein diet. On the low protein diet the figure was even lower, 16.7%. Body weight at conception averaged 243 kg and did not differ significantly among the treatments.

It was concluded that high protein diets caused a faster growth rate. This led to an earlier onset of puberty at a lighter body weight and higher pregnancy rates compared to diets limited in protein content. Oyedipe et al. (1982) suggested that by increasing dietary crude protein content the traits for puberty and conception rates could be

improved in Zebu heifers.

In the Philippines Trong Trung and Escano (1980) did a trial with twenty four yearling crossbred heifers with initial body weights between 119 and 203 kg. The heifers were reported to be dairy heifers, but the breeds crossed were not defined. They were divided into six groups and fed corn-silage-based complete rations containing either 69% or 72% TDN and one of three protein levels; 9% CP , 11% CP or 13% CP. The heifers were fed ad libitum and were kept in individual stanchions, but turned loose twice daily for exercise and observation of estrus behavior. The climate was humid tropical. First standing estrus was used as criterion for onset of puberty.

Protein intake increased significantly, from 420 to 637 g/day, as protein content of the diet increased. Average daily gain was not significantly affected by varying the energy level in the diets, but showed a significant rise from 0.6 kg to 0.7 kg as the protein level was raised from 9.0% CP to 11.0% CP. No further increase in daily gain was observed when dietary crude protein level was increased to 13.0%. Heifers on the low-protein ration consumed less DM, 4.65 versus 4.90 kg/day, compared with those fed high protein rations. However, the difference was not significant. No significant difference was detected in TDN intake between treatments and average TDN consumption was 3.40 kg/head/day. Feed conversion improved with higher energy (7.79 versus 6.90 kg DM/kg gain) and protein level

(7.94 versus 7.03 kg DM/kg gain) in the diets apart from the diet with 13% CP (7.08 kg DM/kg gain), where no improvement could be seen.

No significant effect of dietary energy or protein level was observed on age and body weight at puberty or first breeding. The heifers averaged 15.6 months of age and 192 kg body weight at first standing estrus. Age at first breeding was 18.8 months and body weight 244 kg. Once the heifers weighed 250 kg they finished the trial.

However, the high protein diet was the most favorable in supporting pregnancy. The number of services per conception dropped from 4.0 to 2.0 to 1.6 going from the low protein to the high protein group. The opposite was seen for percent pregnancies, which increased from 25% to 50% to 100% with a higher dietary crude protein content. Expected age at first calving was calculated to be 27.2 months for heifers on the high protein diet. This was much earlier than the 33.4 months obtained from 12 contemporary herdmates outside the experiment.

Trong Trung and Escano (1980) concluded with recommendations to feed dairy replacement heifers under similar conditions a daily intake of 637 g CP and 3.47 kg TDN. They suggested ad libitum feeding of complete rations with 13% CP and between 69-72% TDN.

In conclusion, results from the three trials are contradictory. Raising the dietary crude protein level decreased age and increased body weight at puberty in the

trial by Oyedipe et al (1982), but no effect was observed by Trong Trung and Escano (1980) or Anderson (1933). Increasing dietary crude protein level from 8% to 19% improved growth rate (Oyedipe et al., 1982) and from 9% to 11% but not up to 13% (Trong Trung and Escano, 1980). Conception rates were improved by increasing dietary crude protein level (Oyedipe et al., 1982; Trong Trung and Escano, 1980). It is noteworthy that the heifers studied were at least a year old at the beginning of all three experiments. Further, the age at puberty independent of dietary treatment in the trials by Anderson (1933) and Oyedipe et al. (1982) was much higher than is desirable.

PLASMA THYROID HORMONE CONCENTRATIONS AS RELATED TO GROWTH RATE AND HEAT STRESS IN CATTLE AND TO DIET COMPOSITION IN BOTH CATTLE AND HUMANS

Information about thyroid hormone concentrations in growing calves is scarce. Only three relevant experiments have been reported. These experiments are the most pertinent to the author's study. They are therefore discussed under one heading, despite the fact that they cover both growth rate under thermoneutral conditions and under heat stress and a comparison between effect of different diets on thyroid activity.

The continuation of the discussion of thyroid activity is divided according to topics and one part of a trial may therefore be reviewed under one heading and another part in a different section.

Thyroid hormone concentrations in growing calves

Thyroid hormone concentrations in growing calves were studied by Kahl et al. (1977). They measured plasma concentrations of thyroxine (T4) and triiodothyronine (T3) in Holstein calves of both sexes from birth through 22 weeks of age. The calves were fed standard calf starter diets and they were not subjected to heat stress.

No differences in thyroid hormone concentrations were

seen between the sexes for the first four weeks when body weights were similar for heifer and bull calves. During the time from six to 22 weeks the bull calves grew at a rate of 0.93 kg/day compared to 0.73 kg/day for the heifer calves. The male calves also had higher plasma T4 and a tendency towards higher T3 concentrations over this period. Only values of thyroid hormone concentrations for female calves are included below since these are most relevant to the author's study.

Concentrations of the two hormones were very high immediately after birth reaching values of 145 ng/ml for T4 and 5.56 ng/ml for T3. Already after a week the original concentrations had fallen by 80% and minimal values of both hormones (29 ng/ml of T4 and 0.68 ng/ml of T3) were measured at six weeks of age. T3 then increased to a concentration 80% higher and equal to a normal adult value, 1.39 ng/ml, when the calves were 18 weeks old. The increase of T4 was 33 to 50 % during the same period but was not significant and concentration was raised to 48 ng/ml. At 22 weeks of age when the final measurement was taken the concentration of T4 was 39 ng/ml and 1.58 ng/ml for T3. The pattern of changes in thyroxine and triiodothyronine were similar but the ratio between them (T4:T3), combined for both sexes, changed over time. It was augmented from 27.1 at birth to 55.2 six weeks later only to decrease to 37.5 as the calves reached 22 weeks of age.

Effects of heat stress on growth rate and T3

concentration were studied by Baccari et al. (1983) in a trial with 5 months old Holstein heifer calves. The experiment was conducted in a climatic laboratory, where the calves were initially subjected to three weeks of thermoneutral conditions. Then followed controlled heat stress (32.5 to 34 °C)for five weeks before a return to thermoneutral conditions for four weeks. The last period was included for study of postheat compensatory effects. The calves were fed 3.6 kg/day/calf of a calf ration concentrate with approximately 20% CP and given alfalfa cubes ad libitum.

Feed conversion increased and the concentration of T3 decreased with heat stress. During the period after heat stress T3 concentration and daily weight gains increased significantly showing strong compensatory responses. Average daily gain declined from almost 1 kg/day under thermoneutral conditions to 0.7 kg/day during heat stress and then increased to 1.45 kg/day during a second thermoneutral period. Feed intake was similar during thermoneutrality and heat stress and was 5.6 and 5.8 kg/day respectively. Intake was augmented to 7.2 kg/day when conditions of thermoneutrality were reinstated after heat stress. Feed efficiency decreased during heat stress and went from 6.07 kg DM/kg gain under thermoneutral conditions to 8.75 kg DM/kg gain when the calves were exposed to heat stress. Its lowest value , 5.19 kg DM/kg gain, was registered during the postheat period. T3 values averaged

1.65 ng/ml with thermoneutrality, while the concentration decreased to 1.16 ng/ml under heat stress and reached an average value of 2.20 ng/ml with the return to thermoneutral conditions. A significant positive correlation (r=0.91) between T3 and daily weight gains were found.

Baccari et al (1983) concluded that the experiment demonstrated negative effects of heat stress on plasma T3. It also showed a postheat compensatory effect of increased growth rates and higher plasma T3 concentrations. A positive correlation between plasma T3 and growth rate was found and it was suggested that a higher concentration of T3 will be related to a higher growth rate.

A study of how tropical climate affect growth rate in calves was conducted by Reese (1983) under humid subtropical conditions in Mexico. Purebred Holstein heifer calves were either grazing on star grass pasture only or supplemented with concentrate or concentrate plus Monensin. Average temperature and humidity during the trial was 26^{o}C and 74% relative humidity. The supplemented groups grew at a rate of 550 g/day for the concentrate group and 580 g/day for the concentrate + Monensin group, while non-supplemented calves only gained 200 g/day. Plasma T3 concentrations of the calves followed the growth rate and measured 1.07 ng/ml, 1.21 ng/ml and 0.76 ng/ml for the respective groups.

Conclusions about thyroid hormone concentrations in growing calves can be drawn from the three trials. Plasma T4 and T3 concentrations are high at birth and changes in

their concentrations occur over the first 18 to 22 weeks before normal adult values are established (Kahl et al., 1977). Heat stress, whether simulated in a climatic laboratory (Baccari et al., 1983) or humid subtropical conditions (Reese, 1983), depresses plasma T3 concentration. Plasma T3 concentration appears to have a positive relationship with growth rate (Baccari et al., 1983; Reese, 1983).

Effect of heat stress on thyroid activity in dairy cows

The majority of studies of the effect of heat stress on thyroid activity and other physiological parameters have been conducted in climatic laboratories and not in the tropics. A number of experiments have been reported from the Missouri climatic laboratory, where Johnson and collegues are the principal investigators (Baccari et al., 1983, Johnson and Yousef, 1966, Yousef et al., 1967, Yousef, Kibler and Johnson 1967, Vanjonack and Johnson, 1974 and Magdub et al., 1981).

Effects of heat stress on thyroid activity in the bovine can be further understood by studying experiments conducted with dairy cows. A trial with non-lactating Holstein cows by Yousef and Johnson (1966) separated the direct effects of temperature from secondary effects of a reduction of feed intake and body weight gain under heat stress. They measured the disappearance rate of [I^{131}]

thyroxine from blood and the concentration of protein-bound iodine in the plasma of cows exposed to 18°C and 35°C for two weeks of each. One group was fed ad libitum at both temperatures, while the other was given the same amount of feed eaten in a thermoneutral environment at the high temperature by putting refused feed into the rumen through a rumen fistula. The rate of disappearance of [I^{131}] thyroxine measures the rate of thyroxine degradation. The rate of degradation equals the rate of thyroxine secretion from the thyroid gland if the animal is in a steady state. The plasma protein-bound iodine concentration measures the concentration of thyroid hormone in the plasma (Yousef and Johnson, 1967a). Both the rate of disappearance of [I^{131}] thyroxine from blood and the concentration of protein-bound plasma iodine in the plasma were reduced in both groups of cows. This showed that heat exposure alone lower the secretion of thyroid hormone.

It was concluded that heat acclimation caused a decrease in thyroid activity and that this was a direct effect of high environmental temperatures and not primarily due to reduced feed intake.

Yousef, Kibler and Johnson (1967) investigated the influence of time on the changes of thyroid activity during heat stress in Holstein cows. The method for control of feed intake used by Yousef and Johnson (1965) was also used in this trial. After three weeks adjustment period at 18°C and 50% relative humidity the cows were exposed to a week of

38°C (relative humidity remained the same) before the return to thermoneutral conditions. Measurements of thyroxine [I] disappearance rate and plasma bound iodine were taken at 12, 36, 60, 84 and 108 hours exposure of each temperature. It was found that at least 60 hours of heat exposure were required for a significant change in thyroid activity. Readjustment to control concentration after the animals were returned to a thermoneutral environment required 108 hours.

The conclusion was that the thyroid gland adjusts slowly to an increase in environmental temperature and therefore mainly influences the later period of adaptive changes known as acclimation or the compensation stage.

In a later study by Vanjonack and Johnson (1975) 170 lactating Holstein cows were subjected to a short, 18 hours, exposure to 30°C and 50 % relative humidity once during spring and then again during fall. Plasma thyroxine concentrations of the cows exposed to 30°C remained similar to those measured during control conditions of 15°C and no effect of heat stress could be observed. This further confirmed the slow adaption of the thyroid gland to environmental heat exposure.

Decreased plasma concentrations of T4 and T3 and a reduced thyroid secretion under heat stress, were observed in lactating cows by Magdub et al. (1982). In their trial the cows were kept under thermoneutral conditions of 17.6 °C for 10 days before exposure to 31.2°C for 10 days. Plasma

thyroxine concentrations of the cows declined significantly ($P<0.01$) from 79.11 ng/ml under thermo neutral conditions to 66.07 ng/ml under heat stress. Plasma T3 concentrations showed a similar pattern with concentrations decreasing significantly ($P<0.01$) from 1.46 ng/ml at the lower temperature to 0.62 ng/ml under heat stress. The decline of the thyroid hormones was parallel with changes in milk yield, which dropped from 19.3 kg/day to 11.9 kg/day with the increase in temperature.

Magdub et al. (1982) concluded that reduced plasma T4 and T3 during heat was a reflection of a decrease in synthesis of the two thyroid hormones. They suggested that a decrease in TSH occurred due to heat and that a hypothalamic reduction of TSH synthesis must be involved.

Similar results were obtained by Johnson et al. (1966) in a study with lactating cows subjected to a 3-day heat exposure. Preceeding the heat stress was a thermo neutral period at 20°C and after the three day exposure to 31°C the cows were returned to another thermo neutral period at 20°C. During these three periods plasma thyroxine concentrations changed from 51.1 ng/ml to 33.1 ng/ml and back up to 43.2 ng/ml with the change in temperature. Parallel changes in plasma T3 were observed and the concentrations corresponding to the three periods were; 1.2 ng/ml, 0.64 ng/ml and 0.9 ng/ml. Each of the changes in T3 and T4 concentrations were significant on the ($P<0.05$) level.

The experiments with dairy cows demonstrate that heat

has a primary depressing effect on thyroid activity independent of feed intake (Yousef and Johnson, 1966). The adaptive changes in thyroid activity occur slowly and contribute to acclimation. A reduction in thyroid activity can be observed only after at least 60 hours heat exposure and even longer time appears necessary for a return to thermoneutral concentrations of T4 and T3 (Yousef, Kibler and Johnson, 1967). Several experiments with both non-lactating and lactating cows substantiate the findings of significantly depressed thyroid hormone concentrations during prolonged heat stress (Vanjonack and Johnson, 1975; Magdub et al., 1982).

Additional factors affecting thyroid hormone concentrations in dairy cows

Experiments with dairy cows have been used to explain effects of heat stress on thyroid hormone concentrations. In some of the trials additional factors were studied and found to affect thyroid activity. Due to the complexity of factors affecting, and interacting to cause changes in, thyroid hormone concentrations it is of value to point out the additional findings. Some relevant results from trials by Refsal et al. (1984) and Gerloff et al. (1986) are also included below, although dairy cattle in their trials were not subjected to heat stress.

Gerloff et al. (1986) provided important information

about the interrelationship between T4 and T3 and the free fraction of each hormone from a trial with 80 dairy cows from nine herds. It was reported that concentrations of T3 and T4 were well correlated and the free fractions of the hormones reflected concentrations of total hormones. There was no change over time of the percentage of hormone in the free fraction, indicating that thyroid-binding capacity of serum hormones remained constant. Percentage of the respective hormone circulating as free hormone was 0.012 for T4 and 0.228 for T3. The parallelism between the free fractions of the hormones and their total concentrations indicates that in a normal dairy cow total T4 and T3 accurately reflects thyroid status.

Vanjonack and Johnson (1975) found that non-bred lactating cows had a lower T4 concentration than pregnant cows had during their first trimester of gestation (54.9 vs 66.1 ng/ml). Plasma T4 also increased from the first to the second trimester of gestation (73.8 ng/ml) and remained high throughout pregnancy. A difference between thyroid activity in pregnant lactating cows and pregnant heifers seems to exist. Refsal et al. (1984) studied pregnant Holstein heifers in an experiment to see if T4 and T3 would change during pregnancy. Neither of the hormones presented differences among months. The respective levels were 58.6 ng/ml for T4 and 1.36 ng/ml for T3. The discrepancy may be related to the fact that the cows were lactating during the gestation and the heifers were not. This is supported by

further findings by Vanjonack and Johnson (1975), who demonstrated an inverse relationship between plasma T4 and lactational intensity of the cows. High producing cows averaged 49.8 ng/ml of T4, while the medium and low production groups had significantly higher plasma T4 (62.4 vs 75.6 ng/ml). Refsal et al. (1984) made similar observations in a trial with dairy cows and suggested that a negative energy balance, due to high milk production during early lactation, could cause reduced concentrations of thyroid hormones. Decreased concentrations immediately post-partum and then an increase with time was also observed in Holstein cows by Gerloff et al. (1986).

An inverse relationship between age and thyroid hormone concentrations in dairy cows was observed in a field survey of 13 Holstein herds by Refsal et al. (1984). Older cows had lower concentrations of T4 and T3. A seasonal effect was also demonstrated by the survey. Thyroid hormone concentrations were lower during winter than summer. A considerable herd variation in concentrations of thyroid hormones was seen by both Refsal et al. (1984) and Gerloff et al. (1986). It was speculated that several factors such as herd effects confounded by season effects or genotype differences between herds could have caused the variation. Diet could also have been confounded with herd effect. Both energy content and amount of carbohydrates or glucose precursors may have affected results of the trial according to Gerloff et al. (1986).

It can be concluded that apart from heat affecting thyroid activity in dairy cows activity is also influenced by physiological state of the cows (Vanjonack and Johnson, 1975; Refsal et al., 1984; Gerloff et al., 1986). Differences exist between non-bred lactating cows compared to pregnant lactating cows and between pregnant lactating cows and pregnant heifers (Vanjonack and Johnson, 1975). Lactational intensity is inversely related to plasma thyroid hormone concentrations (Vanjonack and Johnson, 1975) and there are also decreases immediately post-partum followed by subsequent increases again with time (Refsal et al., 1984; Gerloff et al., 1986). In addition, thyroid hormone activity is apparently inversely related to age after mature values have been established (Refsal et al., 1984). Large herd variation (Refsal et al., 1984 ; Gerloff et al., 1986) and seasonal effects (Refsal et al., 1984) are also reported.

Effect of level of nutrition and diet composition on thyroid activity in cattle.

Effects of starvation and refeeding of a complete diet on plasma thyroid hormone concentrations in cattle have been studied by Blincoe and Brody (1955). Later, effects of short term fasting, less than 12 hours, and refeeding at various environmental temperatures were investigated by Yousef and Johnson (1967). However, very little data of

effect of diet composition on thyroid activity in the bovine have been reported.

Four different experiments investigating the effects of level of nutrition on thyroid activity in beef cattle were conducted by Post (1965). In the first trial a 52% increase in plasma protein-bound iodine (P.B.I.) was observed in eight group-fed ,cross-bred steers when supplemented with 20 lb/head/day of lucerne hay (16% CP) during eight days compared to a control group grazing poor quality native (spear grass) pasture.

Forty one spayed heifers of British and British - Zebu crosses were used in a the second study, where effect of peanut meal supplementation on growth rates of beef cattle on natural pastures was studied. The supplemented heifers were group-fed approximately 2 lb/head/day of peanut meal. After four months P.B.I. was significantly higher in the supplemented group.

In the next experiment 10 cross-bred growing steers were fed a constant amount of milled lucerne hay (13-15% CP) during a year. The ration was designed for an initial daily gain of 1 lb/head/day and provided only maintenance requirements during the last two months of the trial. Decreases in P.B.I. and thyroid secretion rate paralleled the decline in feed intake per unit metabolic weight as the steers grew until growth stopped after 10 months.

Effects of ad libitum feeding of three different rations, lucerne hay, non-legume hay or ground sorghum

plus non-legume hay, to 18 steers were investigated in the last trial. At the end of the five week trial P.B.I and thyroid secretion rates were lower in steers fed only non-legume hay compared to the other two rations which resulted in similar and higher values. It was reported that daily individual feed intakes could only be roughly estimated and not accurately measured. It was approximately 20.8 lbs for lucerne hay (16% CP), 13.4 lbs for non-legume hay (10% CP) and 8.5 lbs of the same hay when fed in addition to sorghum (11% CP) with an intake of 11.9 lbs.

From the four experiments Post (1965) conluded that various types of improved nutrition had significant effects on thyroid activity. Differences in thyroid activity between animals and seasons could then be explained by differences in quality and quantity of pasture intake. It was further stated that higher and more consistent effects were seen with the higher protein supplements of lucerne hay and peanut meal, but because additional sorghum also caused an effect Post (1965) speculated that maybe energy intake could have an effect too.

More recently Magdub et al (1982) fed lactating dairy cows either a low fiber or a high fiber diet (30% vs. 70% Corn silage) and found no significant differences in plasma T4 or T3 concentrations. A trend was seen for the low fiber group to have lower T4 and higher T3 than the high fiber group. A suggested reason was greater energy turnover in the low fiber group. However, digestible energy

intake was higher for the low fiber than the high fiber group (34.6 vs. 27.6 Mcal/day) and protein content 2.5% higher in the high fiber diet (15.2% vs. 17.7% CP). It was not clearly stated if these differences were significant, but the magnitude indicate that they might have confounded the results.

Gerloff et al. (1986) reported that Holstein cows fed 17 g supplemental inositol had significantly lower plasma T3 concentration than cows receiving a placebo. The respective T3 concentrations were 1.07 ng/ml and 1.19 ng/ml. Average concentration of T4 was not affected by inositol supplementation. It was 31.4 ng/ml for cows given inositol and 34.5 ng/ml for the non-supplemented cows. The investigators found the effect of inositol on plasma T3 unexpected. They conluded that no apparent explanation, consequences and implications existed at the time.

Early trials indicate effects of improved nutriton on thyroid activity (Post, 1965). More recent trials indicate no effect of dietary fiber (Magdub et al., 1982) and an unexplained effect of inositol (Gerloff et al., 1986) on thyroid activity. However, the effects of specific diet composition and quantity of included components such as amount of carbohydrate, protein or fat, on thyroid metabolism in cattle has so far not been studied with both the more refined techniques of radioimmunoassays and under well-controlled conditions .

Effect of level of nutrition and diet composition on thyroid hormone concentrations in humans

The effects of diet composition on plasma thyroid hormone concentrations are peritinent to the results of the author's study. A review of appropriate experiments with human subjects highlights this aspect of thyroid metabolism and is therefore included below.

A decline in T3 concentration can be induced by fasting in both animals (Blincoe and Brody, 1955; Yousef and Johnson, 1967) and humans. Such a decline in T3 and Free T3 was induced in 27 female and 18 male obese euthyroid patients aged 20 to 60 years by four days fasting in a trial by Azizi (1978). The subjects were then refed with either a mixed diet or a carbohydrate diet. This caused a reverse of the change in serum T3 and the concentration returned to control value. Six subjects were refed a protein diet, but this did not cause an increase in T3 concentration. Azizi (1978) stated that the lack of response to protein refeeding was unexpected since protein is largely converted to carbohydrate during a hypocaloric diet. In the same study an increase in T4 and Free T4 concentrations was also seen after fasting and a decrease in their concentrations after refeeding with a mixed or carbohydrate diet. This is not in agreement with several other studies with fasting, refeeding and overfeeding in humans, where no change in T4 concentration was observed under these conditions (Danforth

et al.,1979; Davidson and Chopra, 1979; Spaulding et al., 1976 ; Grant et al.,1978; Glass et al., 1978).

A decrease of T3 concentration was also observed when hypocaloric diets restricted in carbohydrate were fed to human subjects (Danforth et al., 1979; Spaulding et al., 1976). Changes similar to those found during complete starvation also occurred in the concentration of T3 after one week of a protein supplemented fasting diet containing almost no carbohydrate. T3 concentration continued to fall and was at its lowest after six weeks of a protein supplemented fast (Danforth et al., 1979).

Danforth and his group have done several studies, of the effects of diet composition and thyroid hormone concentrations using human subjects. In 1976 this group reported an increase in serum T3 concentration during carbohydrate overfeeding in both lean and overweight volunteers. Overfeeding with fat or an increase in body weight did not influence serum T3 concentration. Danforth et al. (1975) and Danforth et al. (1979) observed that short-term overfeeding (three weeks) of human subjects with either carbohydrate, fat or protein increased metabolic clearance rate and production of T3 leading to increased serum T3 concentrations. These changes in T3 concentrations were detectable within two or three days of overfeeding and reached a new and relatively stable concentration after a week of overfeeding. Thyroxine concentration was unaltered by overfeeding in these two studies.

Davidson and Chopra (1979) conducted another short-term study from which they evaluated the effects of changes in dietary carbohydrate content and excessive caloric consumption on circulating thyroid hormone concentration. In the study six normal weight human subjects were fed five separate diets; three isocaloric diets with 20%, 40% and 80% carbohydrate and two hypercaloric (> 2000 calories) diets with 20% or 40% carbohydrate for five days. The results showed that T4 concentrations was unaltered and the effect of diet remained non significant at the $P<0.05$ level with all diets. A significantly increased T3 concentration was observed after both hypercaloric diets compared to the iso-20% and iso-40% diets and after the iso-80% compared to the iso-20% diet. The highest correlation was found between T3 concentration with total calories ($r=0.68$; $P<0.001$) and a higher correlation with intake of carbohydrate ($r=0.46$; $P<0.025$) than with protein ($r=0.30$; P=NS). This suggested that non-carbohydrate sources as well as carbohydrate sources are important modulators of plasma T3 concentrations in humans. It appeared that in the short term the influence of total calories may actually be more pronounced than that of carbohydrate when at least an adequate amount (200 g) of carbohydrate is ingested daily.

During long-term overfeeding , more than, seven months, of four human subjects carbohydrate appeared to be the key ingredient in the diet affecting serum concentration of T3 (Danforth et al., 1979). The volunteers overfed a mixed

diet with either a low or a high level of carbohydrate and gained a 25% increase in body weight accompanied by a higher serum concentration of T3. An increase in T3 concentration also occurred before weight was gained when a weight maintenance-low carbohydrate diet was replaced by an equal-caloric high carbohydrate diet. No further increase in T3 concentration resulted when the high carbohydrate diet was given after weight was gained and maintenance established on the increased intake. Measurements of thyroid hormones in the long-term overfeeding study were performed after realtively long (four week) periods at stable weight and not while the subjects were gaining weight, as was the case in the short-term studies. The serum concentration of T3 also increased when carbohydrate was isocalorically substituted for fat in the diet.

Danforth et al. (1979) further measured increased thermogenesis in subjects overfed carbohydrate and suggested that metabolic clearance rate and production of T3 might be responsible for this increase in thermogenesis. During starvation the opposite is seen and energy utilization decrease and it is possible that a decreased production of T3 might be reponsible. This group continued to state that it is now recognized that caloric restriction lowers not only T3 concentration but also the nuclear T3 receptor capacity. This in turn suggests that the capacity to bind T3 to its receptor is a function of the nutritional state of the organism. During overfeeding an increase in the

concentration and production of T3 in conjunction with an altered or even an increase in receptor capacity, as suggested by the replenishment of T3 receptor capacity on refeeding could support a role for T3 in the increased energy utilization after overnutrition.

It was also detected (Danforth et al., 1979) that adjustments in pituitary responsiveness occur on overfeeding. In their experiments individuals overfed with carbohydrate accompanied by an increase in T3 concentration, showed no suppression in either basal concentrations of TSH or the peak TSH response to TRH.

Several conclusions about effect of diet composition, and particularly the effect of dietary carbohydrate, on thyroid activity can be drawn from these studies with human subjects. Refeeding with either a mixed or a carbohydrate diet reverses fasting induced-induced changes and causes an increase in serum T3 to control level, while refeeding with a protien diet does not have this effect (Azizi, 1978). A decrease of T3 concentration also occurs during protein supplemented fast containing almost no carbohydrate (Danforth et al., 1979) and after feeding of hypocaloric diets restricted in carbohydrate (Danforth et al., 1979) . Short-term overfeeding with both carbohydrate, fat and protein cause increased meatbolic clearance rate and production of T3, which results in elevations of serum T3 (Danforth et al., 1975). However, during a short time periods of a few weeks influence of total calories is

apparently stronger than that of carbohydrate, when at least an adequate amount of the latter is fed daily (Davidson and Chopra, 1979). During more than seven months of overfeeding, carbohydrate appears to be the key ingredient increasing plasma T3. An effect of fat can also be observed but to a lesser extent than carbohydrate. Protein does not elevate T3 concentration over long periods of overfeeding (Danforth et al., 1979). The mechanism of action for elevations of plasma T3 may be related to increased capacity of binding of T3 to its receptor (Danforth et al., 1979). In almost all reports T4 and Free T4 remain unchanged under conditions of changes in diet composition (Danforth et al., 1979; Davidson et al., 1979; Spaulding et al., 1976; Grant et al., 1978; Glass et al.,1978)

Overall conclusions about thyroid activity as related to growth rate, heat stress and diet composition

In calves plasma thyroid hormones concentrations are high at birth followed by changes during the first 18 to 22 weeks before normal adult values are established. Plasma T3 concentration appears positively related to growth rate and is depressed by natural and artificial heat stress.

The majority of trials demonstrating effect of heat stress on thyroid activity have been conducted in climatic laboratories. Very little data from the tropics is

available on this topic. Thyroid activity in both non-lactating and lactating dairy cows is depressed by heat stress independent of feed intake, although a decrease in feed intake may enhance the effects. Adaptive changes of thyroid activity take place in response to different environmental temperatures. These changes occur slowly and contribute to acclimation.

Information about effect of level of nutrition and diet composition on thyroid activity in cattle is scarce. Improved nutrition apparently increase thyroid activity and starvation has the reverse effect.

Studies with human subjects has demonstrated that energy level as well as the composition of the diet , especially the carbohydrate content, can affect plasma T3 concentrations. An increase of dietary carbohydrate level corresponds to elevated plasma T3 concentration.

MECHANISM FOR ONSET OF PUBERTY IN HEIFERS

A hypothesis to explain the endocrine mechanism for onset of puberty was proposed 25 years ago. At that time it was tested in rats and later in sheep. In the early seventies animal scientists studying puberty began to test the theory in heifers. The mechanism which initiates puberty is still not clear and more trials are needed for a complete understanding of the process. However, what began as a hypothesis for onset of puberty in rats more than two decades ago has greatly increased knowledge of mechanism for onset of puberty in cattle. The hypothesis and subsequent trials in cattle are reviewed in this section.

Ramirez and McCann (1963) offered a possible explanation of the mechansim behind onset of puberty, called the "gonadostat" hypothesis. A decrease in sensitivity to the negative feedback effects of estrogen on the hypothalamic-pituitary centers controlling gonadotropin secretion was proposed to be required for the onset of puberty. By increasing the treshold to the steroid negative feedback secretion of more of pituitary gonadotropin would be allowed and subsequently result in ovarian follicle maturation and ovulation. The hypothesis was first tested in female rats (Ramirez and McCann, 1963) and ewes (Foster and Ryan, 1979).

The "gonadostat" theory was later tested step by step in cattle. In general it was thought that maturation of the

hypothalamus, adenohypophysis and /or ovary may be required for the beginning of cyclic reproductive activity. Evidence for the negative feedback system being functional long before puberty was provided by Odell et al. (1970). A post-castration increase in LH secretion had been observed in a one month old heifer. Then Hobson and Hansel (1972) observed that increases in concentrations of LH followed ovariectomy in older female cattle. The reason for this was elucidated by Beck et al. (1976), who showed that implants of estradiol or progesterone decreased the elevations of LH observed after ovariectomy. This indicated that ovarian steroids had exerted negative feed back on LH secretion. It was further demonstrated that only estradiol in combination with progesterone caused a suppression of LH to concentrations similar to those in intact heifers. The interpretation of this was that a synergistic action of LH and progesterone inhibit LH release in the post-puberal heifer.

Five Brown Swiss females were used in the experiment by Schams et al. (1981). The calves were separated from their dams at birth and housed indoors. No information about feeding regime or weaning of the calves was provided. The animals were bled 3 times/ week from birth until 12 or 14 months of age. Bleeding took place every 6 hours from the first detection of estrus to the completion of the first estrous cycle. First estrus symptoms were noticed at 10, 11 or 14 months of age. The mean LH and FSH values increased

from birth to 3 months and then decreased reaching a nadir at 5 and 6 months. Later a second increase occurred and at 9 months of age another peak could be observed. Four of the five heifers reached puberty and went through their first estrous cycle at 10 and 11 months of age. The fifth heifer cycled for the first time at 14 months. The ovulations were confirmed by rectal palpations. It was concluded that the pituitary gland and ovaries are already able to respond to specific stimuli long before puberty and it is possible that they are involved indirectly due to changes in the feedback system modulating gonadotropin secretion. The pituitary gland is able to release LH and FSH after GnRH treatment at any age even when basal gonadotropin secretion is low. Serum concentrations of both LH and FSH appear to be elevated as the animal approaches puberty. Another conlusion was that ovaries of the prepuberal heifer can be artificially stimulated to ovulate before puberty, because of responsiveness to gonadotropins from an early age (Foote, 1974).

Regulation of LH secretion in prepubertal heifers at different ages was then studied by Schillo et al. (1982) to determine when and by what mechanism an increased treshold to negative feedback action by estradiol occur. Results from three experiments with ovariectomized heifers revealed that suppression of LH concentrations by estradiol lasted longer in the 4 month old heifers than in 8 and 12 month old heifers. Injections of different doses of

estradiol were given to Angus or Angus x Holstein heifers either four or eight months old and prepuberal or twelve months old and at puberty before ovariectomy. The effectiveness of estradiol in reducing numbers of LH pulses was inversely proportional to age, indicating that negative feedback response decreased before the onset of puberty.

The mechanism behind this is not known, but may according to Schillo et al. (1982) be related to uptake of estradiol by target cells of the hypothalamic-pituitary axis, similar to the way Kato et al. (1971) found that hypothalamic uptake of estradiol is greater in prepubertal rats than adult rats. It could also be related to the length of time that estrogen occupied its receptor and biological activity described by Ferguson and Katzenellenbogen (1977). Padmanabhan and Convey (1978) proposed that the inhibitory effects of estradiol on LH release in female cattle were not due to direct effects on the pituitary, because estradiol facilitated, did not inhibit, GnRH-induced release of LH from pituitary cells in vitro. Schillo et al. (1982) claimed that if the site of action is at the hypothalamic level then estradiol influenced both amount and frequency of GnRH release. It has been shown that estrogen increase cyclic GMP concentrations in uterine cells (Keuhl et al. 1974; Nicol et al., 1974) and it is therefore possible that estradiol blocked LH release via direct cytoplasmic action in cells of the hypothalamic-pituitary axis. In one of the experiments

reported by Schillo et al. (1982) both dose and age influenced duration of LH suppression, but only dose influenced magnitude of suppression. Differences in magnitude of suppression may have been related to the number of target cells influenced by estradiol. Duration of suppression may have been related to length of time estradiol-receptor complexes were present in target cells. Cells exposed to larger doses of estradiol would have had these complexes present for longer periods assuming that amount of hormone-receptor complex formed was proportional to dose and dissociation rate of the complex was independent of dose. In addition, association and dissassociation rates of the hormone receptor complex may change with age (Schillo et al., 1982).

The "gonadostat" hypothesis was further validated as the correct theory for the endocrine mechanism of onset puberty in heifers by Day et al. (1984). Two experiments were conducted with prepuberal heifers. The first trial included seven Angus-Hereford heifers subjected to a dietary intake manipulation to synchronize the reproductive state in all heifers on day 0 of the experiment. All heifers were weaned at 6.2 months of age and 180 kg BW and a dietary restriction was then imposed between 289 (9.5 months) and 508 days (16.7 months) of age to keep them prepubertal until the first day of the experiment. Both feed consumption and energy intake was restricted to maintain an average daily gain of 150 g/day and delay onset

of puberty. All heifers were then switched to a high energy diet on day 0 of the experiment. Simultaneously, three heifers were ovariectomized and three both ovariectomized and fitted with an estradiol-17b implant. One heifer remained as control. Over the first 12 days of the experiment dietary energy was gradually increased until the heifers gained 0.91 kg/head/day and was then continued on that level during the rest of the trial. Blood samples were taken from the jugular vein every 12 minutes for 8 hours prior to ovariectomy on day 0 and again bi-weekly through Day 100. Single blood samples were taken from the control heifer every third day to estimate onset of puberty. She was determined to be 578 days (19 months) old at puberty and weighed 306 kg at that time. A rapid increase in LH secretion was observed after ovariectomy in the three heifers subjected only to that treatment. Ovariectomized heifers with the estradiol implant had a continued low secretion of LH after ovariectomy until a rapid increase coinciding with puberty in the control heifer occurred simultaneously in all three heifers.

The second experiment had a similar design, but heifers were permitted to reach puberty spontaneously since the trial did not include any dietary restriction. Sixteen pre-puberal Red Angus-Hereford heifers, weaned at 5.4 months of age and 105 kg BW were used. The trial was initiated when the heifers were 266 days (8.8 months) old and had a body weight of 177 kg. The authors state that

this starting point was selected based on observations of age and weight of heifers of similar breeding during previous years. All heifers were therefore expected to be prepuberal and to reach puberty within 150 days or before 13.7 months of age. They were fed to gain 0.91 kg/head/day during the trial. Six heifers were assigned as controls and reached puberty spontaneously at an average of 384 days (12.6 months) of age and 282 kg body weight. Five heifers were subjected to ovariectomy and five to both ovarectomy and an implant with estradiol-17b. The ovariectomized heifers had a rapid increase in LH secretion after ovariectomy. This was similar to observations in the first trial. A "day of 0 inhibition" was determined in the ovariectomized plus implanted heifers to provide a physiological endpoint for them comparable to the endpoint of puberty for the control heifers. The individual "day of 0 inhibition" was defined as the day when LH secretion ceased to be influenced by estradiol negative feed back. LH secretion increased gradually in ovariectomized heifers with implants and " day of 0 inhibition" coincided with puberty in control heifers. A gradual increase in LH secretion was also observed in the control heifers approaching puberty.

From these trials it can be concluded that the "gonadostat" theory is a likely explanation of the endocrine mechanism for onset of puberty (Ramirez and McCann, 1963). A decrease in sensitivity to the negative feedback effects of estrogen would then be required for

onset of puberty. This would permit increased gonadotropin secretion leading to ovarian follicle maturation and ovulation. Trials with heifers have demonstrated that LH secretion in the prepuberal heifer is responsive to negative feedback and a rapid increase in LH secretion is observed after ovariectomy (Day et al., 1984). The treshold to negative feed back by estradiol increases with age as puberty is approached (Schams et al., 1981; Day et al., 1984; Schillo et al., 1982). Estradiol is shown to cause inhibition by influencing pulsatile mode of secretion of LH and inhibition is dependent on dose of estradiol (Schillo et al., 1982). The number of LH pulses suppressed by estradiol decreased with age. Suggested mechanisms of action of estradiol are direct cytoplasmic action or through hormone-receptor complexes (Schillo et al., 1982). Action of estradiol could then depend on the number of target cells influenced by and amount of estradiol taken up by target cells as well as characteristics of the hormone-receptor complex (Schillo et al., 1982).

PLASMA PROGESTERONE AS RELATED TO PREPUBERAL HEIFERS, TO CYCLING HEIFERS AND COWS IN THE TROPICS AND AS RELATED TO RELEASE OF PROGESTERONE FROM THE ADRENAL CORTEX IN CATTLE

The first of three sections about plasma progesterone is focused on the sexually immature heifer. Already in the prepuberal heifer plasma progesterone is of importance , because it is closely related to onset of puberty. Only a few experiments have investigated sources and concentrations of plasma progesterone in the young heifer approaching puberty. Some aspects remain unclear and all results are not in agreement, but the reports contribute to increased understanding of onset of puberty in heifers.

In the next section plasma progesterone concentrations in normally cycling heifers and cows are reported from different parts of the tropical world. Progesterone concentrations in both Bos Taurus and Bos Indicus cattle are included and concentrations in different breeds are compared. One trial conducted in a temperate climate is reviewed as a frame of reference.

The final section centers around trials demonstrating release of adrenal progesterone, which resulted in increases in systemic plasma progesterone concentrations. Elevations of plasma progesterone by release of adrenal progesterone due to stress is reported to be of such magnitude that they may confound interpretations of plasma progesterone measurments in trials like the ones reviewed below. It is

therefore important to call attention to the fact that all plasma progesterone measured in female cattle may not be of ovarian origin.

Sources and concentrations of plasma progesterone prior to puberty

Prepuberal concentrations of plasma progesterone were determined to be very low, around 300 pg/ml, in six half-sib Angus heifers used in a trial by Gonzalez-Padilla et al. (1975). Six paternal half-sib Angus heifers born within a 45 day period were weaned at six months of age and then fed to gain approximately 150 g/day until 14 to 15 months of age. At this age a blood sample was collected from each heifer and a week later the feeding regime was changed. Amount of feed was continuously increased over eight days until the heifers were eating alfa alfa hay ad libitum and 2 to 3 kg shelled corn/head/day.

Observation of estrus behavior was initiated 40 days before the first blood sampling. After the beginning of blood collection estrous behavior was observed every six hours. Rectal palpations were performed weekly during the sampling period. At 14.5 months of age the heifers had a permanent canula inserted in the jugular vein and blood was collected every six hours daily and every 20 minutes once weekly. Progesterone concentrations were determined in four samples pooled daily.

Before first ovulation in the heifers two distinct increases in plasma progesterone occurred. The priming peak of LH followed the return of the first progesterone peak to base-line value. After the second progesterone peak followed the puberal peak of LH. A transition of baseline concentration of LH from prepuberal to postpuberal concentration appears to take place between the two LH peaks, coinciding with the second progesterone elevation. This indicated that progesterone influences the alterations leading up to a pulsatile relase of LH, which is significant for the cycling heifer and cow. It is possible that a step by step increase in progesterone concentrations induces a gradual change of LH release to its mature pattern. Progesterone may act as a primer for maturation of the hypothalamic-pituitary-ovarian system.

The pubertal LH peak (Day 0) in the heifers occurred 30 to 60 days after the trial began when the heifer were between 15.5 to 16.5 months old. Three of the six heifers did not show estrus before the first corpus luteum was detected by rectal palpation or by serum levels of progesterone. All heifers did show behavioral estrus 18 to 21 days after day 0. Blood collection ended at the first mid-cycle.

Results from the trial indicated, according to the authors, that during the two months prior to puberty circulating levels of pituitary and hypothalamic hormones are not deficient. The cyclic pattern of release of LH is

lacking during this period, but becomes gradually established and appears mediated by progesterone.

In accordance with the results by Gonzalez-Padilla et al.(1975), Schams et al. (1981) found that progesterone concentrations remained low, below 0.1 ng/ml between one and nine months of age, in five Brown Swiss heifers used in the study. It was believed that the low progesterone concentrations in peripheral blood before nine months of age were of adrenal origin. Elevated progesterone concentrations up to 0.9 ng/ml were registered during 8 to 12 days before first estrus. Schams et al. (1981) stated, in agreement with previous findings by Gonzalez-Padilla et al. (1975), that this progesterone rise could play a key role in the establishment of a pulsatile pattern of gonadotropin secretion appropriate for the devlopment of an ovulatory surge. The first estrous cycle was preceeded by the transient increase in progesterone and began with estrus behavior in four of the heifers. Average length of their initial estrous cycle was 19 days. The fifth heifer is reported to have had a rise in plasma progesterone concentration to a maximal value of 1.2 ng/ml for eight days and also progesterone secretion similar to a normal corpus luteum during an 18 day period before first estrus.

Gonzalez-Padilla et al. (1975) further speculated that the first rise in progesterone prior to puberty might be of adrenal origin. In 1979 Berardinelli et al. conducted an experiment to determine source of progesterone prior to

puberty in beef heifers. As a result of the study it was claimed that both increases in plasma progesterone were due to luteinized tissue in the ovary of the prepuberal heifer. Presence of compact luteal tissue measuring 1.5 mm to 6.0 mm was observed in the ovaries of six Angus-Hereford heifers reported to be prepuberal. They had an average age of 13.5 months and their average body weights were 275 kg. These heifers showed one or two transient increases in plasma progesterone concentrations prior to the reported first ovulation. The increases in progesterone were from approximately 0.7 ng/ml to an average of 2.0 ng/ml and they persisted for four to seven days. After detection of the individual elevations in plasma progesterone concentrations the heifers were ovariectomized. Microscopic exam of the ovaries revealed that the luteal tissue was embedded within the ovary and could not be observed grossly on the surface or be palpated. The luteal tissue had differentiated luteal cells, but did not otherwise resemble a corpus luteum. It was a solid mass of round shape and was surrounded by a layer of connective tissue. The small size and lack of both stigma and residual cavity prompted Berardinelli et al. (1979) to speculate that the luteal tissue may have been derived from follicles which had not yet developed an antrum. They further saw no evidence for an entrapped ovum in any of the luteal structures , but they pointed out that these could easily have been missed with intervals of 1800 um between the histological sections of

12um. It was also reported that the control heifer, which was kept intact , ovulated and developed a corpus luteum without prior exhibition of estrus behavior.

Concerning the second elevation of progesterone the two groups were in agreement. Gonzalez-Padilla et al. (1975) had presumed the second progesterone peak to be due to ovarian progesterone secretion. This could then be explained by the priming peak of LH causing an induction of follicles or a corpus luteum, not detected by rectal palpation. The investigators went on to suggest the circulating concentrations of pituitary and hypothalamic hormones are adequate already two months before onset of puberty, but the cyclic pattern of LH is yet undeveloped. Progesterone could be the mediator causing the gradual maturation of phasic release of LH. Also Schams et al. (1981) suggested that the one transient increase in plasma progesterone observed before onset of puberty was due to small releases of LH and FSH and referred back to Gonzalez-Padilla et al. (1975).

Some conclusions can be drawn about plasma progesterone concentrations prior to puberty. Prepuberal concentrations of plasma progesterone are low. They measure 0.1 to 0.3 ng/ml according to Gonzalez-Padilla et al.(1975) and Schams et al.(1981), while 0.7 ng/ml was reported by Berardinelli et al. (1979). Prior to the first ovulation one (Schams et al.,1981) or two (Gonzalez-Padilla et al., 1975; Berardinelli et al. 1979) transient increases in plasma progesterone occur. They last four to

twelve days and reach a magnitude of 0.9 to 1.3 ng/ml. Gonzalez-Padilla et al.(1975) suggested that the first increase was of adrenal origin, while Berardinelli et al. (1979) demonstrated compact luteal tissue in the ovaries of prepuberal heifers. The plasma progesterone increase observed less than two weeks before first ovulation is apparently of ovarian origin. Only Gonzalez-Padilla et al.(1975) thoroughly discussed the importance of the observations. The hypothesis was that a gradual establishment of the pulsatile pattern of LH occurs during the two months prior to onset of puberty. The process, ultimately leading up to the puberal peak of LH, is mediated by progesterone. Observations of the priming peak of LH after the first prepuberal peak in progesterone and the puberal peak of LH after the second increase in plasma progesterone before the first ovulation supported the theory (Gonzalez-Padilla et al., 1975).

Plasma progesterone concentrations in cycling heifers and cows

Progesterone concentrations in peripheral plasma of six Friesian cows were determined by Stabenfeldt et al (1968). This trial was not conducted under tropical conditions but serves as a useful reference for normal plasma progesterone concentrations in mature cows not subjected to heat stress. The cows had completed at least two

lactations and had a normal breeding history. They were kept in a large paddock and fed alfa alfa and sorghum grain. Estrus behavior was observed and the day of psychic estrus determined to be day 0 of the estrous cycle. Daily plasma samples were taken during seven complete estrous cycles. Plasma progesterone concentrations increased from 0.5 ng/ml at estrus to an average of 6.6 ng/ml (6.1 to 10.2 ng/ml) during the peak luteal phase were observed. Day 3 to day 8 of the estrous cycle corresponded to a rapid rise in progesterone concentrations. From day 8 to day 17 progesterone concentration increased at a slower rate. Around day 18 - 20 plasma progesterone concentration decreased by 50 % from one day to the next. One to five days were required between the decline of progesterone and onset of estrus. The researchers remarked that the required time for follicle maturation may vary significantly between individual cows.

In one trial conducted under tropical conditions in Africa plasma progesterone concentrations were of similar magnitude to those measured by Stabenfeldt et al. (1968). Ayedemo and Heath (1980) determined plasma progesterone concentrations in five nulliparous (17 to 25 months old) heifers each of German Brown Swiss, Holstein-Friesian and White Fulani breeds in Nigeria. Apart from grazing star grass during five hours in the morning the heifers were kept loose in shaded pens in open housing the rest of the day. They had constant access to water and salt lick and

received supplemental feed of both cut grass and concentrate. Daily blood samples were collected during two consecutive estrous cycles during the wet and two cycles during the dry season. Plasma progesterone concentrations were below 1 ng/ml from the day before estrus until day 2 or 3. Elevations were observed from day 4 and the highest values, ranging from 3.6 to 7.6 ng/ml, were recorded between days 7 and 15 of the cycle. No significant effect of season on plasma progesterone concentrations were detected, but a breed effect was demonstrated. The Fulani heifers had lower mean progesterone concentrations during the luteal phase than the European breeds (4.5 vs. 5.0 ng/ml).

Breed differences were also observed by Randel (1977) in a trial, where plasma progesterone concentrations in Brahman, Brahman-Hereford and Hereford heifers were compared. It was demonstrated that both the Brahman and Brahman-Hereford heifers had lower plasma progesterone concentrations than the Hereford heifers on day 2 - 11 of the estrous cycle. During this time plasma progesterone in the Zebu and Zebu cross heifers increased from approximately 1.0 to 4.8 ng/ml and in the Hereford heifers from 1.4 to 7.0 ng/ml.

In a later trial Adeyemo (1987) reported plasma progesterone concentrations during normal estrous cycles in 19 postpuberal nulliparous heifers. Six German Brown Swiss, seven Holstein and six White Fulani heifers were

included in the trial. All heifers were exhibiting estrus regularly in cycles of 16 to 23 days. Location of the trial and mangement and feeding of the heifers were similar to the previous trial by Ayedemo et al. (1980). However, plasma progesterone concentrations in the heifers during normal estrous cycles were considerably lower than in the earlier trial. The peak concentrations of progesterone were similar and around 3 ng/ml for all heifers independent of breed. No reason is apparent for the different progesterone concentrations reported in heifers from different trials but of the same breeds and under the same management and climate conditions.

Jimenez et al. (1984) also reported plasma progesterone concentrations in Bos Taurus and Bos Indicus cows. This trial was located in the humid tropics of Mexico and six mature Brown Swiss and ten IndoBrazil cows were used in the experiment. The cows were grazing Guinea grass with access to mineral salts at all times. They were bled three times a week during the months of March and April to establish the pattern of progesterone concentrations during normal estrous cycles. Plasma progesterone in the Brown Swiss cows varied between 0.5 ng/ml to approximately 3.0 ng/ml during the luteal phase, while maximum concentrations were 2.2 ng/ml for the IndoBrazil. This difference was not significant and large individual variation was observed. Progesterone concentrations in the Brown Swiss cows in this trial were in agreement with Adeyemo (1987), while the

IndoBrazil cows had slightly lower values.

However, Vaca et al. (1983) reported higher plasma progesterone concentrations in 20 mature IndoBrazil cows in a trial preceeding that of Jimenez et al. (1984) at the same tropical location. The IndoBrazil cows were maintained on pasture of a type not reported by the authors. The cows were palpated and blood samples collected twice weekly for 7.5 weeks during July and August. From day 5 of the estrous cycle plasma progestrone increased to maximum concentrations of 3.1 ng/ml on days 9 and 10. The discrepancy beteen reported plasma progesterone concentrations in IndoBrazil cows from the two trials could possibly be due to a seasonal effect. The trial by Jimenez et al (1984) was carried out during the latter part of the dry period, while Vaca et al (1983) conducted their trial during the rainy season.

Low concentrations of plasma progesterone in Zebu females were also reported by Agarwal et al. (1977) in a trial with 20 Hariana cows in India. The cows belonged to a university farm. They were maintained in shaded paddocks with access to an open yard, but no other management details were given. Estrus behavior was observed morning and evening and a vasectomized bull and rectal palpation was used to confirm estrus. Blood samples from the jugular vein was collected 12 hours after onset of estrus and at eight to nine additional times during physiologically different stages of the estrous cycle. The cows were

inseminated at the time of heat and thereafter classified into pregnant and non-pregnant cows. Serum progesterone concentrations in the non-pregnant cows were approximately 1 ng/ml on the day of estrus through day three. A gradual increase until day 15 was then observed and the average maximum value was 2.32 ng/ml.

Gauthier and Thimonier (1983) studied effect of season in combination with different feeding and management on reproductive performance of 47 Creole heifers in a two year trial in Guadelope. During the first year from weaning at 120 kg body weight to first breeding at 250 kg the heifers were maintained unsupplemented on Pangola pasture. After the initial year they were confined in pens and fed Pangola grass during the first five months and then Pangola silage and also received an additional molasses-urea mix during the whole time in confinement. It was reported that no significant seasonal differences could be seen in maximum plasma progesterone concentrations. Plasma progesterone was 3.9 ng/ml in July versus 3.6 ng/ml in January. However, rises in progesterone concentrations during the initiation of the luteal phase was more rapid in January: 0.8 ng/ml/day, than in July: 0.6 ng/ml/day. No differences in plasma progesterone concentrations due to changes in feeding and mangement were reported.

Sergent et al. (1983), also working with Creole heifers in Guadelope, investigated whether direct exposure to sunlight would alter the ovarian secretion of progesterone.

Maximum concentrations of progesterone during the luteal phase of the estrous cycle was similar for the two groups (3 to 4 ng/ml) and similar to those previously reported by Gauthier and Thimonier (1983). No further details about the animals or mangement were provided.

It can be concluded that plasma progesterone concentrations in sexually mature cycling heifers and in cows in the tropics are in general lower than under temperate conditions. Maximum concentrations of approximately 2 to 4 ng/ml during the luteal phase are common (Adeyemo, 1987; Jimenez et al., 1984; Vaca et al., 1983; Agarwal et al., 1977; Randel, 1977; Gauthier and Thimonier, 1983; Sergent et al., 1983). This is about half the peak concentrations of plasma progesterone reported in Holstein cows in a temperate climate (Stabenfeldt et al., 1968). Significant breed differences in progesterone concentrations between Bos Taurus and Bos Indicus females are reported (Ayedemo and Heath,1980; Randel, 1977).

Secretion of adrenal progesterone

Secretion of adrenal progesterone has been studied by Balfour et al. (1957). They found that adrenal venous blood in cattle contains at least ten times more progesterone than arterial blood.

Results from an experiment by Gwazdauskas et al. (1972) indicated the possibility of ACTH being the

stimulatory factor in increased concentrations of progesterone in plasma in pheripheral circulation during stressful conditions. Seven mature Holstein cows, which had exhibited at least one estrus and were between 60 and 90 days postpartum were included in the trial. Four of them were between day one and seven of the estrous cycle and two between day nine and 13, while the last was approximately a month pregnant. After a three week adaptation period with regular handling, jugular canula were established. Once daily during two days four cows received 200 IU ACTH intravenously and three cows were injected with saline and on day three the treatments were reversed. Within five minutes after ACTH injection cortisol increased from a pre-treatment concentration of 3.9 ng/ml and reached an average peak of 45.8 ng/ml 25 minutes later. A significant difference in magnitude of cortisol response and time before the maixmum peak was observed between cows. Plasma progesterone showed a significant increase of 2.5 ng/ml within half an hour post-injection of ACTH and return to basal levels was complete 1.5 hours later. A large individual variation in progesterone response existed. However, the ACTH induced increase of plasma progesterone was present in all cows independent of stage of estrous cycle or pregnancy. Since the cows were intact, it was pointed out that the experiment did not differentiate origin of progesterone measured, but 200 IU of ACTH induced increases in systemic plasma progesterone. These increases

were believed to have been of adrenal origin, because the primary target of ACTH is the adrenal gland.

Watson and Munro (1984) further investigated stress release of adrenal progesterone and ovariectomized cows were used in the study. Five non-lactating dairy cows were ovariectomized three months before start of the trial. Results demonstrated that cows injected with 10 and 500 ug ACTH had significantly higher mean plasma progesterone concentrations than control cows receiving saline. Baseline concentrations of plasma progesterone were approximately 0.3 ng/ml. In three of the five cows progesterone concentrations increased to an average of 0.75 ng/ml with the lower dose and to 1.8 ng/ml with the higher dose of ACTH. Increasing the dose of ACTH caused plasma progesterone concentrations to remain elevated during a longer time period, from 105 to 225 minutes, after initiation of post-injection response. A large individual variation in response to ACTH was observed. The two remaining cows did not respond significantly to either dose of ACTH, but had elevated progesterone concentrations in some of the control samples. It was suggested that these increases were due to release of endogenous ACTH in the two animals. Especially one of the cows remained nervous during handling throughout the trial. It was further proposed that the lack of response in these two cows could be due to chronic stimulation of the adrenals, which might have led to refractoriness of the glands. Watson and Munro (1984)

speculated that the doses of ACTH caused production and release of sufficient amounts of adrenal progesterone to interfere with and lower the fertility of cows. This is in accordance with Wagner et al. (1972), who proposed that elevated ACTH-induced plasma progesterone during the early part of the cycle may feed back on the hypothalamus or the pituitary to block normal LH production and release.

According to Abilay et al. (1974) it is also possible that stressful conditions during prolonged heat exposure can result in release of higher amounts of adrenal progesterone than under temperate conditions. Depressed plasma cortisol was observed in 14 months old cycling Guernsey heifers subjected to heat stress during two estrous cycles after an adaptation period. It was proposed that the low concentrations of cortisol reflected inactivity of the 17-hydroxylating enzyme in the adrenal cortex under hot conditions. This enzyme is responsible for the synthesis of cortisol from progesterone. Therefore accumulation of metabolites of progesterone was enhanced. Increased ACTH stimulation from stress may then cause a release of accumulated progesterone, in addition to cortisol, from the adrenal cortex.

It can be concluded that stress causes a release of adrenal progesterone in intact cows (Gwazdauskas et al., 1972) and in ovariectomized cows (Watson and Munro, 1984) as demonstrated by injections of variable doses of ACTH. The amount of adrenal progesterone released in response to

ACTH and duration of the response are dboth apparently dose dependent and the average maximum elevations reported are 1.50 to 2.5 ng/ml and duration between 90 to 225 minutes (Gwazdauskas et al., 1972; Watson and Munro, 1984). Also plasma cortisol concentrations increase in response to injections of ACTH with an average peak concentration reported to be 45.8 ng/ml (Gwazdauskas et al., 1972). There is a large individual variation in both plasma cortisol (Gwazdauskas et al., 1972) and progesterone response to ACTH (Gwazdauskas et al., 1972 and Watson and Munro, 1984). An endogenous release of ACTH can possibly elicit a similar reponse with release of adrenal progesterone and an increase in plasma progesterone concentrations (Watson and Munro, 1984). The elevated plasma progesterone concentrations may interfere with reproduction (Wagner et al., 1972; Watson and Munro, 1984). It also is suggested that more adrenal progesterone may be released if stress occurs under prolonged exposure to heat (Abilay et al., 1974).

ONSET OF PUBERTY AND ESTROUS CYCLES IN HEIFERS IN THE TROPICS AND SOME CHARACTERISTICS PECULIAR TO REPRODUCTIVE PHYSIOLOGY OF ZEBU FEMALES

Age at puberty in heifers varies widely, depending on genetics, nutrition, climate and hormonal status. Heifers of European breeds under temperate conditions and on an adequate plane of nutrition commonly reach puberty at eight to nine months of age. A delay of onset of puberty in temperate breeds under tropical conditions have been reported. It has also been observed that Zebu and Criollo cattle reach puberty six to 12 months later than the European breeds.

In the first part of this section experiments conducted to determine onset of puberty in heifers in the tropics by use of rectal palpation of the ovaries and/or observation of estrus are reviewed. Characteristics of estrous cycles in the trials are also described.

The second part of this section is devoted to female Zebu cattle and some of their particular physiological characteristics, which may influence diagnosis of onset of puberty if this is determined by observations of estrus or by rectal palpation of the ovaries. Since plasma progesterone concentrations in Zebu females were reviewed earlier, that topic is excluded from this section.

Estrous cycles in puberal heifers in the tropics

The ovaries of 252 Brahman heifers and 60 crossbred heifers were examined by Plasse et al. (1968). The heifers originated from three farms in sub tropical north central and south Florida. A high management level was maintained on the farms and the heifers were fed at a norm above average for the area. They were weaned at 205 days of age. Observations were initiated when heifers with complete data were 8 to 13 months old and heifers with incomplete data were between 18 and 26 months old. All heifers were palpated monthly, but total time on the trial was variable among them. Puberty in the trial was defined by the finding of a corpus luteum by rectal palpation.

Among 83 heifers, who completed the trial, puberty was reached between age 14 and 24 months with an average of 19.4 months. Forty one additional heifers, without complete data, suggested that some Brahman heifers reached puberty before 14 months of age, while others were up to 26 months of age before the first corpus luteum could be observed. Neither season of birth nor the location of the ranch influenced age at puberty. Weaning weight, at 205 days, showed a correlation of around -0.40 with age at puberty in both the Brahman and the crossbred heifers. Sexual activity , determined by frequency of corpora lutea and by size and tonus of the uterus, increased during spring and peaked during summer. The seasonal variation was

significant. The investigators indicated temperature, nutrition and presence of bulls as the main influences on the endocrine system leading to a seasonal variation in sexual activity in Brahman heifers.

Gauthier and Thimonier (1983) demonstrated that Creole heifers around 14 months old on pasture without supplement in the humid tropics of Guadeloupe had a cyclic activity decreasing from 40 % in July (rainy season) to 0% in January (dry season). Despite major improvements of both feeding and management during the second year of the trial, seasonality in sexual activity persisted even when the heifers were adequately fed throughout a complete year. From the beginning of the second year they were fed Pangola grass or silage and molasses mixed with urea and confined to pens. This demonstrated that the seasonal variation was due to additonal factors apart from nutrition.

In another trial Plasse et al. (1970) studied reproductive behavior of 53 2-year old pure Brahman heifers raised on pasture. At this age rectal palpation revealed that 27 of the heifers had a small uterus with little tone and were reported as either prepubertal or anestrous. Of the rest a corpus luteum was detected in only 11 heifers. The trial took place under sub-tropical conditions and the heifers were fed according to norm in a controlled feeding scheme. They were fed 3.2 kg/head/day of a mixture of mainly corn with some cottonseed meal and alfa alfa pellets and had free access to coastal Bermuda grass hay and mineral

mix. All heifers were kept in a small field and pasture was limited. Five vasectomized bulls of Angus or Hereford breed were used for estrus detection in the trial.

Average daily gain was 0.38 kg averaged over the year. The only seasonal variation detected in this experiment was incidence of silent estrus, which was higher during winter. More than 25 % of all ovulations during winter were quiet ovulations. The heifers ovulated on the average 15.5 times per year and had 12.2 estrous periods during the same time. Individual variation between heifers was significant for length of estrous cycle, but the average cycle lasted 28.8 days. Three fourths of the estrous cycles ranged from 14 to 28 days. Estrus lasted for an average of 6.7 hours with 65.7% between 2 and 7.5 hours and the majority of estrous periods began during daylight hours. Ovulation occurred on the average 26 hours after the beginning of estrus.

Adeyemo et al. (1979) characterized the estrous cycles in German Brown Swiss , Holstein and White Fulani heifers in the humid, equatorial tropics during 15 months. The Holstein heifers had a significantly shorter estrous cycle, 20.1 days compared to 21 and 21.4 days respectively for the Brown Swiss and the Fulani heifers. Duration of estrus was longer, 16.2 hours, in the Brown Swiss heifers than in the other breeds. It lasted 15 and 14.6 hours respectively in the Holstein and Fulani heifers. Ovulation intervals after the end of estrus was around 14 hours for all breeds. Estrous cycles persisted throughout

the year and no significant seasonality was observed. These investigators suggest that environment did not influence estrous cycles of Brown Swiss, Holstein and White Fulani heifers.

Wiltbank et al. (1962) studied age and weight at puberty in Hereford and Angus heifers and their crosses. All together 64 heifers were used in the trial. The heifers were randomly assigned to one of two different nutritional levels used from weaning, which took place between 127 to 175 days of age, to puberty. Heifers on the high level of feed had access to a feeder with a concentrate of 40% beet pulp and 60% ground shelled corn during 14 hours daily and also received 1.5 kg wheat grass hay. Heifers on the low feeding level were given intermediate wheat hay ad libitum and 0.2 kg of a 40% CP concentrate daily. One sterilized bull was used for estrus detection. During day time he was kept with the heifers on the high nutrition level and during night time with the other group. The first ovulatory estrus was determined to be puberty. Incidence of silent ovulations were reported as neglible.

Average daily gain of crossbred heifers fed on the high plane of nutrition was 0.82 kg and for the purebred heifers 0.72 kg. Corresponding figures on the low plane of nutrition were 0.30 kg and 0.36 kg. Heifers on the high levels of feed, independent of breed, reached puberty at 381 days of age. On the low level of feed puberty was delayed until 424 days of age for crossbred heifers and the

purebred heifers were 572 days old at puberty. The heifers on the high level of feed were heavier at puberty, 330 kg for crossbreeds and 299 kg for purebreeds, than the heifers fed a low level of feed. The latter weighed 254 kg versus 268 kg respectively. When comparing age at puberty between purebred and crossbred heifers difference in growth rate accounted for a large portion of the difference in age at puberty.

In these experiments a late age at puberty is demonstrated in heifers of various breeds in the tropics and sub-tropics. Puberty is reported at an average age of 19.4 months and a range from 14 to 24 months of age in 83 Brahman and crossbred heifers. Detection of a CL by rectal palpation was the criterion used to determine onset of puberty (Plasse et al., 1968). The same criterion for puberty was used in a trial, where 27 of 53 two-year old Brahman heifers were determined to be prepuberal or anestrous and only 11 heifers had a corpus luteum (Plasse et al. 1970). Sixty four purebred and crossbred British heifers reached puberty at 381 days of age on a high level of nutrition and puberty was delayed until 424 and 572 days of age in crossbred and straightbred heifers respectively on a low level of nutrition (Wiltbank et al., 1962). First ovulatory estrus detected with the help of a vasectomized bull and confirmed by rectal palpation was the criterion for onset of puberty.

Seasonality in cyclic activity was observed in two

trials (Plasse et al., 1968; Gauthier and Thimonier.,1983). Variable characteristics of estrous cycles were reported. An average cycle length of 28.8 days and 6.7 hours duration of estrus (Plasse et al., 1968) compared to 20.1 to 21.4 days and 14.6 to 16.2 hours with breed differences indicated by the intervals (Adeyemo et al., 1979) was reported.

Some reproductive characteristics peculiar to Zebu females.

Reproductive characteristics specific for the Zebus were found by Aguilar et al. (1983) when they studied the histology of the Zebu corpus luteum and ovary. Zebus were determined to have a relatively small corpus luteum as well as ovary. This leads to difficulties in accurate detection of the corpus luteum. Accuracy , as defined by Vaca et al. (1983), to be progesterone concentrations exceeding 0.5 ng/ml corresponding to a palpated copus luteum was 76 %. Both Plasse et al (1983) and Irvin and Randel (1978) had previously reported similar findings of the Brahman corpus luteum being smaller by weight and lower in corpus luteum progesterone content than Hereford corpus luteum.

Cuq (1975) reported an experiment with 415 female Zebus, of the Maure and Gobra breeds and their crosses, in

tropical Africa. He found that the the corpus luteum of these Zebus showed a much slower regression than that of European breeds. The Zebu corpus luteum was frequently present and appeared to be functional at the following pro-estrus.

Rhodes et al. (1978) reported further that Brahman cattle have a lower preovulatory LH surge and a shorter time from estrus to ovulation than Hereford females. Time from the preovulatory LH surge to ovulation is the same for the two breeds. Griffin and Randal (1978) ovariectomized Brahman and Hereford cows and studied their release of LH after a high dose of gonadotropin releasing hormone. The Brahman cows released less LH than the Herefords. Fewer estrus behavior responses could be seen in ovariectomized Brahman cows given various doses of Estradiol-17B than in Hereford or crossbreed females , which received the same doses of the hormone. The responses were also delayed to 19.3 hours in the Brahmans compared to the crosses and Hereford cows, which responded after 12.8 and 10.1 hours respectively. This could be an indication of a different biological timing of estrus behavior patterns in the Bos Indicus compared to Bos Taurus (Rhodes and Randel, 1978).

In summary, the reproductive characteristics particular to Zebu females compared to those of European breeds include: A small ovary and coprus luteum, which may be almost completely embedded in the ovary (Aguilar et al. 1983; Plasse et al., 1973) and a low content of

progesterone (Irvin and Randel, 1978). The corpus luteum further shows a slow regression and may be both present and functional at the following pro-estrus (Cuq, 1975). Apart from progesterone content, the other characteristics of the ovary and corpus luteum may cause problems at rectal palpation.

The preovulatory surge of LH is low and a short time passes from estrus to ovulation (Rhodes et al.,1978). A comparatively small amount of LH is released after stimulation by gonadotropin releasing hormone (Griffin and Randel., 1978). Responses to injections of estradiol indicate a different biological timing of estrus behavior in the Zebu female compared to those of European breeds. In Zebu females the reponses are delayed and few signs of estrus behavior observed (Rhodes and Randel, 1978). These features may make detection of estrus more difficult in the Zebu females than those of European breed.

NONPUBERAL ESTRUS AND OVULATION WITHOUT ESTRUS

In this final section on puberty three experiments are reviewed to further illustrate the complexity of onset of puberty. Two reports introduce and present data to support a new concept called nonpuberal estrus - estrus behavior without ovulation in the prepuberal heifer. The two trials were conducted under different feeding regimes. Results from the third experiment demonstrate contradictory events, a very high incidence of ovulation without estrus. The feeding scheme imposed on the heifers in this trial was of a third type. The three experiments contribute to improved understanding of onset of puberty and also provide good examples of the importance of adequate feeding of heifers prior to puberty. None of these trials were conducted in the tropics. However, they are included in the literature review because the information is pertinent to the author's trial and no comparable trials have been reported from the tropics.

Nonpuberal estrus - estrus behavior without ovulation and formation of a corpus luteum in the heifer

A new concept in the context of onset of puberty have been introduced and studied during the last few years (Nelsen et al. 1985; Rutter and Randel, 1986) . Nelsen et

al. (1985) investigated a phenomenon they called nonpuberal estrus (NPE). Nonpuberal estrus was defined as occurrence of behavioral estrus in prepuberal heifers that were not followed by ovulation and formation of a corpus luteum and therefore no corresponding changes in plasma progesterone concentration.

Nelsen et al. (1985) conducted a two-year experiment with all together 360 heifers of seven different crossbreeds weaned at 180 days of age. They were then started on the trial and fed a growing diet ad libitum. On a dry matter basis the diet contained 94.5% corn silage and the rest was barley, soybean meal, urea, minerals and vitamin A. The heifers were kept in group pens with approximately 25 animals and a marker bull per pen. Each heifer was examined three times with 3 to 4 days interval after being marked by a bull. Three criteria were used for puberty; marking by a bull, rectal palpation of a corpus luteum and serum progesterone above 1 ng/ml.

It was demonstrated that an average of 16.6 % of the 360 heifers, although there were differences between the types of crossbreds used, had at least one occurrence of NPE. It was also reported that the average interval between first NPE and puberty was 89 days, but younger heifers had a longer interval to puberty (correlation age at NPE and interval -0.73, $P < 0.01$). Nonpuberal estrus heifers eventually reached puberty at ages and body weights that were not different ($P > 0.10$) from the other heifers.

It was speculated that certain physiological changes may trigger behavioral estrus prior to completion of all changes leading up to puberty.

Rutter and Randel (1986) defined nonpuberal estrus as standing estrus behavior not followed by an elevation in serum progesterone concentration. In contrast to Nelson et al. (1985) they did not perform rectal palpations of the ovaries, but assumed that neither ovulation nor formation of a corpus luteum had occurred if no increase in progesterone was found.

The trial by Rutter and Randel (1986) consisted of 43 Simmental-Hereford heifers 9 to 11 months old. There was a significant difference in initial body weight of the heifers. The heifers were therefore divided into two groups and fed differentially for four months to achieve a target weight of 330 kg at a predetermined date. Heifers weighing up to 240 kg were grouped as light-weight heifers and fed to produce a gain of 1.5 kg/day until the target date. They received 5.5 kg of a corn-cottonseed meal concentrate and Coastal Bermudagrass hay ad libitum. The heavy-weight heifers with body weights above 240 kg were fed 3.7 kg of the concentrate but had free access to the hay. The feeding regime was calculated for 0.7 kg gain/day. After the target date all heifers were fed the ration previously given only to heavy weight heifers. Throughout the study sterile marker bulls were kept with the heifers and estrus behavior was observed for one hour three times daily. The

heifers were apparently kept in group pens. Weekly blood samples were taken before the first behavioral estrus. Basal progesterone concentrations were less than 0.3 ng/ml. Values of more than 1 ng/ml during one or two weekly samples were designated as prepuberal elevations. After first behavioral estrus blood samples were taken daily from day 1 through day 14 of the first and following cycles. Puberal first estrus was determined in retrospect as standing estrus behavior followed by at least two consecutive daily samples with progesterone concentrations above 1 ng/ml.

Rutter and Randel (1986) observed a higher incidence of NPE in their trial than Nelson et al. (1985). A nonpuberal first behavioral estrus was observed in as many as 62.8 % of the heifers. A tendency for fewer light-weight heifers to exhibit a puberal first estrus was observed compared to the heavy weight heifers 31.2% compared to 68.8%. Heifers with a puberal first estrus were older than heifers with NPE, 376 days in comparison to 334 days of age. More heifers with a puberal first estrus had elevated plasma progesterone concentrations prior to puberty, 64.3% versus 20.0%. The plasma progesterone increases were also higher, 2.5 versus 1.2 ng/ml in heifers with a puberal first estrus than in the ones which had NPE. The heifers weighed approximately 290 kg at the first behavioral estrus independent of initial weight and whether first estrus was NPE or puberal.

It was proposed that the higher incidence of NPE in the trial compared to percentage NPE reported by Nelsen et al. (1985) could be due to differences in nutrition or breeds. However, it was not believed that the lower rate of gain of heavy-weight heifers during the initial four months was the reason. Since the heifers had similar body weights ,but showed variation in age at first estrus it was suggested that a target weight may induce a behavioral estrus. It was also thought that if a heifer was too young at first estrus, the maturation process eventually culminating in preovulatory LH surge may be incomplete. The result could then be NPE instead of a puberal estrus.

Both Nelsen et al. (1985) and Rutter and Randel (1986) concluded that NPE is neither an uncommon nor an abnormal event in prepuberal heifers. It was also pointed out by both groups that NPE could bias results in experiments, where behavioral estrus is used as criterion for onset of puberty. Therefore it was suggested that this criterion not be used alone when determining onset of puberty.

No general conclusions can be drawn about NPE from the scarce data presently available, but the main features can be summarized. Non-puberal estrus (NPE) is a new concept defined as a prepuberal heifer showing behavioral estrus not followed by an increase in plasma progesterone concentration (Nelsen et al., 1985; Rutter and Randel, 1986) and also no ovulation or formation of a corpus luteum (Nelsen et al., 1985). At least one NPE has been reported in 16.6 % of

350 crossbred heifers, but frequency of NPE was affected by breed. NPE occurred an average of 89 days before puberty and did not affect age and body weight at puberty (Nelsen et al., 1985). A much higher incidence, 62.8%, was observed in 43 Simmental-Hereford heifers (Rutter and Randel, 1986). The frequency of NPE was higher in heifers starting the trial with lighter body weights and in younger heifers compared to those starting at heavier weights and heifers older at first behavioral estrus (Rutter and Randel, 1986). NPE was further seen as a normal event in prepuberal heifers and one , which may confound determination of onset of puberty if behavioral estrus is the only criteria used (Nelsen et al. ,1985; Rutter and Randel, 1986).

Relationship between incidence of a first ovulation without estrus behavior (silent ovulation) and feeding regimes

Dufour (1975) randomly assigned 34 Holstein heifers approximately 143 days (4.7 months) old and with a body weight of 136 kg to either a moderate (MR) or fast-growing feeding (FR) regime for an initial period of 100 days. It was followed by a final period during which half of each group was subjected to the other feeding regime. The final phase ended with ovariectomy of the heifers after puberty. All heifers were fed corn-silage ad libitum. Amount of

concentrate was calculated for expected gains of 0.45 and 0.91 kg/day for the respective growth rates. They were maintained in a closed barn and group feeding was used. Estrus detection with vasectomized bulls introduced twice daily began when the first heifer reached 160 days of age. Standing estrus was used as criterion for puberty.

The results indicated that not only length of the period during which a fast-growing feeding regime is imposed, but also the time at which it is applied is important. Heifers fed for rapid growth only during the final phase reached puberty at an age (308 days old) similar to that of heifers subjected to the fast-growing regime over both periods (286 days old). On the contrary heifers beginning on the fast-growing treatment and finishing with moderate growth rates were approximately as old (344 days of age) as heifers which continued to grow at a moderate rate during the whole trial (353 days old).

Body weights seemed of higher importance than age for onset of puberty during the final phase of growth, because heifers reached puberty at similar weigths (257 kg FR-FR and 267 kg FR-MR) independent of age. The opposite appears likely for the first growth period, since heifers reached puberty at approximately the same ages (315 days FR-MR and 331 days MR-MR), while body weights differed.

During ovariectomy it was discovered that the first ovulation in most cases had occurred without standing estrus. This was determined by findings of corpora

albicantia in an average of 76.5% of the heifers at ovariectomy. Heifers on the FR-FR treatment had an incidence of 55% corpora albicantia, while 100% of the heifers on the MR-FR regime had ovulated the first time without standing estrus. In heifers finishing the trial with a moderate growth rate, regardless of treatment during the first period, incidence of corpus albicantes was approximately the same as the overall average. No statistical analysis was provided for incidence of corpora albicantia. It was suggested that previous conditioning by hormones secreted by the corpus luteum is necessary for the expression of a puberal estrus. Heifers exhibiting estrus at first ovulation might have had an estrogen concentration of such magnitude that the synergistic action of progesterone, normally necessary for expression of estrous behavior, was not required.

A third of the heifers which did exhibit estrus at first ovulation had an estrous cycle with a duration of less than 10 days. Dufour (1975) suggested this as an indication that the first corpus luteum is not as functional as the subsequently formed corpora lutea.

This experiment demonstrated that a medium or fast-growth feeding regime imposed on prepuberal heifers affected onset of puberty differently and that time when the treatment was imposed also was of importance. It seemed as if onset of puberty was more dependent on age during the initial period of growth and more depndent on weight

during the final growth period. Heifers growing at a fast rate during both periods or initially at a medium rate and then finished with a fast growth rate were of similar age at puberty. These heifers were also younger at puberty than heifers maintained on a medium growth rate throughout the trial and were younger than those finishing with a medium growth rate. However, ovarian activity was also influenced by feeding regime. A first ovulation without standing estrus was observed in 76.5% of all the heifers. The figures for heifers on FR-MR and MR-MR were similar and slightly above 70%. Heifers on MR-FR had a 100% incidence of a first silent ovulation, while only 55% of the heifers on FR-FR were lacking estrus behavior before first ovulation.

Finally, if the two variables, age at puberty and ovarian activity at puberty, are combined it can be observed that although no difference in age at puberty exist between heifers on FR-FR and heifer on MR-FR the latter group apparently had a 100% silent first ovulation compared to only 55% in the heifers growing fast throughout the trials. Not only did they reach puberty at a young age, but they also had the highest percentage of a puberal first estrus.

PRESENT STATE OF KNOWLEDGE ABOUT GROWTH RATE AND ONSET OF PUBERTY IN HEIFERS IN THE TROPICS

From the literature it can be concluded that the concept of puberty in heifers is not well defined. Investigators generally define puberty in each report, but its definition varies widely from trial to trial.

Although it is known that genetic composition and rearing conditions influence onset of puberty in heifers, results from trials where both definition of puberty, genetics of heifers and management conditions differ have frequently been compared.

The literature further shows that in the vast majority of experiments designed to study onset of puberty the heifers have not been started on the trials until they are 12 to 15 months old. In some trials the heifers have even been a year and a half to two years old at the start of the experiment. These ages represent the time when heifers of many breeds have already naturally gone through puberty and become completely sexually mature if they are managed properly.

The effects of nutrition and management on onset of puberty have consistently been overlooked. Heifers have in some trials even been purposedly undernourihed during a period before initiation of the experiment in order to synchronize their onset of puberty.

Information about growth rate and onset of puberty in

heifers of European and Zebu breeds under tropical conditions is scarce. From the studies reported it can be concluded that growth rates are generally low and onset of puberty occurs at older ages compared to what is common in temperate climates. It is generally believed that Bos Taurus heifers can not grow and sexually mature as fast under tropical conditions as in temperate climates and that the commonly used Bos Indicus cattle have a low genetic potential for growth and production. It can also be observed that heifers in the tropics are often inadequately fed and managed. Finally, only very limited data is presently available on hormone concentrations in calves and heifers in the tropics.

No previously reported study from the tropics has investigated onset of puberty in heifers weaned and started on the trial at an eraly age and properly fed and managed from birth through onset of puberty. Therefore, the potential of Bos Taurus and Bos Indicus heifers under tropical conditions has not been accurately evaluated. In order to increase animal production in the tropics it is of high importance to properly study onset of puberty in heifers of different breeds commonly found in the these climate areas.

Previous reports on effect of protein on growth rate and onset of puberty in heifers in the tropics have been inconclusive. It was therefore of value to further investigate this.

Mexico is a country with rapidly growing demands for a significant increase in dairy and beef production to feed the population growing at a rate of 2.5% per year. The tropical parts of the country are a highly underutilized resource for cattle production. Veracruz is one of the states located in the tropics, where cattle production is already of high importance. It was therefore of considerable interest to conduct a trial located in Veracuz. Brown Swiss and Zebu heifers, commonly used in the tropics, were chosen to evaluate the possibility of increasing growth rates and improve age at puberty by providing adequate nutrition and management from birth through onset of puberty. To ensure accuracy in the study it was essential to start the heifers on the trial directly following an early weaning.

Effects of adequate and high dietary crude protein, on growth rate and onset of puberty in Bos Taurus and Bos Indicus heifers in the tropics, were selected as the particular variables to investigate.

MATERIAL AND METHODS

Location of the trial

The experiment was conducted at "La Posta", one of the experiment stations belonging to INIFAP (Instituto Nacional de Investigaciones Forestales y Agropecuarias), the Mexican National Institute for Agricultural Research. The station is located 22 km south of the city Veracruz in the central region of the state of Veracruz (19° 12' north latitude, 96° 11' west longitude).

The state of Veracruz is situated on the east coast of Mexico between 17°8' and 22° 28' north latitude. The border against the Gulf of Mexico is 684 km long. Due to variable topography including both coastal plains and mountainous regions the climate differs widely from one part to another within the state. The central region of the state, around the city of Veracruz, is located at sea level. Annual average temperature in the region is 25°C with 80% relative humidity and 1200 mm rainfall (Barradas, 1980). The climate is characterized by a dry period of six months, November through April and rainfall from May through October. Heavy rains are concentrated to June through September. From November through February frequent

"Nortes", strong northern winds, blow in from the Gulf of Mexico and cause temperature drops.

Source of animals

Twenty four heifers from two different experiment stations were used in the experiment. The twelve Zebu heifers included Six Gyr, four IndoBrazil and two Brahman calves (Table 1). Growth rates and development of sexual maturity were expected to be similar for the Gyr , IndoBrazil and Brahman heifers, because of their common origin. Gyr animals were used as genetic foundation in crossbreeding programs for development of both the IndoBrazil and Brahman cattle (Rouse , 1973; Williamson and Payne, 1978). All the Zebu calves came from the experiment station "Las Margaritas". It has a humid subtropical climate and is situated in the state of Puebla, west of Veracruz. This experiment station is located at 19°45' north latitude and 97°20' west longitude 450 - 500 m above sea level. It has an annual average temperature of 21° C with 90% relative humidity and an average of 2400 mm rainfall . The topography at "Las Margaritas" is irregular and mostly hilly (INIP, 1985) . Four Brown Swiss heifers, 281 SP, 365 SP, 343 SP and 366 SP, came from "Las Margaritas" eight were from the experiment station "Aldama" (Table 1). "Aldama" has a dry tropical climate

TABLE 1. IDENTIFICATION, INITIAL AND FINAL BODY WEIGHTS OF, AND TIME ON TRIAL FOR ZEBU AND BROWN SWISS HEIFERS

HEIFER NUMBER	INITIAL WEIGHT, kg	FINAL WEIGHT, kg	NUMBER OF DAYS ON TRIAL
ZEBU			
DIET I			
51 GYR	93.5	232.5	251
39 IB	98.5	279.0	269
62 GYR	89.5	242.0	251
67 GYR	70.5	253.0	280
60 IB	104.0	275.0	280
66 IB	82.5	224.0	269
AVERAGE	77.4	250.9	267
DIET II			
26 GYR	104.0	262.0	238
59 GYR	95.0	255.0	280
65 IB	97.5	260.0	251
61 GYR	82.5	249.0	280
48 BHM	148.0	310.0	199
38 BHM	100.0	195.0	224
AVERAGE	104.5	255.2	245
ZEBU			
AVERAGE	91.0	253.2	256
BROWN SWISS			
DIET I			
35 SP	103.0	258.0	154
38 SP	102.5	243.0	154
41 SP	91.0	254.0	196
50 SP	69.0	252.0	210
281 SP	109.0	287.0	205
365 SP	116.0	225.0	126
AVERAGE	98.4	253.2	174
DIET II			
39 SP	90.5	254.0	196
44 SP	83.0	250.0	182
45 SP	85.0	247.0	182
366 SP	110.0	250.0	196
37 SP	75.0	270.0	224
343 SP	102.5	285.0	182
AVERAGE	91.0	259.3	194
BROWN SWISS			
AVERAGE	94.7	256.2	184

and is situated in the state of Tamaulipas north of Veracruz. It is located 90 meters above sea level at 22°52' north latitude and 98°15' west longitude and the land is part of a large plain. The maximum, minimum and annual average temperature are 34°C, 15°C and 24°C respectively. Average rainfall is 884 mm per year (INIP, 1985).

All 24 heifers were reared on the two respective stations until weaning at three months of age. The Brown Swiss calves were fed six liters of milk daily and were offered concentrate (Table 2) at ten days of age. The four Brown Swiss calves at "Las Margaritas" were kept in shaded, individual pens rotated daily on grass (Estrella de Africa) pasture and were given concentrate ad libitum. At "Aldama" the eight Brown Swiss calves grazed pasture (Zacate Pangola) and were fed 1 kg/head/day of concentrate.

The Zebu calves were on native pasture with their dams and they were creepfed concentrate (Table 2). Creepfeeding was initiated when the calves were 13 to 45 days old.

At three months of age all calves were weaned and their body weights recorded.

Adaptation conditions

Following weaning all calves were transported by truck to "La Posta". Four trips were needed to bring the Zebus and Brown Swiss from "Las Margaritas". Two trips were made to get the calves from "Aldama". The transport takes four

TABLE 2. COMPOSITION OF PRE-WEANING CONCENTRATES ON A DRY MATTER BASIS FOR CALVES AT "ALDAMA" AND "LAS MARGARITAS" EXPERIMENT STATIONS

INGREDIENT, %	"ALDAMA"	"LAS MARGARITAS"
SOYBEAN MEAL	41.0	14.0
SORGHUM GRAIN	33.0	
COCONUT MEAL		13.0
RICE POLISHINGS		39.0
WHEAT BRAN		15.0
MOLASSES	22.5	17.0
SODIUM CHLORIDE	1.0	1.4
TRACE MINERALS	0.04	
MAGNAPHOSCAL		0.6
total	100.04	100.0
CRUDE PROTEIN, %	22.0	18.0
TOTAL DIGESTIBLE NUTRIENTS, %	70.22	70.0
DIGESTIBLE ENERGY, Mcal	3.08	

hours from "Las Margaritas" and 10 - 12 hours from "Aldama" to "La Posta. Calves from " Aldama" were each given a 5 ml injection of the antibiotic Emicina (Pfizer) before the transport to "La Posta" to help combat the stress from transportation. The calves were weighed and jugular blood samples were taken at arrival to "La Posta". The blood samples were used for hematocrit determination to see if the calves were healthy (Table 3). The micro hematocrit method was used and blood samples in capillary tubes were centrifuged in a SolBat centrifuge with 7.5 cm radius at 11,000 r.p.m. for 15 minutes. Hematocrit determinations from blood samples taken bi-weekly were continued throughout the trial (Appendix: Table I).

During the first week at "La Posta" the calves were kept in quarantine. The Brown Swiss calves were confined to a small pasture with fresh water and concentrate ad libitum. The Zebus were in a separate barn and were fed chopped pasture and concentrate ad libitum. Composition of the concentrates were similar to the respective group's preweaning concentrate or creepfeed (Table 2).

After the first week the calves were moved to the experimental barn and kept in individual pens. The barn was an open-air construction with roof and concrete floor. During September through November 1985 it was rebuilt for the trial. Twenty four pens each measuring 1.32 x 8.92 m and separated from one another by chicken wire were constructed. Each pen had a large concrete feedbunk

TABLE 3. HEMATOCRIT VALUES OF ZEBU AND BROWN SWISS CALVES ON ARRIVAL AT "LA POSTA"

ZEBU (calf number)	HEMATOCRIT (%)	BROWN SWISS (calf number)	HEMATOCRIT (%)
51 GYR	42.0	35 SP	26.0
39 IB	44.0	38 SP	30.5
62 GYR	41.0	41 SP	30.0
67 GYR	42.0	50 SP	28.5
60 IB	50.0	281 SP	30.0
66 IB	45.0	365 SP	33.0
26 GYR	42.5	39 SP	26.0
59 GYR	40.0	44 SP	37.0
65 IB	45.0	45 SP	35.0
61 GYR	43.5	37 SP	28.0
38 BHM	38.5	343 SP	38.0
48 BHM	42.0	366 SP	32.0
AVERAGE	43.0		31.0

covering the front and half a 50 gallon barrel for water.

The first week in the experimental barn the calves were continued on the preweaning concentrate and separately given 2 kg/day of Diet IA containing 12.8 % crude protein. The second week they were given the concentrate and diet IA mixed together. From the third week until starting on the trial all calves were fed only Diet IA ad libitum (Table 4).

Duration of the trial

The calves were placed on the trial between December 23, 1985 and January 20th, 1986. Three calves were replaced March 17th, 1986 (Table 5). There was a 27 to 62 day adaptation period prior to start of the trial. This time varied depending on when the calves arrived from "Las Margaritas" and "Aldama". Heifers were removed from the trial when they reached puberty. This occurred between May and September 1986 (Table 5).

Experimental design

The experimental design was a completely randomized block design with two different treatments and two blocks. The two treatments were Diet I initially containing 12.8% crude protein (T1) and Diet II initially containing 16.4% crude protein (T2). The 12 Brown Swiss heifers and

TABLE 4. COMPOSITION OF EXPERIMENTAL DIETS ON A DRY MATTER BASIS FOR ZEBU AND BROWN SWISS CALVES

INGREDIENT, %	DIET IA*	DIET IIA
SORGHUM SILAGE	20.5	20.5
SUNFLOWER MEAL	16.1	48.6
RICE POLISHINGS	51.4	18.9
MOLASSES	10.0	10.0
MINERAL MIX	2.0	2.0
total	100.0	100.0

DIET IA: 12.8% crude protein, 56.0% dry matter and 3.0 Mcal/kg of digestible energy.

DIET IIA: 16.4% crude protein, 57.3% dry matter and 3.0 Mcal/kg of digestible energy.

* Diets marked "A" are initial diets given to calves with a body weight of 100-150 kg. Diets were then adjusted for every 50 kg live weight gain, according to NRC norms, and marked "B" (150-200 kg BW), "C" (200-250 kg BW) and "D" (250-300 kg BW).

TABLE 5. SCHEDULE OF ZEBU AND BROWN SWISS HEIFERS BEGINNING AND FINISHING THE TRIAL AND WHAT STATISTICAL ANALYSIS THEY WERE INCLUDED IN

HEIFER NUMBER	START, date	FINISH, date
ZEBU		
DIET I		
51 GYR	Dec 23, 1985	Aug 31, 1986
39 IB	Dec 23, 1985	Sept 18, 1986
62 GYR	Dec 23, 1985	Aug 31, 1986
67 GYR	Dec 23, 1985	Sept 29, 1986
60 IB	Dec 23, 1985	Sept 29, 1986
66 IB	Dec 23, 1985	Sept 18, 1986
DIET II		
26 GYR	Dec 23, 1985	Aug 18, 1986
59 GYR	Dec 23, 1985	Sept 29, 1986
65 IB	Dec 23, 1985	Aug 31, 1986
61 GYR	Dec 23, 1985	Sept 29, 1986
38 BHM	Dec 23, 1985	Aug 4, 1986*
48 BHM	March 17, 1986	Sept 18, 1986*
BROWN SWISS		
DIET I		
35 SP	Dec 23, 1985	May 26, 1986
38 SP	Dec 23, 1985	May 26, 1986
41 SP	Dec 23, 1985	July 7, 1986
50 SP	Jan 20, 1986	Aug 18, 1986
281 SP	Jan 6, 1986	July 30, 1986
365 SP	March 17, 1986	July 21, 1986*
DIET II		
39 SP	Dec 23, 1985	July 7, 1986
44 SP	Jan 6, 1986	July 7, 1986
45 SP	Jan 6, 1986	July 7, 1986
37 SP	Dec 23, 1986	Aug 4, 1986
343 SP	Jan 20, 1986	July 7, 1986
366 SP	March 17, 1986	Sept 29, 1986*

All heifers and their performance during the whole trial are included in the ANOVA with a completely randomized block design without repeated measurements.

* Heifers excluded from all ANOVA with a completely randomized block design with repeated measurements. Heifer number 38 BHM was excluded due to illness during part of the time period included in the analysis.

the same number of Zebu heifers made up a block each. Each calf was randomly assigned to treatment within breed.

Statistical model:

$$Y_{ijk} = u + T_i + D_j + (TD)_{ij} + E_{ijk} \qquad (i=1,2)\ (j=1,2)\ (k=1,2,3,4)$$

T_i = dietary protein levels

D_j = breeds (blocks)

TD_{ij} = interaction between protein level * breed

E_{ijk} = residual error

This model was used for analysis of variance of average daily gain, dry matter intake, feed conversion and crude protein intake. It was also used for analyses of thyroid hormone concentrations, age and body weight of the heifers at onset of puberty and plasma mineral concentrations . The analyses included all 24 heifers and their performances during the whole trial.

Analyses using the above model were done both with and without co-variates. The co-variates were "initial body weight" and "number of days on trial". The exceptions were analyses of age and body weight at onset of puberty and plasma mineral concentrations, which were done only without co-variates.

For the randomized block design with repeated

measurements the following model was used:

$$Y_{ijl} = u + T_i + D_j + (TD)_{ij} + E1 + B_l + (TB)_{il} + (DB)_{jl} + E2$$

$(i=1,2)$

$(j=1,2)$

$(l=1...10)$

TB_{il}= interaction protein level * period

DB_{jl}= interaction breed * period

E1 = error due to heifer within breed and treatment

E2 = residual error

The repeated measurements model was used for analyses of average daily gain, dry matter intake, feed conversion and crude protein intake. It was also used for analysis of thyroid hormone concentrations. Since the heifers started and finished the trial at different times, these analyses did neither include all the heifers nor the whole duration or the experiment. Instead an optimal combination of number of heifers and length of time was chosen to include as many observations and as many measurements as possible in the analyses. Feed intake, daily gain and feed conversion were averaged over two week periods and the analyses included 20 heifers and 10 periods. The first period was from January 20th through February 2nd and the last period ended May 26th. Thyroid hormone concentrations were measured bi-weekly. Twenty heifers and 11 times, beginning

with January 20th and finishing with May 26th, were used in the analysis (Table 5).

The general linear model's procedure in Statistical Analysis Systems (SAS) version five (SAS Institute inc., 1985) was used for all statistical analyses of results from the trial.

Diets

The two diets used (Table 4) were designed to meet nutrient requirements recommended by NRC (1978) for dairy heifers gaining 500 g/day (Table 6). The adequate protein diet had an initial crude protein level of 12.8% and the high protein diet contained 16.4 % CP . For heifers on Diet II the amount of crude protein and its percentage of dry matter was modified for higher intakes (Table 6). Diets were isocaloric and identical apart from the difference in crude protein content.

The diets consisted of sorghum silage, sunflower meal, rice polishings, molasses and minerals fed as a complete mixed ration (Table 4). Diets were made by blending all ingredients in a mechanical mixer, a metal drum with a rotating screw. After mixing the diets were kept in nylon or plastic bags. Rice polishings were not available from the end of June through September so wheat bran was used during the last three months of the experiment.

TABLE 6. DAILY NUTRIENT REQUIREMENTS FOR DAIRY HEIFERS GAINING 500 g/day ACCORDING TO NRC (1978)

BODY WEIGHT (kg)	CRUDE PROTEIN (g)	(% of DM)	DRY MATTER (kg)	DIGESTIBLE ENERGY (Mcal)	CALCIUM (g)	PHOS-PHORUS (g)
DIET I						
100-150	360	12.8	2.8	8.35	16	8
150-200	474	11.8	4.0	11.11	17	11
200-250	586	11.3	5.2	14.06	20	13
250-300	678	10.8	6.3	16.49	22	16
DIET II						
100-150	459*	16.4*	2.8	8.35	16	8
150-200	604	15.1	4.0	11.11	17	11
200-250	754	14.5	5.2	14.06	20	13
250-300	876	13.9	6.3	16.49	22	16

* For heifers on Diet II the amount of crude protein and its % of DM has been modified to a higher intake for all four body weight groups.

BIOSAL (Agroquimica) mineral mixture was added to the rations (Appendix: Table II). This mineral mix was analyzed at "La Posta" for calcium and phosphorus content. Calcium content was 27.3% lower than declared (11.27% vs 15.5%) and amount of phosphorus was 83.6% lower (1.67 % vs 10.17%) than stated by manufacturer. Therefore MAGNAPHOSCAL (Bayer) was added to the diets to provide 30 g/head/day (Appendix: Table III) .

The sorghum silage was made at "La Posta" and the amount needed for the whole trial was kept in one bunker silo. All the other feed ingredients were purchased.

Both diets had the same percentage of sorghum silage, molasses and minerals. Amounts of sunflower meal and rice polishings or wheat bran were balanced differently for Diet I and Diet II in order to obtain their respective protein levels. Feed composition was adjusted for each 50 kg live weight gain according to the NRC daily nutrient requirements for dairy heifers (Table 6). The feed was given ad libitum (> 10 % orts daily) and the quantity continuously adjusted to individual intake.

Management routines including collection and preparation of plasma samples

Feed was mixed every day Monday through Friday. On Fridays feed was also mixed for Saturday and Sunday. Feeding was done once daily in the morning after taking

orts. The pens were cleaned and fresh water given to the heifers once a day. After two outbreaks of footrot, one in May and one in June, the floors were covered with wood shavings replaced daily. Due to footrot a clove-bath was built in the area used for estrus detection directly behind the barn. The bath was filled with a solution of copper sulphate, which the heifers walked through daily.

Once every two weeks the heifers were weighed and blood samples from the jugular vein were taken in the morning before feeding. The blood samples were collected in 20 ml Vacutainer tubes (Becton-Dickinson) containing EDTA. Immediately after finishing blood collection all samples were brought to the laboratory and centrifuged for 15 minutes at 4500 r.p.m. in a SolBat centrifuge. Plasma from each sample was divided into 1 ml aliquotes and kept in plastic tubes (Sarstedt). The plasma samples were stored frozen at - 24 ^{o} C. At the end of the trial the frozen samples were brought to the USA for analyses of progesterone, thyroid hormone and mineral content.

Average daily gain, dry matter and crude protein intake and feed efficiency of the heifers were calculated bi-weekly during the trial. A summary of primary data for individual heifers can be found in the appendix (Table IV).

Health care and diseases

All heifers were dewormed monthly with Helmezine (Glaxo) alternated with Valbazen (Smith Kline). They were sprayed with Asuntol+Neguvon (Bayer) against ticks biweekly and an ADE-vitamin injection (GAN-ADE , Squibb) was given to each calf every three months (Table 7).

The heifers were also vaccinated against Blackleg, Malignant Edema, Brucellosis and Pasteurellosis during the trial (Table 7).

The resident veterinarian in charge of the health care of the animals at "La Posta" was also responsible for health care of the heifers in this experiment.

Two outbreaks of footrot took place among the heifers during the trial. The first outbreak in May affected four Brown Swiss heifers. During the outbreak in June seven heifers, both Brown Swiss and Zebu, were infected with footrot. In both cases the heifers with footrot were treated both locally, with Hydrogen peroxide , Copper sulphate and Pezunol , and generally with antibiotics such as Flupen (Trianon), Bisolvon (Anchor) or Trisulfas (Carlo Erba) (Table 8).

TABLE 7. DATES, PROPHYLAXIS AND ROUTINE TREATMENTS OF ZEBU AND BROWN SWISS HEIFERS DURING THE EXPERIMENT

DATES	PROPHYLAXIS OR ROUTINE TREATMENT
1985	
19/12*	Dewormed: 5 ml Helmezine per heifer Vitamin injection: 3 ml GAN-ADE per heifer Vaccination against Blackleg and Malignant Edema
27/12	Sprayed against ticks with Asuntol
1986	
3/1	Sprayed against ticks with Asuntol and Neguvon
17/1	Sprayed against ticks with Asuntol and Neguvon
24/1	Dewormed: 1 ml/20 kg body weight of Helmezine
31/1	Sprayed against ticks with Asuntol and Neguvon
14/2	Sprayed against ticks with Asuntol and Neguvon
6/3	Sprayed against ticks with Asuntol and Neguvon
10/3	Dewormed: 1 ml/20 kg body weight of Helmezine Vitamin injection: 3-5 ml (depending on BW) of GAN-ADE
21/3	Sprayed against ticks with Asuntol and Neguvon
4/4	Sprayed against ticks with Asuntol and Neguvon
15/4	Dewormed: 1 ml/20 kg body weight of Helmezine Vaccination against Brucellosis
17/4	Sprayed against ticks with Asuntol and Neguvon
29/4	Sprayed against ticks with Asuntol and Neguvon
2/5	Vaccination against Pasteurellosis
14/5	Dewormed: 0.0075 ml/kg body weight of Valbazen
15/5	Sprayed against ticks with Asuntol and Neguvon
28/5	Sprayed against ticks with Asuntol and Neguvon
9/6	Dewormed: 1 ml/20 kg body weight of Helmezine Vitamin injection: 4-5 ml (depending of BW) of GAN-ADE
10/6	Sprayed against ticks with Asuntol and Neguvon
24/6	Sprayed against ticks with Asuntol and Neguvon
10/7	Sprayed against ticks with Asuntol and Neguvon
20/7	Dewormed: 0.075 ml/kg body weight of Valbazen
25/7	Sprayed against ticks with Asuntol and Neguvon
7/8	Sprayed against ticks with Asuntol and Neguvon
21/8	Sprayed against ticks with Asuntol and Neguvon
22/8	Dewormed: 1 ml/ 20 kg body weight of Helmezine
26/9	Vaccination against Pasteurellosis
30/9	Vitamin injection: 5 ml of GAN-ADE and 20 ml CuFe-solution per heifer

* Day/month.

TABLE 8. IDENTIFICATION OF HEIFERS SUFFERING FROM FOOTROT DURING ONE OR BOTH OUTBREAKS OF THE DISEASE DURING THE EXPERIMENT

HEIFER NUMBER	DATES OF ILLNESS
38 SP	May 12th - May 23rd
39 SP	May 17th - May 26th
343 SP	May 18th - May 26th June 16th - June 25th
41 SP	May 20th - May 28th June 13th - June 23rd
66 IB	June 8th - June 22nd
62 GYR	June 11th - June 22nd
365 SP	June 12th - June 23rd
65 IB	June 19th - June 27th
48 BHM	June 22nd - JUNE 30th

Symptoms: Infected clove(s) and fever.

Treatment: Local treatment of the clove(s) and antiniotics against the infection.

Onset of Puberty

The onset of puberty was defined as the first ovulation of a heifer as confirmed by a palpable corpus luteum. A plasma progesterone concentration of more than 1 ng/ml in the corresponding plasma sample confirmed the presence of a functional corpus luteum. All plasma samples were analyzed for progesterone concentration at Michigan State University after the field work of the trial was finished.

Procedure for detection of onset of puberty began when the heifers reached eight months of age and/or 180 kg body weight. From then on blood samples were taken and they were weighed and palpated biweekly.

At the same time the daily routine changed to include turning the heifers loose in a fenced area with concrete floor directly behind the pens between 6 - 7 am and 5 - 6 pm. During these hours they were observed for signs of estrus such as nervousness, mooing, mounting of other heifers, allowing themselves to be mounted as well as mucous discharge from the vagina.

Any heifer showing signs of heat was palpated and jugular blood was taken simultaneously in the pen. As the heifers matured and approached puberty all of them were palpated at least once a week to assure detection of ovarian activity. Rectal palpations were generally done in the morning. The detection of a developing follicle meant the heifer was palpated and blood collected once daily, until it

could be determined either that the follicle did mature and an egg was released or it was certain the follicle had undergone atresia without reaching maturity. If a heifer ovulated, she was palpated for a mature corpus luteum on day eight or nine after ovulation. Throughout the trial all palpations were accompanied by blood sampling to enable later confirmation of ovarian structures by corresponding changes in plasma progesterone concentration.

The period of palpations lasted from April through September. During April, May and August the director of "La Posta" was responsible for this task. Due to illness he was replaced by a veterinarian from the department of reproduction during June and July. Both veterinarians have extensive experience palpating ovaries. The author was palpating alongside the two veterinarians throughout the trial and was solely responsible for palpations during September. To test accuracy of rectal palpation "double palpations" were performed. In the beginning this was done by the two veterinarians, who would palpate ovaries of the same heifers and compare results. Another experienced veterinarian also assisted with this quality control. Once the author had gained enough experience at rectal palpation she and one veterinarian routinely performed "double palpations" on a daily basis.

Endpoint of the trial.

A second ovulation confirmed by palption of a corresponding corpus luteum marked the endpoint of the trial for each of the heifers. The experiment was extended to include the second ovulation to demonstrate that the heifers were actively cycling.

Climate registration

The climate was registered daily at the weather station at "La Posta". Temperature, relative humidity, rainfall and sun hours were recorded (Table 9).

Feed analyses

All feed analyses were done in the nutrition laboratory at "La Posta".

Samples of the rations and orts were taken monthly during the trial. Feed analyses included dry matter, crude protein, dry matter digestibility, crude fiber and ash content of the diets. Dry matter and crude protein analyses were done on each individual sample, while the other analyses were done on pooled samples. Samples analyzed for dry matter digestibility, crude fiber and ash were pooled over time within diet and weight group, making the final number of samples for analyses 15.

TABLE 9. A MONTHLY SUMMARY OF AVERAGE DAILY TEMPERATURE, RELATIVE HUMIDITY, SUN HOURS AND RAINFALL AT "LA POSTA" EXPERIMENT STATION FROM OCTOBER 1985 THROUGH SEPTEMBER 1986

MONTH	TEMPERATURE (°C)	(range)	RELATIVE HUMIDITY (%)	(Range)	SUN HOURS (Hrs)	RAIN (mm)
1985						
OCT	25.9	33.0 - 18.0	55	82 - 16	6.4	9.2
NOV	23.9	33.0 - 14.0	52	83 - 10	5.4	0.3
DEC	21.7	33.0 - 11.0	52	80 - 8	3.8	0.0
1986						
JAN	18.6	31.0 - 5.0	46	84 - 2	5.0	0.0
FEB	22.8	35.5 - 10.0	49	82 - 5	5.0	0.0
MARCH	22.8	39.0 - 10.5	45	78 - 1	6.4	1.0
APRIL	26.6	37.5 - 15.0	45	77 - 12	6.0	0.0
MAY	29.0	39.5 - 18.5	55	89 - 20	4.5	1.4
JUNE	28.9	38.0 - 21.0	62	92 - 31	7.5	6.0
JULY	27.5	35.5 - 19.5	70	99 - 41	6.0	7.2
AUG	27.6	36.5 - 21.0	63	94 - 31	5.4	9.3
SEPT	27.5	33.5 - 20.0	77	97 - 56	3.3	11.7

Feed dry matter was determined by drying fifty grams in an oven at 50 to 60 C for 48 hours. Moisture content of the molasses was determined by the titrimetric method (A.O.A.C., 1984) (Appendix: Table V).

Dry sample was ground through a 1 mm sieve before analyses.

Crude protein in the feed and weighbacks was analyzed by the Kjeldahl method according to A.O.A.C (1984) (Appendix: Table V).

Apparent dry matter digestibility, in situ, of the rations was determined by use of the nylon bag technique developed by Mehrez and Orskov (1977) (Appendix: Table VI).

Crude fiber and ash were also determined according to AOAC 1984. In the case of crude fiber analysis the ceramic fiber filter method was used (Appendix: Table VI).

Diet ingredients were analyzed for dry matter and crude protein content (Appendix: Table VII).

Plasma mineral analyses

Plasma mineral profiles from the heifers were analyzed to ensure that the heifers had been given adequate mineral supplementation. Four plasma samples taken every second month from each heifer were analyzed for calcium, phosphorus, magnesium, sodium, copper, iron and zinc. The method of inductively coupled argon plasma emission spectroscopy was used (Brasselton et al., 1981). The

analyses were carried out by the Animal Health Diagnostic Laboratory at Michigan State University.

Plasma progesterone analyses

Plasma progesterone concentrations were analyzed by Radioimmunoassay using the commercial Coat-a-Count Progesterone assay from Diagnostic Products Corporation. The assay was modified for the bovine samples to be analyzed. Standards were made up using progesterone-free bovine plasma to which were added known amounts of progesterone. Bovine plasma from cows at various stages of the estrous cycle were used as quality controls instead of human plasma samples provided with the kit. Concentrations of the quality controls (1.85 ng/ml and 0.25 ng/ml) were adjusted to fit the low amounts of progesterone expected in the plasma samples. Incubation time of the antibody coated tubes after addition of sample and labelled progesterone was modified from 3 hours at room temperature to 24 hours at + 4 ^{o}C. Before counting the samples with a gammacounter, the tubes were rinsed once with double distilled water, which was another modification. Instructions for use of the kit recommends immediate counting without rinsing out the tubes. However, interference is strongly reduced by rinsing the tubes (Personal communication with AHDL personnel at MSU). A total of 751 plasma samples were

analyzed for progesterone concentration.

Sensitivity of the assay, calculated as B/B0 at 90%, was 0.13 ng/ml and the interassay coefficient, averaged over seven assays, for the high quality control (0.25 ng/ml) was 8.1% and for the low quality control (1.98 ng/ml) was 19.2%. Intraassay coefficients for the high quality control averaged 4.2 % for the seven assays. The average coefficient of variation for the low quality control 16.2% (Appendix: Table VIII).

A series of dilutions were made after the modifications of the assay as part of the validation. Dilutions with water, buffer and plasma as well as a series of spiking with progesterone showed good parallelism and no strong protein matrix effect (Appendix: Table IX).

A comparison was made between use of the modified Coat-a-Count progesterone assay with an extraction step (Refsal et al., 1987) versus as a direct assay. Sixty seven samples, 19 from a Brown Swiss heifer (35 SP) and 48 from a Zebu heifer (39 IB), were analyzed with and without an extraction step. The result was a correlation coefficient of 0.98 between the two methods (Appendix: Table X and Figure I). It was concluded that an extraction step was unneccesary when the modified progesterone assay was used.

The assay is highly specific for progesterone. Crossreactivity with other steroids is very low as shown in data provided by the manufaturer: Corticosterone 0.4%, 11-Deoxycorticosterone 1.7%,

11-Deoxycortisol 2.4%, 20a-Dihydroprogesterone 2.0%, 17a-Hydroxyprogesterone 0.3%, 5b-Pregnan-3a-ol-20-one 0.2%, 5a-Pregnan-3,20-dione 0.8% and 5b-Pregnan-3,20-dione 1.3% (Diagnostics Products Corporation, 1986).

Analyses of plasma thyroid hormone concentrations

Two hundred and forty seven plasma samples were analyzed for concentration of thyroxine (T4), free T4, triiodothyronine (T3) and free T3.

Commercially available solid phase component system assays from Becton Dickinson were used for the T4, free T4 and free T3 analyses. The T4 assay was modified from a 10 ul sample size and 45 minutes incubation at room temperature to 30 ul sample and two hours incubation at 37°C. These assays have been previously validated by Gerloff at al. (1985).

For the T3 analyses a liquid phase assay previously validated by Refsal et al. (1984) was utilized. The antibody was obtained from Miles laboratories. A barbitol buffer was used and the I125 came from New England Nuclear. Standards were from Wein laboratories and dextran coated charcoal was used in the assay.

All coefficients of variation and sensitivities, calculated as B/B0 at 90%, for thyroid hormone fractions given below are values calculated over nine assays.

The interassay coefficient for T4 for the high quality

control (37.6 ng/ml) was 8.8% and for the low quality control (32.6 ng/ml) 5.8%. The intraassay coefficients of variation averaged 3.6 % and 4.5 % respectively. Sensitivity of the assay was 0.87 ng/ml (Appendix: Table XI).

The respective coefficients for free T4 (8.1 pg/ml and 6.7 pg/ml) were 6.9% and 9.0% for the high and low quality controls. Average intraassay coefficients were 5.1% and 6.8% and sensitivity was 0.64 pg/ml (Appendix: Table XII).

The values for T3 (1.9 ng/ml and 1.5 ng/ml) were 6.9% for the high quality control and 6.6% for the low quality control. Values for intraassay variation were 5.3% and 4.9%. Sensitivity of the T3 assay was 0.10 ng/ml (Appendix: Table XIII).

Finally, quality controls of free T3 (5.2 pg/ml and 4.6 pg/ml) had interassay coefficients of variation of 5.9 pg/ml and 5.9 pg/ml. Intraassay coefficients were 5.6% and 4.6% and sensitivity was 0.47 pg/ml (Appendix: Table XIV).

Becton Dickinson used a T4 analog in the assay instead of T4. Due to this additional validation of the assay was done. Dilution series of T4 with water, buffer and plasma showed parallelism and a series of add-measure-back with spiked samples had an excellent recovery rate (Appendix: Table XV).

Plasma cortisol analyses

Cortisol concentrations were analyzed in 31 plasma samples from one Zebu heifer, number 60 IndoBrazil. Gammacoat (125I) Cortisol Radioimmunoassay Kit, a solid phase commercial assay from Travenol-Genentech diagnostics, was used with two modifications. The sample size was increased from 10 to 20 ul and incubation prolonged from 45 minutes to two hours at 37° C. The assay, with these modifications , has been previously validated by Roth et al. (1985).

RESULTS

The chapter of results is divided into sections. The first five sections are; average daily gain, dry matter intake, feed conversion, crude protein intake and plasma concentrations of thyroid hormones. First in each section are the results from statistical analyses with the completely randomized block design. These are the overall results of the trial and include all 24 heifers and their performance during the whole trial. Further analyses with the same model and observations included one or two covariates; either "number of days on trial" or "initial body weight" or both of them together. The co-variates, whether used alone or combined, were non-significant for all five variables above. Only results from the analyses without co-variates are therefore reported.

Then follow results of the statistical analyses with repeated measurements. Results of feed intake and feed conversion are presented from 10 two-week periods and the first period is an average from January 20th through February 2nd. Plasma concentrations of thyroid hormones are reported at 11 different times beginning with January 20th called time zero. All analyses with repeated measurements end on May 26th. When a period or time effect was found no

overall conclusions about the 10 periods or 11 times could be drawn, but each period of importance is discussed.

Next come results from the statisitical analyses of age and weight at onset of puberty as determined by rectal palpation of the ovaries. In the subsequent section a comparison is made between determination of onset of puberty in the Brown Swiss heifers by rectal palpations and measurements of changes in plasma progesterone concentrations. Results from primary data of plasma progesterone concentrations in the Zebu heifers are also described.

A final section on hormones compares results from primary data of plasma cortisol concentrations and corresponding progesterone concentrations in 31 samples from one Zebu heifer.

The last section of the chapter presents results from plasma mineral analyses.

No interaction between breed and treatment was found in any of the statistical analyses.

Average daily gain

The Brown Swiss heifers had a higher ($P < 0.0001$) average daily gain than the Zebus, 882 g compared to 611 g (Table 10). There was no effect of dietary crude protein level on average daily gain. Heifers on the diet with 12.8% CP gained 748 g/day versus 745 g/day for heifers on

TABLE 10. OVERALL EFFECT OF BREED AND DIETARY CRUDE PROTEIN LEVEL ON AVERAGE DAILY GAIN, DRY MATTER AND CRUDE PROTEIN INTAKE AND FEED CONVERSION OF 12 ZEBU AND 12 BROWN SWISS HEIFERS

	LS MEAN	STD ERROR	F VALUE	PR > F
AVERAGE DAILY GAIN, g		LS MEAN		
BREED EFFECT				
ZEBU	611	26	56.24	0.0001
BROWN SWISS	882	26		
TREATMENT EFFECT				
12.8% CP	748	26	0.01	0.9764
16.4% CP	745	26		
AVERAGE DAILY DRY MATTER INTAKE, kg				
BREED EFFECT				
ZEBU	4.7	0.15	30.56	0.0001
BROWN SWISS	5.9	0.15		
TREATMENT EFFECT				
12.8% CP	5.0	0.15	10.35	0.0041
16.4% CP	5.6	0.15		
AVERAGE DAILY CRUDE PROTEIN INTAKE, g				
BREED EFFECT				
ZEBU	641	17	41.81	0.0001
BROWN SWISS	801	17		
TREATMENT EFFECT				
12.8% CP	604	17	90.72	0.0001
16.4% CP	838	17		
AVERAGE DAILY FEED CONVERSION, kg DM/kg gain				
BREED EFFECT				
ZEBU	7.8	0.19	16.11	0.0006
BROWN SWISS	6.7	0.19		
TREATMENT EFFECT				
12.8% CP	6.8	0.19	12.60	0.0019
16.4% CP	7.7	0.19		

the diet with 16.4% CP.

A period effect ($P < 0.0021$) on average daily gain was observed, both when breeds and protein levels are compared, over the 10 two-week periods in the repeated measurements analysis (Figure 1 and 2).

Difference in average daily gain between the two breeds was significant during three periods. The Brown Swiss heifers had a higher average daily gain compared to the Zebus during periods two, six and seven; 860 versus 460 g/day ($P < 0.0263$), 1577 versus 869 ($P < 0.0008$) and 611 versus -64 g/day ($P < 0.0362$). During all other periods average daily gain was similar for the two breeds (Figure 1).

No effect of dietary crude protein level on average gain could be seen during any of the 10 periods in the analysis. A trend towards a higher average daily gain for heifers on the 16.4% CP diet compared to those on the diet with 12.8% CP was observed in period four; 720 ± 100 g/day versus 450 ± 91 g/day ($P < 0.0621$). Due to large variation in individual gain the apparent difference between effects of the two protein levels in period nine was not significant (Figure 2).

Average daily dry matter intake

Average daily dry matter consumption was significantly different between both breeds and protein levels (Table

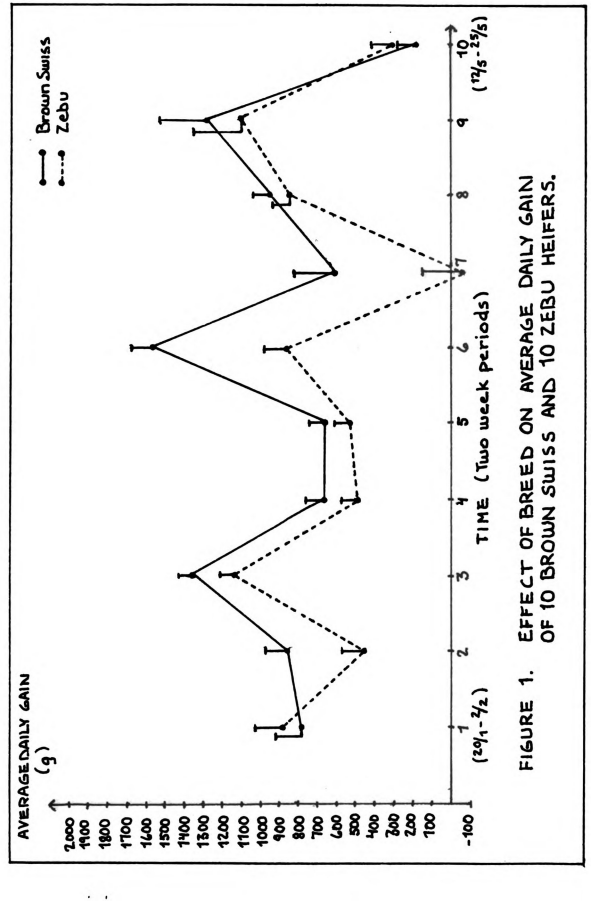

FIGURE 1. EFFECT OF BREED ON AVERAGE DAILY GAIN OF 10 BROWN SWISS AND 10 ZEBU HEIFERS.

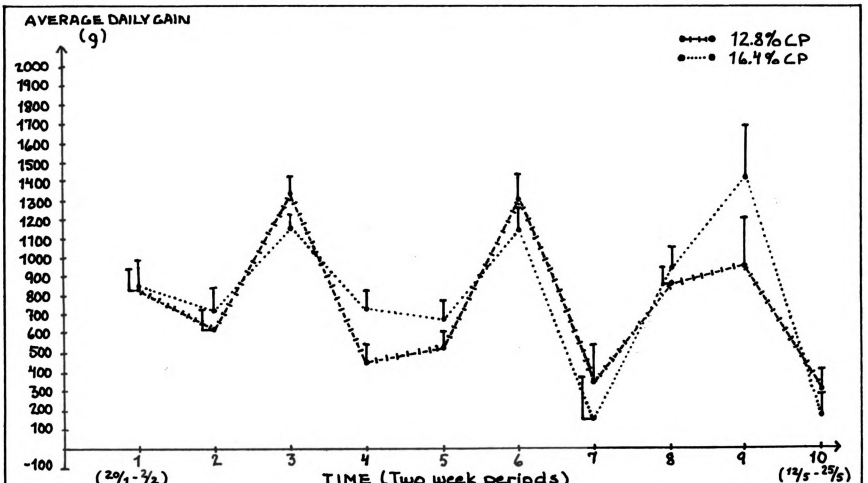

FIGURE 2. EFFECT OF DIETARY CRUDE PROTEIN LEVEL ON AVERAGE DAILY GAIN OF 10 BROWN SWISS AND 10 ZEBU HEIFERS.

10). Brown Swiss heifers had a higher ($P < 0.0001$) dry matter intake, 5.9 kg/day, than the Zebus, which consumed only 4.7 kg/day. A higher ($P < 0.0041$) dry matter intake was also seen on the high protein diet, 5.6 kg/day, compared to the adequate protein diet 5.0 kg/day.

There was a period effect ($P < 0.0001$) on average daily dry matter intake for both breeds and treatments (Figure 3 and 4). This was mainly due to the large decrease in dry matter intake during period seven.

The higher average daily dry matter intake of Brown Swiss heifers compared to the lower intake of the Zebus was significantly different for each of the 10 periods (Figure 3).

Effect of protein level on average daily dry matter intake was significant for periods four, eight and nine. During these periods heifers on the 16.4% CP diet consumed more dry matter than did heifers on the 12.8% CP diet (Figure 4). The same trend was observed during period two, three, five, six and ten ($P < 0.0539$-0.0984). No significant difference could be shown between protein levels during period one and ten.

Average daily crude protein intake

Brown Swiss heifers consumed more ($P < 0.0001$) crude protein than did the Zebu heifers; 801 g/day compared to 641 g/day (Table 10). The effect of dietary crude protein

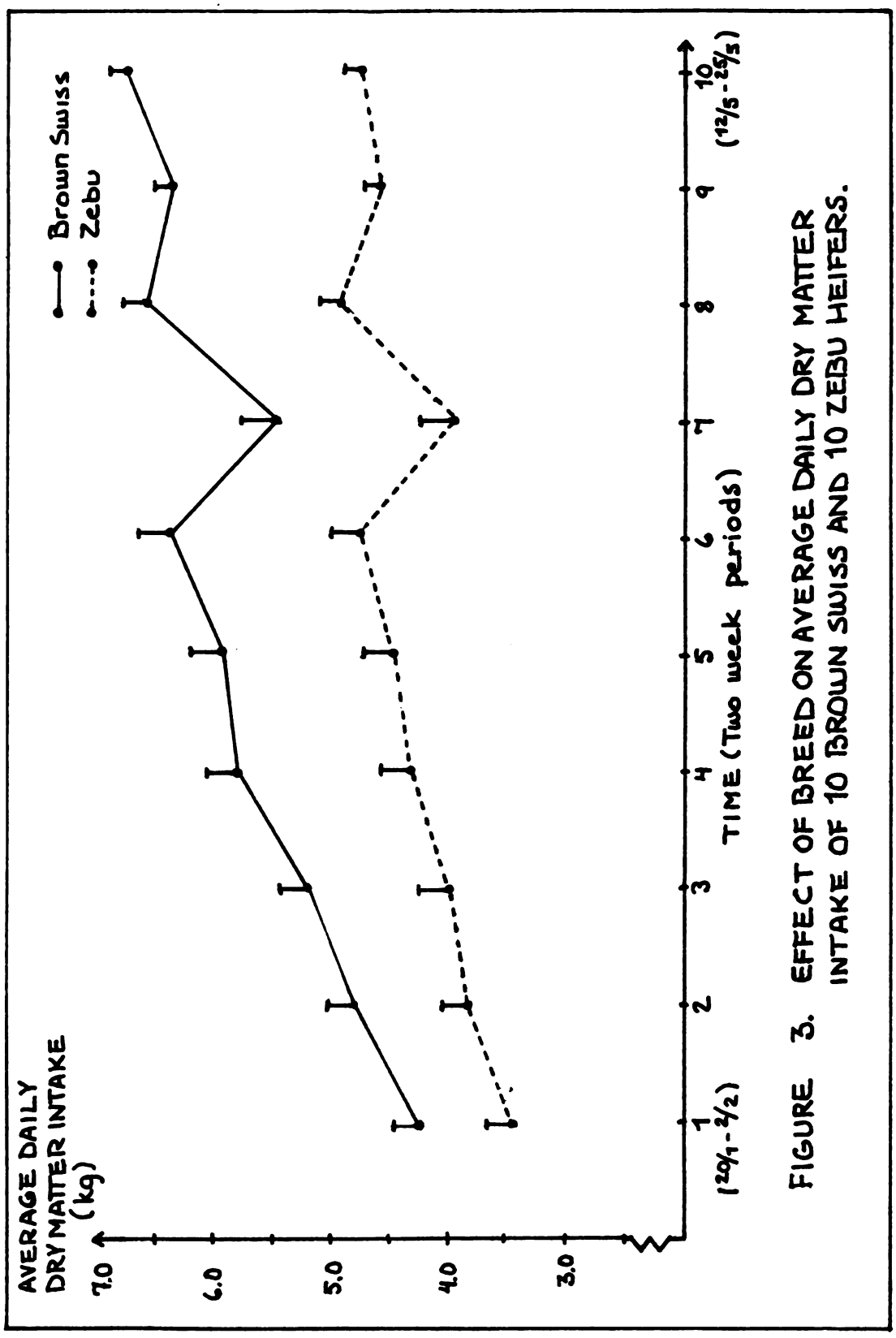

FIGURE 3. EFFECT OF BREED ON AVERAGE DAILY DRY MATTER INTAKE OF 10 BROWN SWISS AND 10 ZEBU HEIFERS.

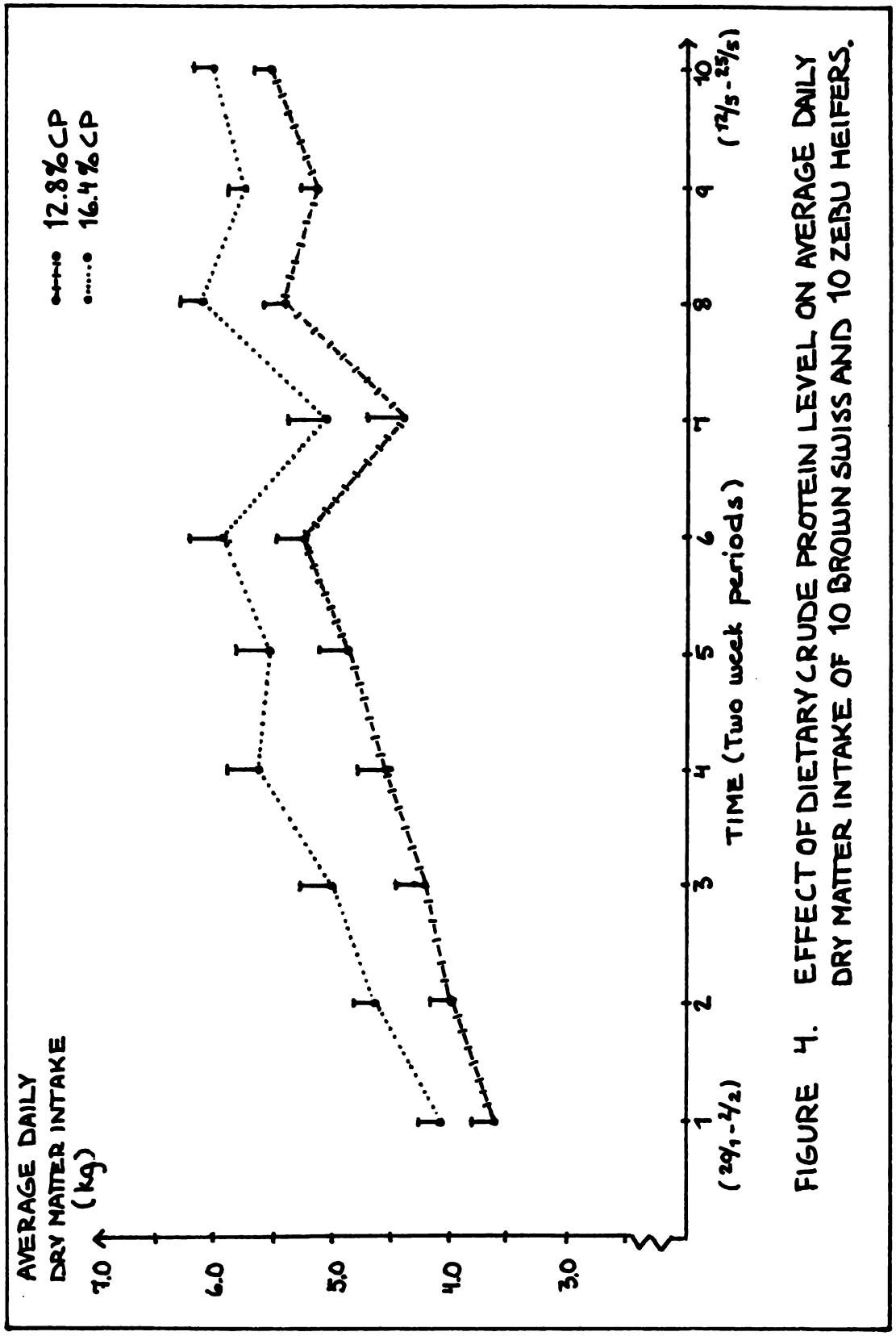

FIGURE 4. EFFECT OF DIETARY CRUDE PROTEIN LEVEL ON AVERAGE DAILY DRY MATTER INTAKE OF 10 BROWN SWISS AND 10 ZEBU HEIFERS.

level on intake of protein was highly significant. Heifers on the high protein diet had an intake of 838 g CP/day versus 604 g CP/day ($P < 0.0001$) for heifers on the adequate protein diet.

A period effect ($P < 0.0001$) on crude protein intake was observed for both Brown Swiss and Zebu heifers and both dietary protein levels. Similar to the period effect on dry matter intake, the large decrease in feed consumption during period seven was the main cause of the period effect on crude protein intake (Figure 5 and 6).

Average daily crude protein intake was higher ($P < 0.0001$-0.0266) for the Brown Swiss heifers compared to the Zebus during all ten periods (Figure 5).

Heifers on the diet with 16.4% CP consumed more ($P < 0.0001$-0.0003) crude protein daily during all ten periods than did the Zebu heifers (Figure 6).

Average daily feed conversion

Average daily feed conversion was different ($P < 0.0006$) between breeds and between treatments (Table 10). Brown Swiss heifers were more efficient in their feed conversion, 6.7 kg DM/kg gain, compared to the Zebu heifers, which needed 7.8 kg DM/kg gain. Heifers on the 12.8% CP diet were also more efficient ($P < 0.0019$) with 6.8 kg DM/kg gain, than heifers on the 16.4% CP diet with 7.7 kg DM/kg gain.

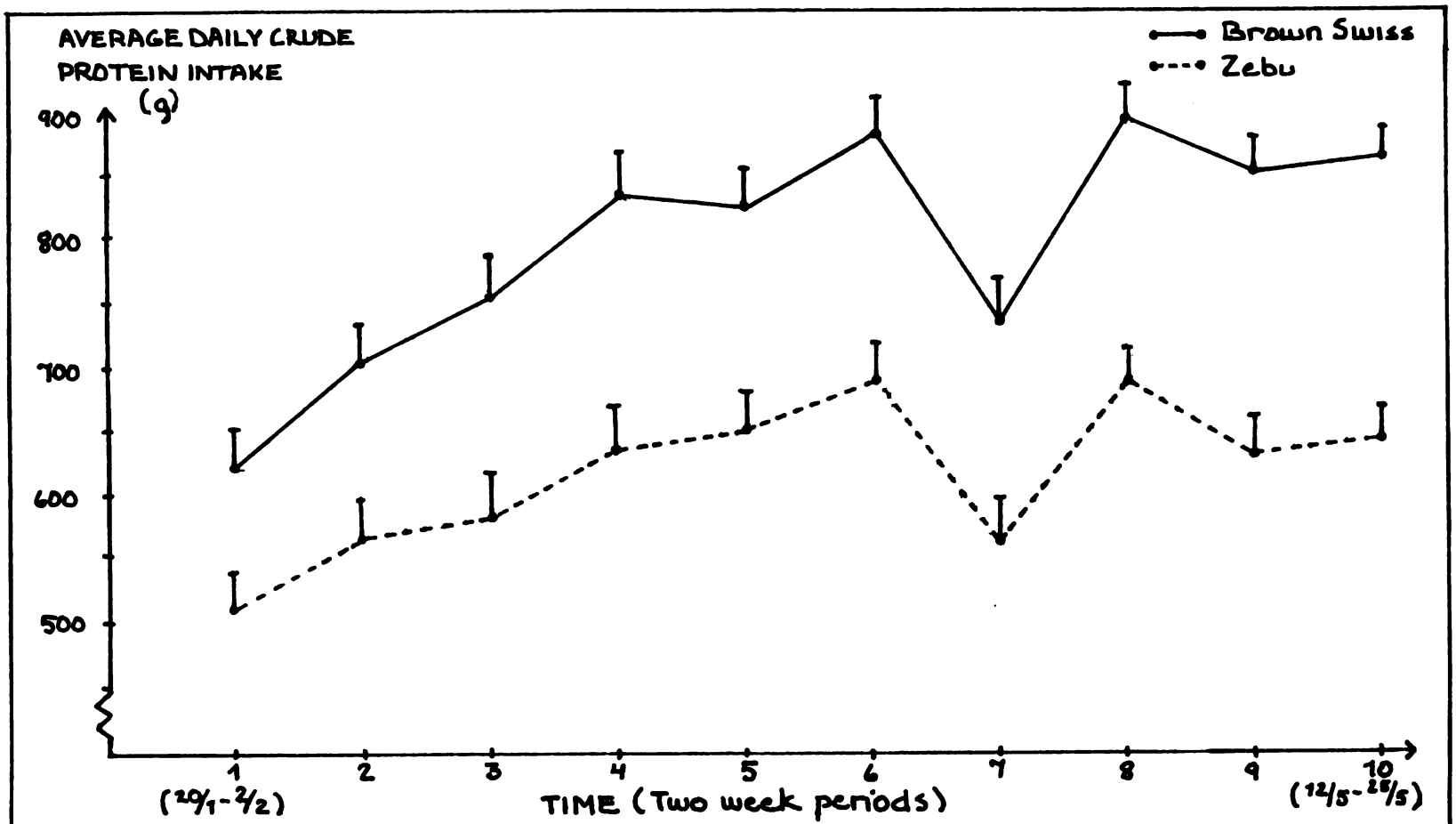

FIGURE 5. EFFECT OF BREED ON AVERAGE DAILY CRUDE PROTEIN INTAKE OF 10 BROWN SWISS AND 10 ZEBU HEIFERS.

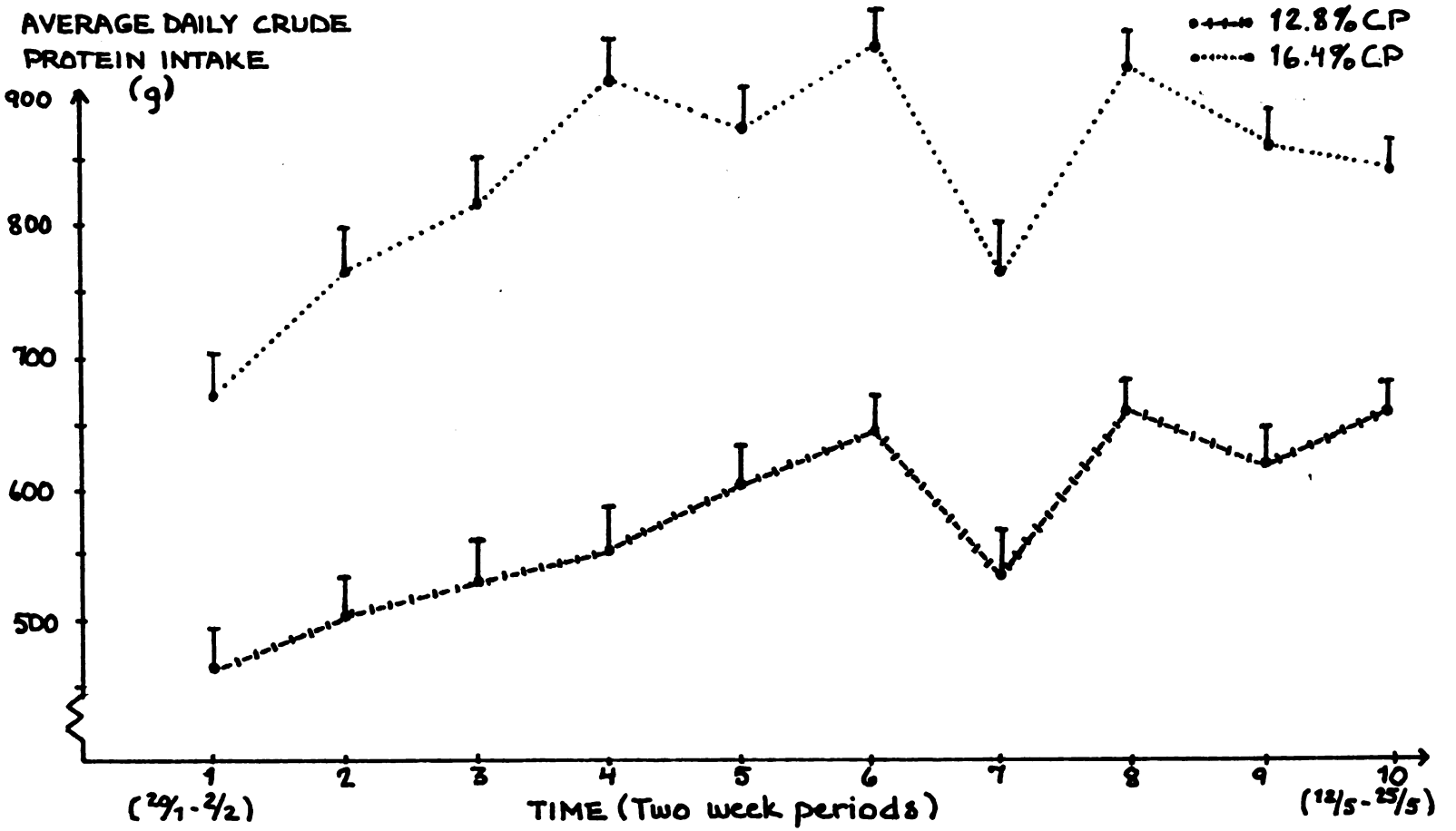

FIGURE 6. EFFECT OF DIETARY CRUDE PROTEIN LEVEL ON AVERAGE DAILY CRUDE PROTEIN INTAKE OF 10 BROWN SWISS AND 10 ZEBU HEIFERS.

There was also a period effect ($P < 0.0024$) on feed conversion of all heifers independent of dietary crude protein level (Figure 7 and 8).

No significant difference in average daily feed conversion could be detected between the Brown Swiss and the Zebu heifers during any period in the repeated measurements analysis (Figure 7). The apparently large difference between the two breeds in period four, -1.96 ± 10.6 kg DM/kg gain for the Brown Swiss heifers compared to 23.1 ± 10.7 kg DM/kg gain for the Zebus, was non-significant. This was due to the large individual variation in feed conversion.

Average daily feed conversion was not significantly affected by crude protein level during nine of the ten periods (Figure 8). It was only during period three that heifers on the adequate protein diet had a more efficient feed conversion than the heifers on the high protein diet; 3.28 ± 0.35 versus 4.38 ± 0.35 kg DM/kg gain ($P < 0.0309$). This was the only period with a difference between treatments and a small indiviual variation in feed conversion. During period seven, nine and ten the apparently large differences in feed conversion were non-significant due to large errors of the least square means.

Plasma concentrations of thyroid hormones

There were no significant differences between Zebu and Brown Swiss heifers in plasma thyroxine (T4), free T4 or

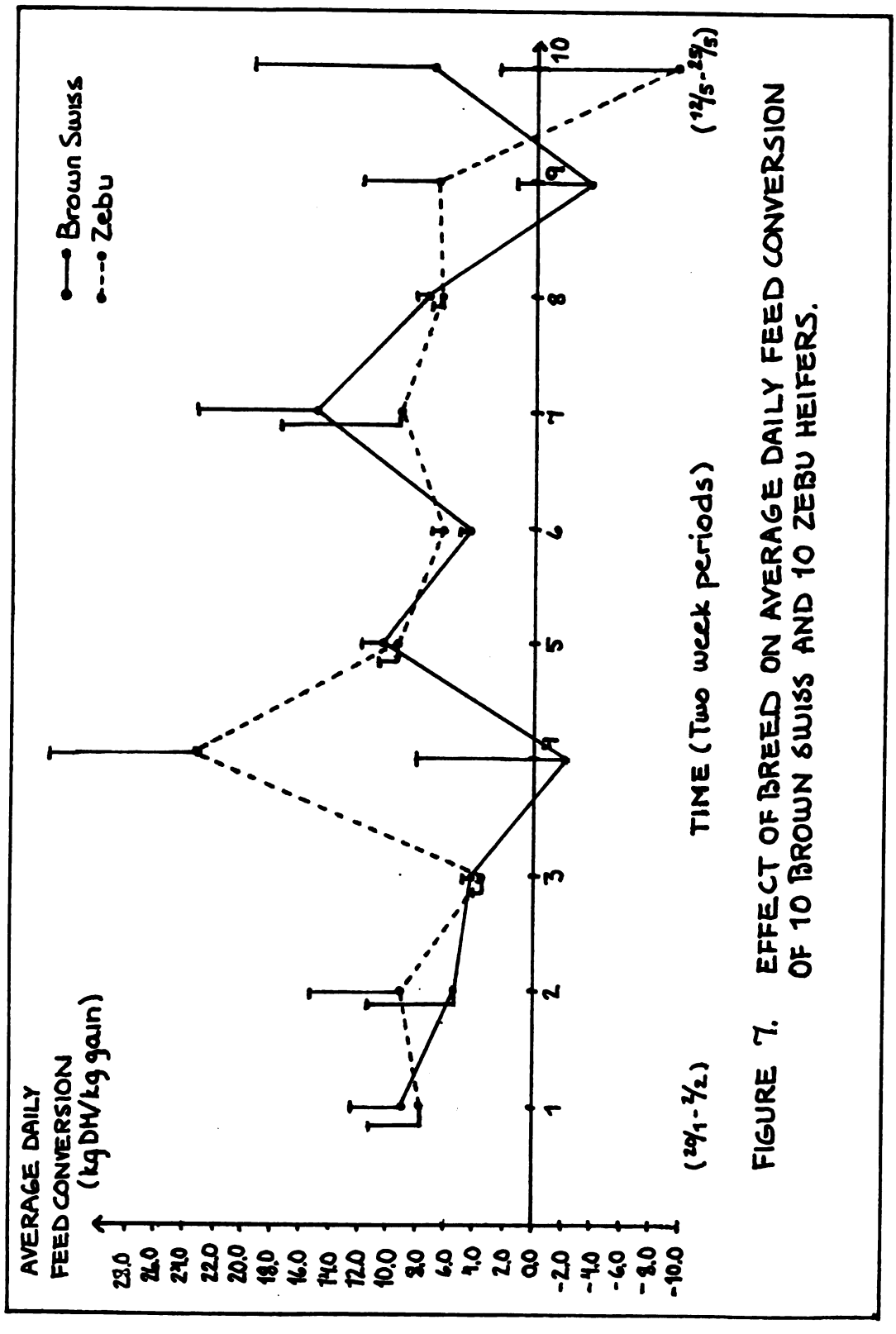

FIGURE 7. EFFECT OF BREED ON AVERAGE DAILY FEED CONVERSION OF 10 BROWN SWISS AND 10 ZEBU HEIFERS.

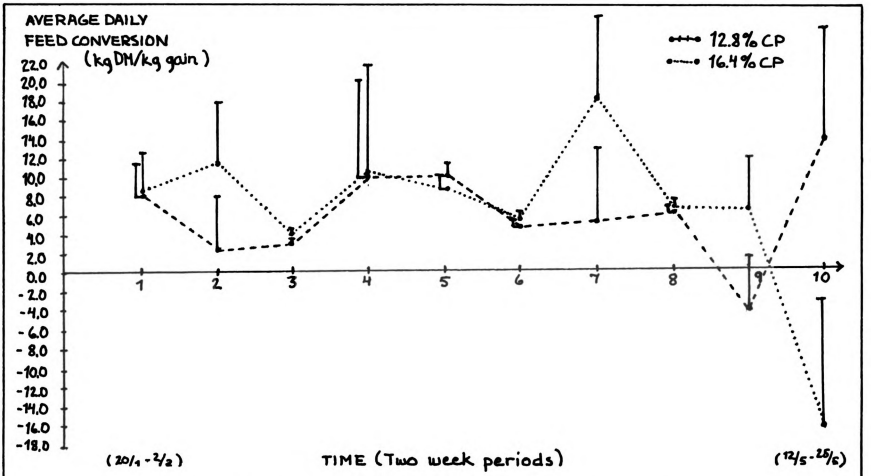

FIGURE 8. EFFECT OF DIETARY CRUDE PROTEIN LEVEL ON AVERAGE DAILY FEED CONVERSION OF 10 BROWN SWISS AND 10 ZEBU HEIFERS.

free triiodothyronine (free T3) concentrations (Table 11). Concentrations of the three hormone fractions were 32.0 ng/ml, 6.1 pg/ml and 5.0 pg/ml for the Zebu heifers and 29.8 ng/ml, 6.1 pg/ml and 4.8 pg/ml for the Brown Swiss heifers. Triiodothyronine (T3) concentrations were 1.8 ng/ml for the Zebu heifers compared to 1.6 ng/ml for the Brown Swiss ($P < 0.068$) indicating a breed difference for this hormone fraction (Table 11).

No significant effect of dietary crude protein level was observed on T4 or free T4 concentrations (Table 11). The concentrations were 30.6 ng/ml and 6.1 pg/ml for heifers on the 12.8% CP diet. Heifers on the 16.4% CP diet had concentrations of 31.2 ng/ml and 6.2 pg/ml respectively. The lower level of protein caused higher concentrations of both T3 ($P < 0.0061$) and free T3 ($P < 0.00196$) than did the high protein level. Plasma concentrations were 1.9 ng/ml compared to 1.5 ng/ml of T3 and 5.4 pg/ml versus 4.4 pg/ml of free T3.

Significant time effects on breeds and protein levels were observed for free T4, T3 and free T3 but not for T4 concentrations (Figure 11 - 16). It can therefore be concluded that there were no overall significant breed or treatment effect on T4 concentrations in the repeated measurements analysis. However, at times zero, one and three a significant breed difference was observed (Figure 9). The concentrations of T4 for the Zebu heifers compared to the Brown Swiss were 33.4 ng/ml versus 26.7 ng/ml ($P <$

TABLE 11. OVERALL EFFECT OF BREED AND DIETARY CRUDE PROTEIN LEVEL ON PLASMA CONCENTRATIONS OF THYROID HORMONES IN 12 ZEBU AND 12 BROWN SWISS HEIFERS

	LS MEAN	STD ERROR LS MEAN	F VALUE	PR > F
PLASMA CONCENTRATIONS OF THYROXINE (T4), ng/ml				
BREED EFFECT				
ZEBU	32.0	1.18	1.71	0.2051
BROWN SWISS	29.8	1.18		
TREATMENT EFFECT				
12.8% CP	30.6	1.18	0.15	0.7033
16.4% CP	31.2	1.18		
PLASMA CONCENTRATIONS OF FREE T4, pg/ml				
BREED EFFECT				
ZEBU	6.1	0.25	0.00	0.9890
BROWN SWISS	6.1	0.25		
TREATMENT EFFECT				
12.8% CP	6.1	0.25	0.08	0.7765
16.4% CP	6.2	0.25		
PLASMA CONCENTRATIONS OF TRIIODOTHYRONINE (T3), ng/ml				
BREED EFFECT				
ZEBU	1.8	0.08	3.70	0.068
BROWN SWISS	1.6	0.08		
TREATMENT EFFECT				
12.8% CP	1.9	0.08	9.28	0.0061
16.4% CP	1.5	0.08		
PLASMA CONCENTRATIONS OF FREE T3, pg/ml				
BREED EFFECT				
ZEBU	5.0	0.26	0.21	0.6542
BROWN SWISS	4.8	0.26		
TREATMENT EFFECT				
12.8% CP	5.4	0.26	6.38	0.0196
16.4% CP	4.4	0.26		

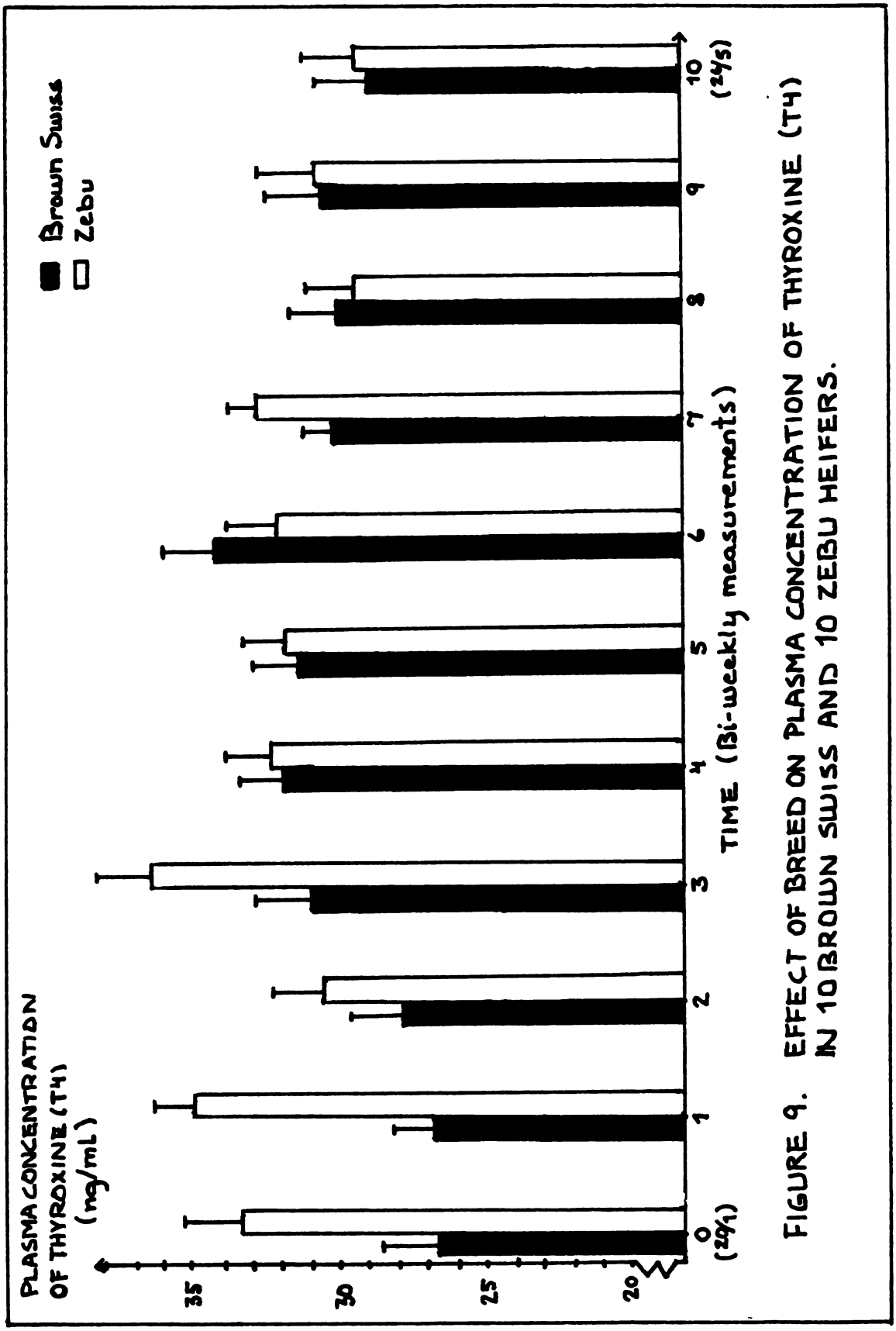

FIGURE 9. EFFECT OF BREED ON PLASMA CONCENTRATION OF THYROXINE (T4) IN 10 BROWN SWISS AND 10 ZEBU HEIFERS.

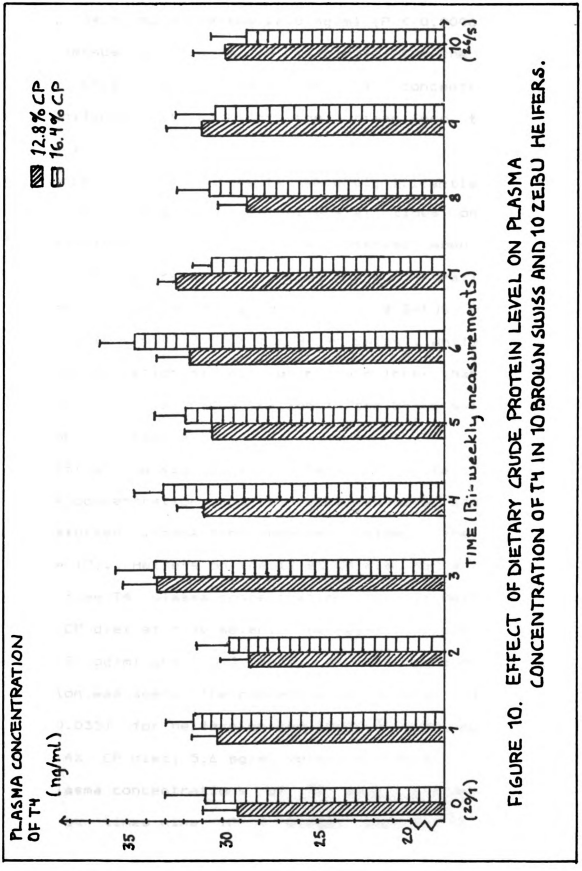

FIGURE 10. EFFECT OF DIETARY CRUDE PROTEIN LEVEL ON PLASMA CONCENTRATION OF T4 IN 10 BROWN SWISS AND 10 ZEBU HEIFERS.

0.027), 34.9 ng/ml versus 26.8 ng/ml ($P < 0.0006$) and 36.4 ng/ml versus 30.8 ng/ml ($P < 0.054$) for the respective times. Effect of protein on T4 concentration was non-significant at each of the times zero through ten (Figure 10).

Concentrations of free T4 were significantly different between Zebu and Brown Swiss heifers at times one and six and a time*breed interaction was observed when comparing these times (Figure 11). At time one concentrations of the hormone fraction were 7.6 pg/ml for the Zebus compared to 5.6 pg/ml ($P < 0.0012$) for the Brown Swiss , while the Zebus had a concentration of 6.6 pg/ml and lower than the 8.0 pg/ml ($P < 0.0038$) concentration in the Brown Swiss heifers for time six (Figure 11).

Similarly a significant effect of protein levels on free T4 concentrations was seen at times seven and eight and a time*breed interaction observed between the two times (Figure 12). Heifers on the 12.8% CP diet had a higher ($P < 0.05$) free T4 plasma concentration than did heifers on the 16.4% CP diet at time seven. The respective concentrations were 6.2 pg/ml and 5.3 pg/l. At time eight the opposite situation was seen. The concentration of free T4 was lower ($P < 0.035$) for heifers on the 12.8% CP diet and higher on the 16.4% CP diet; 5.6 pg/ml versus 6.5 pg/ml.

Plasma concentrations of T3 were different between breeds at times nine ($P < 0.028$) and ten ($P < 0.0421$) (Figure 13). The Zebu heifers had T3 concentrations of

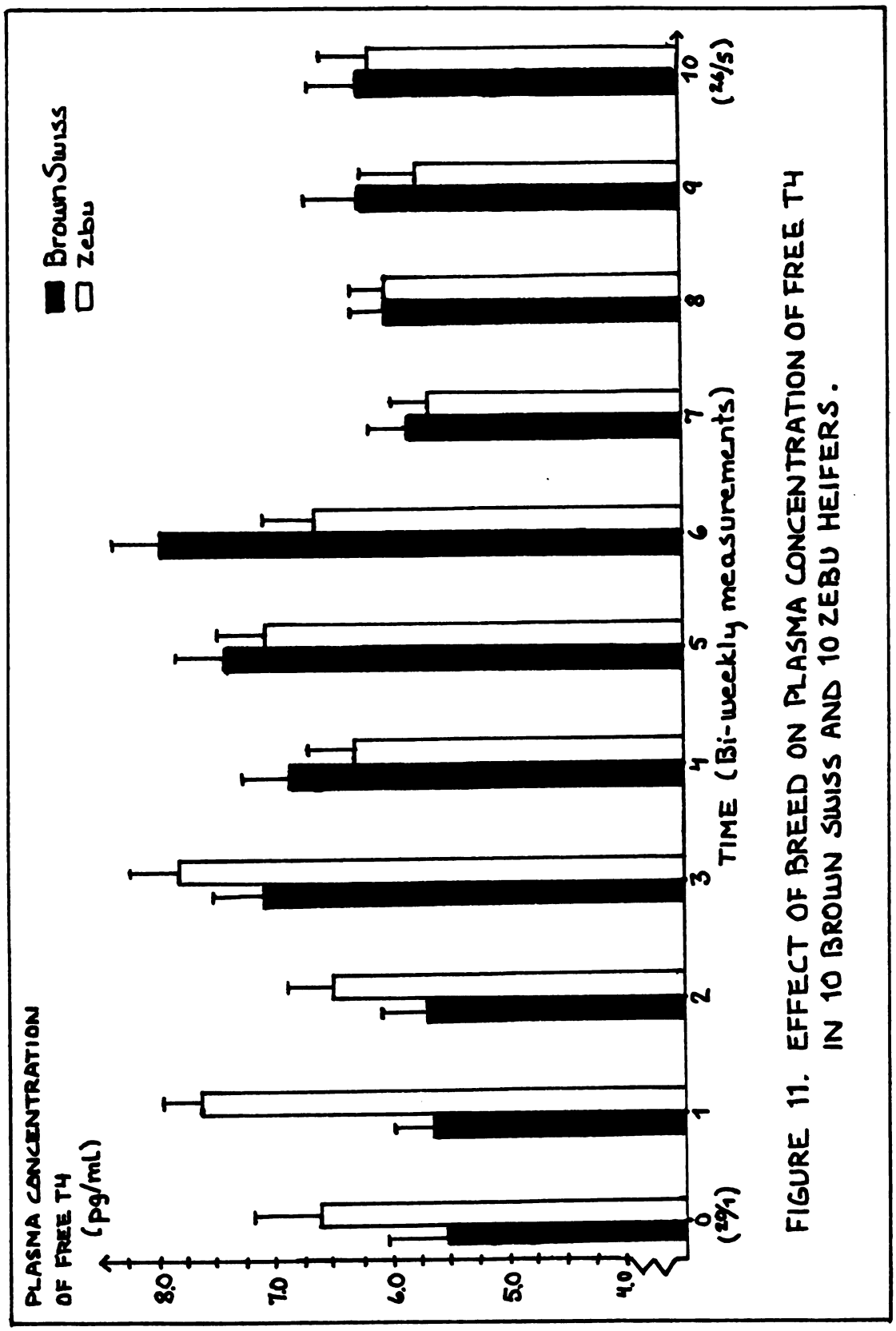

FIGURE 11. EFFECT OF BREED ON PLASMA CONCENTRATION OF FREE T4 IN 10 BROWN SWISS AND 10 ZEBU HEIFERS.

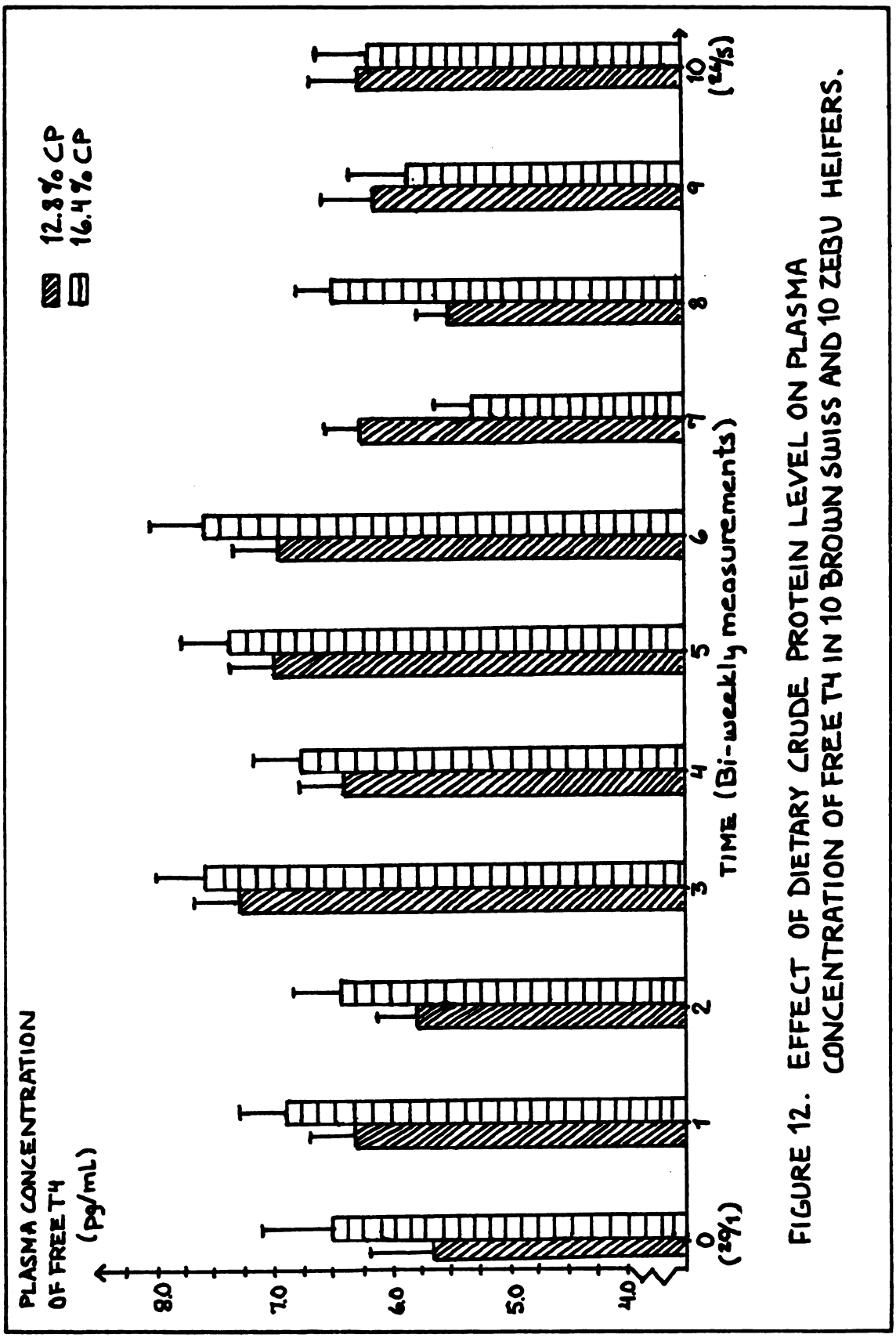

FIGURE 12. EFFECT OF DIETARY CRUDE PROTEIN LEVEL ON PLASMA CONCENTRATION OF FREE T4 IN 10 BROWN SWISS AND 10 ZEBU HEIFERS.

2.0 ng/ml and 1.9 ng/ml at these times, while concentrations in the Brown Swiss heifers were 1.7 ng/ml and 1.5 ng/ml for the same times. Breed differences were non-significant at times zero and three due to a large individual variation in T3 values. The concentrations were 1.8 ± 0.2 ng/ml for the Zebus versus 1.6 ± 0.2 ng/ml for the Brown Swiss heifers at time zero and 2.3 ± 0.3 versus 1.8 ± 0.3 ng/ml for the two breeds at time three (Figure 13).

Differences in T3 concentrations between the two protein levels were seen at times seven ($P < 0.0125$), nine ($P < 0.0612$) and ten ($P < 0.0549$) (Figure 14). At these times higher T3 concentrations were consistently observed for the 12.8% CP diet; 2.2 ng/ml, 2.0 ng/ml and 1.9 ng/ml, compared to the 16.4% CP diet; 1.6 ng/ml, 1.7 ng/ml and 1.5 ng/ml. Due to a large individual variation in T3 concentrations no significant difference between protein levels occurred at time three. Plasma concentration of T3 was 2.3 0.2 ng/ml for the 12.8% CP diet and 1.8 0.3 ng/ml for the 16.4% diet (Figure 14).

The apparently large breed differences in free T3 concentrations at the first four times reached statistical significance only for times one and two (Figure 15). The large individual variation in T3 concentrations at times zero and three made the differences non-significant for these times. Concentrations of free T3 for the Zebu heifers compared to the Brown Swiss at the first four times were; 5.2 ± 0.6 versus 4.2 ± 0.6 pg/ml, 6.0 ± 0.4 versus 4.4

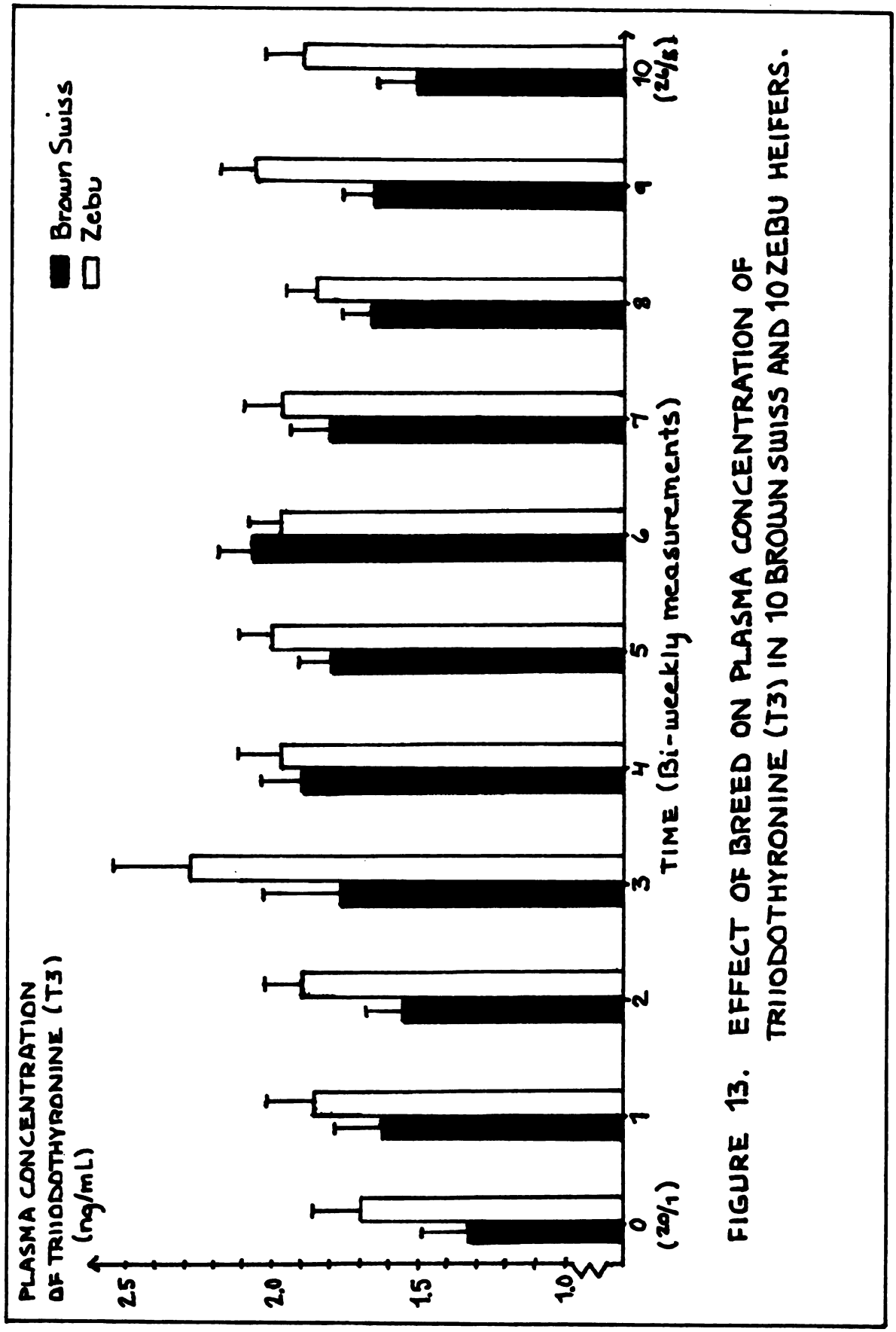

FIGURE 13. EFFECT OF BREED ON PLASMA CONCENTRATION OF TRIIODOTHYRONINE (T3) IN 10 BROWN SWISS AND 10 ZEBU HEIFERS.

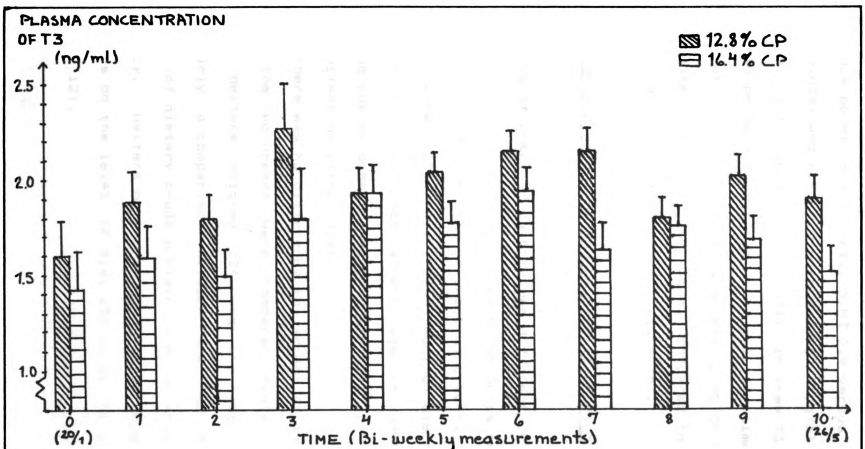

FIGURE 14. EFFECT OF DIETARY CRUDE PROTEIN LEVEL ON PLASMA CONCENTRATION OF T3 IN 10 BROWN SWISS AND 10 ZEBU HEIFERS.

± 0.4 pg/ml (P < 0.0061), 5.8± 0.4 versus 4.7 ± 0.4 pg/ml (P < 0.039) and 6.9±0.5 versus 6.0±0.5 pg/ml (Figure 15).

A difference (P < 0.0004) in free T3 concentrations between protein levels was seen only at time seven (Figure 16). At this time heifers on the 12.8% CP diet had a free T3 concentration of 5.7 pg/ml compared to 4.0 pg/ml for heifers on the 16.4% CP diet (Figure 16).

Age and weight of the heifers at onset of puberty

Age at onset of puberty was different (P < 0.0001) for the two breeds (Table 12). The Brown Swiss heifers were 286 days (9.4 months) old at onset of puberty, while the Zebu heifers were 373 days (12.3 months) old. Dietary crude protein level did not affect age at onset of puberty. Average age at onset of puberty was 330 days (10.9 months) for heifers on either diet.

There was no difference in weight at onset of puberty when the two breeds were compared (Table 12). The Brown Swiss heifers weighed 234 kg and the Zebus 233 kg. Similarly a comparison between the two diets showed no effect of dietary crude protein level on weight at onset of puberty. Heifers on the 12.8% CP diet weighed 234 kg and heifers on the 16.4% CP diet 233 kg at the onset of puberty (Table 12).

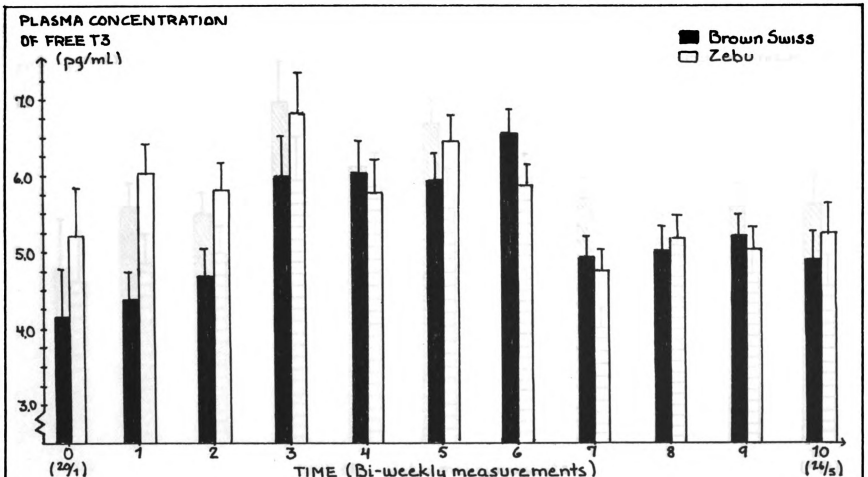

FIGURE 15. EFFECT OF BREED ON PLASMA CONCENTRATION OF FREE T3 IN 10 BROWN SWISS AND 10 ZEBU HEIFERS.

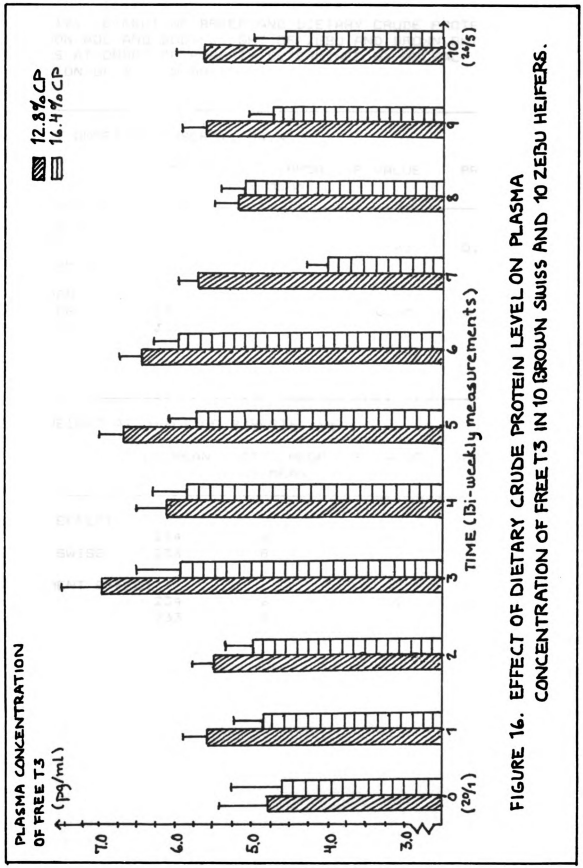

FIGURE 16. EFFECT OF DIETARY CRUDE PROTEIN LEVEL ON PLASMA CONCENTRATION OF FREE T3 IN 10 BROWN SWISS AND 10 ZEBU HEIFERS.

TABLE 12. EFFECT OF BREED AND DIETARY CRUDE PROTEIN LEVEL ON AGE AND BODY WEIGHT OF ZEBU AND BROWN SWISS HEIFERS AT ONSET OF PUBERTY DETERMINED BY RECTAL PALPATION OF THE OVARIES

AGE AT ONSET OF PUBERTY, days

	LS MEAN	STD ERROR LS MEAN	F VALUE	PR > F
BREED EFFECT				
ZEBU	373	7	77.43	0.0001
BROWN SWISS	286	7		
TREATMENT EFFECT				
12.8% CP	330	7	0.00	0.9934
16.4% CP	330	7		

BODY WEIGHT AT ONSET OF PUBERTY, kg

	LS MEAN	STD ERROR LS MEAN	F VALUE	PR > F
BREED EFFECT				
ZEBU	234	8	0.00	0.9592
BROWN SWISS	233	8		
TREATMENT EFFECT				
12.8%	234	8	0.02	0.8897
16.4%	233	8		

Plasma concentrations of progesterone

Five of the twelve Brown Swiss heifers had changes in plasma progesterone concentrations, which corresponded to ovarian structures determined by rectal palpations (Table 13). In four of these five heifers a rise in plasma progesterone (>1.0 ng/ml) corresponded to the second functional corpus luteum determined by palpation of the ovaries. One heifer, number 44 SP, had an elevated progesterone concentration corresponding to a functional corpus luteum during both her first and second estrous cycle as detected by rectal palpation (Table 13).

Plasma samples from the Zebu heifers had consistently very low progesterone concentrations. Four hundred and twelve samples were analyzed and 385 of these had progesterone concentrations of less than 0.4 ng/ml. Twenty four plasma samples contained between 0.4 and 1.0 ng/ml of progesterone. Only three samples had concentrations of more than 1.0 ng/ml of progesterone. Those three samples were baseline samples taken from one heifer, number 60 IndoBrazil, while she was prepuberal. Representative profiles of plasma progesterone concentrations in select Zebu heifers on diet 1 are shown in Figure 17 and on diet 2 in Figure 18.

TABLE 13. A COMPARISON BETWEEN RECTAL PALPATION OF THE OVARIES AND PLASMA PROGESTERONE CONCENTRATIONS TO DETECT FIRST AND SECOND OVULATION IN BROWN SWISS HEIFERS

RECTAL PALPATIONS

HEIFER NUMBER	1ST OVULATION (date)	BODY WEIGHT (kg)	AGE (days)	CL (date)	2ND OVULATION (date)	CL (date)
50 SP	July 3	217	282	July 11	July 19*	July 26
44 SP	June 7*	237	281	June 16	June 22*	July 3
45 SP	May 15	196	251	May 26	June 24*	July 1
39 SP	June 12	242	297	June 9	June 27*	July 5
343 SP	June 24	249	281	July 1	July 16*	July 23

PLASMA PROGESTERONE CONCENTRATIONS

HEIFER NUMBER	1ST** OVULATION (date)	CL (date)	PROGESTERONE (ng/ml)	2ND** OVULATION (date)	CL (date)	PROGESTERONE (ng/ml)
50 SP	July 19*	July 26	1.2			
44 SP	June 7*	June 16	1.1	June 22*	July 2	4.3
45 SP	June 24*	July 1	3.9			
39 SP	June 27*	July 16	5.1			
343 SP	July 21*	July 21	1.1			

* cycles during which ovarian structures detected by rectal palpations corresponded to changes in plasma progesterone concentrations.

** Ovulation number if a progesterone concentration > 1 ng/ml, had been used as criterion for onset of puberty.

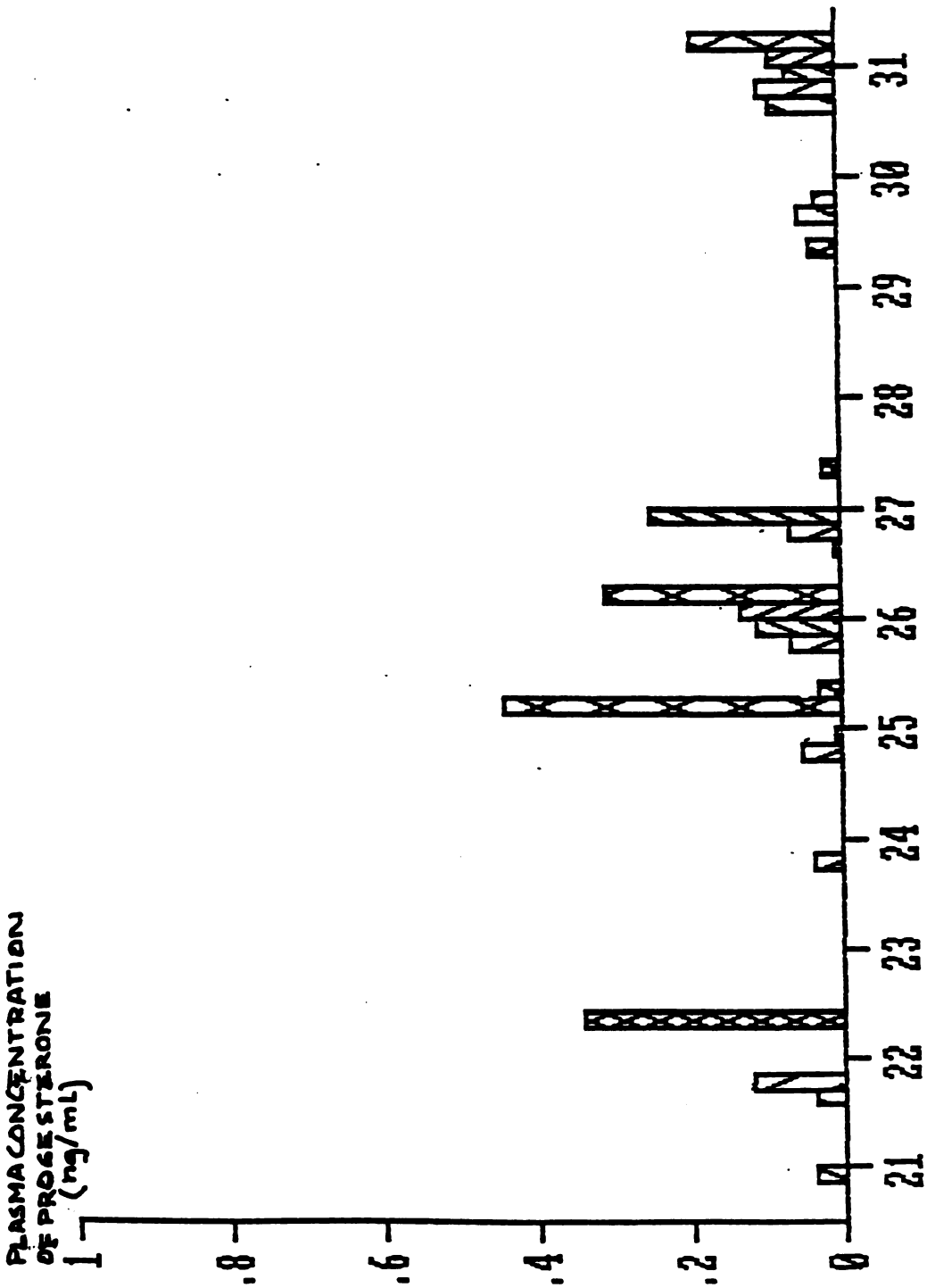

FIGURE 17. REPRESENTATIVE PROFILES OF PLASMA PROGESTERONE CONCENTRATIONS IN SELECT ZEBU HEIFERS ON DIET I. EACH SET OF BARS REPRESENT ONE HEIFER.

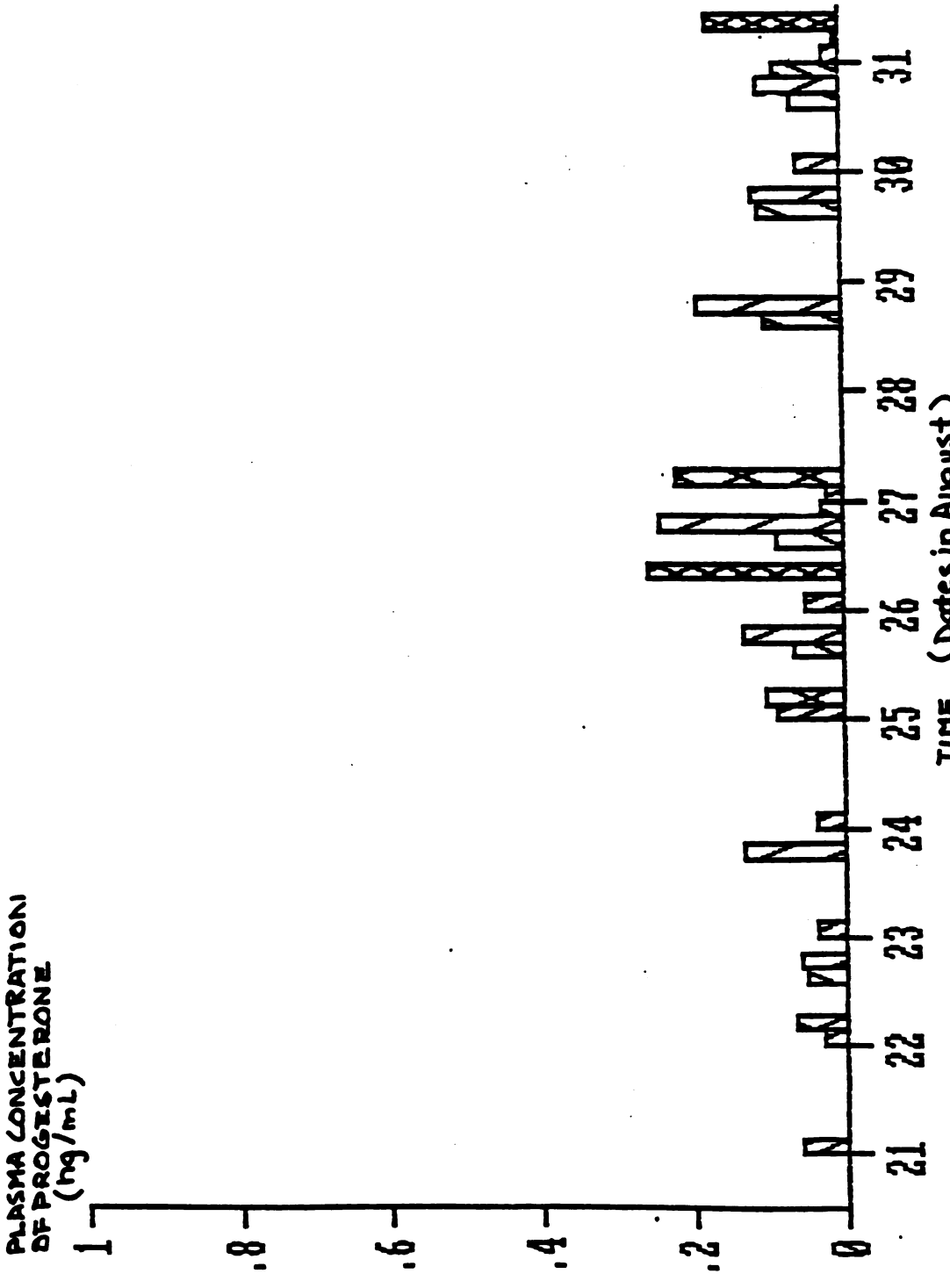

FIGURE 18. REPRESENTATIVE PROFILES OF PLASMA PROGESTERONE CONCENTRATIONS IN SELECT ZEBU HEIFERS ON DIET II. EACH SET OF BARS REPRESENT ONE HEIFER.

Plasma concentrations of cortisol

Analysis of cortisol in 31 plasma samples from one Zebu heifer, number 60 IndoBrazil, showed very high concentrations of the hormone in the majority of samples (Figure 19). Twenty two of the plasma samples had cortisol concentrations above 20 ng/ml and in 11 of these the concentrations of cortisol was above 30 ng/ml. Normal concentrations of cortisol in heifers were between 5 and 10 ng/ml (Zinn et al., 1986). A comparison was made between cortisol concentrations and plasma progesterone concentrations in the these samples (Figure 19). Changes in cortisol concentrations corresponded to changes in plasma progesterone concentrations. It was especially obvious for the two samples, in which cortisol concentrations peaked to 49.3 ng/ml and 56 ng/ml respectively and plasma progesterone increased to 2.6 ng/ml and 3.0 ng/ml. These two samples are the prepuberal samples mentioned in the section above. Plasma concentrations of both cortisol and progesterone decreased in samples taken later during the trial.

Plasma concentrations of minerals

No significant effect of breed or dietary crude protein level could be observed on plasma concentrations of calcium, copper, sodium and zinc (Table 14). Calcium concentrations were 9.9 to 10.0 mg/dl for the Zebus and the Brown Swiss

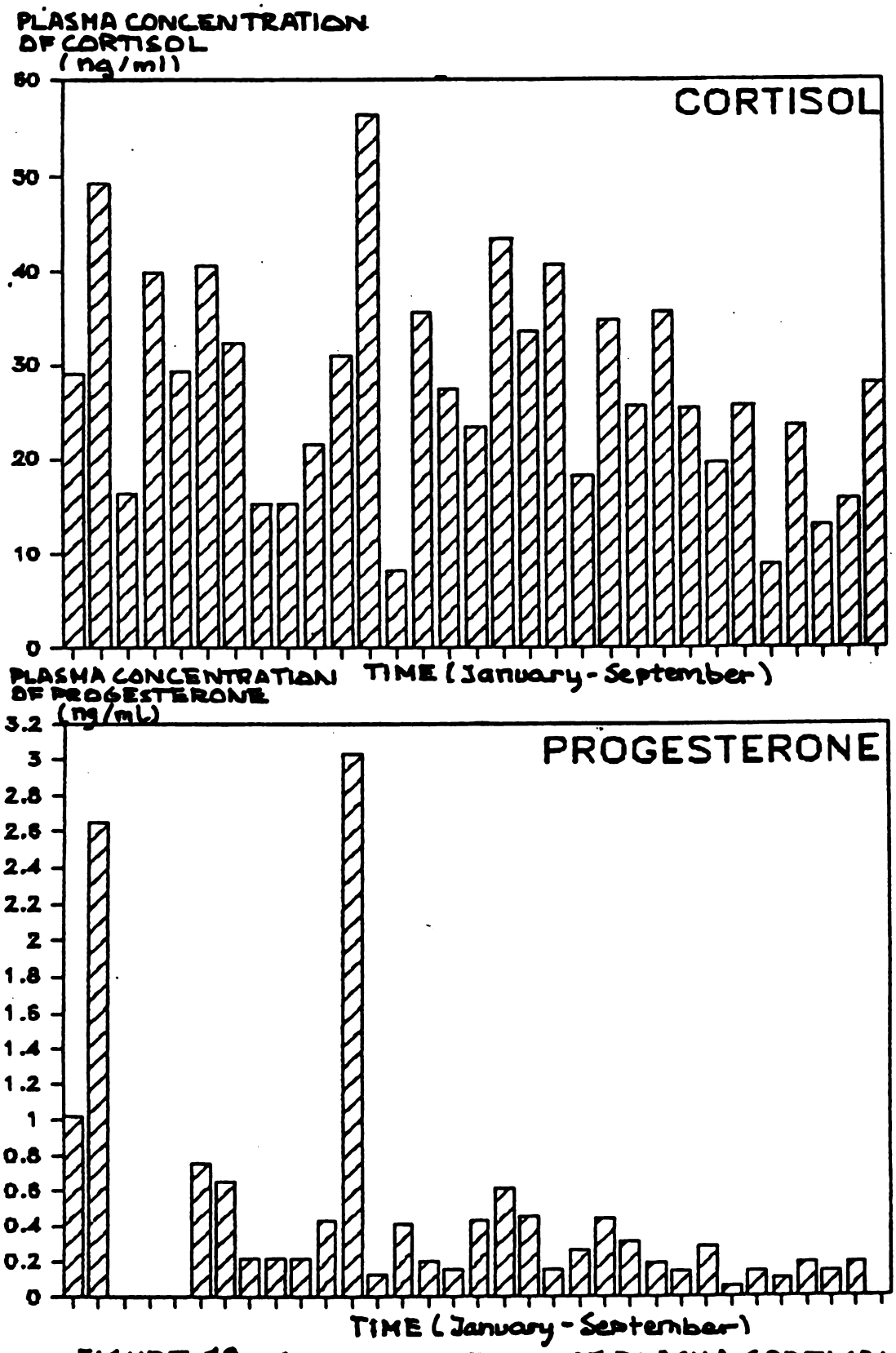

FIGURE 19. CONCENTRATIONS OF PLASMA CORTISOL AND PROGESTERONE IN SAMPLES TAKEN WITH IRREGULAR INTERVAL FROM HEIFER NUMBER 60 INDOBRAZIL.

TABLE 14. EFFECT OF BREED AND DIETARY CRUDE PROTEIN LEVEL ON CONCENTRATIONS OF PLASMA MINERALS IN ZEBU AND BROWN SWISS HEIFERS

		PLASMA MINERALS, mg/dl			
MINERAL	FACTOR	LS MEAN	STD ERR LS MEAN	F VALUE	PR < F
CALCIUM	ZEBU	10.0	0.11	0.17	0.6817
	BROWN SWISS	9.9	0.11		
	12.8%	10.0	0.11	0.10	0.7582
	16.4%	9.9	0.11		
PHOSPHORUS	ZEBU	7.5	0.17	13.49	0.0014
	BROWN SWISS	6.6	0.17		
	12.8%	7.3	0.17	5.88	0.0244
	16.4%	6.7	0.17		
MAGNESIUM	ZEBU	2.2	0.06	0.93	0.3469
	BROWN SWISS	2.3	0.06		
	12.8%	2.4	0.06	6.05	0.0227
	16.4%	2.2	0.06		
SODIUM	ZEBU	336	5	0.56	0.4640
	BROWN SWISS	331	5		
	12.8%	339	5	2.12	0.1604
	16.4%	329	5		
COPPER	ZEBU	0.16	0.05	1.20	0.2858
	BROWN SWISS	0.08	0.05		
	12.8%	0.16	0.05	1.15	0.2965
	16.4%	0.08	0.05		
IRON	ZEBU	0.22	0.01	2.76	0.1116
	BROWN SWISS	0.19	0.01		
	12.8%	0.22	0.01	4.56	0.0447
	16.4%	0.19	0.01		
ZINC	ZEBU	0.27	0.02	3.40	0.0794
	BROWN SWISS	0.22	0.02		
	12.8%	0.27	0.02	2.33	0.1420
	16.4%	0.23	0.02		

and for both dietary crude protein levels. Copper concentrations were 0.16 ± 0.05 mg/dl in the Zebu heifers and 0.08 ± 0.05 mg/dl in the Brown Swiss. Heifers on the 12.8% CP diet had a concentration of 0.16 ± 0.05 mg/dl and the ones on the 16.4% CP diet a concentration of 0.08 ± 0.05 mg/dl. Concentrations of sodium were 329 to 339 mg/dl for both breeds and treatments. Zinc concentration in the Zebu heifers was 0.27 ± 0.02 mg/dl and in the Brown Swiss 0.22 ± 0.02 mg/dl. Heifers on the adequate protein level had a zinc concentration of 0.27 mg/dl compared to 0.23 mg/dl for those on the high protein diet..

Concentration of total phosphorus was measured in the plasma samples. The values were then converted to inorganic phosphorus by use of the factor 0.43 (Stowe et al, 1985) . The concentration of phosphorus was higher ($P < 0.001$) in the Zebu heifers with 7.5 mg/dl compared to 6.6 mg/dl in the Brown Swiss (Table 14). There was also an effect ($P < 0.0195$) of dietary crude protein level on plasma phosphorus. Heifers on the lower protein diet had a phosphorus concentration of 7.3 mg/dl and heifers on the high protein diet had a concentration of 6.7 mg/dl.

Magnesium and iron concentrations were different between diets ($P < 0.0244$ and $P < 0.0447$) (Table 14). A higher magnesium concentration of 2.4 mg/dl was seen on the 12.8% CP diet, while the concentration on the 16.4% CP diet was 2.2 mg/dl. Iron concentration was also higher on the adequate protein diet, 0.22 mg/dl while concentration on the

high protein diet was 0.19 mg/dl. There was no breed effect on either magnesium or iron concentrations. The concentrations of magnesium were 2.2 mg/dl in the Zebus and 2.3 mg/dl in the Brown Swiss and the concentrations of iron 0.22 mg/dl and 0.19 mg/dl in the two breeds.

DISCUSSION

The discussion is divided in 12 sections. The first two sections follow the results presented in the previous chapter. In the first section the results of feed intake and growth rate are discussed. The second section is focused on observations made about plasma thyroid hormone concentrations. Age and weight at onset of puberty is discussed in the next section and plasma mineral concentrations in the subsequent one.

The following two sections concentrate on effects of handling stress. The first is centered around its effect on performance of the heifers and the other around effect of handling stress on plasma cortisol and progesterone concentrations. In the following section attention is brought to problems found with prior research on puberty and it leads up to the concept of puberty, which is the subject of the subsequent section.

Early weaning and the use of silage-based rations to feed young calves are then discussed in two more practically oriented sections. The Coat-a-Count progesterone assay used in the experiment is given attention in a subsequent section. The last section is focused on practical implications of the results found in this experiment.

Results of average daily gain, dry matter and crude protein intake and feed conversion

Average daily gain of Zebu and Brown Swiss heifers was very satisfactory (611 and 882 g) . Their daily gain was two to four times higher than common under grazing conditions in the study area and many other parts of the tropics. Dietary crude protein level had no effect on average daily gain (748 and 745 g). The heifers were gaining weight at maximum capacity on the given diets, therefore crude protein was not a limiting factor.

A higher average daily crude protein intake of heifers on the 16.4% CP diet compared to those on the 12.8% diet was planned in the design of the experiment. Heifers on the high protein diet consumed 838 g CP/day compared to 604 g CP/day on the adequate protein diet. Some of that difference can be attributed to slightly higher dry matter intake (5.6 versus 5.0 kg/day) for heifers on the high crude protein diet. It is possible that the higher crude protein content made the diet more palatable.

The more efficient feed conversion of heifers on the adequate protein diet compared to the high protein diet (6.8 versus 7.7 kg DM/kg gain) demonstrated a disadvantage of increasing the dietary crude protein level. The additional crude protein in the 16.4% CP diet was no doubt oxidized. Excess protein, with its high heat increment, may have decreased feed efficiency (Shirly,1986). These

observations are in agreement with feed efficiency reported in dairy heifers in the humid tropics of the Philippines (Trong Trung and Escano, 1980). An improved feed efficiency was reported when dietary crude protein was increased from 9% to 11%, but no further improvement was seen when the protein level was elevated to 13%. Feed efficiency was further similar to that seen in Zebu and Brown Swiss heifers in the present trial (7.8 and 6.7 kg DM/kg gain).

The greater feed intake, higher average daily gain and better feed efficiency of the Brown Swiss heifers compared to the Zebus were expected. The Zebus (Bos Indicus) are known to be slowly maturing and metabolically less efficient cattle than Brown Swiss (Bos Taurus). The digestive tract of Zebus has a smaller metabolic weight and their feed consumption is lower than that of European breeds.

A better understanding of what affected feed intake and growth rate of the heifers was obtained by studying individual two week periods of the trial. Several factors were found to have an effect on average daily gain and feed conversion, while dry matter and subsequent crude protein intake was more stable during the course of the experiment (Figure 1,2 and 7,8).

Average daily gain and feed conversion vary inversely in the graphs, a peak in gain corresponds to a decrease in the feed conversion graph. In the trial an increase in

average daily gain corresponds to an improved feed efficiency. Heifers of both breeds, independent of crude protein level in their diet, were similarly affected by the prophylaxis and routine treatments (Table 7) given during the trial. During periods two and nine no treatments interfered with the daily routine and average daily gain of the heifers increased significantly. In period six another increase in average daily gain was observed, for which there is no obvious explanation. A major decrease in average daily gain occurred over period seven. This can be explained by a change in daily routine that included turning the heifers loose for observation of estrus behavior and the beginning of rectal palpations. The heifers were also sprayed against ticks, dewormed and vaccinated against brucellosis in period seven. Finally another plunge in daily gain took place during period ten. This coincides with the time of the first outbreak of footrot, spraying against ticks and deworming. Two Zebu heifers also had diarhoea lasting three days during this period.

As the heifers grew their dry matter and crude protein intake increased steadily over time (Figure 3 - 6). Daily dry matter and crude protein consumption were in general not affected by prophylaxis and routine treatments. The exception was period seven when the start of estrus

detection and rectal palpations as well as several routine treatments of the heifers caused feed intake to drop drastically.

Plasma concentrations of thyroid hormones

The only trend towards a breed difference in plasma concentrations of thyroid hormones was the higher concentration ($P < 0.068$) of T3 in the Zebus compared to the Brown Swiss (1.8 versus 1.6 ng/ml). The abscence of a breed difference indicates that the Brown Swiss heifers were well adapted to the tropical climate. European breeds are generally known to have lower concentrations of thyroid hormones in the tropics (Howes et al.,1962; Chaiyabutr et al., 1977; Cowley et al. 1971) and under controlled heat stress (Singh et al., 1981; Blincoe and Brody, 1955; Blincoe 1958) than Criollo or Zebu cattle. The Brown Swiss heifers in this experiment were all born to dams several generations younger than the original Brown Swiss cattle imported from temperate climate to the two experiment stations in the tropics. Since then acclimatization has apparently continued over time. High temperatures are known to depress thyroid function in cattle (Baccari et al., 1983; Yousef and Johnson ,1966; Yousef, Kibler and Johnson, 1967; Magdub et al., 1981; Hurley et al. 1981). In the present trial plasma concentrations of thyroxine were lower than reported in heifers under temperate climate

conditions (Schillo, Hansen et al., 1982; Resal et al., 1984; Kahl et al., 1977). However, plasma concentrations of triiodothyronine were similar to temperate climate values (Baccari et al., 1983; Kahl et al., 1977; Hurley et al..,1981) or even slightly higher than reported from a temperate climate trial (Refsal et al., 1984). It was also considerably higher than previously reported in heifers in tropical Mexico (Reese, 1983). The comparatively high T3 concentrations in Zebu and Brown Swiss heifers may be related to their high growth rates, since plasma concentration of T3 have been demonstrated to be positively correlated to growth rate (r=0.91) (Baccari et al., 1983).

Plasma concentrations of T4 and free T4 were not affected by dietary crude protein level or feed intake, which is in agreement with previous findings in other species (Danforth et al., 1979; Davidson and Chopra, 1979; Spaulding et al., 1979; Grant et al., 1978; Glass et al., 1977).

However, plasma concentrations of T3 and free T3 were significantly higher in heifers on the 12.8% CP diet compared to the 16.4% CP diet. A change in T3 concentration while T4 concentration remained constant may be due to the fact that any change in T4 production rate will be balanced by an alteration in T4 catabolism to T3. During fasting or overfeeding the change in conversion rate of T4 to T3 result in only minor alterations of T4 concentrations and significant changes in T3 concentrations (Grant et al.,

1978).

It is suggested that the significant effect of diet on T3 and Free T3 plasma concentrations in this trial was due to a carbohydrate effect and not a protein effect. The 12.8% crude protein diet contained a higher level of carbohydrate, with its higher content of rice polishings, than the 16.4% crude protein diet. The adequate protein diet held 51.4% rice polishings, while the high protein diet contained only 18.9% of this ingredient. An increased conversion from T4 to T3 , due to increased activity of 5'-deiodinase is seen , among other conditions, as an effect of carbohydrate overfeeding. That a carbohydrate effect was seen in this trial is supported by research conducted with human subjects (Danforth et al., 1975; Danforth et al., 1979; Davidson and Chopra, 1979; Azizi, 1978; Spaulding et al., 1976). A positively correlated effect of dietary carbohydrate on plasma concentration of T3 was also more pronounced in a long-term (> 7 months) study than in shorter trials (Danforth et al., 1979). The effect on T3 was attributed to an increased metabolic clearance rate and production of T3 without changes in the serum concentration or metabolism of T4. In the same trial overfeeding with protein did not influence plasma T3.

The importance of higher T3 and free T3 concentrations observed in heifers on the adequate protein diet appears related to an increased metabolic efficiency. Growth rates were identical on both diets, but heifers on the 12.8% CP

diet had a significantly lower dry matter and crude protein intake. The reason for identical growth rates was the improved feed efficiency in heifers on the adequate protein diet. Since T3 is the biologically more potent of the two thyroid hormones, an increase in its plasma concentration may reflect elevated metabolic activity. This is further supported by the observation that also free T3 was significantly higher in the heifers and the fraction not bound to protein is the active hormone. A reduction in thyroid activity is thought to be a defense mechanism reducing metabolic demands in the presense of high catabolic functions. In the heifers overfed with carbohydrate the opposite situation may have existed and an increase in metabolic activity promoted.

Another possibility would be that T3 and free T3 concentrations were decreased on the high protein diet reflecting a decreased metabolic efficiency, due to the heat increment produced by additional dietary protein.

Further understanding of what affected plasma thyroid hormone concentrations in the heifers was obtained by observing changes during 11 bi-weekly measurements. A study of each time when measurements were taken demonstrated that the Zebus either had significantly higher concentrations than the Brown Swiss of both bound and free fractions of T3 and T4, or showed a consistent trend towards higher plasma concentrations of these, at times zero through three (Figures 9-16). The lower thyroid

hormone concentrations in the Brown Swiss at these times could be due to a depression of thyroid activity caused by heat stress, which affected the young, immature Brown Swiss calves more than the Zebus at the same age and maturity. As the calves grew the acclimatization of the Brown Swiss seemed to improve. The breed difference disappeared after the first four measurements of thyroid hormone concentrations and no breed difference was observed in the overall results discussed above.

At time six a higher concentration of free T4 (Figure 13) was found in the Brown Swiss compared to the Zebus. The increase in free T4 concentration in the Brown Swiss heifers corresponded to a highly significant peak in their average daily gain, 1577 g/day during this period (Figure 1).

Plasma T3 was higher in Zebu heifers than Brown Swiss at times nine and 10, but no explanation was found for the difference in T3 concentration. During times nine and 10 average daily gain was similar for the breeds. Dry matter and crude protein intake, although different between the breeds, increased and decreased in the same manner for both breeds. Feed conversion was inconsistent for the breeds at the two times and gave no clues to the difference observed in T3 concentrations .

An effect of protein on free T4 and free T3 concentrations was observed at time seven and on free T4 at time eight. At time seven significantly higher free T4 and free T3 concentrations corresponded to the lower

dietary crude protein level. At time eight the opposite was seen for free T4. Heifers on the high protein diet now had the higher plasma concentration of free T4 compared to those on the lower protein diet. As discussed previously the heifers experienced excessive stress, which caused all of them a major decrease in average daily gain and feed intake during period seven. In contrast feed efficiency was significantly decreased only in heifers on the high protein diet over this period. The large decrease in free T4 and free T3 concentrations from time six to time seven in heifers on the 16.4% CP diet and their significantly lower free T4 and free T3 concentrations at time seven could be due to the high specific dynamic action of protein. The additional heat increment produced by meatbolism of the higher amount crude protein in this diet could have caused the decrease in feed conversion during stress. Consequently the less efficient feed conversion in heifers on the high protein diet was reflected in their lower free T4 and free T3 concentrations. At time eight the heifers, independent of dietary treatment, showed good average daily gain, feed intake and feed conversion and the effect of dietary crude protein on free T4 concentration was reversed. The additional crude protein in the 16.4% CP diet was beneficial for free T4 concentration. Plasma free T4 was significantly higher in heifers with the higher protein intake at time eight.

Also a higher plasma T3 concentration was observed for

heifers on the 12.8% CP diet compared to those on the 16.4% CP diet at time seven. The concentration of T3 did not change in heifers on the lower protein diet from time six to time seven. The significant effect of protein at time seven is entirely due to the decrease in plasma T3 from time six to time seven in heifers on the high protein diet. Despite this discrepancy between T3 concentration compared to the free fractions of T4 and T3 it is logical to assume that this difference too was due to the high specific dynamic action of protein.

Both at time nine and ten a trend towards higher T3 and free T3 concentrations could be detected in heifers on the lower protein diet compared to those on the high protein diet. The situation is similar to the comparison between the two breeds at the same times, which was commented on above. In the same way as with the breeds average daily gain, feed intake or feed conversion during corresponding periods do not help to explain the difference between treatments. On the contrary both average daily gain and feed conversion were better on the high protein diet at time nine, while feed conversion improved slightly on the lower protein diet at time ten and average daily gain was similar for heifers on either diet at that time.

After close study of the individual thyroid hormone measurements it can be concluded that average daily gain, feed intake and feed conversion showed changes corresponding to changes in thyroid hormone concentrations,

but no consistent pattern was detected. More likely all four variables in interrelationship affected thyroid activity. Apparently any of the variables may have a more pronounced influence on thyroid activity at a certain measurement depending on specific circumstances at the time. This was exemplified at several times above.

Age and weight of the heifers at onset of puberty

Onset of puberty in both Brown Swiss and Zebu heifers occurred very much earlier than common in the tropics of Mexico or elsewhere in the tropics. It is therefore of value to compare results of the present trial to what is common on farms as well as on experiment stations in the tropics, and also under temperate climate conditions.

The Brown Swiss heifers were 286 days old (9.4 months) when they attained puberty. Under temperate conditions this is a common age at puberty for Brown Swiss heifers. No previous experiments have reported puberty in a European breed at such a low age in the tropics. Even under well-managed experimental conditions in sub-tropical Mexico 86 Brown Swiss heifers in a puberty study at "Las Margaritas" were 432 days old (14.4 months) at puberty (INIP, 1985), which is five months older than age at puberty of the Brown Swiss heifers in this trial.

The Zebu heifers reached puberty at 373 days of age (12.3 months) or approximately six months earlier than

common under tropical conditions. A study, already in progress for several years, designed to improve age at puberty in Zebu heifers (IndoBrazil) by ensuring continuous adequate nutrition and management on sown pastures is being conducted under experimental conditions in the Mexican tropics (INIFAP 1986 and 1987). They have so far reported an average age at puberty of 15 months in 112 heifers. This is three months older than age at puberty of Zebu heifers in this experiment. There are no reports of Zebu heifers going through puberty under tropical conditions at such an early age. The breed difference in age at onset of puberty was expected since Bos Taurus mature earlier than Bos Indicus.

Weight at onset of puberty was similar for both breeds. The Brown Swiss weighed 234 kg and the Zebus 233 kg. These body weights are much lower than commonly reported in Brown Swiss ands Zebu heifers in the Mexican tropics. The heifers were also considerably lighter at puberty when compared to the previously described 86 Brown Swiss and 112 Zebu heifers, which had an average body weight at puberty of 283 kg and 268 kg respectively (INIP, 1985; INIFAP, 1986, 1987). Heifers in the present trial reached puberty at both an earlier age and a lower body weight than observed in the trials above. This is in agreement with findings by Dufour (1975). On the contrary Wiltbank et al.(1969) and Oyedipe et al. (1982) reported that heifers going through puberty at a younger age were

heavier and that heifers reaching puberty at a higher age weighed less.

Body weights at puberty of the Brown Swiss and Zebu heifers were identical in absolute terms (233 kg). A better understanding of what stage of development Brown Swiss and Zebu heifers have reached at onset of puberty can be obtained by expressing body weights at puberty as the ratio between absolute body weight at puberty and expected mature cow weight and compare the two breeds. The Brown Swiss breed is considered one of the larger dairy breeds and a mature cow in normal body condition can be expected to weigh around 590 kg (Rouse, 1973). A mature Zebu cow in normal body condition will not be as heavy as a Brown Swiss cow , but can be expected to weigh around 420 kg (Rouse, 1973; Williamson and Payne, 1978). In the present experiment the Brown Swiss heifers averaged 40% and the Zebus 55% of their expected mature body weights at the onset of puberty. This would indicate that a Zebu heifer has to reach a later stage of development than a Brown Swiss heifer before maturity of the physiological processes leading to puberty occurs. Maybe this is an adaptive mechanism to ensure that the Zebu heifer is in a strong physiological condition and therefore can survive adverse environmental conditions before she becomes pregnant.

No effect of dietary crude protein level on age or weight at puberty of the heifers was seen in this experiment. It appeared that the heifers were growing and

sexually maturing at maximal rates with the adequate protein diet and the additional dietary crude protein provided by the high protein diet could not further enhance either process. Support for this observation is given by Trong Trung and Escano (1980), who reported no improvements in age or body weight at puberty of heifers whether they were fed 9%, 11% or 13% crude potein ad libitum in total mixed rations. All heifers were approximately 15.5 months old and had an average body weight of 191 kg. Contrary to results of both the present trial and the one by Trong Trung and Escano (1980), Oyedipe at al. (1982) found that increasing the dietary crude protein level in isocaloric diets from 8% to 13.4% and to 19% affected age and weight at puberty in Zebu heifers in Africa. A higher level of dietary crude protein corresponded to a younger age and heavier weight at puberty. A likely explanation of the observations is that neither group of heifers were growing and sexually maturing at maximum rate. Therefore their development was enhanced by the additional crude protein in the diet. This is further supported by the old ages and decreasing body weights at puberty of heifers on either diet. Age at puberty decreased from 704 days to 570 day of age and the body weights decreased from 207 kg to 162 kg with increased dietary crude protein.

It can be concluded that a significant breed difference in age at puberty was seen , while body weights at puberty were identical for the Zebu and Brown Swiss

heifers. When body weight at puberty was expressed as percent expected mature cow weight of the respective breeds, a calculated 15% relative difference in weight at puberty was observed between Brown Swiss and Zebu heifers. This could be interpreted as an adaptive mechanism causing the Zebu heifer to develop further and thereby become more resistent to adverse environmental conditions before she can be bred .

The dramatic decrease in age and weight at puberty of both breeds compared to those commonly observed in the tropics were due to three factors. Number one was placing all the calves on the trial at an early age. The second factor was uninterupted supply of feed of adequate quantity and quality during the whole time period through onset of puberty. Finally, the third factor was adequate daily management of the heifers.

This experiment has demonstrated that it is possible to bring Brown Swiss heifers to puberty at the same age uncer tropical conditions as they normally reach puberty in temperate parts of the world. It is also the first report of Zebu heifers in the tropics attaining puberty when only a year old.

Plasma concentrations of minerals

Plasma concentrations of all minerals except zinc were within normal range for both breeds, independent of dietary

crude protein level. Zinc concentrations were similar to an average concentration of 0.23 0.04 mg/dl measured in sera from 607 bovines with the same method, Inductively Coupled Argon Plasma emission spectroscopy (ICAP) (Stowe et al., 1985). They were also within the normal range given for zinc by Underwood (1977). However, these zinc concentrations are twice or three times the values reported by Stout et al. (1976) and Puls (1981). Minnick et al. (1982) found that serum concentrations of zinc above normal values were due to contact with rubber-stoppered vacutainer tubes (Becton, Dickinson & Co, Rutherford, NJ). This was also a possible explanation given for the high zinc values in the study by Stowe et al. (1985). The same explanation seems likely for the high zinc values in this experiment. Vacutainer tubes were used and the blood samples came in contact with the rubber stoppers, when anticoagulant in the tubes was mixed with the blood.

A breed difference ($P < 0.0014$) was observed in plasma concentration of inorganic phosphorus, which was higher in the Zebu heifers than in the Brown Swiss. There was also a treatment difference in plasma phosphorus. This difference can be attributed to high plasma phosphorus concentrations in the Zebus on the 12.8% CP diet compared to the ones on the 16.4% CP diet. The reasons for the difference are not apparent.

Plasma mineral concentrations found in the Brown Swiss and Zebu heifers in this trial are compared to those

TABLE 15. A COMPARISON BETWEEN PLASMA MINERAL CONCENTRATIONS IN ZEBU AND BROWN SWISS HEIFERS AND CONCENTRATIONS REPORTED BY BARRADAS (1980) AND MONROY (1982) FROM PASTURE TRIALS WITH CROSSBRED HEIFERS IN THE STATE OF VERACRUZ

PLASMA MINERAL CONCENTRATIONS, mg/dl

MINERAL	ZEBU	BROWN SWISS	DAIRY CROSS*	BEEF CROSS**
CALCIUM	10.0 ± 0.11#	9.9 ± 0.11	9.5 ± 0.11	8.0 ± 0.21
PHOSPHORUS	7.5 ± 0.17 [a]	6.6 ± 0.17 [b]	5.8 ± 0.12	5.0 ± 0.20
MAGNESIUM	2.2 ± 0.06	2.3 ± 0.06	2.6 ± 0.04	2.1 ± 0.06
COPPER	0.16±0.05	0.08±0.05	0.06±0.001	0.05±0.0015
IRON	0.22±0.01	0.19±0.01	0.21±0.003	0.18±0.006
ZINC	0.27±0.02	0.22±0.02	0.098±0.002	0.104±0.003
SODIUM	336 ± 5	331 ± 5		

* Pangola and Guinea grass pasture and native or Zebu cattle crossbred with Holstein and/or Brown Swiss (Barradas, 1980)

** Pangola, Guinea, Zacate and Star grass pasture and native cattle crossbred with Zebu or Brown Swiss for commercial beef production (Monroy, 1982)

\# Standard deviation

a and b = statistically different values ($P < 0.0014$)

reported by Barradas (1980) and Monroy (1982) from pasture trials with heifers in the State of Veracruz in Table 15. Plasma mineral concentrations, apart from those of phosphorus and zinc, found in the three trials are similar. Phosphorus concentrations are higher for both breeds in this trial than in the experiments by Barradas (1980) and Monroy (1982). Concentrations in the Zebu and Brown Swiss heifers are within the range, 6 to 8 mg/dl, reported to be normal for young cattle under one year of age (NRC, 1978). The values reported in crossbred heifers by Barradas (1980) and Monroy (1982) fall below the range for young animals and are within the normal range of 4 to 6 mg/dl, for adult cattle (NRC, 1978). The difference in zinc concentrations found in this experiment compared to the ones found by Barradas (1980) and Monroy (1982) is most likely due to a difference in handling of the blood samples. Their reported zinc values are in agreement with Stout et al. (1976) and Puls (1981).

Plasma sodium concentrations were within the normal range, 306 to 354 mg/dl, reported by Simesen (1980). Barradas (1980) and Monroy (1982) did not report sodium values.

It was concluded that Zebu and Brown Swiss heifers were fed an adequate level of the seven measured minerals, because mineral status was reflected in normal plasma concentrations of those elements.

Effect of "handling stress" on performance of the heifers

It is obvious from the results of this trial that additional handling, apart from the daily routine, and changes in daily management affected the heifers. This phenomenon will be referred to as "handling stress" . Average daily gain was most easily and consistently affected. Fluctuations from period to period reflected the amount of handling. Illnesses like outbreak of footrot also affected gain. The best illustration of effect of "handling stress" on both the Brown Swiss and the Zebu heifers is period seven when the combination of prohylaxis, routine treatments and major changes in daily management affected all four variables related to feed intake of the heifers and also their plasma thyroid hormone concentrations. Average daily gain of the heifers decreased drastically. Dry matter and crude protein intake also decreased markedly and feed efficiency fell dramatically for the Brown Swiss and slightly for the Zebus. Plasma concentration of T4 and T3 were slightly lowered in all heifers, while their free T4 and free T3 concentrations showed a significant decrease at this time.

Effect of "handling stress" on release and plasma concentration of cortisol and adrenal progesterone in heifers

Heifer number 60 IndoBrazil provided an excellent example of stress induced release of adrenal progesterone in a prepuberal heifer. Plasma concentrations of 1, 2.6 and 3.0 ng/ml respectively of progesterone were found in three blood samples taken in January and February for determination of baseline concentration of plasma progesterone. Number 60 did not reach puberty until August the same year. The only possible source of the progesterone in these plasma samples was the adrenal cortex. Since number 60 was the most nervous and easily excitable heifer, and the only one not to become docile during the course of the trial, a release of adrenal progesterone due to stress was suspected. The suspicion was confirmed by the results of plasma cortisol analysis of her samples previously analyzed for progesterone content.

Plasma cortisol concentration is known to show a wide variation within a short time and any kind of stress, such as handling and blood sampling, cause significant increases in plasma concentration of the hormone (Dickson, 1985). More than 70% of the samples had cortisol concentrations above 20 ng/ml and half of these were above 30 ng/ml. These concentrations indicated stress levels of the hormone compared to a normal concentration of 5 to 10

ng/ml (Zinn et al.,1986). Variation of plasma cortisol values corresponded positively to variation in plasma progesterone concentrations. The hypothesis of a stress induced release of adrenal progesterone was convincingly confirmed by cortisol peaks of 49.3 ng/ml and 56 ng/ml corresponding to 2.6 ng/ml and 3.0 ng/ml of plasma progesterone. Obviously the adrenal cortex can release considerable amounts of progesterone (Balfour et al., 1957) when the animal is under intensive stress (Watson and Munro, 1984; Wagner at al., 1972; Gwazduskas et al., 1972; Abilay et al., 1974).

The concentrations of this progesterone was higher than the generally accepted cut off point of 1.0 ng/ml used to detect a functional corpus luteum in female cattle (Stabenfeldt et al., 1968; Schams et al., 1981; Rutter and Randel, 1986; Nelsen et al., 1985; Adeyemo et al., 1980; Adeyemo, 1987; Agarwal et al., 1977). There are important implications of this observation when it comes to use of plasma progesterone concentrations for diagnosis of stage of estrus cycle in heifers and cows. Adrenal progesterone may be confounded with ovarian progesterone and incorrect conclusions be drawn about time of ovulation in female cattle. The amounts of adrenal progesterone released might be enough to lower fertility of cows (Watson and Munro, 1984; Wagner et al.,1972) through feed back of progesterone during the early part of the cycle on the hypothalamus or pituitary to block normal LH production

and/or release (Wagner et al., 1972). This would provide a possible explanation for the extremely low conception rates obtained in the Zebu cattle in Mexico (Galina, 1986) .

Thus, it becomes important to emphasize that female cattle should be frequently and gently handled to avoid both confounded results of diagnosis of stage of estrous cycle by plasma progesterone concentrations and a decreased fertility of the cows. Attention to this phenomenon is needed since it may affect many research projects designed to study different aspects of female reproduction in cattle. Particularly projects in developing countries, where it is common that cattle are infrequently handled and when handled are subjected to much acute stress, may be affected by stress release of adrenal progesterone. An additional factor in the tropical and developing countries is that Zebu cattle are often the most common type of bovines in those areas and they are nervous and more easily excitable than cattle of European breeds.

Following the reasoning above, the author cannot support the use of a concentration of 0.5 ng/ml progesterone as a confirmation of a functional corpus luteum. This criteria is consistently used by Galina et al. in Mexico (Galina, 1986; Orihuela et al,1983; Vaca et al,1983; Jimenez et al., 1984). Even the use of 1 ng/ml, previously cited as a much more generally accepted criteria for a functional corpus luteum, may be inadequate to ensure that adrenal progesterone does not interfere with

diagnosis of stage of estrous cycle. Further studies are needed to clarify the effects, frequency and magnitude of release of adrenal progesterone in female cattle in the tropics. Until then it is strongly suggested that the criteria of 1 ng/ml of plasma progesterone, as a cut-off point for confirmation of a functional corpus luteum, be continued. In addition it should not be the only criteria but should be used together with rectal palpation of the ovaries.

Problems with prior research on onset of puberty in heifers

Three main problems with prior research on puberty can be identified. To begin with it is apparent that there is no consistent terminology and understanding of the process of puberty in previous trials concerned with sexual maturity in heifers. Comparisons have continuously been made between trials independent of whether the objective of the investigation is onset of puberty, ongoing puberty or time when the heifers have gone through puberty and are completely sexually mature. Definitions of puberty vary widely between trials (Plasse et al., 1968; Wiltbank et al., 1962; Nelsen et al., 1985) and may also be determined in retrospect (Rutter and Randel, 1986).

Secondly the late age of heifers when started on a trial to determine what affects onset of puberty. The

heifers are often very close to natural puberty in both age and body weight (Plasse et al.,1968; Rutter and Randel, 1985; Trong Trung and Escano, 1980). In this type of trials previous history of feeding and management is generally not reported.

The last problem is nutrition of the heifers before and during the trials for study of onset of puberty. It has been demonstrated that nutrition affects onset of puberty (Wiltbank et al., 1962; Dufour, 1975; Oyedipe et al., 1982). Yet heifers in some trials heifers are purposedly undernourished to delay onset of puberty and onset of puberty investigated in the same trial. They were also switched from one level of feed to another during these trials (Day et al., 1984;, Gonzalez-Padilla et al., 1975) not as a treatment but to synchronize point in time of puberty.

Physiology of puberty

Observations in this trial and various physiological phenomena related to puberty reported in the literature reinforce the importance of the structural changes on the ovaries detected prior to and at onset of puberty in the Brown swiss and Zebu heifers. It is therefore warranted to provide a description of the findings by rectal palpations during that period. Already during the months immediately preceeding puberty, heifers of both breeds had clearly

palpable ovarian structures determined to be growing follicles. Several "waves" of growing follicles were detected in both Zebu and Brown Swiss heifers before onset of puberty. Usually it was one small follicle at a time, but sometimes two or more were palpated on the ovary. Daily palpations demonstrated that a single follicle developed in size over the next few days, before undergoing atresia during the following days. Follicles found during the period preceeding puberty were smaller in size than those at onset of puberty and did not mature and rupture. Thus no changes in plasma progesterone concentrations were detected corresponding to the occurrence and disappearance of these follicles. Estrus behavior, such as mounting and vaginal discharge of mucus were further signs of the approaching puberty in both breeds. At the onset of puberty the follicles appearing on the ovary generally grew to a larger size than during the prepuberal follicular "waves". Once a follicle was fully matured it ruptured and afterwards a fossa was felt on the ovary. Eight to nine days later a corpus luteum was palpated. It was only in one of the Brown Swiss heifers and in none of the Zebus that an increase in plasma progesterone concentration corresponded to the first palpated corpus luteum. During the second estrous cycle the expected changes in plasma progesterone concentration occurred in five of the Brown Swiss heifers. In the Zebu heifers, follicles, ovulations and corpora lutea did not correspond to changes in plasma

progesterone concentrations. They remained consistently very low during either estrous cycle.

It should be emphasized that onset of puberty in the heifer is a complex physiological process occurring gradually due to a mechanism still not completely understood (Ramirez and McCann, 1963; Scillo et al., 1982; Day et al., 1984). A new concept, nonpuberal estrus, have been introduced and described during the last few years (Nelsen et al., 1985; Rutter and Randel, 1986) Physiologdical findings contradicting accepted facts have also been reported (Dufour, 1975; Berardinelli et al., 1979).

Before continuation of this discussion it is essential to point out that the term "nonpuberal estrus" is missleading according to the author. By using the term "puberal" the impression is given that this occurs in the puberal heifer. Also despite the negation placed before "puberal" it indicates an event, which is to some degree related to functional fertility. Neither of this is correct, since it is clearly stated that this is an occurrence in the prepuberal heifer and it is solely a descriptive term of a type of behavior observed in immature heifers approaching puberty. The author would prefer to call it "prepuberal estrus behavior" to avoid missinterpretations. In the following discussion it will therefore be called "prepuberal estrous behavior ".

From a thorough study of the literature and

observations in the trial , it can be concluded that puberty is apparently an even more complex process than previously believed. During the time from the first indications of its onset until the process of puberty is completed, and the heifer has attained complete sexual maturity, a series of events has to take place and become synchronized. That process seems to take considerable time and it is suggested that clear distinctions be made between onset of puberty, puberty and complete sexual maturity.

A complete characterization of the process of puberty could include the appearance of six main features. Before presenting a plausible scheme for puberty it is noteworthy that at present it seems as if the chronological order for occurrence and characteristics of the events may vary due to nutrition and management (Gonzalez-Padilla et al., 1975; Berardinelli et al., 1979; Plasse et al., 1968; Wiltbank et al., 1962; Dufour, 1975) and genetics (Adeyemo et al., 1979; Aguilar et al., 1983; Nelsen et al., 1985; Rutter and Randel., 1986) and possibly additional factors. However, a basic order of the events can be indicated. It is suggested that onset of puberty begins with prepuberal estrus behavior and/or palpable ovarian follicles. Subsequent events could be either a puberal estrus or a silent ovulation. Either of the two should be succeeded by a detectable ovarian fossa. Afterwards three alternatives appear possible in the ovary; no

development of luteal tissue, formation of compact luteal tissue distinct from corpus luteum or development of a corpus luteum, which is at least to some degree functional. At present it is indicated that only three events are required for attainment of complete sexual maturity. They seem to be development of mature ovarian follicles, ovulation and formation of a functional corpus luteum. A heifer can be said to have gone through puberty and to have reached complete sexual maturity when ovulation occurs regularly and each ovulation is followed by development of a functional corpus luteum. By then ovulation will be preceeded by estrus in most heifers.

The objective of this trial was to study the onset of puberty in Brown Swiss and Zebu heifers. It was demonstrated that heifers at the onset of puberty may complete at least one estrous cycle before an ovulation followed by development of a functional corpus luteum occur. This is an indication that heifers at the onset of puberty, go through one or more estrous cycles before events required for complete sexual maturity form a well established pattern. It is speculated that ovulation without subsequent development of a functional corpus luteum is due to an incompletely synchronized follicle population at the time of ovulation. It has been observed that the group of follicles, which develop but never completely mature, seems to play an important role in the synchronization of estrus and ovulation (Hafez, 1978) and

may also affect the ovary after ovulation.

A hypothesis would be that the majority of heifers at the onset of puberty go through one or more estrous cycles. During these the physiological events lack the necessary synchronization for complete sexual maturity. Eventually the required events will be synchronized. However, the apppearance and chronological order of events and time period necessary to obtain synchrony may depend on nutrition, management, genetics, climate and possible other factors.

Early weaning

Early weaning of both Zebu and Brown Swiss calves is an important feature of the present experiment. To ensure an accurate and thorough evaluation of the effect of adequate nutrition and management on growth rate and onset of puberty in the heifers, their development had to be controlled with adequate nutrition and management and the animals monitored closely from the earliest age possible. For this reason the calves had to be weaned and started on the trial as young as possible. Both the Zebu and Brown Swiss calves were therefore weaned at once already at three months of age. It is of value to point out how this age at weaning relates to common practice for weaning of these breeds in the Mexican tropics.

Weaning of Zebu calves at three months of age is

highly unusual in the Mexican tropics. Both there and in many other parts of the tropics the common age at weaning of Zebu calves is eight to nine months. Even the experiment stations belonging to INIFAP, where improved management practices are continuously being tried out, wean Zebu calves at this age. In the present trial weaning of the Zebu calves at three months of age was facilitated by creepfeeding them concentrate from shortly after birth. At the time of weaning their daily intake of concentrate was 1 kg/head, which made the transition from nursing on pasture to total mixed rations and complete confinement easier. Once on the trial high quality silage-based rations and fresh water ad libitum, shaded pens, close supervision and access to veterinary care if needed ensured the health and good growth of the young calves. Results from this experiment proved that early weaning of Zebu calves can be done succesfully in the Mexican tropics and is feasible when adequate nutrition and management of the calves can be provided.

Also Brown Swiss calves are commonly weaned at an age beyond three months of age. Usually they are six months or older on farms in tropical Mexico or elsewhere in the tropics. Contrary to the management of Zebu calves on the INIFAP experiment stations, Brown Swiss calves are, however, weaned at three months of age under experimental conditions. Dairy farms with purebred cattle in the tropics of Mexico have in general a higher level of management than farms

with Zebu cattle. With the better infrastructure available on the farms with purebred dairy cattle early weaning of the calves at three months of age is becoming a possibility in the Mexican tropics. It is presently being tried out extensively with purebred calves within INIFAP and this trial added yet more data to that continuously collected on the experiment stations.

Use of silage-based rations for young and early weaned calves in the tropics

Feeding young calves silage-based rations is not a common feature in the tropics. The author does not know of any other trial in the Mexican tropics, where this has been done. Results from the present experiment showed that the calves readily accepted and achieved good gains on complete rations based on sorghum silage given to them from three months of age. Further support for feeding silage-based diets to young animals is given by trials conducted in temperate climates (Hammes et al., 1968; Leaver, 1973) and in the tropics of Colombia (Moore and Cock, 1983). A brief description of the experiment in Colombia is warranted to further emphasize the value of feeding silage to young calves in the tropics . Moore and Cock (1983) fed 18 grade Zebu steers cassava silage for 112 days after weaning at five months of age. Initial body weights of the calves were 97.7 kg and their average daily

gains were 405 g/day and 472 g/day respectively when daily rations of cassava silage ad libitum, 0.5 kg dried cassava chips and a mineral mix or this ration and an additional 0.25 kg cotton seed meal were given. This feed was readily accepted by the calves. Quality of the cassava silage was reported to be excellent and comparable to corn silage. It had 24.05% CP, 19.7% DM and 29.36% fiber. Cassava chips and/or cottonseed meal was/were given to provide additional energy.

It can be concluded, from the present trial and others reported, that silage-based rations can be succesfully fed to early weaned calves and offers a possibility to obtain a good growth rate when rearing young calves in the tropics.

It is possible today, in the tropics of Mexico, to advise farmers with good infrastucture on their farms to consider an earlier than common age of weaning of their calves. Farmers who can wean their calves during the rainy season and provide pasture of good quality as well as at least a mineral supplement would be the target group for early weaning of their calves. They could possibly wean their calves at five to six months of age instead of the prevalent weaning at eight to nine months of age.

Coat-a Count Progesterone assay procedure

The Coat-a-Count Progesterone assay procedure used in this experiment is officially endorsed by the International Atomic Energy Agency (IAEA) in Vienna for use in developing countries (Personal communication with Dr. R Nachreiner, 1987 and 1988, and Dr. E Mather 1988, Veterinary Clinic at Michigan State University). Spring of 1987 IAEA ordered 100 000 antibody coated tubes for progesterone analysis from Diagnostics Products Corp. From that time on this assay procedure has been used in experiments to determine plasma progesterone concentrations in domestic species under different conditions in a large number of developing countries. The International Atomic Energy Agency recommend modifying the assay procedure the way it was modified in this trial.

It is very important to set up international standards and criteria for RadioImmunoAssays. By doing so results from experiments conducted in different parts of the world can be compared without bias and correct conclusions can be drawn. For many chemical analysis different methods give the same results, but this is not true for progesterone assays. A large number of assays, such as liquid phase assays with different antibodies as well as commercially available solid phase kits of variable quality are used around the world. Each assay procedure has its own characteristics. Unless the citations refer to work with

the same assay, it is possible that apparent differences between progesterone concentrations are due to different assays and no real differences exist. By promoting widespread use of the Coat-A-Count Progesterone assay procedure the International Atomic Energy Agency is able to establish international standards for plasma progesterone and make results from trials in various parts of the world readily comparable. Results from this trial add a piece of valuable information, on plasma progesterone concentrations at onset of puberty in Brown Swiss and Zebu heifers in the humid tropics, to the data base presently being developed.

However, the possibility of release of adrenal progesterone due to stress of the animals at the time of blood sampling calls for caution when introducing Coat-A-Count Progesterone assay procedure to the developing countries. Unless the cattle are frequently handled and behave calmly there is a risk of faulty diagnosis of stage of estrous cycle when using this assay as well as any other RadioImmunoAssay. Due to its simplicity the new tool of the solid phase RadioImmunoAssay is rapidly becoming available for research in reproductive physiology in cattle of the developing world. Even though this assay procedure is simple to use, the common management level of cattle in the third world may not be adequate for correct use of this technology.

Practical implications

Before discussing the implications of this trial on cattle production in the Mexican tropics, two assumptions generally accepted as evidence for the difficulties in increasing cattle productivity in the tropics should be examined. It is important to do so, because results from the present trial give clear indications of their inaccuracy. One assumption is that cattle of temperate breeds do not grow, mature or produce on a similar level in the tropics as they do under temperate conditions. Results of this trial show that a growth rate and sexual maturation of Brown Swiss heifers comparable to what is seen in temperate climate zones can be obtained in the humid tropics. It is logical to believe that heifers with this rate of development would also have a comparable productivity during their adult life. The second assumption often put forward against rapid development of cattle production in the tropics is the low genetic potential of the Zebu cattle, which are the predominant type of cattle in many parts of the tropics. The Zebu heifers in this experiment grew at an average rate over the complete trial of more than 600 g/day. There were growth peaks of 1100 g/day for the whole group during two different two week periods. They also reached puberty at 12 months of age. These results are comparable to the development of beef heifers under temperate conditions. This trial has showed

that in the tropics adequate nutrition and management of growing calves results in performances comparable to those observed in dairy and beef heifers reared under temperate climate conditions. It can be concluded that in the tropics nutrition and management of young cattle are the factors limiting their performance and not the tropical climate per se or the genetic potential of local Bos Indicus cattle.

Mexico has 37.8 million cattle (FAO, 1984). Nine million cattle are considered to be dairy cattle. Only 12.4% of them are estimated to be kept in complete confinement while 87.6% are kept in semi-confinement or on pasture (Fernandez- Baca et al., 1986). Dairy farms managing their herds in complete confinement are commonly located in the temperate climatic zones, while milk production under the more extensive forms is mostly found in the low tropical areas. If the cows are kept entirely on pasture seasonal milking, where milking is restricted to the most favorable season of the year, is a characteristic feature. Milk production from systems using semi-confinement and milk production based only on pasture contribute 44% of Mexico's total milk production. Average annual milk production per cow under the semi-confinement system is 460 l and under the seasonal milking system 360 l (Fernandez-Baca et al., 1986).

Mexico, with its variety of climates, offer very good opportunities for significant increases in livestock

productivity. Many developing countries are not so fortunate. In the case of Mexico the expansion and highly technological development of dairy industry in the temperate zones around the larger cities and, most prominently, surrounding Mexico City must be continued and recieve strong support. When taking into consideration that Mexico City alone has an estimated population of 18 million people at present and the annual populatation growth of the country is 2.5%, it is easy to realize that additional efforts are needed to supply the Mexican population with milk. A rapid development of the dairy industry in the Mexican tropics , where a large number of the cattle is located, provides a clearly feasible solution to the problem.

At present only 5% of 4.5 million cattle in the state of Veracruz are pure bred, but 2.0 million are considered dairy cattle (INIP, 1981). This indicates the proportion of cattle contributing to milk production in one of the tropical states, where livestock production is of importance. If a substancial increase in milk and beef production is to be achieved in the Mexican tropics over the next five to ten years, factors limiting cattle productivity must be clearly identified. Information from the present trial about the potential in the tropics of purebred Brown Swiss and Zebu cattle when provided adequate nutrition and mangement help to fill the gap of knowledge. It would be of great value to conduct a similar experiment

with crossbred (F1) Brown Swiss-Zebu heifers, since crossbred cattle are presently the most common cattle type in the Mexican tropics.

The Mexican tropics is a highly underutilized resource for cattle production. This is especially true for milk production , but it is also true for beef production. In the present trial it has been demonstrated that inadequate nutrition and management of exisiting herds is likle to be the main constraint. Inadequate nutrition affects all aspects of production. These effects are malnutrition of the young calf and slow and irregular gains of all growing cattle. Weight losses occur during the dry period and body weight gain occurs only during the rainy season. As a consequence sexual maturity is delayed and first calving takes place at four years of age. Average milk production is only around 400 kg/lactation and the calving interval amounts to 16 to 20 months. This is not acceptable in light of the potential existing for cattle production in the Mexican tropics. It has been shown in this trial that when nutritional and management constraints are removed purebred heifers in the tropics can grow and mature at a rate, where they reach puberty at nine to 12 months. As a result they can be bred at 12 to 15 months of age and produce their first calf at two years of age. This is a highly significant improvement compared to what is now common. Strong and consistent support from the Mexican government is crucial to alleviate the constraints of

tropical cattle production and promote the necessary, rapid increase in milk and beef production feasible from the tropical parts of Mexico.

It can be concluded that if nutritional and mangement constraints are removed from cattle production in the Mexican tropics the potential exists for milk and beef production comparable to that in temperate climates. This can be achieved by applying the technology already available.

SUMMARY AND CONCLUSIONS

Summary

The objective of the trial was to study the effect of protein on growth rate and onset of puberty in heifers in the humid tropics of Mexico. Twelve Zebu and twelve Brown Swiss calves were weaned at three months of age, confined in individual pens and assigned to either an adequate protein (12.8% CP) or high protein (16.4% CP) diet until puberty. Diets consisted of sorghum silage, sunflower meal, rice polishings, molasses and minerals and were fed ad libitum as total mixed rations.

Rectal palpation of the first corpus luteum was used as criterion for onset of puberty. However, a progesterone concentration above 1.0 ng/ml confirmed the presence of a functional corpus luteum.

Age at puberty was 9.4 months for the Brown Swiss heifers and 12.3 months for the Zebus, a reduction of three to six months compared to what is common in the study area. Body weights at puberty were lighter than previously reported in Zebu and Brown Swiss heifers, 233 versus 234 kg. There was no effect of dietary crude protein level on either age or weight at puberty.

The Zebu heifers had an average daily gain of 611 g. Their dry matter and crude protein intake was 4.7 kg/day and 641 g/day and the feed conversion 7.8 kg DM/kg gain. Corresponding figures for the Brown Swiss heifers were 882 g, 5.9 kg/day, 801 g/day and 6.7 kg DM/kg gain.

Growth rates of the Zebu and Brown Swiss heifers were two to three times higher than common in the study area and comparable to growth rates reported in temperate climates.

The Brown Swiss had a significantly higher average daily gain and feed intake and were also more efficient feed converters than the Zebus. Since the Zebus are known to be slowly maturing animals, a characteristic which may be part of their adaptation to environmental stress, breed differences were expected.

There was no effect of dietary crude protein level on average daily gain. The heifers were gaining weight at maximum capacity and crude protein was not a limiting factor. Heifers on the 16.4 % diet had a higher feed intake than those on the 12.8% diet. The high protein diet may have been more palatable. A less efficient feed conversion was observed on the 16.4% CP diet, which may have been due to the additional heat increment from excess dietary protein.

Plasma mineral concentrations were measured and determined to be within normal range.

Plasma concentration of T4 and free T4 were 32 ng/ml and 6.1 pg/ml in the Zebu heifers. They had a T3

concentration of 1.8 ng/ml and the free T3 concentration was 5.0 pg/ml. The Brown Swiss heifers had plasma thyroid hormone concentrations of 29.8 ng/ml T4, 6.1 pg/ml free T4, 1.6 ng/ml T3 and 4.8 pg/ml free T3.

The only breed difference observed was in plasma T3 concentration, where a trend towards a higher ($P < 0.068$) value was observed for the Zebus. Lack of a breed difference indicate that the Brown Swiss heifers were well acclimatized to tropical conditions. Plasma T4 was lower than recorded under temperate conditions. However, plasma T3 and free T3 were similar to concentrations in temperate climates. This is probably due to high growth rates of the heifers, since a positive correlation between plasma T3 concentration and growth rate has been reported.

Heifers on the adequate protein diet had significantly higher concentrations of both T3 and free T3 than heifers on the high protein diet, possibly due to the higher carbohydrate content of the 12.8% CP diet. This is supported by reports from studies with human subjects.

Only five of the Brown Swiss heifers and none of the Zebus had a functional corpus luteum during their first or second estrous cycle . It is suggested that onset of puberty can occur without the development of a functional corpus luteum. Apparently a heifer may go through at least one estrous cylce before the first functional corpus luteum is developed.

Plasma progesterone concentrations of 1.0 to 3.0 ng/ml

were found in one of the Zebu heifers at prepuberal age. It was determined that this progesterone was of adrenal origin and released due to handling stress.

Conclusions

Reports of age and body weight at puberty are in many cases not comparable, due to lack of a generally accepted definition of puberty in heifers. More research is warranted to clarify the concept of puberty, so that appropriate trials can be conducted in different parts of the world and accurately compared. The basic requirements for correct determination of onset of puberty in heifers are an early start of the calves on the trial and adequate nutrition and mangement provided continuously through puberty. Data about onset of puberty in heifers in the tropics is scarce.

Secretion of adrenal progesterone due to handling stress may in female cattle interfere with accurate diagnosis of stage of estrous cycle determined by plasma progesterone concentration. It is also possible that it results in reduced fertility. Release of adrenal progesterone could provide a possible explanation for the low pregnancy rates in Zebu cattle in the Mexican tropics. Research in the area of stress-induced release of adrenal progesterone in Zebu cattle is recommended to elucidate the effects of this source of plasma progesterone

on estrous.

Given adequate nutrition and management Zebu and Brown Swiss heifers in the Mexican tropics have growth rates and reach puberty at ages comparable to those reported in dairy and beef breeds in temperate climates. Zebu and Brown Swiss heifers reaching puberty at 9.4 and 12.3 months can be bred approximately six months later and produce their first calf at 24 to 27 months of age. This represents a considerable improvement from the age of 36 to 48 months now common in the study area. With adequate government support the Mexican tropics can make important contributions to dairy and beef production to help feed the country's rapidly increasing population. The significant increase needed in dairy and beef production can be achieved by application of already available technology.

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APPENDIX

TABLE I. PRIMARY DATA OF AVERAGE HEMATOCRIT VALUES FOR ZEBU AND BROWN SWISS HEIFERS

HEIFER NUMBER	HEMATOCRIT VALUE, %	HEIFER NUMBER	HEMATOCRIT VALUE, %
ZEBU DIET I		ZEBU DIET II	
51 GYR	40.6 ± 4.7*	26 GYR	40.8 ± 3.0
39 IB	39.0 ± 5.6	59 GYR	33.5 ± 3.6
62 GYR	36.0 ± 5.6	65 IB	37.7 ± 4.7
67 GYR	35.2 ± 6.6	61 GYR	36.9 ± 4.3
60 IB	39.0 ± 3.8	48 BHM	32.8 ± 2.0
66 IB	33.5 ± 5.1	38 BHM	31.0 ± 7.6
AVERAGE	37.2 ± 5.2	AVERAGE	35.4 ± 4.2
ZEBU AVERAGE**	36.3 ± 4.7		
BROWN SWISS DIET I		BROWN SWISS DIET II	
35 SP	30.8 ± 4.8	39 SP	26.9 ± 4.2
38 SP	36.5 ± 3.1	44 SP	31.3 ± 4.9
41 SP	32.3 ± 5.3	45 SP	32.2 ± 5.5
50 SP	29.4 ± 3.4	37 SP	28.0 ± 3.9
281 SP	30.3 ± 3.3	343 SP	34.0 ± 4.9
365 SP	28.3 ± 3.4	366 SP	27.3 ± 3.5
AVERAGE	31.3 ± 3.9	AVERAGE	30.0 ± 4.5
BROWN SWISS AVERAGE**	30.6 ± 4.2		

* Standard deviation.
** Both breed averages are in agreement with the literature value of 27 to 50.5 % for beef cattle less than a year old reported by Schalm (1965). They are also similar to average values found in calves and heifers in Veracruz by Barradas (1980), 35.4% (27.4% - 42.4%), and Monroy (1982), 33.1% (26.2% - 41.2%).

TABLE II. COMPOSITION OF BIOSAL* MINERAL MIX

MINERAL	%
CALCIUM OXIDE	10.76
CALCIUM	7.70
PHOSPHORUS PENTAOXIDE	11.70
PHOSPHORUS	5.06
CHLORINE	18.48
SODIUM	12.00
IRON	0.60
MAGNESIUM	0.081
COPPER	0.036
ZINC	0.024
IODINE	0.011
COBALT	0.003
POTASSIUM	0.003
EXCIPIENT	33.542
total	100.000

* Agroquimica, S.A. de C.V.

TABLE III. COMPOSITION OF MAGNAPHOSCAL* MINERAL MIX

MINERAL	%
PHOSPHORUS	17.5
SODIUM	12.9
MAGNESIUM	3.4
CALCIUM	5.6
EXCIPIENT	60.6
total	100.0

* Bayer.

TABLE IV. PRIMARY DATA OF AVERAGE DAILY GAIN, DRY MATTER INTAKE, FEED CONVERSION AND CRUDE PROTEIN INTAKE OF ZEBU AND BROWN SWISS HEIFERS

HEIFER NUMBER	ADG (g)	DM INTAKE (kg)	FEED CONVERSION (kg DM/kg gain)	CP INTAKE (g)
ZEBU				
DIET I				
51 GYR	554	4.47	8.07	549
39 IB	671	4.58	6.82	529
62 GYR	608	4.30	7.08	529
67 GYR	652	4.23	6.50	518
60 IB	611	4.91	8.04	589
66 IB	526	3.91	7.44	483
AVERAGE	604	4.40	7.32	533
DIET II				
26 GYR	664	5.60	8.43	815
59 GYR	571	5.10	8.92	759
65 IB	647	4.99	7.71	729
61 GYR	595	4.72	7.93	710
48 BHM	814	6.30	7.74	893
38 BHM	424	3.72	8.68	595
AVERAGE	619	5.07	8.23	750
ZEBU				
AVERAGE	612	4.74	7.78	642
BROWN SWISS				
DIET I				
35 SP	1006	5.96	5.92	716
38 SP	912	5.47	6.00	670
41 SP	832	5.48	6.59	668
50 SP	871	5.16	5.92	634
281 SP	868	6.07	6.99	727
365 SP	865	5.15	5.95	634
AVERAGE	892	5.55	6.23	675
DIET II				
39 SP	834	6.62	7.93	967
44 SP	918	5.99	6.53	888
45 SP	890	6.05	6.80	919
37 SP	870	6.32	7.26	995
343 SP	1003	6.08	6.07	890
366 SP	714	6.16	8.62	901
AVERAGE	872	6.20	7.20	927
BROWN SWISS				
AVERAGE	882	5.88	6.72	801

TABLE V. DRY MATTER AND CRUDE PROTEIN CONTENT OF EXPERIMENTAL DIETS AND ORTS CONTAINING RICE POLISHINGS VERSUS WHEAT BRAN

DIET NUMBER	DIETS CONTAINING RICE POLISHINGS				DIETS CONTAINING WHEAT BRAN			
	DRY MATTER (%)	SD	CRUDE PROTEIN (%)	SD	DRY MATTER (%)	SD	CRUDE PROTEIN (%)	SD
IA	56.0	± 0.9	12.8	± 0.5	56.2	± 0.6	13.3	± 0.5
IB	51.1	± 1.2	12.1	± 0.5	49.6	± 0.4	12.6	± 0.4
IC	44.3	± 1.2	11.9	± 0.9	42.8	± 1.4	11.6	± 0.7
ID	39.5	± 1.4	11.0	± 0.4	40.8	± 1.0	11.0	± 0.8
IIA	57.3	± 1.4	16.4	± 0.8	56.8	± 1.0	17.0	± 1.0
IIB	50.0	± 0.5	15.3	± 0.9	48.6	± 0.8	15.0	± 0.7
IIC	44.9	± 1.5	13.2	± 1.2	44.2	± 1.2	13.6	± 0.4
IID	*		*		40.6	± 1.0	11.6	± 0.3
ORTS								
IA	56.0	± 1.3	12.4	± 1.0	56.1	± 0.9	13.8	± 1.0
IB	54.0	± 1.2	11.8	± 1.1	48.4	± 2.2	13.6	± 0.7
IC	46.8	± 1.6	11.6	± 1.1	45.1	± 0.8	11.8	± 0.4
ID	42.6	± 0.6	10.6	± 0.5	38.2	± 0.1	11.1	± 0.3
IIA	57.0	± 1.2	17.6	± 0.9	58.0	± 0.3	18.9	± 2.0
IIB	51.4	± 0.6	15.1	± 0.4	48.6	± 0.5	16.2	± 0.8
IIC	45.0	± 1.2	13.4	± 1.3	40.6	± 2.3	15.1	± 0.3
IID	*		*		35.2	± 1.8	14.1	± 0.8

Diets marked I = diets with adequate protein according to NRC norm for dairy heifers gaining 500 g/day.
Diets marked II = high protein diets.
Diets marked A = 100-150 kg BW, B = 150-200 kg BW, C = 200-250 kg BW and D = 250- 300 kg BW.

* Diet IID was not in use before the change from rice polishings to wheat bran.

TABLE VI. CRUDE FIBER AND ASH CONTENT AND IN SITU DIGESTIBLE DRY MATTER OF EXPERIMENTAL DIETS CONTAINING RICE POLISHINGS VERSUS WHEAT BRAN

DIET NUMBER	DIETS CONTAINING RICE POLISHINGS (%)	SD	DIETS CONTAINING WHEAT BRAN (%)	SD	DIET NUMBER	DIETS CONTAINING RICE POLISHINGS (%)	SD	DIETS CONTAINING WHEAT BRAN (%)	SD
CRUDE FIBER									
IA	10.8	± 0.4	14.7	± 0.9	IIA	10.4	± 0.1	14.3	± 0.8
IB	12.0	± 0.4	14.6	± 1.3	IIB	13.4	± 0.6	14.3	± 1.0
IC	17.8	± 1.9	15.6	± 0.9	IIC	16.8	± 0.0	17.9	± 0.4
ID	17.8	± 1.3	19.3	± 0.6	IID	*		22.3	± 0.6
ASH (single samples)									
IA	8.6		8.6		IIA	7.9		7.7	
IB	9.1		7.8		IIB	8.2		8.6	
IC	9.2		8.0		IIC	8.4		8.4	
ID	8.7		9.1		IID	*		8.4	
DIGESTIBLE DRY MATTER									
IA	53.6	± 2.6	50.3	± 2.3	IIA	52.2	± 0.4	49.6	± 1.0
IB	50.2	± 1.5	52.2	± 2.8	IIB	49.2	± 0.4	53.1	± 2.0
IC	51.1	± 2.2	47.6	± 3.4	IIC	51.6	± 2.7	50.8	± 1.4
ID	47.4	± 2.6	51.6	± 1.4	IID	*		48.2	± 2.6

* Diet IID was not in use before the change from rice polishings to wheat bran.

TABLE VII. DRY MATTER AND CRUDE PROTEIN CONTENT OF INGREDIENTS IN THE EXPERIMENTAL DIETS

INGREDIENT	DRY MATTER (%) SD	CRUDE PROTEIN (%) SD
SUNFLOWER MEAL	91.6 ± 0.4	31.0 ± 1.3
RICE POLISHINGS	91.5 ± 0.4	13.4 ± 1.2
SORGHUM SILAGE	22.8 ± 2.1	6.2 ± 0.09
MOLASSES a)	79.2 ± 1.3	3.1 ± 0.01
b)*	82.5 ± 3.2	4.9 ± 0.5
WHEAT BRAN	90.3 ± 0.3	14.7 ± 0.2

* The molasses used during the last trimester of the trial had a different composition.

TABLE VIII. VALIDATION OF THE MODIFIED COAT-A-COUNT PROGESTERONE RADIOIMMUNOASSAY PROCEDURE: INTRA- AND INTERASSAY VARIATION AND SENSITIVITY OF SEVEN ASSAYS

PLASMA PROGESTERONE CONCENTRATIONS, ng/ml
HIGH QUALITY CONTROL (HQC)

ASSAY NUMBER	FRONT HQC	BACK HQC	AVERAGE HQC	STD DEV	INTRA CV, %
1	1.86	1.84	1.85	0.01	0.5
2	2.12	2.27	2.20	0.11	5.0
3	1.87	1.97	1.92	0.07	3.6
4	1.85	1.77	1.81	0.06	3.3
5	2.07	2.11	2.09	0.03	1.4
6	1.95	1.88	1.91	0.05	2.6
7	1.89	2.27	2.08	0.27	13.0
AVERAGE			1.98		4.2
AVERAGE STD DEV			0.16		
INTER CV, %			8.1		

PLASMA PROGESTERONE CONCENTRATIONS, ng/ml
LOW QUALITY CONTROL (LQC)

ASSAY NUMBER	FRONT LQC	BACK LQC	AVERAGE LQC	STD DEV	INTRA CV (%)	INTRAASSAY SENSITIVITY (ng/ml)
1	0.23	0.20	0.22	0.02	9.1	0.14
2	0.29	0.28	0.28	0.01	3.6	0.14
3	0.23	0.29	0.26	0.04	15.4	0.13
4	0.30	0.16	0.23	0.10	43.5	0.13
5	0.25	0.28	0.26	0.02	7.7	0.12
6	0.26	0.19	0.22	0.05	22.7	0.14
7	0.31	0.36	0.34	0.04	11.8	0.13
AVERAGE			0.26		16.2	0.13
AVERAGE STD DEV			0.05			
INTER CV, %			19.2			

TABLE IX. VALIDATION OF THE MODIFIED COAT-A-COUNT PROGESTERONE RADIOIMMUNOASSAY PROCEDURE: DILUTIONS WITH WATER, BUFFER AND PLASMA AND SPIKING WITH PROGESTERONE

PLASMA PROGESTERONE, %	
HIGH QUALITY CONTROL (HQC) 3.48 ng/ml	LOW QUALITY CONTROL (LQC) 1.12 ng/ml
WATER DILUTIONS	WATER DILUTIONS
1:2 156%	1:2 111%
1:4 214%	1:4 139%
*	1:8 100%
AVERAGE 185%	AVERAGE 117%
BUFFER DILUTIONS	BUFFER DILUTIONS
1:2 152%	1:2 105%
1:4 178%	1:4 146%
*	1:8 86%
AVERAGE 165%	AVERAGE 112%
PLASMA DILUTIONS	PLASMA DILUTIONS
1:2 113%	1:2 131%
1:4 103%	1:4 138%
1:8 114%	1:8 206%
AVERAGE 110%	AVERAGE 158%
SPIKING WITH PROGESTERONE	SPIKING WITH PROGESTERONE
HQC + 2.5 ng/ml 85%	LQC + 2.5 ng/ml 81%
HQC + 5.0 ng/ml 86%	LQC + 5.0 ng/ml 86%
HQC + 10.0 ng/ml 97%	LQC + 10.0 ng/ml 95%
AVERAGE 89%	AVERAGE 87%

* No 1:8 dilutions were made with water or buffer.

TABLE X. CORRELATION BETWEEN PROGESTERONE CONCENTRATIONS IN PLASMA SAMPLES ANALYZED WITH VERSUS WITHOUT AN EXTRACTION STEP USING THE MODIFIED COAT-A-COUNT PROGESTERONE RADIOIMMUNOASSAY PROCEDURE

	PLASMA PROGESTERONE, ng/ml				
	EXTRACTION	DIRECT		EXTRACTION	DIRECT
SAMPLES	0.20	0.28	CONT.	0.00	0.12
FROM	0.15	0.47	39 IB	0.01	0.08
HEIFER	0.10	0.10		0.00	0.08
NUMBER	0.10	0.13		0.25	0.26
39 IB	0.12	0.13		1.50	1.97
	0.69	0.75			
	0.04	0.06	SAMPLES	0.44	0.06
	0.11	0.13	FROM	0.54	0.31
	0.06	0.11	HEIFER	0.23	0.19
	0.35	0.25	NUMBER	0.36	0.20
	0.02	0.07	35 SP	0.19	0.21
	0.05	0.04		0.19	0.16
	0.02	0.03		0.40	0.17
	0.14	0.14		0.50	0.14
	0.06	0.02		0.22	0.19
	0.07	0.21		0.09	0.18
	0.05	0.02		0.10	0.17
	0.06	0.06		0.07	0.15
	0.05	0.06		0.05	0.02
	0.05	0.05		0.02	0.05
	0.10	0.02		0.13	0.09
	0.12	0.09		0.33	0.53
	0.12	0.01		4.92	6.05
	0.06	0.08		0.46	0.21
	0.00	0.07		1.94	1.85
	0.00	0.04			
	0.00	0.03	REGRESSION OUTPUT:		
	0.00	0.04			
	0.25	0.44	CONSTANT:		-0.00438
	0.06	0.10			
	0.04	0.03	STD ERROR OF Y ESTIMATE:		
	0.06	0.10			0.126527
	0.01	0.04			
	0.05	0.03	R :		0.981659
	0.00	0.05			
	0.04	0.05	NO OF OBSERVATIONS:		67
	0.08	0.01			
	0.03	0.09	DEGREES OF FREEDOM:		65
	0.02	0.11			
	0.02	0.15	X COEFFICIENT:		0.970859
	0.02	0.12			
	0.06	0.17	STD ERROR OF COEFFICIENT:		
	0.00	0.08			0.023386

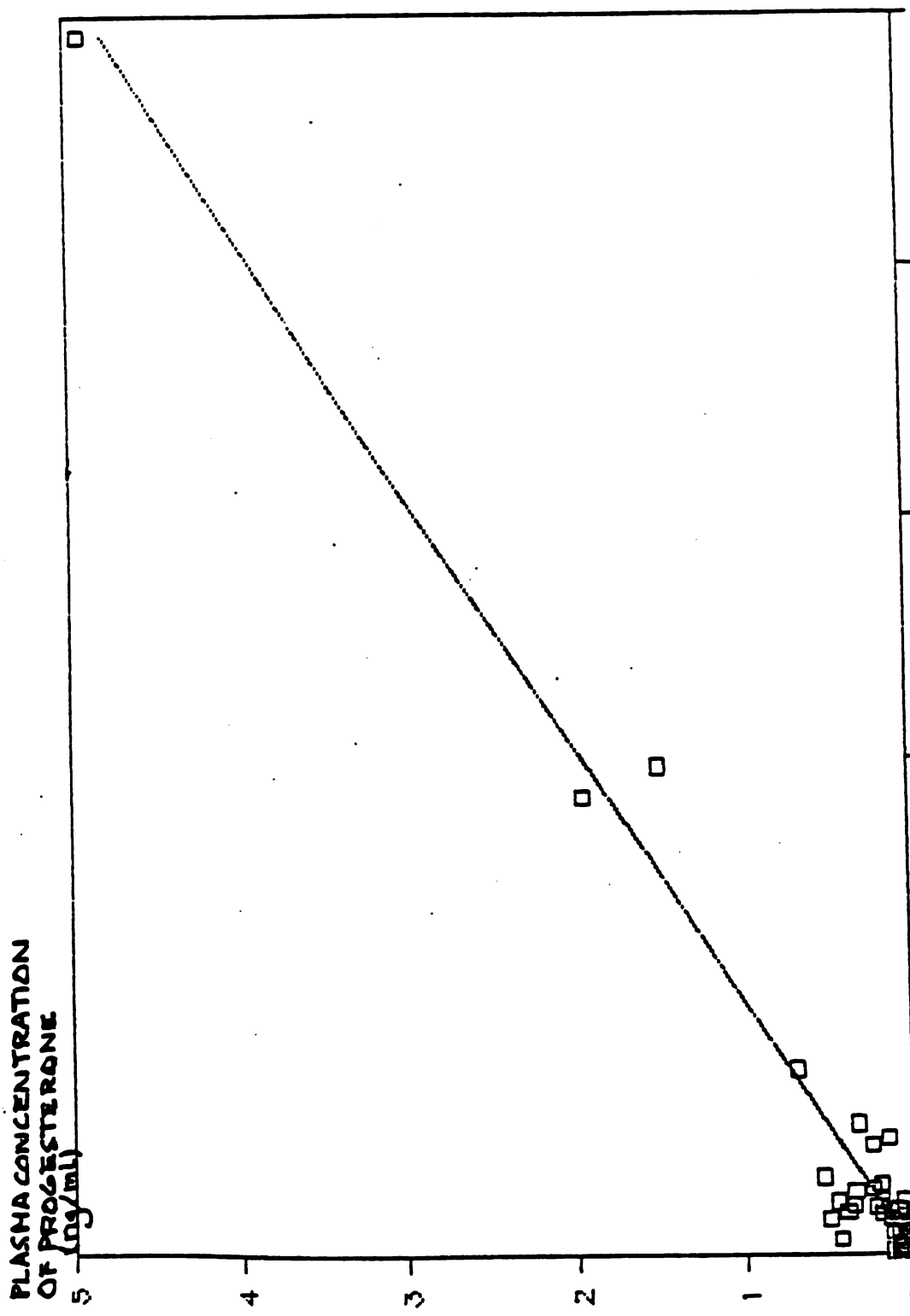

FIGURE I CORRELATION BETWEEN PROGESTERONE CONCENTRATIONS IN PLASMA SAMPLES ANALYZED WITH VERSUS WITHOUT AN EXTRACTION STEP USING THE MODIFIED COAT-A-COUNT RADIOIMMUNOASSAY PROCEDURE.

TABLE XI. VALIDATION OF THE THYROXINE (T4)* RADIOIMMUNOASSAY PROCEDURE: INTRA- AND INTERASSAY VARIATION AND SENSITIVITY OF NINE ASSAYS

PLASMA THYROXINE (T4) CONCENTRATIONS, ng/ml
HIGH QUALITY CONTROL (HQC)

ASSAY NUMBER	FRONT HQC	BACK HQC	AVERAGE HQC	STD DEV	INTRA CV, %
1	41.49	41.89	41.69	0.28	0.7
2	32.01	32.26	32.14	0.18	0.6
3	40.45	38.66	39.56	1.26	3.2
4	35.20	37.93	36.56	1.93	5.3
5	39.93	40.02	39.98	0.06	0.2
6	36.17	36.27	36.22	0.07	0.2
7	37.39	30.50	33.94	4.87	14.3
8	40.38	37.77	39.08	1.84	4.7
9	40.01	37.98	39.00	1.44	3.7
AVERAGE			37.57		3.6
STD DEV			3.32		
INTER CV, %			8.8		

PLASMA THYROXINE (T4) CONCENTRATIONS, ng/ml
LOW QUALITY CONTROL (LQC)

ASSAY Number	FRONT LQC	BACK LQC	AVERAGE LQC	STD DEV	INTRA CV (%)	INTRASSSAY SENSITIVITY (ng/ml)
1	34.92	34.74	34.83	0.13	0.4	0.90
2	34.59	26.84	30.72	5.48	17.8	0.86
3	31.93	30.90	31.42	0.73	2.3	0.74
4	33.85	31.27	32.56	1.82	5.6	0.82
5	33.27	32.27	32.77	0.71	2.2	0.98
6	32.28	32.12	32.20	0.11	0.3	0.92
7	32.69	33.16	32.92	0.79	2.4	0.86
8	33.69	31.94	32.82	1.24	3.8	0.92
9	34.45	31.74	33.10	1.92	5.8	0.84
AVERAGE			32.59		4.5	0.87
STD DEV			1.89			
INTER CV, %			5.8			

* The kit contained a T4 analog instead of T4.

TABLE XII. VALIDATION OF FREE THYROXINE (FREE T4) RADIOIMMUNOASSAY: INTRA- AND INTERASSAY VARIATION AND SENSITIVITY OF NINE ASSAYS

PLASMA CONCENTRATIONS OF FREE T4, pg/ml
HIGH QUALITY CONTROL (HQC)

ASSAY NUMBER	FRONT HQC	BACK HQC	AVERAGE HQC	STD DEV	INTRA CV, %
1	8.08	8.53	8.30	0.32	3.8
2	7.89	6.54	7.22	0.95	13.2
3	8.06	8.46	8.26	0.28	3.4
4	8.33	8.37	8.35	0.03	0.4
5	8.36	8.12	8.24	0.17	2.1
6	8.13	8.57	8.35	0.31	3.7
7	8.00	7.57	7.78	0.30	3.8
8	7.28	8.02	7.65	0.52	6.8
9	7.94	8.96	8.45	0.72	8.5
AVERAGE			8.07		5.1
STD DEV			0.56		
INTER CV, %			6.9		

PLASMA CONCENTRATIONS OF FREE T4, pg/ml
LOW QUALITY CONTROL (LQC)

ASSAY NUMBER	FRONT LQC	BACK LQC	AVERAGE LQC	STD DEV	INTRA CV (%)	INTRAASSAY SENSITIVITY (pg/ml)
1	6.42	6.69	6.56	0.19	2.9	0.68
2	6.19	8.76	7.48	1.82	24.3	0.70
3	6.62	6.49	6.56	0.09	1.4	0.72
4	6.70	6.79	6.74	0.06	0.9	0.66
5	6.90	6.41	6.66	0.35	5.2	0.59
6	6.65	7.53	7.09	0.62	8.7	0.62
7	6.35	6.71	6.53	0.25	3.8	0.54
8	5.93	6.74	6.34	0.57	9.0	0.66
9	6.45	6.98	6.72	0.37	5.5	0.59
AVERAGE			6.74		6.8	0.64
STD DEV			0.61			
INTER CV, %			9.0			

TABLE XIII. VALIDATION OF TRIIODOTHYRONINE (T3) RADIOIMMUNOASSAY PROCEDURE: INTRA- AND INTERASSAY VARIATION AND SENSITIVITY OF NINE ASSAYS

PLASMA TRIIODOTHYRONINE (T3) CONCENTRATIONS, ng/ml
HIGH QUALITY CONTROL (HQC)

ASSAY NUMBER	FRONT HQC	BACK HQC	AVERAGE HQC	STD DEV	INTRA CV, %
1	2.02	1.74	1.88	0.20	10.6
2	1.88	1.80	1.84	0.06	3.3
3	2.03	1.99	2.01	0.03	1.5
4	1.89	1.82	1.86	0.05	2.7
5	1.97	1.81	1.89	0.11	5.8
6	1.84	1.76	1.80	0.06	3.3
7	2.00	2.24	2.12	0.17	8.0
8	1.92	1.76	1.84	0.11	6.0
9	1.86	1.70	1.78	0.11	6.2
AVERAGE			1.89		5.3
STD DEV			0.13		
INTER CV, %			6.9		

PLASMA TRIIODOTHYRONINE (T3) CONCENTRATIONS, ng/ml
LOW QUALITY CONTROL (LQC)

ASSAY NUMBER	FRONT LQC	BACK LQC	AVERAGE LQC	STD DEV	INTRA CV (%)	INTRAASSAY SENSITIVITY (ng/ml)
1	1.46	1.63	1.54	0.12	7.8	0.10
2	1.41	1.60	1.50	0.13	8.7	0.10
3	1.48	1.55	1.52	0.05	3.3	0.10
4	1.57	1.62	1.60	0.04	2.5	0.10
5	1.41	1.77	1.59	0.25	15.7	0.10
6	1.40	1.38	1.39	0.01	0.7	0.10
7	1.53	1.53	1.53	0.00	0.0	0.11
8	1.43	1.43	1.43	0.00	0.0	0.10
9	1.45	1.56	1.50	0.08	5.3	0.09
AVERAGE			1.51		4.9	0.10
STD DEV			0.10			
INTER CV, %			6.6			

TABLE XIV. VALIDATION OF FREE TRIIODOTHYRONINE (FREE T3) RADIOIMMUNOAASSAY PROCEDURE: INTRA- AND INTERASSAY VARIATION AND SENSITIVITY OF NINE ASSAYS

PLASMA CONCENTRATIONS OF FREE T3, pg/ml
HIGH QUALITY CONTROL (HQC)

ASSAY NUMBER	FRONT HQC	BACK HQC	AVERAGE HQC	STD DEV	INTRA CV, %
1	5.30	5.39	5.34	0.06	1.1
2	5.48	4.94	5.21	0.38	7.3
3	5.28	5.85	5.56	0.40	7.2
4	5.26	5.71	5.48	0.32	5.8
5	5.36	4.51	4.94	0.60	12.1
6	5.02	5.28	5.15	0.18	3.5
7	5.19	5.50	5.34	0.22	4.1
8	5.09	*	5.09	*	*
9	5.00	5.28	5.14	0.20	3.9
AVERAGE			5.25		5.6
STD DEV			0.31		
INTER CV, %			5.9		

PLASMA CONCENTRATIONS OF FREE T3, pg/ml
LOW QUALITY CONTROL (LQC)

ASSAY NUMBER	FRONT LQC	BACK LQC	AVERAGE LQC	STD DEV	INTRA CV (%)	INTRAASSAY SENSITIVITY (pg/ml)
1	4.44	4.43	4.44	0.01	0.2	0.50
2	4.80	4.62	4.71	0.13	2.8	0.50
3	4.66	4.81	4.74	0.11	2.3	0.52
4	4.62	5.16	4.89	0.38	7.8	0.54
5	4.56	3.89	4.22	0.47	11.1	0.42
6	4.32	4.60	4.46	0.20	4.5	0.37
7	4.60	4.85	4.72	0.18	3.8	0.48
8	4.54	*	4.54	*	*	0.47
9	4.53	4.27	4.40	0.18	4.1	0.46
AVERAGE			4.57		4.6	0.47
STD DEV			0.27			
INTER CV, %			5.9			

* Missing values.

TABLE XV. VALIDATION OF THE THYROXINE (T4)* RADIO-IMMUNOASSAY PROCEDURE: DILUTIONS WITH WATER, BUFFER, PLASMA AND SPIKING WITH THYROXINE

PLASMA THYROXINE, %			
ZEBU PLASMA		BROWN SWISS PLASMA	
QUALITY CONTROL 30.6 ng/ml		QUALITY CONTROL 34.8 ng/ml	
WATER DILUTIONS		WATER DILUTIONS	
1:2	117%	1:2	100%
1:4	104%	1:4	88%
1:8	112%	1:8	75%
AVERAGE	111%	AVERAGE	88%
BUFFER DILUTIONS		BUFFER DILUTIONS	
1:2	117%	1:2	110%
1:4	124%	1:4	104%
1:8	136%	1:8	120%
AVERAGE	126%	AVERAGE	111%

PLASMA THYROXINE, %	
BOVINE PLASMA	
QUALITY CONTROL (QC) 64.0 ng/ml**	
SPIKING WITH T4	
QC + 10 ng	100%
QC + 25 ng	105%
QC + 50 ng	105%
QC + 75 ng	102%
QC + 100 ng	100%
AVERAGE	102%

* The kit contained a T4 analog instead of T4.

** Quality control from the Animal Health Diagnostic laboratory at Michigan State University.