MAATALOUDEN TUTKIMUSKESKUS
AGRICULTURAL RESEARCH CENTRE

KOTIELÄINHOIDON TUTKIMUS-LAITOKSEN TIEDOTE N:o 13

INSTITUTE OF ANIMAL HUSBANDRY, REPORT N:o 13

Research reports of NKJ hormone symposium

Korpilampi, Finland
4—5 January 1979

Edited by Vappu Kossila and Ritva Mäkelä

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FOREWORD

Scandinavian countries, Denmark, Finland, Norway and Sweden, have done hormone studies in domestic animals since 1970, when the first inter-Scandinavian hormone project NKJ-22 "Studies on the relationships between hormonal activity and productional capacity in domestic animals" was started. This project ended in 1975. During the years 1976-1978, the Scandinavian countries had the second inter-Scandinavian hormone project NKJ-34 "Continuation for the studies on the relationships between hormonal activity and productional capacity in domestic animals". During these projects, a radioimmunoassay method was developed for several hormones. Hormone levels and their relation to the productional capacity were investigated mainly in cattle, sheep and pig.

The end meeting of the project NKJ-34 was kept in Forest Lake Hotel, Espoo, Finland, January 4th 1979. In connection with the end meeting, the hormone symposium was arranged January 5th 1979. This book contains the end reports presented by the research groups of the project NKJ-34 in the end meeting as well as the lectures given in the symposium. The papers in this book deal with the results from the inter-Scandinavian hormone studies in domestic animals. Also hormone studies outside the NKJ-projects are reported.

Helsinki, April 10th 1979

Vappu Kossila and Ritva Mäkelä

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SCIENTIFIC PROGRAM

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Thursday
4.1.1979

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  Norway. Standal, N..... E1-4

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- Hormone receptors and their clinical significance. Kontula, K. (Finland)..... F1-10
- Hormonal control of fluid balance. Eriksson, L. (Finland)..... G1-6
- Peptide hormones in TRH and LRH stimulation tests. Karonen, S.-L. (Finland)..... H1-5
- Prolactin in bovine blood, milk and urine. Mäkelä, R. and Kossila, V. (Finland)..... I1-9
- Correlation of plasma prolactin level to some plasma enzymes and other plasma parameters in Ayrshire bulls. Tanhuanpää, E., Mäkelä, R. and Kossila, V. (Finland). J1-4
- LH and testosterone, repeatability and variation in three pairs of monozygous bulls. Oltner, R., Lundström, K. and Edqvist, L.-E. (Sweden)..... K1-21
- Age-dependent testosterone-related growth in bulls. Sundby, A. (Norway)..... L1-2
- A simplified RIA for androstenone in boar fat. Andresen, Ø. (Norway)..... M1-3
- Some physiological aspects with regard to 5α-androsterone and boar taint. Lundström, K. (Sweden)..... N1

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Chairman Prof. L. Ekman

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- Plasma corticosteroid levels in laying hens. Effect of two different blood sampling techniques and of rough handling of the animals. Blom, A.K. and Eskeland, B. (Norway) P1-6
- Prostaglandin and bovine reproduction. Edqvist, L.-E. (Sweden) Q1
- Oestrus synchronization in sheep using oral MAP-treatment for 10 days only. Velle, W. and Helle, O. (Norway) R1-3
- Thyroid morphology and growth performance of young progeny groups of AI bulls. Kossila, V., Riihonen, A. and Puntila, M.-L. (Finland) S1-23
- Thyroxine degradation, thyroxine, free thyroxine and thyrotropine levels in young bulls during feeding and starvation conditions. Tveit, B. and Almlid, T. (Norway) T1-9
- Genetic variation in thyroxine degradation rate and plasma cholesterol concentration in bovine. Edfors-Lilja, I., Kruse, V., Andresen, B.B. and Gahne, B. (Sweden) U1-13
- Thyroxine degradation lines of pigs selected for rate of gain and thickness of backfat. Standal, N., Tveit, B. and Dahl, P.M. (Norway) V1-9
- Hormonally induced changes in the lactational performance and blood components of dairy cows. Kossila, V., Luomajärvi, U. and Luomajärvi, A. (Finland) X1-27
- Closing of the Symposium. Prof. A. Neimann-Sørensen (Denmark)

SLUTREDOGÖRELSE FÖR DEN SVENSKA DELEN AV NKJ-PROJEKT NR 34. SAMBANDET MELLAN HUSDJURENS HORMONAKTIVITET OCH DERAS PRODUKTIONSEGENSKAPER

1. Den svenska delen av NKJ-projekt 34 startade 1975-07-01. Projektet understöddes ekonomiskt t o m 1978-06-30. Arbetet med projektet beräknas vara avslutat 1978-12-31.

2. För den svenska delen av NKJ-projekt 34 har medel beviljats av Statens Råd för Skogs- och Jordbruksforskning enligt följande:

Anslagsbeteckning	Tid	Anslag, kr
A 4072/B 3192	1975-07-01 -76-06-30	150.000
A 4419/B 3328	1976-07-01 -77-06-30	200.000
A 4806/B 3483	1977-07-01 -78-06-30	215.000
		565.000

3. Anslagsmottagare:

Prof. Lars Ekman Bitr. prof. Lars-Eric Edqvist	Institutionen för klinisk kemi, Sveriges Lantbruksuniversitet, Uppsala
Bitr. prof. Bo Gahne Docent Kerstin Lundström	Institutionen för husdjursförädling och sjukdomsgenetik, Sveriges Lantbruksuniversitet, Uppsala

I arbetet med projektet har dessutom deltagit:

Veterinär Maj-Britt Egelrud (1975)
Veterinär Roland Oltner (1976-1978)
Agronom Inger Edfors-Lilja (1976-1978)

4. Projektet är en direkt fortsättning på NKJ-projekt nr 22 (1972-07-31 - 1975-06-30), till vilket beviljades totalt 150.000 kronor. Projektet har haft som målsättning att finna hormonparametrar, som är korrelerade med viktiga produktionsegenskaper hos nötkreatur, svin och får. De svenska undersökningarna har koncentrerats till fyra områden som redovisas i det följande.

5. LH OCH TESTOSTERON, REPRODUCERBARHET OCH VARIATION UNDER UPPVÄXTPERIODEN HOS TJUR

Bakgrund

En betydande variation i den perifera blodplasmakoncentrationen av LH och testosteron föreligger hos tjur. En adekvat skattning av nivåerna av dessa båda hormon kan endast göras om relativt täta blodprover tas under ett dygn. Injektion av Gn-RH (gonadotropin-releasing-hormone) resulterar i frisättning av LH ifrån hypofysen och det frisatta LH-hormonet stimulerar testikeln till ökad testosteronproduktion. Genom att en stor mängd LH-hormon på detta sätt frisättes under en längre period utplånas koncentrationsvariationen av både LH och testosteron. Genom att administrera en hög dos Gn-RH fås en maximal frisättning av LH med en åtföljande troligen maximal stimulering av testosteronproduktionen.

I föreliggande studie har tjurar tillförts Gn-RH under ungtjursperioden och resulterande LH och testosteronnivåer har bestämts.

Material och metoder

Tre par homozygota tjurtvillingar av SRB-ras insattes i försöket vid en ålder av cirka 4 månader. Efter intravenös injektion av 2 mg Gn-RH uttogs blodprover med 15 minuters intervall under 2 timmar. Därefter uttogs blodprover var 30:e minut under cirka 6 timmar. För att utröna den ostimulerade dagliga variationen av LH och testosteron uttogs 6-10 blodprover under ett 8 timmars intervall dagarna före och efter stimuleringen. Varje tvillingpar har testats minst 5 gånger med 2 till 3 månaders mellanrum. Vid försökets avslutande hade varje par uppnått en minimiålder av 16 månader.

Resultat

LH och testosteron (ostimulerade värden):

(1) LH-koncentrationen uppvisade en signifikant negativ linjär regression med åldern under observationsperioden.

(2) Testosteronkoncentrationen i perifert blod uppvisade under motsvarande tid en signifikant positiv linjär regression med ålder.

LH och testosteron (stimulerade värden):

(1) LH-värden efter stimulering, både koncentration och duration av svaret, skiljer sig ej påtagligt emellan olika åldrar.

(2) Medeltalskurvan för LH-koncentrationerna efter stimulering uppvisade ett påtagligt bifasiskt förlopp. En första topp uppnåddes efter 30 minuter, varefter nivån sjönk för att därefter stiga och uppnå maximum efter $1\frac{1}{2}$ - 2 timmar. Den prestimulerade LH-nivån (basalnivån) erhölls i allmänhet 5-8 timmar efter injektionen av Gn-RH.

(3) Den maximala LH-nivån som nås efter stimulering är avsevärt högre än den högsta nivån som uppnås vid icke stimulering.

(4) De uppmätta testosteronvärdena efter stimulering visar en kontinuerlig ökning med stigande ålder.

(5) Maximal testosteronkoncentration uppnås ca 1 timme efter stimulering. Maximal nivå bibehålles i cirka 3 timmar. Med tilltagande ålder bibehålles förhöjda nivåer under längre tid efter stimulering (upp till 8 timmar).

(6) Den maximala testosteronnivån som nås efter stimulering är signifikant korrelerad till de maximala ostimulerade testosteronvärdena ($r = 0,84$; $P < 0,001$).

(7) Efter injektion av Gn-RH erhölls höga partiella korrelationer mellan olika skattningar av testosteronsvaret. Således var maximumvärdet korrelerat till hormonsvarets "yta" (ytan av området mellan baslinje och stimuleringskurva) samt till medelvärdet av alla ingående mätvärden på stimuleringskurvan ($r = 0,84$ resp. $0,95$; $P < 0,001$).

Korrelationer av samma storleksordning erhölls för motsvarande värden för LH. De partiella korrelationerna mellan LH och testosteron var däremot låga och icke signifikanta.

(8) Reproducerbarhetsskattningar för de olika måtten på testosteronsvaret var relativt höga. För exempelvis maximivärdet efter stimulering var reproducerbarheten mätt som intraklasskorrelation 0,62±0,19. Motsvarande skattning av reproducerbarheten för LH var betydligt lägre (0,38±0,22). Reproducerbarheten för ostimulerade maximala testosteron- och LH-värden var 0,15 och 0,12 respektive.

Konklusion

Gn-RH stimulering ger en reproducerbar skattning av maximala testosteronkoncentrationen i blodplasman. Även maximala LH koncentrationen skattas relativt bra, medan däremot ostimulerade värden uppvisar låg reproducerbarhet. Följande enkla provtagningsmetodik ger god uppfattning om de maximala hormonkoncentrationerna: två-tre blodprover uttages fördelade i intervallet $1\frac{1}{2}$ - $2\frac{1}{2}$ timme efter injektionen av Gn-RH. Vid stor spridning i ålder måste ålderskorrektion införas.

LH HOS LAMM

LH-nivån i blodplasman hos lamm har i utländska undersökningar haft ett positivt samband med tackornas fruktsamhet. Målsättningen för våra LH-undersökningar var att bestämma LH hos lamm efter seminbaggar för att kunna bestämma baggarnas nedärvning av LH aktivitet och på så sätt kunna förutsäga fruktsamheten hos deras döttrar.

Blodprov togs från ca 500 lamm vid en månads ålder från fyra olika besättningar med pälsfår. I en besättning togs även blodprov vid upprepade tillfällen från samma lamm (1-5 gånger). Lammen härstammade från 26 olika fäder av vilka de flesta var seminbaggar. De flesta avkommegrupperna fördelade sig på alla besättningarna.

Totalkorrelationen mellan LH-värden för samma djur vid olika tidpunkter var mycket låg och i de flesta fall inte signifikant. En stor variation i LH förelåg också mellan djur (0,0-48,0 ng/ml). Mellan besättningar och kön förelåg signifikanta skillnader medan ingen signifikant effekt kunde påvisas av fader, lammets ålder vid provtagningen, moderns ålder och kullstorlek.

Konklusionen av undersökningen blir att den ostimulerade LH-nivån i blodplasman hos lamm varierar alltför mycket hos enskilda djur för att det skall vara möjligt att påvisa eventuella skillnader mellan olika avkommegrupper.

KOLESTEROLKONCENTRATION I PLASMA I RELATION TILL TILLVÄXTHASTIGHET OCH SLAKTKROPPSSAMMANSÄTTNING

Bakgrund

Kolesterol är en viktig beståndsdel i cellerna, försubstans till steroidhormonerna och medverkar vid fettransporten i plasma. Kolesterolkoncentrationen i plasma har hos de flesta arter visat sig ha en ärftlig variation och samband med tillväxthastighet har påvisats i vissa utländska undersökningar. Målsättningen i vår undersökning har varit att bestämma den genetiskt betingade variationen i kolesterolkoncentrationen i plasma hos nötkreatur samt dess samband med tillväxthastighet och slaktkroppssammansättning, för att eventuellt få fram fysiologiska parametrar av värde för avelsarbetet på nötkreatur. Dessutom har sambanden mellan olika mått på thyroideas aktivitet, kolesterolkoncentrationen i plasma samt tillväxt undersökts. Även vissa undersökningar av enzymet alkaliskt fosfatas har ingått.

Resultat

Kolesterolkoncentrationen i plasma har analyserats i blodprover från två material, dels 247 kalvar av SRB-ras samt SRB x SLB-korsningar av båda könen, dels 247 ungtjurar av raserna RDM och SDM från den danska individprövningsstationen

Egtved. Hos de danska djuren bestämdes dessutom thyroxinnedbrytningen per dygn. Blodprov togs, för båda djurmaterialen, en till tre gånger per djur vid olika åldrar.

Kolesterolkoncentrationen var lägre för tjurarna jämfört med kvigorna. Både kolesterolkoncentration och thyroxinnedbrytning ökade med ökande ålder. När thyroxinnedbrytningen mättes per 100 kg kroppsvikt sjönk den emellertid med ökande ålder. Reproducerbarheten mellan provtagningar gjorda vid olika åldrar var för kolesterolkoncentration mellan 0.2 och 0.5 samt för thyroxinnedbrytning 0.3, både när den mättes per djur och per 100 kg kroppsvikt. De säkraste skattningarna för kolesterolkoncentration och thyroxinnedbrytning erhölls när medeltal från ett eller flera provtagningstillfällen användes. Arvbarheten för kolesterolkoncentration skattades då till 0.78±0.28 resp. 0.33±0.28 för de två djurmaterialen. För thyroxinnedbrytning per djur beräknades arvbarheten till 0.57±0.23 och för thyroxinnedbrytning per 100 kg kroppsvikt till 0.52±0.28. Den genetiska korrelationen mellan thyroxinnedbrytning per djur och per 100 kg kroppsvikt var hög (0.92±0.07), liksom motsvarande fenotypiska korrelation (0.88). De genetiska korrelationerna mellan tillväxthastighet och kolesterolkoncentration var 0.80±0.28 resp. 0.42±0.48 för de två djurmaterialen. Motsvarande fenotypiska korrelationer var 0.25 samt 0.11. De genetiska korrelationerna mellan thyroxinnedbrytning och kolesterolkoncentration var positiva men ej signifikanta. Den genetiska resp. fenotypiska korrelationen mellan tillväxthastighet och thyroxinnedbrytning per djur var 0.76±0.30 och 0.33. Motsvarande korrelationer för thyroxinnedbrytning per 100 kg kroppsvikt var 0.63±0.40 och 0.08.

Sambandet mellan kolesterolkoncentrationen i plasma och olika mått på slaktkroppssammansättningen studerades hos de svenska djuren. Positiva genetiska korrelationer erhölls mellan kolesterolnivå och olika fettmått, negativa mellan kolesterolnivå och olika mått på köttinnehåll. Det var dock omöjligt att dra några säkra slutsatser eftersom korrelationerna, beroende på höga medelfel, ej var signifikanta.

Sambandet mellan mjölkavkastning resp. mjölksammansättning och kolesterolkoncentration undersöktes dessutom hos mödrarna till de svenska kalvarna. Inga signifikanta samband erhölls dock.

Konklusioner

Slutsatsen av undersökningen blir att kolesterolkoncentrationen i plasma och den dagliga thyroxinnedbrytningen teoretiskt sett skulle kunna utgöra selektionskriterier vid indirekt selektion för tillväxt, men att i praktiken detta knappast är möjligt på grund av att prov behöver tas vid upprepade tillfällen och miljön behöver standardiseras i hög grad. Dessutom är sambandet med andra fysiologiska funktioner oklart.

ANDROSTENON OCH ORNELUKT HOS SVIN

Bakgrund

Okastrerade hangrisar (ornar) har ett bättre foderutnyttjande och en köttigare slaktkropp än kastrerade grisar. Problemet med ornarna är att kött och späck från vissa djur har en obehaglig lukt och smak (ornelukt). Den viktigaste substansen som förorsakar ornelukt är en steroid, androstenon. Denna bildas i testiklarna och lagras upp i fettvävnaden. Målsättningen med undersökningarna har varit att komma fram till säkrare skattning av enskilda ornars androstenonproduktion, så att dessa värden eventuellt skulle kunna användas för en selektion mot ornelukt.

Resultat

För att standardisera provtagningen har ornar provocerats till bildning av androstenon och testosteron genom intravenös injektion av HCG. För att undersöka om det är möjligt med tidig stimulering och därmed tidig test av androstenonbildningen har HCG-injektion utförts på 30 ornar vid både 30 och 85 kg levande vikt. Alla djur fick en ökning av androstenon- och testosteronkoncentrationen i plasma efter injektion av HCG både vid 30 kg och 85 kg. Steroidkoncentrationerna i plasma vid 30 kg var emellertid ej korrelerade med de värden

som uppmättes i späck och plasma före och efter HCG-stimulering vid 85 kg.

Utan HCG-stimulering var androstenonkoncentrationen i plasma inte signifikant korrelerad till androstenonvärdena i ryggspäck vid 85 kg. Efter HCG-stimulering erhölls däremot höga korrelationer mellan androstenon i plasma och ryggspäck. Liknande samband erhölls också mellan androstenon - och testosteronkoncentrationerna i samma plasmaprov.

En enstaka betäckning ledde inte till någon ökning av androstenoninnehållet i ryggspäcket hos 4 undersökta ornar. Responsen i plasmakoncentrationerna av androstenon- och testosteron varierade mellan djur. Hos ytterligare ett djur där upprepade prover togs under 24 tim. erhölls mycket stor variation i plasmakoncentrationerna av androstenon och testosteron. Korrelationen mellan de två steroiderna var låg och icke signifikant.

HCG-stimulering gör troligen att icke-genetiska faktorer som påverkar koncentrationen av androstenon i ryggspäck eller plasma får mindre betydelse. HCG kan därför användas som ett standardiseringsmedel vid ev. selektion mot ornelukt eller androstenon. Däremot kan tydligen inte androstenonproduktionen vid slaktmogen ålder förutsägas på grundval av androstenonbildningen vid 30 kg även om HCG-stimulering används.

I en annan undersökning studerades sambandet mellan ornelukt, androstenon och fettsyrasammansättning hos 104 ornar slaktade vid olika slaktvikter. Korrelationer mellan subjektiva mått på ornelukt och androstenonkoncentrationen i ryggspäck var av storleksordningen 0,4-0,7. Vissa kombinationer av fettsyror i ryggspäck svarade för ca 30% av variationen i ornelukt och androstenon för lantrasornar. Däremot bidrog inte fettsyramönstret till att förklara variationen i ornelukt och androstenon hos yorkshireornar. Då man tar hänsyn till fettsyramönstret förutom androstenonhalten ökar dock inte förklaringsgraden nämnvärt för ornelukt. Eftersom det både finns späckprover som har hög halt androstenon trots låg orneluktsintensitet och prover som har låg androstenonhalt trots stark

ornelukt finns det med stor sannolikhet andra substanser än androstenon som bidrar till att modifiera resp. förstärka intrycket av ornelukt. Sambanden mellan fettsyror, androstenon och ornelukt skiljer sig också i de två undersökta raserna.

6. De resultat som framkommit inom ramen för NKJ-projektet utgör i flera avseenden viktiga bidrag till att öka förståelsen för vissa betydelsefulla fysiologiska funktioner i samband med husdjurens produktionsegenskaper. Genom projektet har nödvändig metodik för hormonbestämningar kunnat etableras. Det gäller framför allt bestämning av LH, testosteron och androstenon. Med hjälp av den utvecklade metodiken för hormonstimulering är det exempelvis nu praktiskt möjligt att i en normalpopulation undersöka tjurarnas LH- och testosteronproduktion. Detta kommer att få betydelse för framtida undersökningar av dessa hormoners inverkan på tillväxt- och fertilitetsegenskaper både hos nötkreatur och får. Androstenonbestämningarna har redan prövats på fältmaterial i samband med individprövning av ornar och vissa uppfödningsförsök av ornar. Genom ytterligare förenklingar av metodiken, som gjorts av Andresen, kan androstenonbestämningarna bli ett viktigt led i en eventuell selektion mot ornelukt. I projektets slutskede har en ny aspekt i samband med hormonundersökningen kommit in i bilden. Det gäller förekomsten och betydelsen av hormonreceptorer i olika vävnader, vilket förmodligen kommer att behöva beaktas i framtida undersökningar.

7. Av de undersökningar som gjorts inom NKJ-projektet kommer för nötkreaturens del vissa hormonundersökningar att fortsätta inom ramen för det s.k. hållbarhetsprojektet. Androstenonundersökningarna kommer att utföras i mindre omfattning genom att samarbete pågår med Köttforskningsinstitutet vad beträffar ornelukten. Dessutom ansökes nu om forskningsanslag för att undersöka androgenreceptorer i relation till tillväxt hos svin.

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Uppsala 1978-10-30

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END REPORT OF THE FINNISH PART IN THE NKJ-PROJECT 34

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Duration - The Finnish part in the NKJ-project 34 started January 1st, 1976 and ended December 31st, 1978.

Background - Prolactin was selected for the studies because of its numerous biologic actions in the vertebrates. Nearly one hundred biologic actions are known today (TURNER & BAGNARA 1976). They can be classified roughly into five groups as follows: (1) actions on integument and derivatives, especially mammary gland, (2) actions related to reproduction and parental care, (3) actions related to growth, (4) actions related to osmoregulation and ionoregulation and (5) actions related to fat and carbohydrate metabolism as well as energetics. Prolactin is considered the most versatile of all adenohypophyseal hormones.

In 1970, prolactin studies in bovine were introduced as the Finnish part for the inter-Scandinavian hormone project NKJ-22 by prof. Kalle Maijala. During the project NKJ-22 (1970-1975), the Finnish participants in the NKJ-34 developed a radioimmunoassay (RIA) for bovine blood prolactin. In the inter-Scandinavian hormone project NKJ-34, the assay was applied to milk and urine. Furthermore, prolactin was determined in the semen of certain bulls. The reliable RIA was the most valuable tool for the prolactin studies in the NKJ-project 34.

STUDIES ON PROLACTIN RIA

Reliability of the RIA - Sensitivity of the RIA developed by the authors (MÄKELÄ 1973, MÄKELÄ et al. 1975, MÄKELÄ & KOSSILA

1975, MÄKELÄ & KOSSILA 1976a, MÄKELÄ & KOSSILA 1976b) was 0.1 ng. The cross-reactivity of the antiserum with bovine growth hormone (GH), follicle stimulating hormone (FSH), luteinizing hormone (LH) and thyroid stimulating hormone (TSH) as well as synthetic arg-8-vasopressin and thyrotropin releasing hormone (TRH) were studied. None of these hormones interfered with the prolactin determinations. The dilution curves of the blood and milk were parallel to the standard curve. The dilution curve of the urine was not completely parallel to the standard curve. Recoveries of the prolactin added into the blood, milk and urine were about 90, 110 and 50 %, respectively. The intra-assay and inter-assay variations of the determinations were 8 and 14 %, respectively.

RIA for ovine prolactin, too? - It is possible that the RIA developed for bovine prolactin is suitable for ovine prolactin as well. The antiserum, produced against bovine prolactin, reacted immunologically with ovine hormones almost to the same extent as with the respective bovine hormones (MÄKELÄ & KOSSILA 1976b). The antiserum against bovine prolactin gave a sensitive standard curve for ovine prolactin, too. Ovine GH, FSH, LH and TSH did not interfere with the standard curve of ovine prolactin.

PROLACTIN STUDIES IN CATTLE

Prolactin levels in blood - Prolactin levels in the blood of 15 animals, including calves, heifers and cows, were followed for shorter or longer periods during the years 1976-1978. The majority of the animals were originated from the feeding tests carried out by the late professor A.I. Virtanen's research group. Blood samples were taken monthly with a needle; a catheter was used for hourly or more frequent sampling.

Prolactin levels of the calves (♀ and ♂) were rather low (< 25 ng/ml) until the puberty. Large fluctuations in blood prolactin were observed in the heifers from 9 to 12 months of age. At that age, prolactin level might exceed 100 ng/ml. At about mid-pregnancy, prolactin levels increased, in general. The increase was, perhaps, a little more marked in heifers than in cows. At the time of parturition, prolactin levels were highest. Periparturient prolactin values in blood could be more than 500 ng/ml. Milking was found to stimulate prolactin release

into the blood. Prolactin response to milking seemed to be more significant in the early stage than in the late stage of lactation. Diurnal rhythmic variation was observed in the prolactin level of lactating cows. Prolactin levels in the blood were generally low in the morning and gradually rose towards the afternoon (MÄKELÄ & KOSSILA 1979).

In a pair of Ayrshire twins, prolactin levels in the blood were followed for one year and a half (Figure 1). During that period, the animals received the same feeds; Helka received proteins as protected and Elsa non-protected. Helka had mostly a little higher prolactin levels in her blood than Elsa. On the whole, average prolactin levels of the twins were of the same order. Physiological changes were rather simultaneous in the twins. Changes in the prolactin levels of the twins were simultaneous, too. Furthermore, they were parallel. In the mid-pregnancy, a prolactin peak was observed in both animals. A prolactin peak generally connected to the parturition was not found in this case, because the blood samples were not taken in the immediate vicinity of the parturition. A prolactin peak in the early stage of lactation may be associated partly to the lactation and partly to the season. At the end of the test period, prolactin levels in the blood of the twins differed from each other. Some physiological differences between the animals were found later on.

Excretion of prolactin into milk, urine and sperma - Prolactin levels in milk were studied in two non-pregnant cows in the late stage of lactation. During the ten-day test period, mean prolactin levels were 3.4 and 4.3 ng/ml in the morning as well as 4.5 and 6.9 ng/ml in the evening, respectively (MÄKELÄ & KOSSILA 1979). Evening milk contained more prolactin than morning milk. The diurnal rhythm observed in milk was in agreement with the diurnal rhythm observed in blood. Daily excretions of prolactin were 45.0 and 76.4 µg/day in milk of the two cows studied, on the average.

Furthermore, the prolactin levels in the colostrum and milk samples from one cow were measured adter parturition. The colostrum from the first milking (some hours after parturition) contained prolactin as much as 320 ng/ml. Within five days after parturition the prolactin concentration had decreased to the

level of about 10 ng/ml. The diurnal rhythm was found in milk prolactin from the fourth day on after parturition.

Prolactin or immunologically prolactin like activity was found in urine (MÄKELÄ & KOSSILA 1979). During the ten-day test period, prolactin levels in the urine of the two non-pregnant cows in the late stage of lactation were rather constant and low, about 1 ng/ml. In both cows, daily excretions of prolactin was about 15 µg/day in urine.

Prolactin was determined in the sperma plasma from ten bulls in Salpausselkä AI-station. Five sperma samples were taken in July 1975 and five in November 1976. Seasons seemed not to have any effect on the prolactin levels in sperma. The levels ranged from 3.8 to 7.5 ng/ml, being 5.5 ng/ml, on the average. Excretion of prolactin in an ejaculate varied from 19.3 to 52.5 ng.

Prolactin response to TRH - TRH is known to stimulate prolactin release from the adenohypophysis to the blood circulation. About 1 µg of TRH/kg live weight was intravenously administered to 7 animals in various physiological states.

In each animal studied, prolactin response to TRH in blood was maximal 20 minutes after the TRH administration. The most marked responses (345 and 176 ng/ml) were observed in the two non-pregnant cows in the late stage of lactation (MÄKELÄ & KOSSILA 1979). Responses were observable during three hours after the TRH administration. Prolactin responses to TRH were also rather clear (about 80 ng/ml) and long-lasting (four hours) in the pregnant heifer triplets. Prolactin responses to TRH in blood were the smallest (38 and 75 ng/ml) in the non-pregnant cow twins in the early stage of lactation. The responses lasted only one hour and a half after the TRH administration.

Prolactin response to TRH in milk was studied in two non-pregnant cows in the late stage of lactation (MÄKELÄ & KOSSILA 1979). Responses in milk were clear (16.8 and 14.0 ng/ml), but slighter than in blood. The cow with greater response in blood had greater response in milk than the other cow.

Prolactin response to TRH in urine was studied in the two cows mentioned above. Responses were not observed in urine, at all (MÄKELÄ & KOSSILA 1979).

PROLACTIN STUDIES IN BULLS

Purpose of the bull studies - The purpose of the bull studies was to investigate the relationship of prolactin to the semen characteristics and to some blood constituents of bulls. Relation between prolactin and growth intensity of the young bulls is under statistical studies. Relationship between prolactin and productional capacity of the daughters of the AI-bulls tested will be studied later on when results of the progeny tests are available.

Bull material and analyses - During the years 1974-1978 (between October and January) 405 blood samples were taken from 316 bulls, mostly of Ayrshire breed. Of these, 60 were young bulls and the rest AI-bulls. They were sons of 48 sires; seven/sire, on the average. Blood samples were collected from AI-bulls in Salpausselkä, Pirkkala and Rauhalinna AI-stations and from young bulls in Humppila bull station (Table 1). Blood samples were taken three times from 12 bulls, twice from 65 bulls and one from 239 bulls. Blood samples were mostly taken with a needle. Heparinized blood plasma was generally used for prolactin analyses. From 120 blood samples, both plasma and serum prolactin was analyzed. Outside the NKJ-projects NKJ-22 and 34, also other blood constituents of the bulls were analyzed. Hematocrit value, hemoglobin, some minerals and trace elements were determined in the Agricultural Research Centre, Institute of Animal Husbandry. Some enzymes and other blood constituents were analyzed in the College of Veterinary Medicine (TANHUANPÄÄ et al. 1979). Statistical calculations were done by the Computing Service Unit of the Agricultural Research Centre.

Prolactin levels in the blood of bulls - On the whole, prolactin concentrations of the bulls varied between 0 and 260 ng/ml, being 15 ng/ml, on the average. Older bulls had higher prolactin levels in their blood than young bulls. Simple correlation between prolactin level and age of the bulls was -0.15 ($P < 0.01$, $n = 345$).

Mean prolactin concentration in the blood of bulls in each station and each year was about 15 ng/ml (Table 1). Exceptions were the blood samples from Salpausselkä AI-station in 1976 (4.5 ng/ml) and from Pirkkala AI-station in 1978 (35.6 ng/ml).

Effect of sampling technique on blood prolactin - Blood sampling techniques investigated in this study had non-significant effect on the prolactin level of bulls (KOSSILA et al. 1975). Prolactin concentrations were similar in plasma and serum, on the average (Table 1).

Prolactin and semen characteristics - Relationship of prolactin was studied to the following semen characteristics: volume of ejaculate, sperm count, motility of sperms, number of sperms alive, deep freezing quality of sperms, non-return per cent of the inseminations made with the sperma. Furthermore, the relation between blood prolactin and sexual behaviour of the bulls was investigated. Observations of the semen characteristics and sexual behaviour of the bulls were made by the personel in the AI-stations mentioned. Table 2 shows simple correlations of prolactin to the said traits in three bull groups, the mean prolactin levels of which were of different order. The levels in the group I, II and III were 10.9, 15.2 and 22.2 ng/ml, respectively.

Correlations of prolactin were statistically significant to the volume of ejaculate in the group III, to the sperm count in the group I, to the number of sperms alive in the groups I and II and to the non-return per cent of the inseminations made with the sperma in the group III. Although the correlations were not, in general, very high, the blood prolactin level seemed to have either an advantageous or disadvantageous effect on each trait studied.

Prolactin level obviously had stimulatory influence on sexual behaviour of the bulls. When the mean prolactin level of the bull groups increased, the libido of the bulls increased as well. Increasing amounts of prolactin in the blood of the bulls seemed to have negative influences on the quality of fresh sperma: volume of ejaculate, sperm count and number of sperms alive. Because disadvantageous changes occurred both in the volume of

ejaculate and in the sperm count, high prolactin levels obviously interfered with the production of semen plasma as well as spermatogenesis. On the other hand, high prolactin levels improved fertilizing ability of frozen sperma. It could be concluded from the fact that high non-return per cent of the inseminations made with the sperma was connected with high prolactin levels in the blood.

Prolactin and minerals - Simple correlations between plasma prolactin and minerals are given in bull groups in Table 3. Prolactin was highly significantly correlated to inorganic phosphorus in each bull group studied (I-VII). Correlations of prolactin to magnesium were mostly negative and often significant (I-IV). Correlations between prolactin and calcium were non-significant. Prolactin had a significant negative correlation in one bull group (I) and significant positive correlations in two bull groups (V and VI). Correlation of prolactin to sodium was significant and positive in one bull group (VII).

Prolactin and trace elements - Simple correlations of plasma prolactin and trace elements are shown in Table 4. Significant correlations of prolactin to iron were positive (VI and VII), while significant correlations of prolactin to copper were negative (II and IV-VI). Prolactin was not significantly correlated to zinc in any bull group studied.

Prolactin, enzymes and other blood constituents - Correlations of prolactin to plasma enzymes and some other parameters were studied. Results are reported by TANHUANPÄÄ et al. (1979).

CONCLUSIONS

During the inter-Scandinavian hormone projects, a RIA for bovine prolactin was developed by the Finnish participants. Prolactin levels in the blood, milk, urine and sperma of the Finnish cattle were determined by the RIA. Prolactin levels were measured in various physiological states. Factors affecting on the secretion of prolactin were investigated. Furthermore, relationships of prolactin to the semen characteristics and blood constituents of bulls were studied. These studies are not yet completed. Much data are stil waiting for further statistical treatments. Prolactin studies in bovine will be continued on the national level.

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FIGURE 1. PROLACTIN LEVELS IN THE BLOOD SERUM OF THE AYRSHIRE TWINS

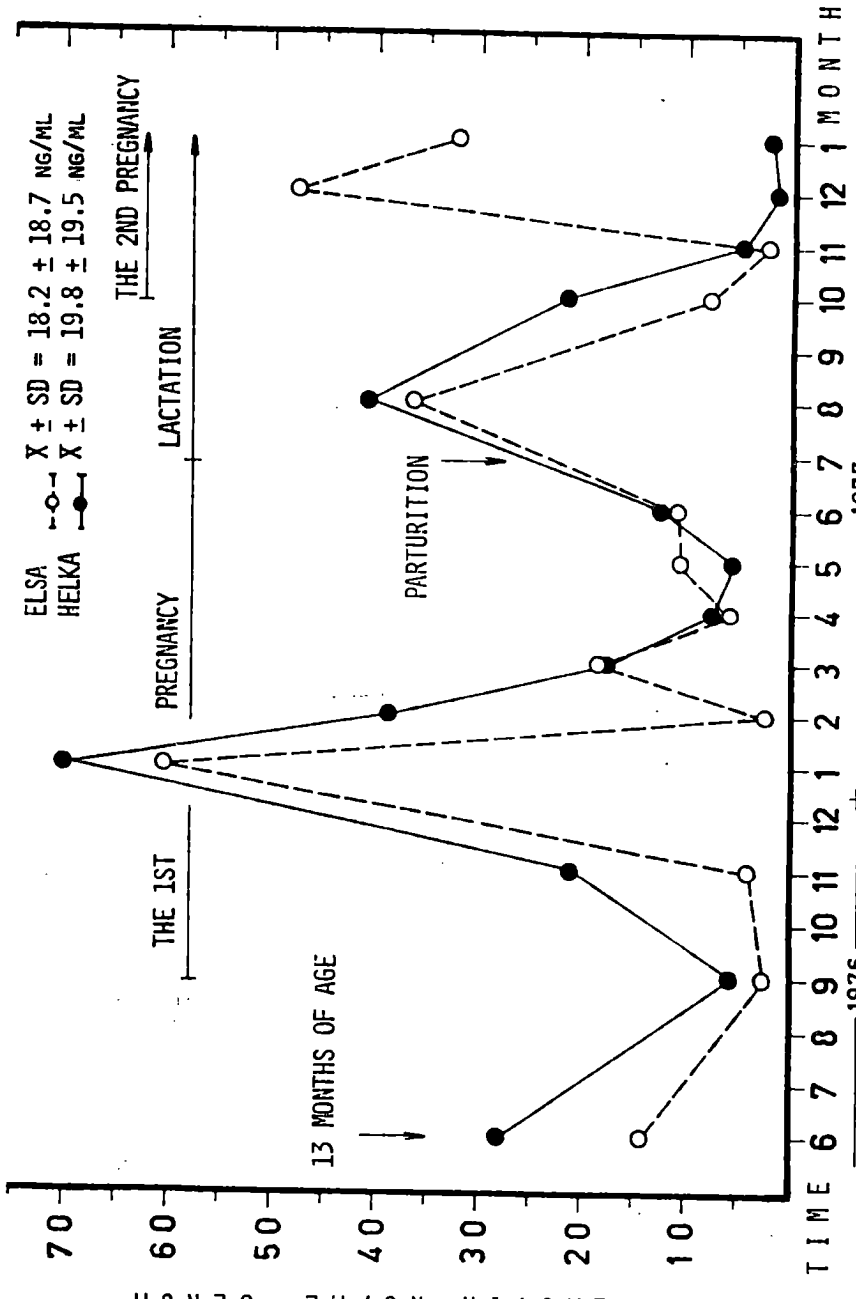

TABLE 1. MEAN PROLACTIN LEVELS (± SD) IN THE BLOOD OF THE BULLS IN VARIOUS STATIONS AND IN VARIOUS YEARS

SAMPLES			MEAN PROLACTIN (± SD) NG/ML		
BULL STATION	SAMPLING TIME	NUMBER OF BULLS	PLASMA FROM EAR VEIN	PLASMA FROM V. JUGULARIS	SERUM FROM V. JUGULARIS
1) AI-BULLS					
SALPAUSSELKÄ	27.11.1974	30	14.9 ± 13.5	14.7 ± 19.8	NO SAMPLE
- " -	05.12.1975	87	NO SAMPLE	NO SAMPLE	15.4 ± 22.9
- " -	13.12.1976	60	- " -	4.7 ± 2.4	4.3 ± 2.4
- " -	16.11.1977	60	- " -	NO SAMPLE	13.5 ± 11.2
PIRKKALA	07.12.1977	48	- " -	- " -	35.6 ± 50.7
RAUHALINNA	23.01.1978	60	- " -	- " -	11.4 ± 21.9
2) YOUNG BULLS					
HUMPPILA	18.10.1976	60	NO SAMPLE	15.5 ± 18.3	16.5 ± 17.5
TOTALLY		405			

Table 2. Simple correlations of prolactin to the sexual behaviour and semen quality of AI-bulls.

Bull group	Libido	Volume of ejaculate	Sperm count	Motility of sperms	Number of sperms alive	Deep freezing qual. of sperms	Non-return-%
I	-0.09	+0.03	+0.17 *	+0.02	-0.16 *	+0.02	-0.15
II	-0.05	-0.01	-0.04	-0.08	-0.11 *	-0.04	+0.08
III	+0.15	-0.30 **	-0.06	-0.11	-0.06	-0.12	+0.28 **

I = S75, S76 (n = 147; $\bar{X}$ = 10.9 ng/ml)

II = S74, S75, S76, S77, P77, R78 (n = 345; $\bar{X}$ = 15.2 ng/ml)

III = P77, R78 (n = 108; $\bar{X}$ = 22.2 ng/ml)

* = $P < 0.05$

** = $P < 0.01$

Table 3. Simple correlations of prolactin to plasma minerals in various bull groups.

Bull group	Na	K	Ca	Mg	P
I	+0.03	-0.12 *	-0.04	-0.17 **	+0.21 ***
II	-0.01	-0.11	-0.03	-0.16 **	+0.22 ***
III	+0.06	-0.11	-0.06	-0.17 **	+0.22 ***
IV	+0.01	-0.09	-0.05	-0.16 **	+0.23 ***
V	-0.00	+0.15 *	-0.04	-0.10	+0.31 ***
VI	+0.06	+0.22 **	+0.03	-0.10	+0.32 ***
VII	+0.36 ***	+0.10	-0.09	+0.03	+0.31 ***

I = S75-77, H76, P77, R78 (n = 375)
II = S74-77, H76, P77, R78 (n = 405)
III = S75-77, P77, R78 (n = 315)
IV = S74-77, P77, R78 (n = 345)
V = S75-76, H76 (n = 207)
VI = S74-76 (n = 177)
VII = S75-76 (n = 147)

* = $P < 0.05$
** = $P < 0.01$
*** = $P < 0.001$

Table 4. Simple correlations of prolactin to plasma trace elements in various bull groups.

Bull group	Fe	Cu	Zn
I	+0.01	-0.10	+0.01
II	-0.01	-0.12 *	-0.01
III	+0.03	-0.11	+0.00
IV	+0.01	-0.13 *	-0.02
V	+0.05	-0.21 **	-0.04
VI	+0.16 *	-0.22 **	-0.10
VII	+0.29 ***	+0.10	+0.08

See the foot notes in table 3.

SLUTRAPPORT FOR DEN DANSKE DEL AF NKJ-PROJEKT 34
UNDERSØGELSER OVER SAMMENHAENGEN MELLEM HUSDYRENES HORMONAKTIVITET OG DERES PRODUKTIONSEGENSKABER

1. Den danske del startede 1.4.1976 og sluttede 31.3.1977. Der har dog også efter sidstnaevnte dato vaeret kontakt med projektets øvrige deltagere.

2. Til den danske del er bevilget:

237.000 kr.	for	perioden	1.4.1976 -	31. 3.1977
10.000	-	-	1.4.1977 -	31. 3.1978
10.000	-	-	1.4.1978 -	31.12.1978

3. Deltagere i den danske del: vid. ass. Viggo Kruse og prof. A. Neimann-Sørensen (projektleder for hele projekt 34), Afd. for forsøg med kvaeg og får, Statens Husdyrbrugsforsøg, Roligħedsvej 25, 1958 Københan V.

4. Projektet er en videreførelse af NKJ-projekt 22. Omkring 1970 blev det muligt relativt let og billigt at måle de meget lave koncentrationer af hormoner, der forekommer i husdyrenes blod og vaev. Da hormoner udøver en overordnet regulerende funktion i forbindelse med alle livsytringer, blev en undersøgelse sat i gang (NKJ 22) med henblik på åt undersøge sammenhaenge mellem hormonparametre og produktionsegenskaber. De danske undersøgelser har vedrørt de i det følgende naevnte områder.

5. Nedbrydning af thyroxin hos individprøvetyre

Målingerne af thyroxinnedbrydningen hos individprøvetyre på forsøgsstationerne Eqtved og Ålestrup ophørte i begyndelsen af 1978, idet et tilstraekkeligt antal tyre dermed var undersøgt. Det drejer sig om følgende antal af henholdsvis SDM, RDM og Jerseyrace: 160, 180, 100. Omkring to trediedele af de udtagne prøver (SDM, RDM) blev faerdiganalyseret i 1976, og en foreløbig opgørelse omfattende 278 tyre blev udarbejdet samme år. Det blev blandt andet vist, at heritabiliteten for thyroxinkoncentrationen var 0,82 (middelfejl 0,31), mens den for thyroxinnedbrydningen kun var 0,28 (middelfejl 0,25). De resterende prøver vil vaere faerdiganalyseret med

udgangen af 1978. Det samlede datamateriale vil herefter blive bearbejdet, og foreløbig publikation udarbejdet. Der foreligger indtil nu kun ydelsestal for en del af døtregrupperne efter de tidligst afprøvede tyre, og endelige beregninger over sammenhaegen mellem målene for thyroxin og tyrenes avlsvaerdi vil først kunne beregnes i løbet af ca. 2 år.

6. Heritabiliteten for plasmathyroxin hos svin samt korrelationen mellem plasmathyroxin og vaeksthastighed, foderforbrug, slagte- og kødkvalitet

I samarbejde med dr. A. Just, Afd. for forsøg med svin og heste, og med støtte fra Statens jordbrugs- og veterinaervidenskabelige Forskningsråd (513-6514) er der foretaget undersøgelser til bestemmelse af heritabiliteten for plasmathyroxin og korrelationen mellem plasmathyroxin og produktionsegenskaber hos svin. Der er udført thyroxinbestemmelser på ca. 3000 plasmaprøver, og datamaterialet vil blive analyret i naer fremtid.

7. Sammenhaengen mellem plasmakolesterol og tilvaeksthastighed og slagtekropssammensaetning

Der er over den svenske del af NKJ 34 udført undersøgelser over heritabiliteten af plasmakolesterol samt over sammenhaengen mellem plasmakolesterol og egenskaberne tilvaeksthastighed og slagtekvalitet på en vaesentlig del af prøverne fra individprøvetyrene. Resultaterne indgår i den svenske slutredegørelse.

8. Analysetekniske undersøgelser

Praecise målinger spiller en stor rolle i undersøgelser af ovennaevnte art. Der er udført et betydeligt arbejde for at fremstille og karakterisere et godt antiserum til anvendelse i forbindelse med den udviklede analysemetode for thyroxin. Det fremstillede antiserum anvendes i laboratoriet hos to af projektets øvrige deltagere, ligesom det anvendes i flere andre laboratorier verden over.

Der er udviklet en ny metode til karakterisering af antisera, som skal bruges i radioimmunanalyser. Ligeledes er der udviklet to metoder til at forbedre allerede producerede antisera

i laboratoriet.

9. Mange af resultaterne opnået inden for NKJ-projekt 22 og 34 viser, at der er korrelationer af lav størrelsesorden mellem forskellige hormoner og produktionsegenskaber. Korrelationer som går i samme retning, som det kunne forventes på basis af vor viden inden for fysiologi og biokemi. To væsentlige forhold gør det imidlertid på nuvaerende tidspunkt vanskeligt at basere udvalget af avlsdyr på hormonmålinger. Det ene er, at mange produktionsegenskaber, såsom tilvaekst, maelkeydelse m.v., er af polygenisk natur og staerkt miljøpåvirkelige. Selv et nøje kundskab til én eller få af faktorerne gør det derfor ikke muligt at udvaelge avlsdyr med vaesentlig sikkerhed. Det andet er, at det for mange hormoners vedkommende kan vaere vanskeligt at opnå sikre skøn over den gennemsnitlige koncentration i blodet. Dette skyldes ikke analysemetodernes usikkerhed, men de store koncentrationssvingninger, der forekommer i blodet for de fleste hormoners vedkommende.

Der er dog grund til at påpege, at visse hormoner og hormonlignende stoffer viser staerk sammenhaeng med vigtige produktionsegenskaber, således progesteron → brunst, draegtighed; androstenon → ornelugt.

10. Publikationer

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NORGES VETERINÆRHØGSKOLE
Institutt for fysiologi

SLUTTRAPPORT

om

NKJ prosjekt nr. 34: Undersøkelser av sammenhengen mellom dyrenes hormonaktivitet og deres produksjonsegenskaper

fra

Institutt for fysiologi
Norges veterinærhøgskole

1) NKJ prosjekt nr. 34 ble som en videreføring av NKJ prosjekt nr. 22 påbegynt 1/1 1976 og varer frem til 31/12 1978. Det består for vårt institutts vedkommende av 3 delprosjekter, som nedenfor vil bli beskrevet hver for seg.

2) De årlig bevilgede beløp til den norske del av prosjektet fordeler seg som følger:

	Bevilget	Brukt
1976: nkr.	338.300	252.230
1977: nkr.	345.000	275.250
1978: nkr.	388.500	?

Midlene fordeler seg på lønn til personale og til driftsutgifter mellom NVH og NLH.

3) Deltakere i prosjektet har fra NVH vært:

Professor dr. Weiert Velle, ansvarlig leder.

Vitenskapelig assistent Tata Ringberg Lund-Larsen (delprosjekt veksthormon/somatomedin) frem til 1/3 1977 da hun sluttet for å gå over i et prosjekt ved University of Fairbanks, Alaska.

Forskningsassistent cand. real. Anne Sundby (delprosjekt testosteron/tilvekst).

Forsker dr. Øystein Andresen
(delprosjekt androstenon/kjønnslukt hos råne) frem til 1/7 1977 da han gikk over i stilling ved et annet institutt.

Av disse har Anne Sundby hele tiden vært lønnet med midler bevilget til prosjektet, mens Tata Ringberg Lund-Larsen og Øystein Andresen i prosjektperioden har vært lønnet over høgskolens ordinære budsjett. På prosjektet var det videre over prosjektbevilgningen lønnet to ingeniører, hvorav den ene sluttet 1/6 1977. Fra dette tidspunkt har kun en ingeniør assistert i arbeidet, idet to av de tre forskere på prosjektene da hadde sluttet ved vårt institutt.

I Prosjekt veksthormon/somatomedin

Tata Ringberg Lund-Larsen

(1) Bakgrunn

Veksthormon fra hypofysen er en forutsetning for normal vekst. Tidligere undersøkelser bl.a. ved vårt institutt (Blom o.a.) hadde imidlertid vist at veksthormonnivået i plasma undergår store variasjoner og influeres av flere forhold. En eventuell sammenheng mellom plasmaverdier for veksthormon og produksjonsegenskaper er derfor vanskelig å fastslå. Veksthormonet utøver imidlertid virkninger via somatomediner, peptider som dannes i leveren under innvirkning av veksthormon. Somatomedinnivået (-aktiviteten) i plasma fluktuerer mye mindre enn veksthormonnivået, og vil derfor være av stor interesse å kartlegge i relasjon til bl.a. tilvekst.

Følgende problemstillinger ble søkt belyst med sikte på forhold hos storfe og svin:

1) Hvordan er sammenhengen mellom veksthormon og somatomedin i plasma?
2) Er det positiv sammenheng mellom somatomedin-nivå i plasma og kroppsvekt under fysiologiske forhold?
3) Vil i tilfelle somatomedin-aktivitet bestemt på et tidlig tidspunkt kunne gi indikasjon på dyrets endelige kroppsstørrelse?
4) Hvor mange analyser pr. dyr må i tilfelle undersøkes for med rimelig sannsynlighet å kunne fastslå en representativ

gjennomsnittlig somatomedinverdi for ut fra denne å estimere fremtidig tilvekst?

(2) Resultater

På høypoeng- og lav-poeng griser ved NLH ble det i samarbeid med H. Bakke foretatt en undersøkelse som viste at hurtigvoksende, magre griser i gjennomsnitt hadde høyere somatomedinverdier enn langsomtvoksende, fete griser ($p<0,05$). Kontrollgruppens dyr lå midt mellom. Også veksthormonverdiene lå høyest hos høypoeng-grisene. (Ref. 1)

Undersøkelsen av somatomedin-aktivitet hos oksekalver på forskjellige alderstrinn i relasjon til tilvekst, muskelutvikling, lineær vekst (høyde og brystomfang) og fôrutnyttelse ga følgende resultater: Somatomedin-aktiviteten fra 4-5 månedersalderen var positivt korrelert ($p<0,05$) til tilvekst, høyde og brystomfang, og negativt korrelert til fôrforbruk pr. kg tilvekst, alle 3 parametre målt ved nådd 12 måneders alder. (Ref. 2)

Undersøkelser over døgnvariasjoner i plasma veksthormon og plasma somatomedin ble foretatt på en gruppe på 58 ungokser. For veksthormon ble det funnet store svingninger, med lave verdier like før eller under fôring, slik som tidligere beskrevet av A. Blom et al. ved vårt institutt. Somatomedin-nivået holdt seg derimot relativt konstant gjennom perioden kl.01^{00}-15^{00}. Også i dette materiale ble det funnet signifikant ($p<0,05$) positiv korrelasjon mellom somatomedin-nivå og tilvekst. Også veksthormon viste en viss positiv korrelasjon til tilvekst. (Ref. 3 og 4)

Som et biprodukt av dette prosjektet ble det, fordi vi hadde metodikken innarbeidet, også anledning til å studere relasjonen somatomedin/dvergvekst hos 3 schäfer-hunder. Alle 3 hadde meget lavt somatomedin-nivå i plasma, til tross for at kun den ene hadde patologiske forandringer i hypofysen. (Ref. 5)

(3) Vurdering av resultatenes betydning

Vi mener i dette delprosjektet å ha vist:

1) Positiv sammenheng mellom somatomedin-nivå i plasma og kroppsstørrelse hos dyr i vekst, hos både svin og storfe.

2) Måling av somatomedin-nivå i plasma på et tidlig tidspunkt i dyrets liv vil gi en viss indikasjon på dyrets tilvekstpotensiale.
3) Den lave døgnvariasjon i somatomedin-aktivitet hos ungokser tyder på at et begrenset antall prøver pr. dyr skulle være tilstrekkelig til angivelse av en representativ gjennomsnittlig somatomedin-verdi for dyret.
4) Det vil imidlertid være nødvendig å forbedre analysemetoden for somatomedin-verdier i plasma, før det kan trekkes noen endelig konklusjon om sammenheng mellom tilvekst og somatomedin-aktivitet hos husdyr.

Instituttet har for tiden ingen som arbeider med somatomedinmålinger. Det foreligger derfor i øyeblikket ikke planer om fortsettelse av prosjektet.

II Prosjekt testosteron/tilvekst

Anne Sundby

(1) Bakgrunn

Dette er en videreføring av NKJ prosjekt nr. 22. Hos hanndyr har det intakte dyr større veksthastighet og bedre fôrutnyttelse enn kastrater. Påvisning av eventuelle forskjeller i testosteron-nivå i plasma, eller i sekretorisk kapasitet for testosteron, vil derfor kunne ha interesse i avlsmessig sammenheng, idet det er viktig å finne parametre for seleksjon av avlsdyr i så ung alder som mulig.

Følgende forhold har vært søkt belyst:

1) På bakgrunn av de tidligere påviste store døgnvariasjoner i plasma testosteron-nivået å utvikle metoder som muliggjør sammenligning av testosteron-verdier mellom dyr basert på en enkelt eller få blodprøver.
2) På basis av sammenlignbare analyseverdier for plasma testosteron å undersøke eventuell tilvekst, fôrforbruk m.v. hos ungokser under ellers mest mulig standardiserte betingelser.
3) Årstidens og andre faktorers eventuelle innflytelse på plasma testosteron-nivået.

(2) Resultater

Tidligere er det vist (ref. 6) at variasjonskoeffisientene for plasma testosteron som ligger på 36-70% hos ubehandlede dyr kan reduseres til 5-14% etter injeksjon av 6000 I.E. HCG (humant chorion-gonadotropin). Denne observasjon dannet utgangspunkt for en systematisk undersøkelse på et stort oksemateriale der en studerte nærmere hvilke doser av HCG som ville være aktuelle å benytte.

Injeksjon av 375-6000 I.E. HCG til 7 måneder gamle okser ga ingen doseavhengig respons i de første 9 timene etter injeksjonen. HCG induserte imidlertid også en sekundær stigning i plasma testosteron, og mellom HCG-dose og denne sekundære, maksimale testosteron-verdi ble det funnet høy korrelasjon (r = +0,9). Også varigheten av det forhøyede testosteron-nivå var doseavhengig. (Ref. 7).

Effekten av PMSG (pregnant mare serum gonadotrophin) ble også undersøkt og viste seg å gi lignende respons som HCG, men med noe senere innsettende og mer langvarig virkning. (Ref. 7)

HCG og PMSG er glukopeptider og har derfor potensielt antigene egenskaper. I et annet arbeid ble effekten av gjentatt HCG-injeksjon på samme dyr studert med henblikk på såvel respons i testosteron-nivå som på antistoff-utvikling. Annen gangs HCG-injeksjon (6 uker etter første) ledet hos 3 av 4 okser til betydelig raskere retur av plasmatestosteron enn etter første injeksjon, samtidig med utvikling av målbare mengder antistoffer mot HCG i plasma. Disse resultater var uavhengige av injeksjonsmåten for HCG. (Ref. 8)

Hos ungokser ved testingsstasjonen Egtvedt, Danmark, fantes gjennomsnittlige og maksimale plasma testosteron-verdier å være signifikant negativt korrelert til fôrforbruket pr. kg tilvekst, r var henholdsvis -0,52 ($p<0,05$) og -0,67 ($p<0,01$). (Ref. 2)

I et større oksemateriale fra testingsstasjonene Øyer og Klepp i Norge, er gjennomsnittlig og maksimalt plasma testosteron-nivå sammenholdt med verdier 5 og 7, eller kun 7 timer etter en enkelt injeksjon av 750 I.E. HCG til de samme dyrene (140). Korrelasjonskoeffisienten for sammenhengen mellom maksimalt

testosteron-nivå før HCG og nivå 5 og 7, h.h.v. 7 timer etter HCG-injeksjonen lå på 0,7-0,8. Variasjonskoeffisienten for verdiene 5 og 7 timer etter HCG-injeksjon var 11%. (Ref. 9).

På dette grunnlag ble testosteronmålinger foretatt på enkeltprøver tatt 7 timer etter en HCG-dose for ialt 330 ungokser. De maksimale verdier ble funnet i 8-månedersalderen, og falt sammen med kurvene for maksimal tilvekst.

Korrelasjonen mellom plasma testosteron og tilvekst i prøvetakingsmåneden var for 8 måneder gamle okser +0,46 ($p<0,001$) for et materiale undersøkt i 1976, og +0,33 ($p<0,001$) for et materiale målt i 1978. For hele perioden (3-12 måneder) var $r = +0,37$ ($p<0,001$). Undersøkelsene er enda ikke avsluttet.

Årstidsvariasjoner i plasma testosteron-nivå er påvist hos avlsokser, med signifikant lavere gjennomsnittsnivå i oktober ($p<0,001$) og desember ($p<0,05$) enn i februar, juni og august. (Ref. 10)

Hemikastrasjon av 3 måneder gamle oksekalver ga ingen forskjell hverken i tilvekst eller testosteron-nivå sammenlignet med intakte dyr. (Samarbeid med Ø. Andresen)

Sulting leder til signifikant fall i plasmatestosteron hos okse. (Samarbeid med T. Almelid, NLH)

(3) Vurdering av resultatenes betydning

Vi mener, ut fra de undersøkelser som er foretatt, å kunne trekke følgende konklusjoner:

1) Hos normale, ubehandlede okser er døgnvariasjonene i plasma-testosteron-nivå spontant så store at det må tas svært mange prøver av hvert dyr innen en kortere periode dersom en skal få grunnlag for sammenligning av gjennomsnittsverdier mellom dyr.
2) Da det er høy positiv korrelasjon mellom spontane maksimumsverdier for testosteron og de verdier man måler 7 timer etter injeksjon av en liten dose HCG, kan imidlertid sammenligning mellom dyr likevel forenkles til å bygge på analyse av en enkelt prøve.

3) Brukes standardisert prosedyr HCG-injeksjon med påfølgende testosteron-analyse på blodprøve tatt 7 timer senere, finner en høy signifikant korrelasjon mellom plasmatestosteron og veksthastighet hos ungokser i perioden 3 til 12 måneder.
4) HCG gir hos de fleste dyr opphav til antistoffer som vil kunne interferere med testosteron-responsen i tilfelle man måtte ønske å gjenta målingene.

Prosjektet har gitt flere interessante resultater, men det synes ikke å være grunn til å fortsette undersøkelsene i samme spor som hittil da en neppe vil komme stort lenger når det gjelder plasma-testosteron i relasjon til tilvekst. Imidlertid er det, når det dreier seg om hormon-effekter, ikke bare hormonet som er bestemmende for effekten, men også reseptorfunksjonen. Som nasjonalt prosjekt har vi derfor nå søkt om midler til nærmere undersøkelser over testosteron-reseptorer i forskjellige vev, spesielt med henblikk på svin. Dette er et prosjekt,som hva materiale angår, ikke er betinget av nordisk koordinering. Det har også mer karakter av grunnforskning, og vi håper det skal bli mulig å arbeide med dette spørsmål som en naturlig videreføring av vårt delprosjekt testosteron/tilvekst under NKJ proskekt nr. 34.

III Prosjekt androstenon - kjønnslukt hos råne

Øystein Andresen

(1) Bakgrunn

Prosjektet er en videreføring av NKJ prosjekt nr. 22.
Råner har i flere henseender egenskaper som gjør dem velegnet til bruk i produksjonen av svinekjøtt. Sammenlignet med purker og kastrerte hanner produserer de således mer kjøtt og mindre fett. Ved siden av å produsere et kvalitativt bedre slakt, kommer også råner godt ut i en sammenligning av fôrutnyttelse og veksthastighet.

På grunn av forekomst av ranelukt, har en imidlertid hittil ment at råneslakt er uegnet som råstoff ved fremstilling av de fleste produkter av svinekjøtt.

Steroidet androstenon er den vesentligste komponent i ranelukten.

Formålet med prosjektet har vært:

1) Utvikle metoder for kvantitering av androstenon i plasma og fettvev, herunder også raske og enkle metoder som kan brukes ved rutinebedømmelse av råneslakt.
2) Skaffe til veie kunnskap om androstenons normale produksjon, nivå i plasma og fettvev og også faktorer som stimulerer og hemmer dets produksjon.
3) Undersøke sambandet mellom ránelukt og androstenonnivå i fettvev.
4) Finne metoder for å redusere androstenonnivået i fettvev og dermed gjøre det mulig å bruke råner i produksjonen av svinekjøtt.

(2) Resultater

Når det gjelder virksomheten frem til 1976, viser en til "Sluttrapport for den norske del av NKJ prosjekt nr. 22".

Undersøkelsen over androstenon og testosteron hos råner under vekst og kjønnsmodning (11) ble fullført i 1976. En fant at androstenon-og testosteron-nivåene i plasma følger hverandre ganske nøye (r = 0,64), og videre at androstenonnivåene er betraktelig høyere enn testosteronnivåene. Det viste seg også at androstenonnivåene i plasma og fettvev er sterkt korrelert (r =+0,78).

Undersøkelsen over hvilken effekt parring har som stimulus for androstenon- og testosteron-sekresjon (12) ble også fullført i 1976. Det viste seg at det var en viss forskjell fra dyr til dyr, men gjennomgående fant en at parring var ledsaget av økende steroidnivåer i perifert plasma.

I et forsøk på å redusere androstenonsekresjonen og samtidig bibeholde en viss testosteronsekresjon, fjernet en den ene testikkel på 3 ca. 1 måned gamle råner, og fulgte så androstenon-konsentrasjonen i fettvev hos disse dyrene under vekst og kjønns-modning. Samtidig hadde en med en kontrollgruppe som besto av 5 intakte dyr.

Resultatene viste at androstenon-nivåene hos de hemikastrerte og intakte dyrene ikke var særlig forskjellige. Da dyrene ble slaktet ved ca. 90 kg levende vekt, fant en at vekten av den

ene testikkel hos de hemikastrerte dyrene var svært lik den samlede vekt av begge testikler hos de intakte dyrene (484 g hos hemikastrater og 458 g hos intakte dyr). Det histologiske bilde av testiklene hos de to gruppene var også ganske likt.

Undersøkelsen over genetiske forskjeller mellom råner i testikulær endokrin aktivitet har fortsatt. Undersøkelsen foretas i samarbeid med dr.agro. Per Jonsson, Landøkonomisk Forsøgslaboratorium, København. Arbeidet er ikke helt avsluttet, men en beregning på grunnlag av en begrenset del av materialet tyder på at arvbarheten for androstenonkonsentrasjon i fettvev hos råner med levendevekt 90 kg er ca. 0,12.

En rask og meget enkel metode for bestemmelse av androstenon i fett er utarbeidet (15). Metoden har stor kapasitet idet en tekniker ganske enkelt kan måle androstenon i duplikat i 100 prøver pr. dag.

(3) Annen virksomhet

Syv av artiklene som er kommet som et resultat av prosjektet Androstenon - kjønnslukt hos råne, ble i 1977 sammenstillet til et doktorarbeid og forsvart for den veterinærmedisinske doktorgrad (13).

Etter oppdrag fra Norges landbruksvitenskapelige forskningsråd ble det i 1977 utarbeidet en utredning med tittel: Råneslakt som råvare ved fremstilling av kjøttvarer (14).

(4) Vurdering av resultatenes betydning

1) Viktig resultat av dette delprosjekt er den metodeutvikling som har ført til at man kan bestemme androstenon i såvel blod som fettvev.
2) Den seneste forenkling av androstenonbestemmelsen i fettvev vil gjøre det mulig å introdusere kvantitative målinger som rutine i slakteriene og åpner derfor for en mer differensiert vurdering av råneslakt enn det som hittil har vært mulig.
3) Undersøkelsene over arvbarheten for rånelukt viser foreløpig skuffende lave verdier, men undersøkelsen var likevel nødvendig for å vite hvor vi står.

Det er et sterkt ønske om å få fortsette arbeidet omkring

androstenon og råtnelukt i Norge, men det er enda ikke avgjort i hvilken form dette skal skje. En vil i alle fall forsøke å videreføre det gode samarbeid en har hatt under NKJ prosjekt nr.22 og 34 med

Institutionen för husdjursförädling,
Sveriges Lantbruksuniversitet,

Landøkonomisk Forsögslaboratorium,
København,

Slakterienes Forsögslaboratorium,
Roskilde.

NKJ prosjekt nr. 34: Publikasjoner fra Institutt for fysiolgi, Norges veterinærhøgskole.

Prosjektet veksthormon/somatomedin

1) Ringberg Lund-Larsen, T. og Bakke, Haavard. 1975. Growth hormone and somatomedin activities in lines of pigs selected for rate of gain and thickness of backfat. Acta Agric. Scand. 25, 231-234.

2) Ringberg Lund-Larsen, T., Sundby, A., Kruse, V. og Velle, W. 1977. Relation between growth rate, serum somatomedin and plasma testosterone in young bulls. J. Anim. Sci. 44, 189-194.

3) Ringberg, T. 1978. Serum somatomedin - a measure of prospective growth capacity in bull calves ? J. Anim. Sci. Submitted.

4) Ringberg, T. 1978. Diurnal variation of growth hormone in bull calves. Acta Agric. Scand. Submitted.

5) Ringberg Lund-Larsen, T. og Grøndalen, J. 1976. Ateliotic dwarfism in the german shepherd dog. Acta vet. scand. 17, 293-306.

Prosjektet testosteron/tilvekst

6) Sundby, A., Tollman, R. and Velle, W. 1975. Long-term effect of HCG on plasma testosterone in bulls. J. Reprod. Fert. 45, 249-254.

7) Sundby, A. and Farahat, A. 1978. Plasma testosterone in bulls - Response to various doses of HCG and PMSG. Acta endocr. (Kbh.) 88, 793-800.

8) Sundby, A. and Torjesen, P.A. 1978. Plasma levels of testosterone in bulls - Response to repeated HCG injections. Acta endocr. (Kbh.) 88, 787-792.

9) Sundby, A. and Velle, W. 1978. Age, Weight and HCG on plasma testosterone in bulls. J. Anim. Sci. In press.

10) Sundby, A. and Tollman, R. 1978. Plasma testosterone in bulls - Seasonal variation. Acta vet. scand. 19, 263-268.

Prosjektet androstenon/kjønnslukt hos råne

11) Andresen, Ø. 1976. Concentrations of fat and plasma 5α-androstenone and testosterone in boars selected for rate of body weight gain and thickness of back fat during growth, sexual maturation and after mating. J. Reprod. Fert. 48, 51-59.

12) Andresen, Ø. 1976. 5α-androstenone and testosterone in peripheral plasma of the boar during and following copulation. Acta vet. scand. 17, 475-487.

13) Andresen, Ø. 1977. Radioimmunological estimation of 5α-androstenone and studies on its occurrence in boar peripheral plasma and fat, with some reference to plasma testosterone levels. Thesis, Veterinary College of Norway, Oslo.

14) Andresen, Ø. 1977. Råneslakt som råvare ved fremstilling av kjøttvarer. Norges landbruksvitenskapelig forskningsråd, utredning nr. 89, Oslo. Stensil, 29 sider.

15) Andresen, Ø. 1978. A rapid radioimmunological evaluation of the androstenone content of boar fat. Acta endocr. (Kbh.) Submitted for publication.

Videre gjør en oppmerksom på at flere publikasjoner er under forberedelse. Spesielt gjelder dette for delprosjektet "Testosteron/tilvekst".

Sluttrapport for NKJ prosjekt 34

Delprosjekt Institutt for husdyravl
Norges Landbrukshøgskole
Nils Standal

Prosjektet kom som en fortsettelse av prosjekt NKJ 22, og har vart fra 1976-1979.

Den norske delen av prosjektet har vært delt på to institusjoner: Intitutt for fysiologi, Norges Veterinærhøgskole og Institutt for husdyravl, Norges Landbrukshøgskole. De årlige bevilgninger til den norske delen av prosjektet er det redegjort for ved rapport fra NVH.

Deltakere i delprosjektet ved Institutt for husdyravl har vært Øystein Joakimsen (1976) og Torbjørn Almlid (1977 og 1978), som har vært lønnet på prosjektet. Dessuten har følgende personer som har vært lønnet av andre midler i større eller mindre grad deltatt i prosjektet: Borghild Tveit, Håvard Bakke, Per Monrad Dahl, Ågot Eggum og Nils Standal.

Bakgrunn for prosjektet.

De økonomisk viktige egenskapene slik de manifesterer seg hos våre husdyr representerer sluttresultatet av en rekke kompliserte biokjemiske prosesser. I regulering av disse prosessene spiller hormonene en viktig rolle. Det er derfor rimelig å anta at en del av den variasjonen vi finner mellom dyr f.eks. i mjølkeproduksjonsevne, kjøttproduksjonsevne osv. har sin årsak i ulik hormonproduksjon. Dersom en kunne finne slike sammenhenger, og dersom de kvantitative relasjonene var sterke nok, ville dette kunne gi oss muligheter til å effektivisere avlsarbeidet. Det ville særlig kunne bli betydningsfullt for kjønnsbegrensede egenskaper. Denne problemstillingen var bakgrunnen også for NKJ-prosjekt 22 som var forløper for NKJ-prosjekt 34.

Under NKJ-prosjekt 22 ble metodikken for studium av thyroideafunksjonen hos okser utviklet, og sammenhengen mellom thyroxinnedbryting og veksthastighet ble undersøkt. Det ble også utført en stor del av felt- og laboratoriearbeidet for en undersøkelse av

sammenhengen mellom thyroxinnedbryting og melkeproduksjon. Dette arbeidet, og andre sider av hormonenes virkning på produksjonsegenskapene er undersøkt i NKJ-prosjekt 34.

Resultater.

a) Det er funnet en svak, men klart positiv sammenheng mellom avkomsundersøkelse for døtrenes melkeproduksjon og tyroxinnedbryting hos okser ved 4-12 eller 12-16 måneders alder. Det er laget en foreløpig rapport om resultatene (Joakimsen, 1975). En er kommet til at den metoden som brukes for avkomsundersøkelse for melkeproduksjon ikke lenger er fullt tilfredsstillende. Det arbeides med å forbedre avkomsundersøkelsene, og en håper å kunne publisere de endelige resultatene for sammenhengen med thyroxinnedbrytingen i nærmeste framtid. I dette arbeidet er en imidlertid avhengig av arbeidet med avkomsundersøkelsene.
Trijodthyroninnivået hos okser viste en dårligere sammenheng med døtrenes melkeproduksjon enn thyroxinnedbrytingen.

b) Det er utviklet en metode for bestemmelse av fritt thyroxin, og en for bestemmelse av TSH (Thyroideastimulerende hormon) i plasma. Disse metodene har særlig vært benyttet i forbindelse med et ketoseprosjekt, men har også vært benyttet innenfor et delprosjekt der en har studert virkningen av to dagers faste på blodparametre hos okser.

c) Virkningen av 2 dagers faste på serumnivå av veksthormon, thyroxin, insulin, acetoacetat, glucose og NEFA er undersøkt i to faseperioder. Også tyroxinnedbrytingen er undersøkt under vanlig fôring og under faste. Det er laget en foreløpig rapport om resultatene (Almlid, 1977). Ellers vil det bli publisert mer utførlige resultater med det første. Ved symposiet i Finland 4. januar 1979 vil det bli publisert en rapport om virkningen av faste på thyroideahormonene og TSH.
Det var planlagt å utvide denne undersøkelsen til et større antall potensielle seminokser på testingsstasjonen i Øyer, men søknaden om å benytte disse oksene ble avslått av Avlslaget for Norsk Rødt Fe.

d) Serumnivået av thyroxin og trijodthyronin hos selekterte svinegrupper er undersøkt. Det ble funnet signifikant forskjell mellom linjene i T_3 nivå, men ikke for T_4. Regresjonene på tilvekst og fôrutnytting innen linje var ikke signifikant (Bakke og Tveit, 1976).

e) Det er gjennomført en undersøkelse for å bestemme nedbrytingen av thyroxin og trijodthyronin hos selekterte svinegrupper. Målingene ble foretatt på 12 griser ved 40-50 [1] seleksjonsgrupper fra et seleksjonsforsøk ved instituttet. Resultatene er ikke endelig oppgjort, men det ser ikke ut til å være forskjell mellom gruppene i nedbryting av de to hormonene. Rapport vil bli gitt på symposiet i Finland 4. januar 1979.

f) Analysen av sammenhengen mellom veksthormonnivå og veksthastighet hos okser er publisert (Joakimsen og Blom, 1976).

g) Instituttet satte i gang et delprosjekt for å undersøke om en kunne måle fosterets innvirkning på melkeproduksjonen hos mora via bestemmelse av østradiol og progesteron hos søyer på 140. drektighetsdag. En stipendiat fra Nederland, Cora Bakker arbeidet en god del med disse analysene. Det var imidlertid store problemer med analysemetodene, og de videre planene med prosjektet ble droppet. Det er skrevet en intern rapport om arbeidet.

Vurdering av resultatenes betydning.

Det er påvist en klar positiv sammenheng mellom tyroxinnedbryting hos seminokser, og døtrenes melkeproduksjon. Som påpekt av Joakimsen (1975) er det imidlertid uklart om seleksjon vil ha en gunstig effekt på effektiviteten i melkeproduksjonen. Siden thyroideahormonene har en sterk effekt på stoffskiftet kan det tenkes at slik seleksjon kan føre til høgere vedlikeholdsbehov, og dermed til en mindre effektiv melkeproduksjon. Denne problemstillingen har ført til at det er satt i gang et større forsøk med formål å studere genetisk variasjon i thyroxinnedbryting og sammenhengen med fôrutnyttelse i melkeproduksjonen. Prosjektet omfatter 20 avkomsgrupper, og vil gå i 7-8 år.

1) kg lev.vekt fra hver av to

Prosjekt 34 har ellers ført til utvikling av en rekke metoder, og til grunnleggende kunnskaper om variasjonsårsaker når det gjelder hormonnivå.

En kan neppe si at det utover det som er nevnt har ført til resultater som er direkte nyttbare i praktisk seleksjon.

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HORMONE RECEPTORS AND THEIR CLINICAL SIGNIFICANCE

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Introduction

The development of radioactive hormone derivatives with a high specific activity facilitated the discovery of cellular hormone receptors about ten years ago. These molecules specifically recognize and bind the hormone and, as a consequence of this interaction, can lead to changes which ultimately result in a biological response in the cell. The cellular steps proposed to be involved in hormone action are schematically shown in Fig. 1.

According to the cellular location, three distinctive types of hormone receptors can be separated (Table 1, Fig. 1): Protein and peptide hormones as well as neurotransmitters initially bind to receptors on cell membrane. Steroid hormones, including vitamin D, are thought to freely penetrate the cell membrane and thereafter to bind to cytoplasmic receptors. It is suggested that the cellular recognition of thyroid hormones occurs at the nuclear level.

The various types of hormone receptors have some common characteristics (Table 2). They display clearcut structural requirements as concerns the ligand in question, their presence is limited to target tissues, the affinity towards the hormone is very high and their cellular amount is limited, usually of the order of 10 000 receptors/cell. The binding reaction is relatively rapid and reversible, and is finally correlated to a biological event in the cell.

Receptors on the cell membrane

The initial event in the action of peptide hormones (for review, see ref. 1), such as glucagon, gonadotrophins, ACTH and growth hormone is

the binding of the hormone to a specific receptor at the cell membrane (Fig. 1). The hormone-receptor interaction in some way activates the so-called effector, usually the enzyme adenylate cyclase, which in turn promotes the formation of intracellular second messenger or cyclic AMP. The cell contains an important group of regulatory enzymes, called protein kinases. This enzyme consists of two subunits, regulatory and catalytic; the complex as such is inactive. The cyclic AMP formed binds to the regulatory subunit of the enzyme which triggers the dissociation of the two subunits. The catalytic subunit now works as activated protein kinase and catalyzes the fosforylation of various cellular protein components, for instance enzymes. Depending on its nature, the fosforylated enzyme shows an enhanced or diminished activity, and in this way, the initial hormonal stimulus results in an altered cellular function.

Cytoplasmic receptors

Steroid hormones freely diffuse through the cell membrane whereafter they bind to a cytoplasmic receptor (Fig. 1). The complex needs an activation process which renders it capable of interacting with a nuclear acceptor complex, situated on chromatin. This in some way activates the genome which leads to enhanced RNA synthesis and finally, to enhanced protein synthesis in the cytoplasm.

The function of steroid receptors have recently been the subject of intensive investigation (for review, see 2). This molecule is absolutely required for the ultimate action of a steroid to occur. So, there is a rule: no steroid receptor → no steroid responsiveness, although the reversal is not necessarily true. Several theories of the regulatory mechanisms of steroid receptor complexes have been presented, and two hypotheses - activation of chromatin template or activation of RNA polymerase by the steroid-receptor complex - have earned most favor.

Receptors at the nuclear level

Much less is known about the actions of thyroid hormones (for review, see ref. 3). Most investigators seem to agree that triiodothyronine (T_3), and not thyroxine, is the cellular mediator of thyroid hormone action. It is not known if specific cytoplasmic binding proteins for T_3 exist, but the answer probably is not. T_3 is thought to freely move through the whole cell (Fig. 1) reaching finally the nucleus where it binds to a receptor situated on chromatin. What happens then is not known, but similar events to those in steroidal action have been speculated to ensue. It is of interest to note that thyroid hormone receptors also exist in mitochondria. One could assume that these receptors have something to do with the thermoregulatory action of thyroid hormones.

Receptor measurement

The methods for the measurement of various hormone receptors are based on the ability of the cellular subfraction containing the receptor to bind a radioactive hormone derivative. An example is shown in Fig. 2, which schematically illustrates the steps involved in estrogen receptor determination. After surgical removal, a piece of breast tumor tissue is immediately frozen and can be thereafter stored several months at -70 centrigrades. For the analysis, the tumor piece is pulverized and homogenized and a cytosol fraction is prepared by ultracentrifugation. Radioactive estradiol-17β is added to the cytosol, and after separation of bound and unbound radioactivity (e.g. by activated charcoal), the amount of receptor-bound ligand can be calculated and an estimate of the concentration of receptor binding sites obtained.

Clinical applications of the receptors

For a biochemist, hormone receptors serve as unique models for studies on regulation of eukaryotic cell function. Some important clinical applications have already emerged, and these are shortly discussed in the following.

Hormone-dependent cancer

For about 10 years ago it was found that a considerable amount of human mammary tumors contain estrogen receptor. Later on, several groups were able to confirm that their presence in a tumor sample was positively correlated to a response obtained by endocrine maneuvers, the administration of antiestrogens or surgical removal of hormone-producing glands. McGuire found (4) that even better prediction was obtained when both estrogen and progestin receptors were measured.

The current opinion (4) of the relationship between steroid receptor status in advanced breast cancer and a favorable response to endocrine treatment is depicted in Table 3. Thus, if both receptors are lacking, the patient has no chance to benefit of endocrine therapy, and if both receptors are present (as is the case in about 50 per cent of all patients), there is an about 70-80 per cent possibility to get an objective response with endocrine therapy. It seems at present that steroid receptor measurements constitute by far the most reliable predictive method when directing patients with advanced breast cancer to the areas of either endocrine or cytotoxic treatment. There are also promising data on the use of progestin receptors in endometrial cancer (5) and corticoid receptors in leuchaemia (6) for similar purposes.

Testicular feminization: a congenital receptor defect

Since the discovery of hormone receptors, several congenital deficiencies of hormone receptors have been described, the most well-known of which is probably the testicular feminization syndrome. This disease is an x-linked genetic disorder. The affected patient is genetically male but shows a female appearance. The condition is characterized by a complete insensitivity to endogenous or exogenous androgens, and the biochemical explanation is the complete lack of intracellular androgen receptor (7).

Insulin receptors and resistance to insulin action

The hormone receptor defect can also be acquired. The level of insulin receptor in peripheral lymphocytes of normal and very obese persons is an example. Obesity is a condition which is clinically characterized by a more or less severe insulin resistance. It appears that the insulin receptor level is markedly lowered in lymphocytes of obese persons (8). It is noteworthy that these persons restore their cellular insulin binding capacity towards normal after complete fasting (8). The mechanism of this variation in receptor level has been carefully studied in animal models and in cell culture. The results clearly indicate that it is not the overweight as such which lowers insulin receptor level but it is chronic hyperinsulinemia, which is associated with a continuous caloric excess (8). Thus, insulin itself lowers the concentration of its own receptor, and this bears an extremely important clinical significance. If an overweighed diabetic patient displays an impaired balance, the right way to treat the condition is not to increase his insulin dose but to carefully restrict his caloric intake. The concept that hormone regulates its own receptor level seems to be almost universal and its applicable to both protein, steroid and thyroid hormones.

Anti-receptor antibodies

Traditionally, hormone resistance has been attributed to circulating hormone antibodies. One form of hormone resistance comes from a complete or partial lack of the receptor, as discussed above. A new type of hormone resistance has emerged during the recent few years, and that is the presence of antibodies directed towards a hormone receptor. Circulating anti-receptor antibodies have been described in at least three disease entities (9): Basedow's disease, the syndrome of diabetes mellitus associated with acanthosis nigricans and myasthenia gravis. In the latter two conditions, receptor antibodies usually act as pure antagonists, blocking insulin action in diabetes and acetylcholine action in myasthenia. In Basedow's disease, the situation is different. In a normal person the pituitary gland secretes TSH which binds to its receptor in the thyroid gland. Upon this stimulus thyroid gland secretes thyroid hormones which, in turn, exert a well-known feed-back action at the level of pituitary. In Basedow's disease, there exist large amounts of circulating antibodies towards TSH receptor. These antibodies behave as agonists, and so

they mimic TSH action. As a consequence, overstimulation with no feed-back control results and the clinical picture of hyperthyroidism is seen.

Conclusions

The initial biochemical event in hormone action is the binding of the hormone to a specific receptor component in the target cell. Clinically, a most interesting feature of these receptors is the variation of their concentration in various physiological and pathological conditions. In a way, this has opened a new era in endocrinology because it indicates that the basis of endocrine dysfunction can also exist at the target cell level.

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Table 1. Cellular location of hormone receptors.

1. Receptors on the cell membrane
Peptide hormones
- hypothalamic hormones
- hypophyseal hormones
- insulin and glucagon
Neurotransmitters
- catecholamines
- acetylcholine
2. Receptors in the cytoplasm
- Steroid hormones
- Vitamin D
3. Receptors in the cell nucleus
- Thyroid hormones

Table 2. Common characteristics of hormone receptors

Hormone specificity
Target cell specificity
Low capacity
High affinity
Binding kinetics reversible
Correlation to a biological function

Table 3. The presence of estrogen (ER) and progesterone (PR) receptor in tumor tissue of patients with advanced (metastatic or recurrent) breast cancer and the objective response to endocrine therapy.

Receptor status	Objective response (per cent of patients)
ER- PR-	0-10
ER- PR+	?*
ER+ PR-	30-40
ER+ PR+	70-80

* Very few patients belong to this category

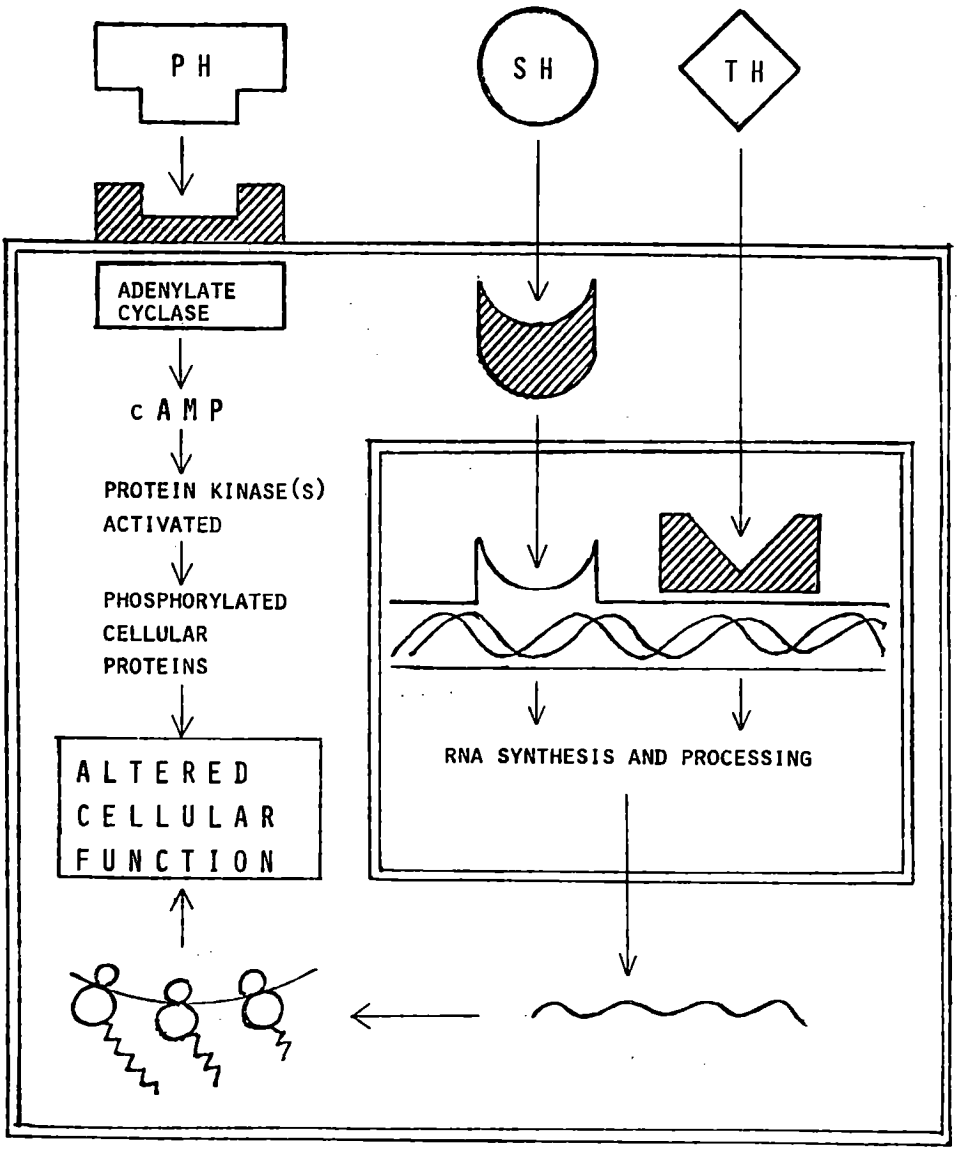

Fig. 1. Mechanism of action of peptide hormones (PH), steroid hormones (SH) and thyroid hormones (TH)

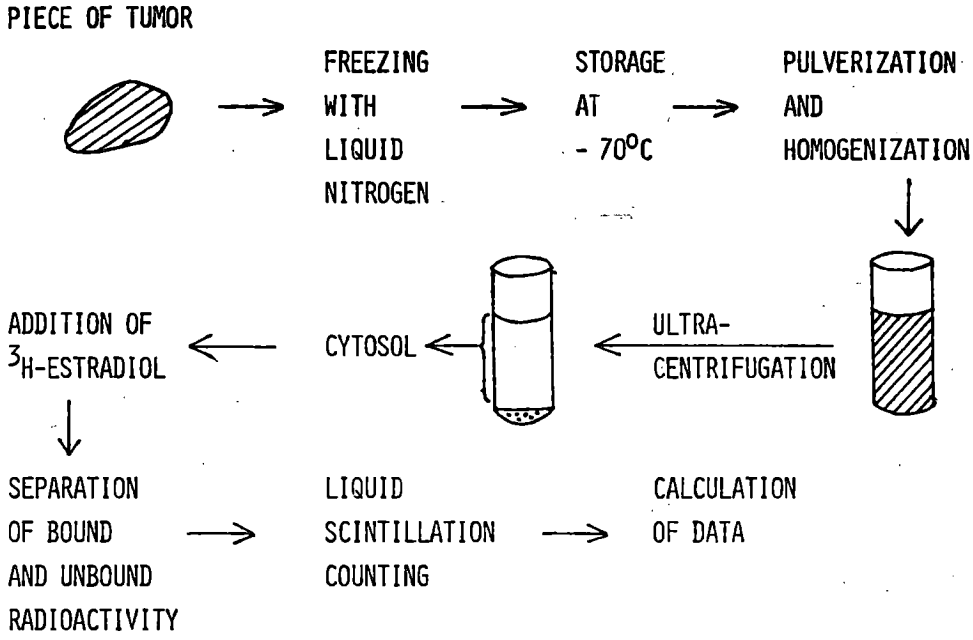

Fig. 2. Measurement of estrogen receptor in a sample of human breast cancer

HORMONAL CONTROL OF FLUID BALANCE

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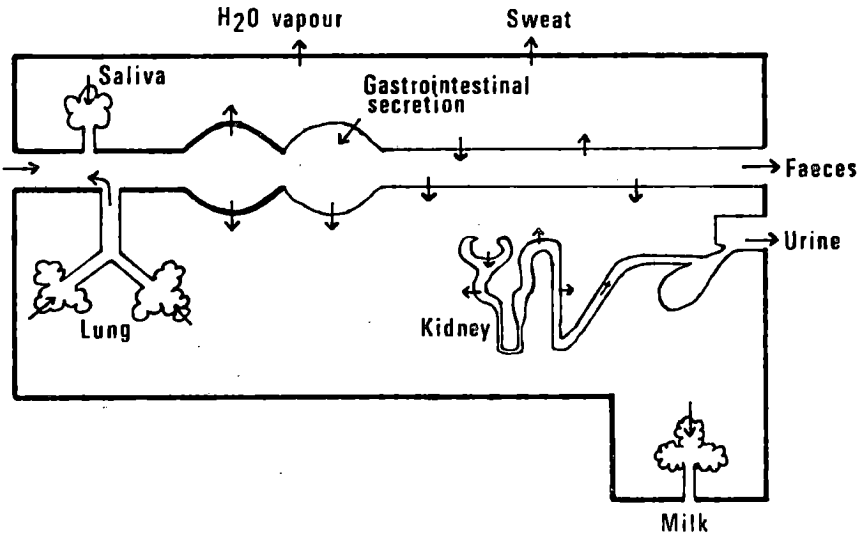
A living mammal contains 55-75% water. One third of the water, not including the ingesta, is outside the cells, s.c. extracellular fluid, and two thirds is intracellular. The main cation of the extracellular fluid is Na^+ and that of the intracellular fluid K^+. Normal tissue functions depend on the precise maintenance of the composition of the extracellular fluid which constitutes the internal environment of the animal. The intake and output of water and electrolytes must be in equilibrium.

The organism obtains fluid by ingestion of water and food, and from metabolism. Water and electrolytes are lost through urine, faeces and sweat. Water is also lost with expired air and through the skin by evaporation. In lactating animals the loss of water and minerals in milk can be quite remarkable. In ruminants the circulation of water and electrolytes mainly from the salivary glands into the alimentary tract and back into blood is many times greater than their intake through food and drinking water.



The fluid balance is effectively controlled by kidneys. Many hormones influence the kidney function. But also purely physical factors and renal nerves participate in this regulation.

The antidiuretic hormone or vasopressin from the posterior pituitary is the main hormone that controls renal water excretion. Vasopressin is a nonapeptide. It has arginine as the 8th amino acid except pigs, which have lysine vasopressin. Vasopressin increases the water permeability of the collecting ducts in the kidney, so that water is reabsorbed in the hypertonic renal medulla. Vasopressin activates the membrane-bound adenyl cyclase and increases the cyclic AMP. As a result, permeability changes. Vasopressin acts rapidly. In the presence of the hormone only small amounts of concentrated urine is excreted. Vasopressin is synthesized by neurons of the supraoptic nuclei in the hypothalamus and transported together with neurophysin down their axons into the posterior pituitary and stored there. Its release into the systemic circulation is controlled by several mechanisms.

Both the concentration and the volume of extracellular fluid affect the release of vasopressin. When the osmolality and sodium concentration increase, more vasopressin is released into the circulation. On the other hand, when the osmolality decreases, e.g. after drinking a great quantity of water, vasopressin release is suppressed and the excess water is excreted in diluted urine. Professor B. Andersson in Stockholm has successfully investigated the central regulation of the kidney function and that of thirst. He uses conscious goats with permanent cannulae in the brain ventricular system. The osmo- (or Na^+-)receptors that control vasopressin release and thirst are located in the hypothalamus, possibly near the anterior wall of the third brain ventricle (Andersson, 1977). The results in our studies in Helsinki with the same experimental model agree with those of Andersson (Eriksson, 1976; Fyhrquist et al., 1979). By using radioimmunoassay for plasma vasopressin (Fyhrquist et al., 1976) we have found that the increase in the Na^+-concentration of the cerebrospinal fluid by infusing hypertonic NaCl elevates plasma vasopressin concentration and induces antidiuresis in hydrated goats. On the other hand, the lowering of Na^+-concentration by infusing isotonic fructose suppresses plasma vasopressin and renal water excretion increases.

Also the volume of extracellular fluid controls the vasopressin level. The decrease of volume stimulates the release of the hormone and urine water loss diminishes, whereas volume expansion suppresses vasopressin. Changes in volume are reflected in the blood volume which is monitored by 1) receptors in the low pressure system of the circulation (e.g. left atrium and pulmonary vessels), 2) baroreceptors and 3) juxtaglomerular apparatus in the kidney and activation of the renin-angiotensin system. Infusions of angiotensin II increase the plasma vasopressin concentration, but the physiological significance of this is not yet generally accepted.

Aldosterone is the chief mineralocorticoid of the adrenal cortex and it is of great importance for the retention of Na^+. It increases the reabsorption of Na^+ in the renal tubuli simultaneously facilitating urine K^+ excretion. It also lowers the ratio of Na^+ and K^+ in saliva and increases Na^+ reabsorption in the colon, thus reducing Na^+ loss in the alimentary tract. Aldosterone increases Na^+ transport by stimulating the messenger RNA and protein synthesis. This explains the long latent period.

In sodium deficiency the secretion of aldosterone is increased by several mechanisms. The most important is the renin-angiotensin system. The juxtaglomerular cells in the kidney release proteolytic enzyme renin which splits the angiotensinogen (or renin substrate, an α_2 globulin) in plasma into angiotensin I. This is further converted into angiotensin II which stimulates aldosterone synthesis. Increase of plasma K^+ or decrease of plasma Na^+ in sodium deficiency can stimulate directly aldosterone synthesis in the adrenal cortex.

Renin secretion from the juxtaglomerular cells is controlled by many local factors, especially by blood pressure in the renal artery. Obviously, the central nervous system also modifies renin secretion (see Davis & Freeman, 1976). For example, the infusion of hypertonic NaCl into the brain ventricles of the goat decreases plasma renin activity, whereas dilution of Na^+ in the cerebrospinal fluid by the infusion of isotonic fructose increases plasma renin activity (Eriksson & Fyhrquist, 1976).

Many hormones, whose chief target organ is not the kidney, influence renal function. Due to their other effects it is often difficult to settle the mechanism of action. Besides, the

great differences of species in some hormonal responses further confuse the interpretation. See e.g. the reviews of Katz & Lindheimer, 1977 and Schrier & Berl, 1975.

Catecholamines highly influence the cardiovascular system and it is difficult to distinguish the direct renal effects from the circulatory ones. The response depends on the dose and the route of application. The role of catecholamines in the regulation of fluid balance is yet a subject of dispute. Noradrenaline given intravenously increases the renal water excretion either due to the suppression of vasopressin release or by inhibiting the action of vasopressin in the renal tubuli. A decrease in urine Na^+ excretion is often seen. Contrary to α-adrenergic stimulation by noradrenaline, the β-adrenergic stimulation has been found to induce antidiuresis.

Prostaglandins. Prostaglandins (of the series E and A) are found in the renal medulla. They may act as local modulators of the response to other hormones and alter the intrarenal hemodynamics. The prostaglandins (of the series E) cause diuresis and natriuresis in the kidney by inhibiting vasopressin action and dilating blood vessels.

Glucocorticoids. Glucocorticoid-deficient men and animals have an impaired ability to excrete a water load. The mechanism of this abnormality is still the subject of much debate. Possibly glucocorticoids are necessary for effective filtration in the glomeruli and for water impermeability of the collecting duct in the absence of vasopressin. In high doses, glucocorticoids also exert mineralocorticoid action, leading to salt and water retention.

Thyroid hormones are important for normal kidney function, obviously due to their calorigenic effect. In hypothyroid patients a delayed diuretic response to water load is frequently observed. The mechanism of this defect is not clear. Total exchangeable Na^+ is increased in myxedematous man, whereas e.g. a hypothyroid rat excretes Na^+ in excess.

Prolactin is important for fluid homeostasis in lower vertebrates. Prolactin appears to conserve both water and electrolytes also in mammals (e.g. Burstyn, 1978). However, there are conflicting reports which may partly depend on impurities in the hormone preparations. Prolactin obviously has no great physiological role in the electrolyte homeostasis of the normal

mammal, but may be partly responsible for the increase in body fluid in pregnancy.

Estrogens and testosterone have a slight Na^+-retaining effect, presumably through promoting Na^+ reabsorption by the renal tubuli.

Progesterone. There are considerable differences of species in the renal effect of progesterone. It is clearly natriuretic in the human. However, in many laboratory animals, its action is equivocal, mostly weakly antinatriuretic.

Parathyroid hormone has its primary function as a regulator of calcium and phosphorus metabolism. In kidneys it promotes renal excretion of phosphate. Some increase in Na^+ and K^+ excretion is simultaneously seen.

Calcitonin increases, not only the renal excretion of phosphate, calcium and magnesium, but also that of Na^+ and K^+.

The maintenance of a proper internal milieu and of an adequate blood volume is so essential for life that nature has secured it through a multiplicity of mechanisms. There is a highly complicated and, only partly clarified, interaction between these different factors.

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Peptide hormones in TRH and LPH stimulation tests

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TRH (thyrotropin releasing hormone) increases the secretion rate of thyroid stimulating hormone (TSH) from pituitary gland thyrotropic cells and it also has a stimulatory effect on prolactin secretion.

The primary sequence of TRH is:

pyroGlu-His-Pro-NH_2

The control of pituitary gonadotropin secretion by the hypothalamus has been found to be due to a decapeptide with the primary sequence:

pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

In addition to the LH-releasing ability it also stimulates the secretion of FSH.

These two peptides are used in experimental as well as clinical studies.

The use of ^{3}H-TRH has revealed that receptor affinity is specific for TRH. The biologically inactive TRH analogue pyroGlu-His-oMe and synthetic LRH do not compete for ^{3}H-TRH bound to its receptor.

In comparing the activity of LRH and LRH analogues it has been found that the arrangement of the amino acids at the carboxyl terminal appears to be decisive for recognition of LRH by its receptor amd that His-Trp is necessary for receptor activation.

There is no general agreement on the actual mechanism of action of hypothalamic adenohypophysiotropic hormones.

In our laboratory the localization of releasing hormone receptor was performed by immunoperoxidase staining and the concentration of releasing hormone in the receptor was determined by using the method of Härkönen et al.

For several years in clinical studies the TRH and LRH stimulation tests have been used for testing the hypothalamic-pituitary-gland axis. The tests are performed by the intravenous injection of TRH (200 ug) or LRH (100 ug). The response is determined

by comparing basal serum concentration of TSH and prolactin or FSH and LH and the values at 20 min and 60 min after the injection.

Determination of plasma peptide hormones is carried out by the method of Karonen et al., where a double antibody solid phase is used. LH, FSH and prolactin were iodinated by using solidified lactoperoxidase. The benefits are as follows: It allowed use of pH that best maintains the immunoreactivity of the peptides ; After iodination the enzyme was easily separated from the mixture by centrifuging down the solid.

The RIA was designed to give as large a range as possible and plasma dilution curves were shown to result in curves similar to the standard curves. Thus we were able to dilute our plasma samples with the assay buffer if values lay outside the chosen range.

Pituitary tropic hormone concentrations in the plasma after stimulation are illustrated as a function of time. Because of the log-normal distribution of the response curves the geometric means of increments are given as they are more meaningful than standard deviation.

TRH and LRH are rapidly excreted in the urine and plasma enzymes quickly inactivate the remaining releasing hormone. The dose-related secretion of pituitary hormones is manifested as a rapid rise and slow fall back to the basal level in one hour. Clinically important findings after stimulation are low or late responses when occuring together with low basal values of circulating hormones and point to a hypothalamic or pituitary disorder. A high basal values of prolactin with amenorrhea and galactorrhea indicates a prolactin secreting microadenoma. However, prolactin secretion is very sensitive to autonomic stimuli. The influence of stress must therefore be minimized and the collection of samples should happen after ten in the morning to eliminate sometimes high morning values.

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TABLE 1. RESPONSES DURING THE TRH AND LRH STIMULATION TESTS

	TIME	MEAN SD	MEAN OF INCREMENTS	GEOMETRIC MEAN OF INCREMENT
LH (MEN)	0′	17.2 ± 4.0	69.9 ± 34.7	60.0
U/L	20′	86.8 ± 36.6		
LH (WOMEN)	0′	23.9 ± 10.5	97.1 ± 61.9	77.0
U/L	20′	120.9 ± 62.1		
PROLACTIN	0′	50.1 ± 24.5	72 ± 55	58
µG/L	20′	127.2 ± 60.5		
TSH	0′	2.9 ± 1.2	12.8 ± 7.9	10
MU/L	20′	15.6 ± 7.9		

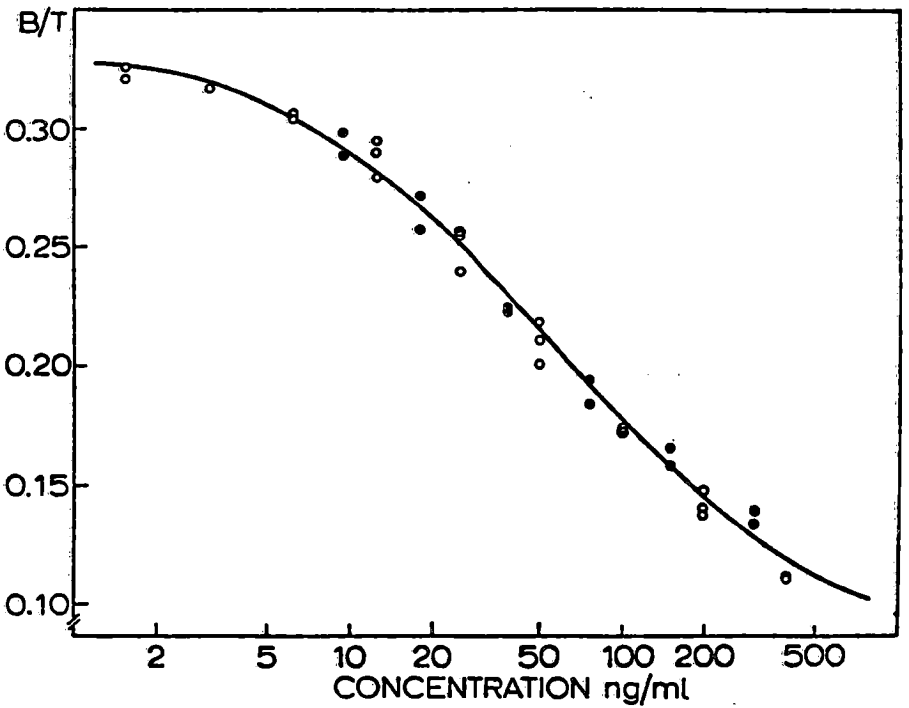

FIG. 1. PLASMA DILUTION CURVE WITH PLASMA FROM A GALACTORRHEA PATIENT (● — ●) AND THE CORRESPONDING DILUTION CURVES WITH PURIFIED HUMAN PROLACTIN (○ — ○)

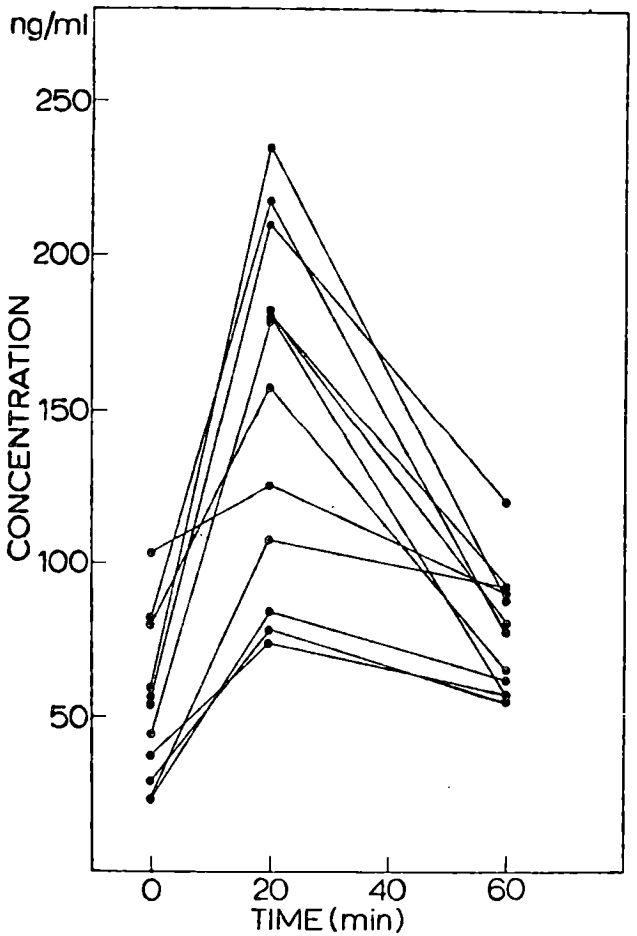

FIG. 3. PLASMA PROLACTIN RESPONSES DURING THE TRH-STIMULATION TEST CARRIED OUT IN NORMAL POLICLINICAL CONDITIONS

PROLACTIN IN BOVINE BLOOD, MILK AND URINE

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INTRODUCTION

Blood, milk and urine have been reported to contain prolactin (SCHAMS 1974, MÄKELÄ & KOSSILA 1976, MALVEN & McMURTRY 1974, MALVEN 1977, NADER et al. 1975). The Finnish participants in the inter-Scandinavian hormone project (NKJ-34) measured prolactin in these three biologic fluids of bovine before and after TRH stimulation. The purpose of the study was to investigate to what extent prolactin in milk and urine reflect that in blood.

Blood sampling is laborious, especially if hundreds or even thousands of animals are to be studied. Blood sampling may stress animals and stress, in turn, can increase the prolactin level in blood (RAUD et al. 1971, SCHAMS 1974). If the average prolactin level in blood could be predicted on the basis of the level in milk or urine, difficulties connected with blood sampling would be avoided.

MATERIALS AND METHODS

Test animals and samples

During a ten-day test period, in August 1977, the prolactin levels in blood, milk and urine from two non-pregnant Ayrshire cows (Appeli and Sipuli) were studied for seven days before and three days after TRH stimulation. The younger cow (Appeli) produced less milk (11.6 kg/day) than the older cow (Sipuli) (15.3 kg/day). Both cows were in the ninth month of lactation. The cows received the same feeds; a feeding level was adjusted according to the milk yield. Feeding was started daily at 7 a.m. and 1 p.m.

All the blood samples were taken via catheter from vena

jugularis. During the first seven test days the interval of the sampling was one hour. On the eight test day, when the TRH stimulation test was carried out, the sampling interval varied from 10 minutes to one hour. Blood samples were collected daily between 8 a.m. and 4 p.m. Heparinized plasma was used for prolactin determinations.

Two milk samples were taken daily in connection with the milkings carried out between 7 and 8 a.m. as well as 2 and 3 p.m. Whole milk was used for analyses.

Urine was collected totally and weighed twice a day: between 7 and 8 a.m. as well as 3 and 4 p.m. It was centrifuged before the analysis.

Prolactin determinations

All the samples were analyzed by the radioimmunoassay developed by MÄKELÄ & KOSSILA (1976). The dilution curves of the blood and milk samples were parallel to the standard curve. The dilution curve of the urine samples was not completely parallel to the standard curve. Recoveries of the bovine prolactin added into blood, milk and urine were 90, 110 and 50 %, respectively. Synthetic TRH (Thyrefact, Hoechst AG) did not cross-react with the antibodies against bovine prolactin.

TRH stimulation test

TRH, which is a thyrotropin-releasing hormone, is also known to release prolactin from the adenohypophysis into the blood circulation in bovine (SCHAMS 1974). In the TRH stimulation test the release of prolactin was stimulated by injecting about 1 µg of TRH/kg live weight into vena jugularis in both cows.

RESULTS

Prolactin levels before TRH stimulation

In blood

The average prolactin levels in the blood of the two cows were 14 and 23 ng/ml during seven days (8 samples/day). The older cow with a higher milk yield showed a higher prolactin level in

blood than the younger cow with a lower milk yield. Fig. 1 shows mean hourly variations in the blood prolactin level of the two cows during the first seven test days. Variations in both cows were marked hourly and daily. Prolactin levels tended to be low in the morning, gradually rising towards the afternoon. This is in agreement with the observations made by KOPROWSKI et al. (1972).

In milk

The average prolactin levels in the milk of the two cows were 3.4 and 4.3 ng/ml in the morning and 4.5 and 6.9 ng/ml in the evening, respectively. The cow with a higher blood prolactin also had a higher milk prolactin compared to the other cow. Evening milk contained more prolactin than morning milk, on the average. This rhythm is in agreement with the diurnal rhythm of prolactin observed in blood (Fig. 1). Total variation in an animal was smaller in milk than in blood. The prolactin content of evening milk secreted during daytime was compared with the average prolactin content of the blood samples collected during the same period. Prolactin content (ng/ml) in the evening milk was 30 % of that in the blood in both cows.

In urine

Prolactin or immunologically prolactin like activity (IPLA) was found in the urine. The average daily prolactin levels in urine were rather constant, about 1 ng/ml, during the ten day test period. Variations in the prolactin or IPLA level in the urine failed to reflect those in the blood. Furthermore, no difference could be observed between morning and evening urine. The prolactin levels in urine were about 5 % of those in blood.

Prolactin levels after TRH stimulation

In blood

TRH significantly increased the prolactin levels in the blood of the two cows studied (Fig. 3). Maximum levels (345 and 176 ng/ml) were found 20 minutes after TRH administration. Increases in the prolactin level after the TRH administration were about 70-fold in one cow and 10-fold in the other cow, compared to

the staring levels. The cow with a higher average prolactin level in blood had a smaller response to TRH stimulation than the other cow. Three hours after the TRH administration, the prolactin levels in blood returned to the initial level. The pattern of prolactin response to TRH was similar in both cows, but the extent of the response was different.

In milk

TRH adminstration also increased the prolactin level in both cows (Fig. 2). The increase was observed only in the milk obtained at subsequent milking (about five hours after TRH administration). Being due to TRH administration, the prolactin level in milk rose from 4.5 to 16.8 ng/ml in one cow and from 6.9 to 14 ng/ml in the other cow. The cow with a greater response in blood had a greater response in milk as well.

In urine

TRH administration failed to affect the prolactin or IPLA level in urine.

DISCUSSION AND CONCLUSIONS

A comparison of the prolactin levels in blood, milk and urine

A comparison of the prolactin levels in the bovine blood, milk and urine are summarized in Table 1. The radioimmunoassay used for the prolactin determinations was sensitive and specific for blood and milk, but not very reliable for urine. Furthermore, it is questionable, whether the immunologically prolactin like substance in urine was prolactin, its metabolic fragment or something else (GALA et al. 1975). The relative concentrations of prolactin in blood, milk and urine were 100, 30 and 5 %, respectively. Variations in the prolactin level between animals could be found in blood and milk, but not in urine. Variations in the prolactin level in an animal were great in blood. This observation is an additional reason to study the suitability of other biologic samples for predicting the average prolactin level in blood. Variations in the prolactin level in an animal were moderate in milk and small in urine. Prolactin levels varied diurnally rhythmically in blood and milk, but not in urine.

Diurnal variations in blood and milk were parallel. Prolactin response to TRH was significant in blood and slight in milk, but none was observed in urine.

Suitability of the milk and urine prolactin level for predicting an average prolactin level in blood

Prolactin levels in milk reflected prolactin levels in blood before and after TRH stimulation in the two cows studied. It seems possible that an average prolactin level in blood could be predicted on the basis of the prolactin level in milk. The milk prolactin level was 30 % of the blood prolactin level in the two cows in the late stage of lactation. Before an average prolactin level in blood could be reliably predicted on the basis of the prolactin level in milk, the relation between blood and milk prolactin levels in the cows in various stages of lactation as well as in various cows in the same stage of lactation had to be established.

The prolactin levels in urine were not related to the prolactin levels in blood in the two cows studied. Thus, urine samples seem to be unsuitable for predicting the average blood prolactin level in non-pregnant cows in the late stage of lactation.

SUMMARY

Prolactin levels in blood, milk and urine were measured by the radioimmunoassay developed by the authors from two non-pregnant Ayrshire cows in the 9th month of lactation. The levels were measured before and after TRH stimulation test during a ten-day test period. The purpose was to study to what extent the prolactin level in milk and urine reflects that in blood. Blood and milk prolactin could be assayed reliably, but not urine prolactin. The relative concentrations in blood, milk and urine were 100, 30 and 5 %, respectively. Differences in the prolactin level between animals could be observed in blood and milk prolactin, but not in urine. Variations in an animal were great in blood, moderate in milk and small in urine. Diurnally rhythmic variations could be observed in blood and milk prolactin, but not in urine prolactin. Rhythmic variations in milk were parallel to those in blood. Prolactin response to TRH was significant in blood and

slight in milk, but not observed in urine. The degree of response in milk was related to that in blood.

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Table 1. Comparisons of relative prolactin concentrations of the cow's blood, milk and urine.

	Prolactin concentration		
	Blood	Milk	Urine
Reliability of RIA	Good	Good	Poor
Relative concentrations	100 %	30 %	5 %
Variation between animals	Observed	Observed	None
Variation in an animal	Great	Moderate	Small
Diurnal variation	Rhytmic	Rhytmic	None
Response to TRH	Significant	Slight	None

FIGURE 1. DIURNAL VARIATION, AS AN AVERAGE OF SEVEN DAYS, IN BLOOD PROLACTIN OF TWO LACIATING COWS

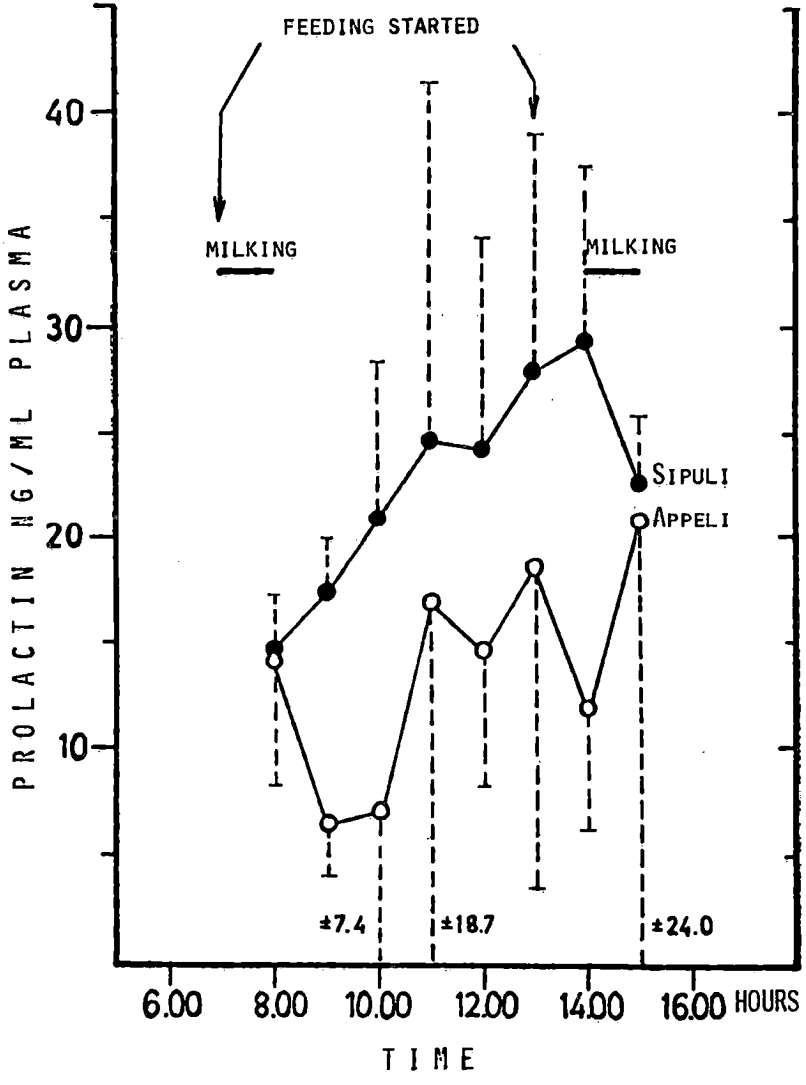


FIGURE 2. PROLACTIN LEVELS IN MORNING AND EVENING MILK OF TWO LACTATING COWS BEFORE AND AFTER TRH-STIMULATION

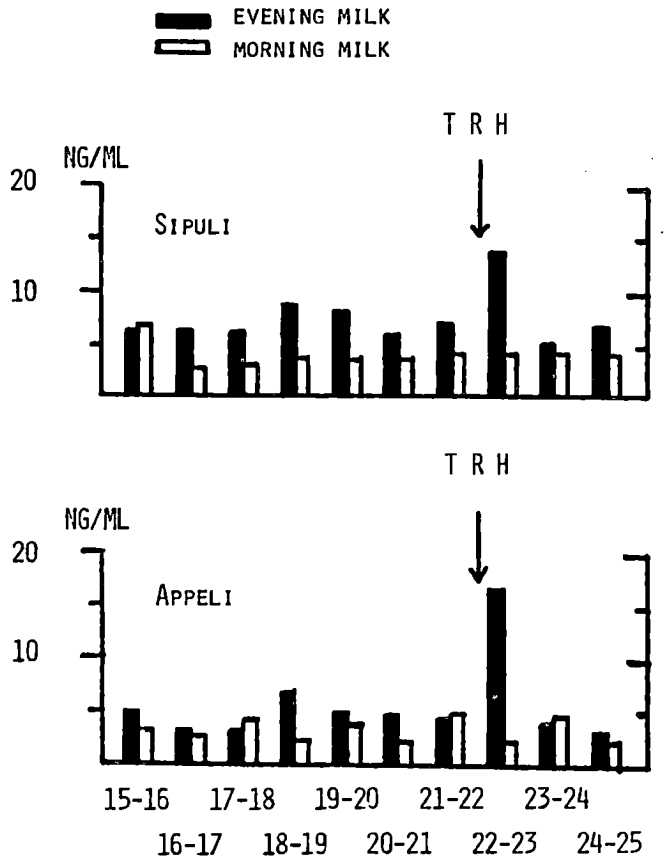
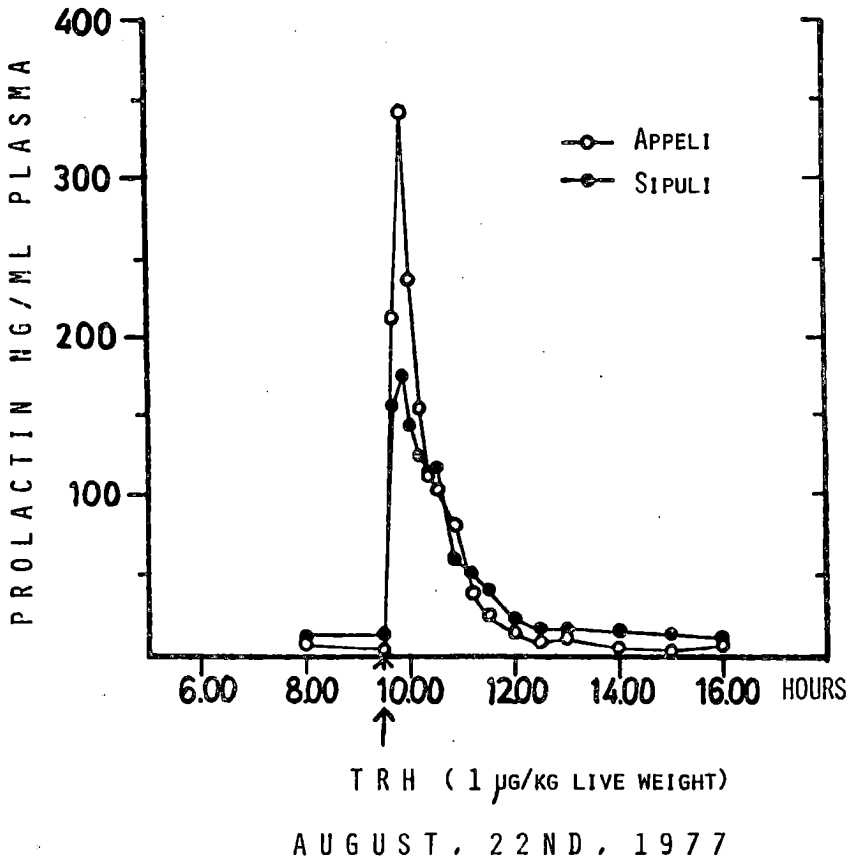


FIGURE 3. PROLACTIN RESPONSE TO TRH IN BLOOD OF TWO LACTATING COWS



CORRELATION OF PLASMA PROLACTIN LEVEL TO PLASMA ENZYMES AND OTHER PLASMA PARAMETERS IN FINNISH ARTIFICIAL INSEMINATION BULLS

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Some plasma hormone and enzyme levels can be used as indicators of either hormone secretion rates or enzymatic activities in the animal body. Since hormone and enzymes play important role in physiological processes essential from the point of view of animal production, it might be worthwile to study these factors in breeding animal.

Material

Blood samples were collected altogether from 168 artificial insemination bulls (AI-bulls) 16 Nov. 1977 at Salpausselkä (n=60), 7 Dec. 1977 Pirkkala (n=48) and 23 Jan. 1978 Rauhalinna (n=60) AI-stations.

Methods

Prolactin was determined using RIA-method of Mäkelä and Kossila (1976, 1977). Enzyme activities of alkaline phosphatase (AP), aspartateaminotransferase (ASAT), alanineaminotransferase (ALAT), creatinephosphokinase (CK), gamma-glutamyltranspeptidase (gamma-GT) and lactatedehydrogenase (LDH) in blood plasma were determined using Gilford-3500 computer directed analyzer at +37^{o}C according to recommendations of the Scandinavian Committee on Enzymes. Also blood urea nitrogen (BUN),plasma bilirubin, cholesterol, creatinine and total protein were estimated with the same apparatus. Correlations were calculated at the Computing Service Unit of Agricultural Research Centre.

Results and discussion

Average live weights, age and blood values of AI-bulls are given in Table 1.

Prolactin concentrations show great variations in all the blood samples taken from bulls at different AI-stations. There were some animals with high and some with very low prolactin level.

Pirkkala bulls were youngest and they had highest prolactin gamma-GT and plasma urea nitrogen levels but lowest ALAT, ASAT, CK, LDH and creatinine levels on the average.

Rauhalinna bulls had highest AP while Salpausselkä bulls had highest CK, creatinine and total protein, respectively.

Simple correlations between prolactin and serum enzymes are given in Table 2. gamma-GT and CK correlated somewhat with prolactin levels.

Figures in Table 3 show that prolactin correlated positively with plasma urea nitrogen ($P < 0.05$) and creatinine.

Conclusions

Although the prolactin studies in the bulls are preliminary, there seems to be some correlations between plasma prolactin levels and gamma-glutamyltranspeptidase and to some extent creatinephosphokinase activities, too. Similarly the correlation of prolactin and plasma BUN and creatinine can be noticed.

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Table 1. Average live weight, age, prolactin, some plasma enzymes and other constituents of AI-bulls at three different bull stations.

	S-77	P-77	R-78
	$\bar{X}\pm SD$	$\bar{X}\pm SD$	$\bar{X}\pm SD$
LIVE WT, (kg)	454±28.2	466±36.8	467±31.5
AGE, (months)	28.1±7.8	25.9±10.4	29.2±6.4
PROLACTIN (ng/ml)	11.2±13.5	29.0±29.0	11.1±20.3
AP (U/l)	174.3±66.8	193.5±95.1	214.6±74.7
ALAT "	32.1±5.5	29.7±7.2	32.3±9.5
ASAT "	67.4±20.6	58.9±25.2	66.5±10.7
CK "	126.7±46.8	72.7±24.8	121.7±39.2
gamma-GT (U/l)	23.8±11.3	28.2±9.7	21.6±4.8
LDH "	3108±526	2372±582	3187±943
BUN (mmol/l)	3.14±0.62	4.33±0.84	3.38±0.42
BILIR. (µmol/l)	-	8.6±2.3	8.5±2.2
CHOL. (mmol/l)	4.3±1.0	-	3.7±0.7
CREAT. (µmol/l)	153.9±22.3	143.0±28.9	146.1±21.9
TOT.PROT. (g/l)	84.5±7.2	78.2±6.9	76.4±4.1

S-77 = Salpausselkä Bull Station 1977 (n=60)
P-77 = Pirkkala 1977 (n=48)
R-78 = Rauhalinna 1978 (n=60)

Table 2. Correlation of prolactin to some enzyme activities in plasma of AI-bulls

	Prolactin 1)				
Enzyme	I	II	III	IV	V
Alkaline phosphatase	-.05	-0.5	-.20^{x}	-.04	-.19
Aspartateaminotranferase	-.04	-.04	+.08	-.04	+.19
Alanineaminotranferase	-.06	-.05	-.02	-.06	-.03
Creatinephosphokinase	-.15	-.16	+.12	-.09	+.38^{xx}
gamma-Glutamyltranspeptidase	+.24^{xx}	+.24^{x}	+.09	+.30^{xx}	-.02
Lactatedehydrogenase		-.10			

1) I = S-77 + P-77 + R-78 (n=168)
II = S-77 + P-77 (n=108)
II = S-77 + R-78 (n=120)
IV = P-77 + R-78 (n=108)
V = R-78 (n=60)

x = P< 0.05

xx = P< 0.01

xxx= P< 0.001

Table 3. Correlation of prolactin with some plasma parameters of AI-bulls

	Prolactin 1)				
Parameter	I	II	III	IV	V
Plasma urea nitrogen	+.30^{xxx}	+.30^{xx}	+.14	+.28^{xx}	+.14
Bilirubin				+.07	+.17
Cholesterol			-.01		-.01
Creatinine	+.03	+.02	+.31^{xxx}	-.04	+.24
Total protein	+.07	+.05	+.08	+.20^{x}	+.05

1) See the footnotes of table 2

LH AND TESTOSTERONE, REPEATABILITY AND VARIATION IN THREE PAIRS OF MONOZYGOUS GROWING BULLS

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INTRODUCTION

It is well established, that peripheral blood plasma levels of testosterone and LH in the bull exhibit a pronounced daily variation. Thus an accurate description of the concentration of these two hormones can only be obtained if a blood sampling schedule involving frequent sample collection is applied. The administration of Gn-RH (gonadotropin-releasing hormone) results in a pronounced discharge of LH from the pituitary gland, and this hormone stimulates the gonads to an increased testosterone output. During the immediate hours following such a stimulation with Gn-RH the daily variation in the LH and testosterone levels are abolished. By administering a relatively high dose of Gn-RH, a maximum LH discharge is likely to occur, followed by a maximal testosterone output.

Testosterone is known to play an important role in the behavioral aspect of reproduction, in development and maintenance of the male secondary sex characters and in maintenance and regulation of testicular function. Testosterone also excerts a well established anabolic action. Theoretically it might be possible to correlate differences in e.g. growth rate, food conversion and reproductive performance among individual bulls to the levels of LH and testosterone. This possibility will only exist if the hormonal patterns or response to stimulation show a high repeatability.

The aim of the present study was to repeatedly investigate the hormonal levels of LH and testosterone before and after stimulation with Gn-RH in three pairs of monozygous twin bulls from about 4 to 18 months of age.

MATERIALS AND METHODS

Three pairs of monozygous twin bull calves of the Swedish Red and White Breed (SRB) were used. The animals were housed together in the same stable during the whole experimental period and were fed according to conventional feeding standards. The age of the animals varied between 4 and 5.5 months when entering the experiment.

Blood samples for hormone analyses were obtained according to the following sampling schedule:

i. 5-10 blood samples were obtained at intervals of 1-1.5 hours the day before injection of Gn-RH

ii. 2 mg Gn-RH (Novo A/S, Copenhagen, Denmark) was administered intravenously. Blood samples were obtained every 15 min. for the first 90 min. and thereafter every 30 or 60 min. during the following 6 hours (totally 14 samples)

iii. for one or two days after the administration of Gn-RH 5-10 blood samples per day were obtained at intervals of 1-1.5 hours.

Each of the pair of bulls was submitted to at least 5 of the above described tests carried out at intervals of 2-3 months.

The blood samples were obtained through puncture of the jugular vein and collected into heparinized vacutainer tubes (Becton-Dickinson, Stockholm, Sweden). Immediately upon collection the blood samples were centrifuged and the blood plasma was collected and stored at -18^{o}C until assayed.

Blood plasma levels of LH were determined by radioimmunoassay. This assay system utilized an antisera to ovine LH (Kindly donated by Dr. G.D. Niswender, Colorado State University, Fort Collins, USA). Bovine LH (NIH LER 1716-2) was used for iodination which was achieved utilizing the chloramine-T procedure. The standard preparation constituted of bovine LH (NIH LH B-9; the authors are indebted to NIH for supplying the LH used for standard and for iodination). Separation was done by utilizing a second antibody to rabbit gamma-globulin coupled to a solid phase (DASP® Organon, The Netherlands).

Testosterone was determined by radioimmunoassay. The antiserum was raised in sheep immunized with testosterone-3-(O-carboxymethyl)oxime-bovine-serum albumin. The assay procedure used has been described previously (Sanwal et al., 1974). The antiserum showed significant crossreaction with 5α-dihydro-testosterone (61%) and with androstenedione (5%).

All hormone measurements were carried out in duplicate, and the means of the duplicate determinations have been used in the statistical evaluation of the results. The within assay precision is given in Table 1.

Statistical methods

The effects of twin pair and age were estimated using the Statistical Analysis System (Barr et al., 1976). To describe the data the following models were used:

Model I (all observations per day of testing were used in the calculations)

$$y_{ijkl} = \mu + t_i + a_{ij} + c_k + e_{ijkl}$$

where

y_{ijkl} = the $ijkl$th observation

μ = general mean

t_i = effect of the ith twin pair (i=1,2,3)

a_{ij} = effect of the jth animal within the ith twin pair (j=1,2)

c_k = effect of the kth age class (k=1,2,...6)

$(tc)_{ik}$ = effect of the interaction between the ith twin pair and the kth age class

$(ac)_{ijk}$ = effect of the interaction between the ijth animal and the kth age class

e_{ijkl} = residual term with variance σ_e^2

The effect of animal was regarded as random and the others as fixed. The various ages were divided into age classes as described in Table 2.

Model II (one observation per day of testing was used in the calculations)

$$y_{ijk} = \mu + t_i + a_{ij} + b_1 x_{ijk} + b_2 x_{ijk}^2 + e_{ijk}$$

where the elements have the same meaning as before and in addition:

b_1, b_2 = linear and quadratic regressions on age of testing

x_{ijk} = age of testing of the ijkth individual

The repeatabilities of the LH and testosterone concentrations were estimated as the intraclass correlation (t) estimated from the components of variance from analysis with both Model I and II. When the repeatabilities were estimated, both the effects of twin pair and animal were regarded as random.

$$t = \sigma_t^2 + \sigma_a^2 \,/\, \sigma_t^2 + \sigma_a^2 + \sigma_e^2$$

where

σ_t^2 = component of variance for twin pair

σ_a^2 = component of variance for animal

σ_e^2 = residual variance

Standard errors for the intraclass correlations were calculated with the approximative formula described by Falconer (1960).

RESULTS

Unstimulated hormone levels

Overall means, standard deviations and range for LH and testosterone for the various age classes without and with Gn-RH stimulation are presented in Table 2. Unstimulated testosterone levels in age group 1 varied between 0.66 and 12.14 nmol/l with a mean of 3.10 nmol/l. A considerable variation in testosterone levels existed between the pairs of bulls in age group 1. Two of the pairs had their daily variation of plasma testosterone levels investigated at an age of 133 and 137 days of age. None of these animals showed any pronounced variation in the testosterone level.(range 0.90 to 3.50 nmol/l). The last pair in age group 1 was tested at an age of 157 days. Both of these bulls showed a pronounced variation in the testosterone level with concentrations varying from 0.69 to 12.14 nmol/l. With advancing age peaks of greater magnitude started to occur. In age class 2 all of the animals showed pronounced daily fluctuations in the peripheral testosterone concentration resulting in increasing mean values (Table 2; Fig. 1). Irrespective of age of the bull the between peak testosterone concentration returned to the same low level of about 2 nmol/l or less. As can be seen from Fig. 1 the rise of the mean testosterone level was not linear. A common feature in all bulls was a relatively fast increase in testosterone concentration between 6 and 7.5 months of age. Thereafter the level remained relatively unchanged or in some cases even decreased, with the mean testosterone level for age class 3 being lower than for age classes 2 and 4 (Table 2; Fig. 1). From 9.5 months of age and onward the mean testosterone level increased continously in most bulls.

In contrast to testosterone concentrations, marked fluctuations in unstimulated plasma LH level was observed in the bulls of age class 1 (Table 2; Fig. 1). Age class 2 had a lower mean value and a narrower range for LH as compared to age class 1. Thus when the testosterone concentration started to rise at about 5.5 months of age the corresponding LH levels dropped. After 7.5 months of age further decrease in the LH concentration

was slow. As for testosterone individual variations in the pattern of plasma LH with time was observed. However, the above given general pattern was quite consistent in most of the animals (Table 2; Fig. 1).

Peak distribution of unstimulated LH and testosterone concentrations during the day was not constant either between or within bulls. However, when the values for the six bulls in all age classes and at different times of the day were computed together a pattern emerged. Mean plasma levels of LH was found to be lowest in the morning and around 14.00 h, with an elevated concentration between. Testosterone levels followed those of LH with a time lag of about 1 hour (Fig. 2).

Statistical analysis according to Model I (all observations) showed that the twin pairs did not differ significantly in LH or testosterone concentrations (Table 3). When only one value per day was used in the calculations (Model II) the pairs of bulls differed significantly in maximal testosterone and in maximal and mean LH levels (Table 4). Using this statistical model the effect of animal was non-significant. The linear regression on age for unstimulated mean and maximum values was significant for both LH and testosterone but with opposite signs (Table 4).

The repeatability calculated from Model I was found to be low for both hormones. When Model II was used, the repeatability was higher than with Model I, but still relatively low (Table 5).

The partial correlation between LH and testosterone (estimated from Model I using all observations) was low but significant ($r=0.10$; $P<0.05$). Low and non-significant relationships between LH and testosterone were found when the mean and maximum values per testing day were used (Table 6). The partial correlation between the mean and maximum values for LH as well as for testosterone, was high (Table 6).

Stimulated hormone levels

The administration of Gn-RH resulted in a pronounced release of LH in all age classes (Table 2). Already 15 min. after injection of the releasing hormone LH concentrations were markedly elevated (Fig. 3). After around 45 min. an LH peak occurred which was followed by decreased concentrations and thereafter at around 2 hours a second peak was found. Thereafter the LH levels decreased continously and pretreatment base-line levels were reached about 6 hours after injection. The plasma concentration of LH measured after stimulation with Gn-RH was more than 20 times higher than the non-stimulated LH levels (Table 2). The LH response differed significantly between age classes (Table 3) but showed no tendency to increase or decrease with age. Thus statistical analysis using Model II revealed that both the linear and the quadratic regressions of LH on time were non-significant for the variables studied (Table 4).

The two pair of bulls in age class 1 which showed no daily variation in their unstimulated testosterone levels did not respond with increased testosterone levels after Gn-RH administration performed at an age of 134 and 144 days. In all other cases testosterone levels started to increase 30 min. after the injection of Gn-RH (Fig. 3). This increase continued for about 1 hour after which the level reached a maximum which was maintained for about 3-4 hours (Fig. 3). Thereafter the level decreased continuously and reached pretreatment levels around 7 hours after the injection of releasing hormone. When comparing the pretreatment testosterone pattern with the pattern obtained the day after the stimulation with Gn-RH no residual effects of the treatment could be detected.

The maximum testosterone concentration obtained after stimulation was highly correlated to the maximum level found in the unstimulated animal the day before or after injection of Gn-RH ($r=0.84$; $P \leq 0.001$).

The mean peripheral plasma level of testosterone after stimulation increased continously with age (Table 2; Fig. 4). When all testosterone measurements after stimulation were used in the statistical analyses (Model I) twin pairs did not differ significantly while animals within pairs showed a significant difference ($P \leq 0.001$; Table 3). Calculations according to Model II (Table 4) revealed significant differences only for the maximal testosterone levels between pairs ($P \leq 0.05$). Both the linear and quadratic regression on age was significant for mean and maximum testosterone levels as well as for the area under the testosterone response curve after Gn-RH stimulation (Table 4).

The area under the LH and testosterone curves after stimulation were determined in all bulls at different ages and was found to be correlated to the corresponding maximum and mean values (Table 6). The partial correlations between the variables studied were high for LH as well as for testosterone ($r=0.80-0.95$; $P \leq 0.001$). No significant correlations were found between LH and testosterone variables after stimulation (Table 6).

Repeatabilities for LH and testosterone were determined after Gn-RH stimulation. For both hormones a substantial increase in the estimated values were obtained in comparison with repeatability estimates from unstimulated animals (Table 5).

Although differences in weight gain between pairs were small, they were statistically significant ($P \leq 0.001$). The difference between animals were, however, not significant ($P > 0.05$) and the twin bulls had similar growth curves. After correction for age no significant partial correlations were found between LH and weight gain, or between testosterone and weight gain.

DISCUSSION

The changes in the unstimulated LH pattern found here with high levels in the 4-5.5 month old bull calves followed by lower concentrations, agrees favourably with data presented by Lacroix et al. (1977). Other investigations have revealed

either no change of the level (Odell et al. 1970; Karg et al., 1976) or an increase of the LH concentration after about 6 months of age (McMillan & Hafs, 1968; Rawlings et al., 1972; Gombe et al., 1973; Moss & Moody, 1974). A similar release pattern of LH as described here for the growing bull has also been reported to occur in the prepubertal ram (Courot et al., 1975). Since LH is released in a pulsatile manner both in adult (Katongole et al., 1971) and young bulls (Gombe et al., 1973) a sampling schedule allowing frequent blood sampling is an absolute prerequisite for an adequate description of the release pattern of the hormone. The above mentioned differences in opinion concerning the LH pattern in the growing bull is probably due mainly to differences in sampling intensity.

Less differences of opinion are existing on the pattern of testosterone in young bulls. Thus, in agreement with data presented here, plasma levels of testosterone have been reported to increase in bulls during their first year of life (Rawlings, 1972; Karg et al., 1976; Secchiari et al., 1976; Lacroix et al., 1977). A stepwise increment of the testosterone level as desribed in the present paper was reported by Karg et al. (1976) and Lacroix et al. (1977).

Short term variations in the blood plasma concentrations of LH and testosterone have been reported previously (Katongole et al., 1971; Sanwal et al., 1974; Thibier, 1976*a*; Karg et al., 1976; Haynes et al., 1977; Lacroix, 1977; Sundby & Tollman, 1978). The same general pattern with about two peaks of testosterone during the day is found by most authors. Some variation exists, however, on the time of occurrence of the peaks. This discrepancys might be due to differences in environmental factors such as light, season of the year, feeding regimens etc.

The LH response after stimulation with Gn-RH has in most cases been reported to be dependent on the dose level administered, age of the bull, and the immediate pre-experimental endocrine situation (Golter et al., 1973; Schams et al., 1974; Dermody et al., 1976; Thibier, 1976*b*; for a review see Pelletier, 1976).

Schams et al. (1974) found that Gn-RH dosages varying between 0.05 and 1.5 mg caused a dose dependent linear increase in LH. Dermody et al. (1976) tested dosages from 0.1 to 10 mg per animal and reported the logarithm of the serum LH response to be linearly related to intravenous dosages of Gn-RH from 0.1 to 2.5 mg while the same maximum serum LH concentrations were observed following doses of 2.5 to 10 mg. The dose used here is high when considering the weight of the animal. The LH levels measured after stimulation in the present study are somewhat higher or similar to those reported by Dermody et al. (1976) when administering Gn-RH doses ranging from 2.5 to 10 mg per animal. It is thus reasonable to assume that the dose of releasing hormone used in this study provoked a maximum LH discharge from the pituitary.

The LH pattern after the injection of Gn-RH to bulls of 2, 4 and 6 months of age have been investigated by Mongkonpunya et al. (1975). These authors found the magnitude and duration of the increase of serum LH to be similar at all ages of bulls and at all doses of Gn-RH administered (0.2, 0.4 and 0.8 mg/animal), except that the duration of the LH response was prolonged when administering the highest dose of Gn-RH. This finding is in agreement with the result of the present study where the LH pattern obtained after the stimulation with Gn-RH, although being significantly different between age classes showed no tendency to increase or decrease with age and thus remained roughly the same throughout the experimental period.

The massive LH discharge induced through the administration of Gn-RH increased the testosterone production and abolished its pronounced daily variation. However, the two pair of bulls in age class 1 which showed no daily variation of unstimulated testosterone levels did not respond with elevated testosterone levels after the Gn-RH stimulation. Similar results were obtained by Mongkonpunya et al. (1975) who reported serum testosterone to be increased about threefold in response to increased LH release after Gn-RH in 6-month-old bulls but not in 2- or 4-month-old bulls. This lack of response in the young

bulls might be due to immaturity of the testicles in such a way that e.g. sufficient amounts of receptors for LH are not present. In relation to the high levels of LH seen in young unstimulated bulls it is tempting to assume that these LH levels have a programming function in inducing LH receptors in the target tissue. When a sufficient amount of receptors are present, the testicular tissue is able to respond with a testosterone production in response to the LH stimulus. In the adult bull the sensitivity of the testis for LH seems to be much higher than in the young animal, as in the adult individual relatively small elevations in LH concentrations result in marked changes of the testosterone level.

The significant difference between animals found after stimulation for both LH and testosterone (using Model I) indicated that the twins had a somewhat different response curve. When only the mean, maximum or area values were used, the between animal variation was non-significant. From this small material, no conclusions can be drawn about the magnitude of the genetic influence on the hormonal levels.

The daily variation in the concentration of the two hormones studied makes it difficult to calculate e.g. mean and maximum levels unless a very frequent bleeding schedule is employed. This is further supported by the low repeatability of 0.011 and 0.014 for unstimulated LH and testosterone levels, respectively. After Gn-RH stimulation, however, relatively high repeatability estimates were found, especially when calculated for the mean, maximum and area values (Model II). The correlation between the different measurements (mean, maximum, area) were high for LH as well as for testosterone. It can be concluded that the most convenient measurement can be chosen as an estimate of the hormonal levels after stimulation.

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Table 1. *Precision of the testosterone and LH measurements*

Hormone	Range	No of duplicate determination	Mean ($\bar{x}$)	$\sqrt{D}$ [a]	$\frac{\sqrt{D} \cdot 100}{\bar{x}}$ (C.V.,%)
Testosterone, nmol/l	0.0- 3.9	476	2.19	0.33	15.1
	4.0- 9.9	295	6.58	0.71	10.8
	10.0-19.9	195	14.34	1.49	10.4
	20.0-29.9	123	24.37	1.85	7.6
	>30.0	74	34.36	1.86	5.4
LH ng/ml	0.0- 0.9	450	0.41	0.23	54.7
	1.0- 4.9	315	2.07	0.29	14.0
	5.0-19.9	86	11.07	0.88	7.9
	20.0-39.9	121	31.96	3.26	10.2
	40.0-59.9	121	47.62	4.57	9.5
	>60.0	82	82.33	14.92	18.1

[a] $D = \Sigma d^2/2n$, where

d = difference between duplicate measurements

n = number of duplicate determinations

Table 2. *Overall mean, standard deviations (S.D.) and range of LH and testosterone for the various ages without and with Gn-RH stimulation*

Treatment and age, class (month)	LH, ng/ml			Testosterone, nmol/l		
	Mean	S.D.	Range	Mean	S.D.	Range
Without Gn-RH						
1 (4-5.5)	1.85	1.87	0.00- 14.00	3.10	2.04	0.66-12.14
2 (6-7.5)	0.98	1.08	0.00- 5.25	5.52	5.64	0.19-29.64
3 (8-9.5)	1.01	0.75	0.00- 4.30	4.83	4.19	0.56-24.66
4 (10.5-12)	0.85	0.92	0.00- 5.90	6.92	8.11	0.44-36.04
5 (13-14.5)	0.39	0.52	0.00- 2.65	7.03	5.97	0.57-27.50
6 (15.5-18)	0.64	0.61	0.00- 3.20	9.32	8.14	1.04-35.09
With Gn-RH						
1 (4-5.5)	39.19	31.02	0.00-157.00	4.72	3.43	0.64-14.41
2 (6-7.5)	29.41	19.83	0.30- 74.5	11.38	6.86	1.31-27.69
3 (8-9.5)	51.64	40.04	1.50-176.50	12.29	6.87	0.57-26.65
4 (10.5-12)	37.82	26.77	0.80-117.00	20.60	11.77	1.80-49.35
5 (13-14.5)	31.85	17.54	1.60- 71.50	21.36	9.34	2.09-38.88
6 (15.5-18)	44.82	22.29	6.65- 98.00	25.53	8.86	3.32-38.88

Table 3. *Levels of significance for the effects studied without (1) and with (2) Gn-RH stimulation (Model I)*

Hormone and treatment	Level of significance				
	Twin pair	Animal	Age class	Twin pair x age class	Animal x age class
LH					
(1)	n.s.	n.s.	***	***	n.s.
(2)	n.s.	*	***	n.s.	*
Testosterone					
(1)	n.s.	n.s.	***	***	n.s.
(2)	n.s.	***	***	***	n.s.

Levels of significance: n.s. = not significance ($P>0.05$);
* = $P\leq 0.05$; ** = $P\leq 0.01$; *** = $P\leq 0.001$

Table 4. *Levels of significance for the effects studied without (1) and with (2) Gn-RH stimulation (Model II)*

Hormone and treatment	Level of significance				b-value	
	Twin pair	Animal	Regression			
			Linear	Quadratic	Linear	Quadratic
LH, (1)						
mean	*	n.s.	***	n.s.	-0.0034	
maximum	*	n.s.	**	n.s.	-0.0077	
LH, (2)						
mean	n.s.	n.s.	n.s.	n.s.		
maximum	*	n.s.	n.s.	n.s.		
area	n.s.	n.s.	n.s.	n.s.		
Testosterone, (1)						
mean	n.s.	n.s.	***	n.s.	0.0160	
maximum	*	n.s.	***	n.s.	0.0454	
Testosterone, (2)						
mean	n.s.	n.s.	***	**	0.1376	-0.0001
maximum	*	n.s.	***	***	0.2338	-0.0002
area	n.s.	n.s.	***	*	0.0062	-0.0000

Levels of significance: n.s. = not significant ($P>0.05$);
* = $P\leq 0.05$; ** = $P\leq 0.01$; *** = $P\leq 0.001$

Table 5. *Repeatabilities estimated as intraclass correlations (±standard errors) for LH and testosterone*

	Without Gn-RH stimulation		With Gn-RH stimulation	
	LH	Testosterone	LH	Testosterone
Model I	0.011±0.013	0.014±0.015	0.033±0.028	0.204±0.109
Model II				
mean	0.105±0.097	0.186±0.129	0.388±0.216	0.602±0.189
maximum	0.067±0.080	0.264±0.151	0.379±0.216	0.618±0.185
area	-	-	0.272±0.209	0.536±0.203

Table 6. *Partial correlations between LH and testosterone estimated from Model II. Correlations without Gn-RH stimulation above the diagonal and with Gn-RH below*

	LH			Testosterone	
	mean	max	area	mean	max
LH					
mean	-	0.81 ***	-	-0.08 n.s.	-0.12 n.s.
maximum	0.94 ***	-	-	0.03 n.s.	0.04 n.s.
area	0.95 ***	0.87 ***	-	-	-
Testo-sterone					
mean	-0.18 n.s.	-0.19 n.s.	-0.14 n.s.	-	0.86 ***
maximum	-0.13 n.s.	-0.08 n.s.	-0.12 n.s.	0.88 ***	-
area	-0.22 n.s.	-0.24 n.s.	-0.13 n.s.	0.94 ***	0.80 ***

Levels of significance: n.s. = not significant ($P>0.05$);

* = $P\leq 0.05$; ** = $P\leq 0.01$; *** = $P\leq 0.001$

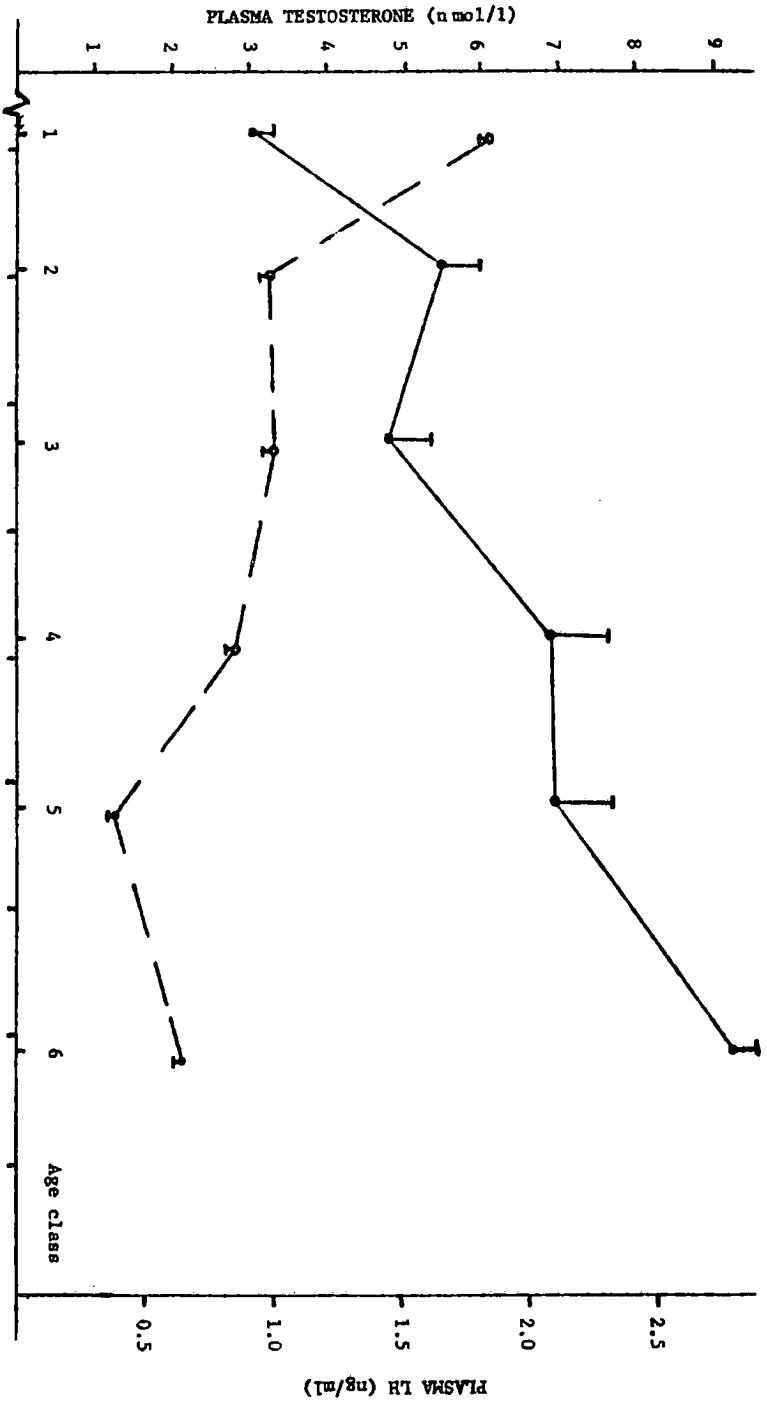

Fig. 1. LH (o——o) and testosterone (●——●) in the various ages (age class or days). Mean±S.E. for the six bulls.

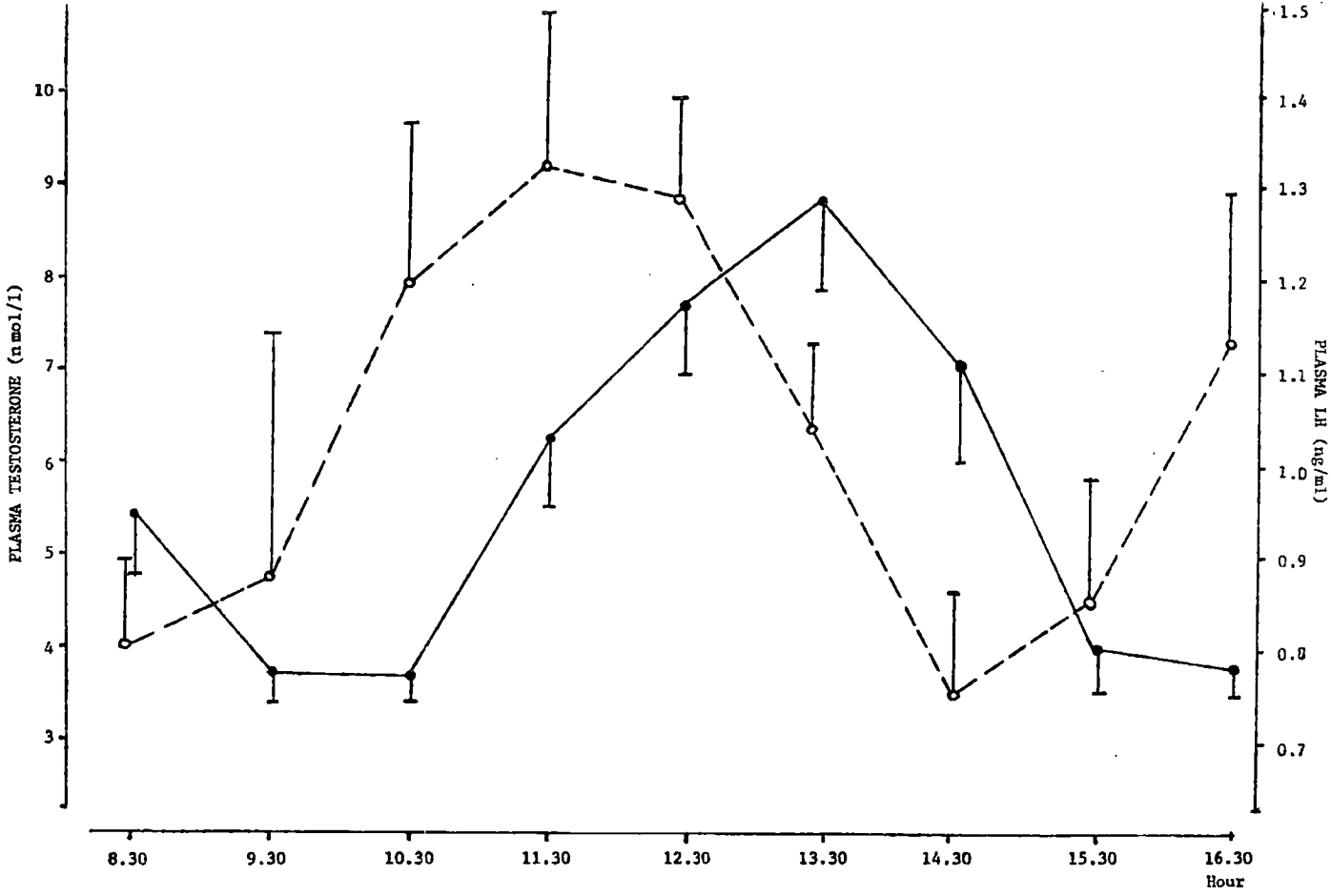

Fig. 2. LH (o----o) and testosterone (●——●) levels during the day (mean±S.E.) for the six bulls in all ages.

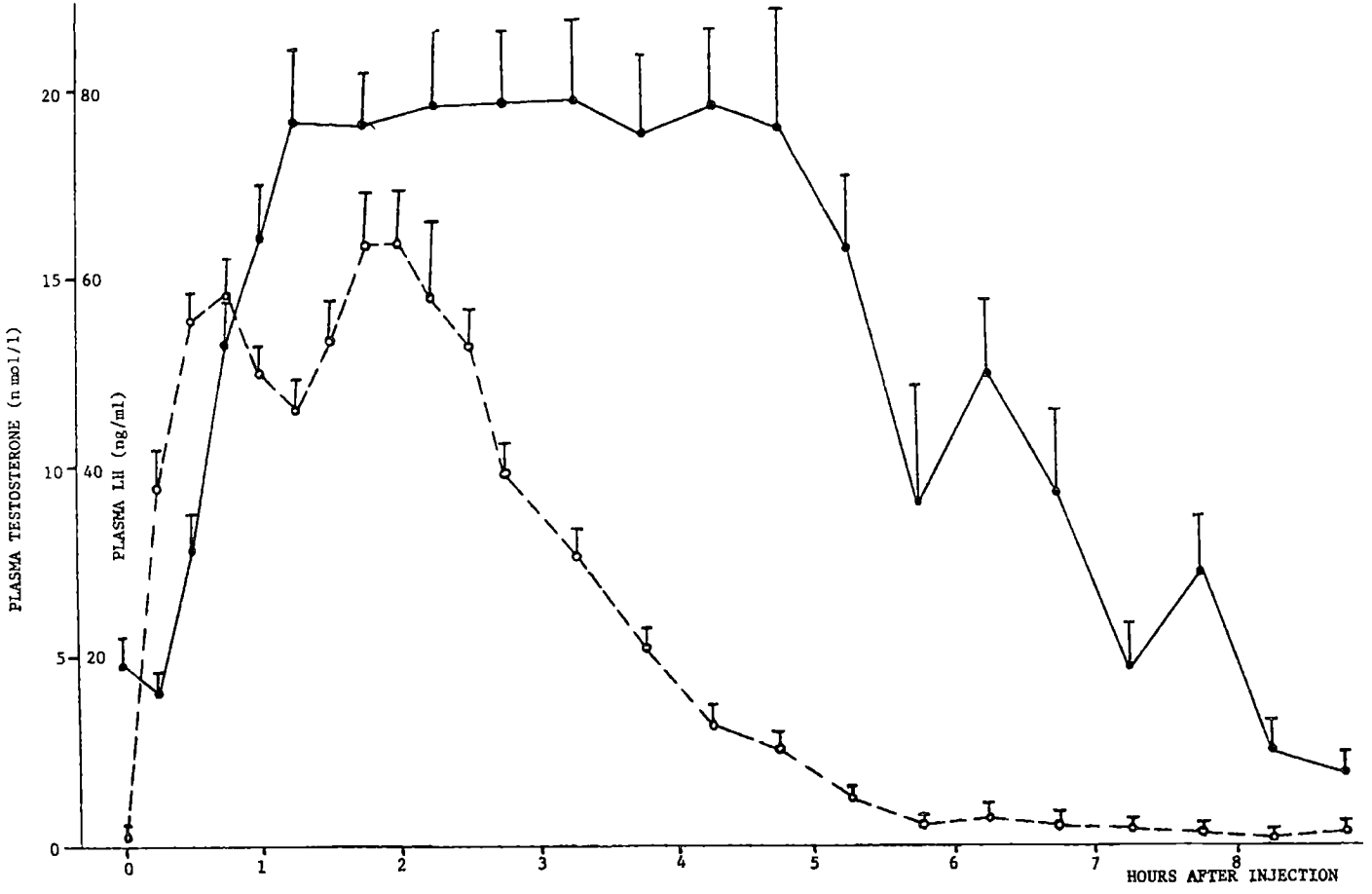

Fig. 3. LH (o----o) and testosterone (●——●) response after i.v. injection of 2 mg Gn-RH (time 0). Mean±S.E. for the six bulls, each stimulated on five different occasions.

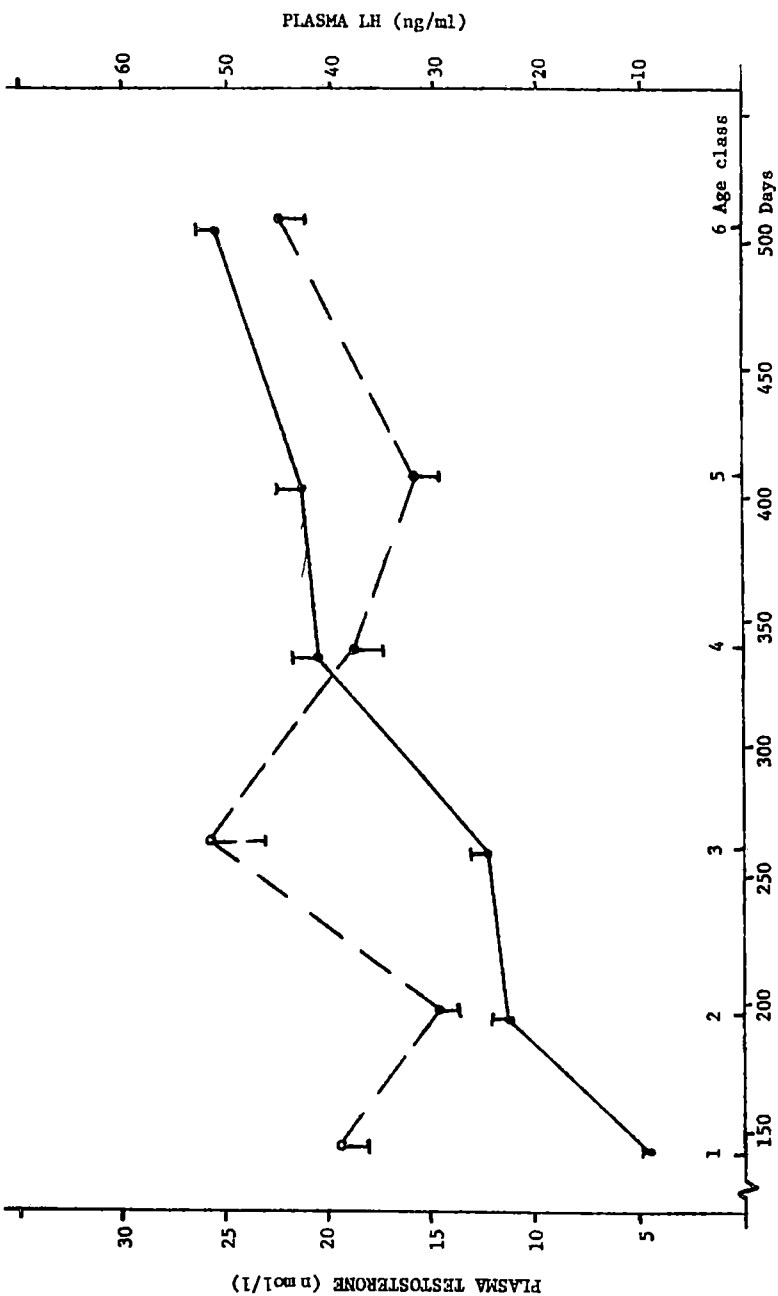

Fig. 4. LH (o——o) and testosterone (●——●) response after 2 mg Gn-RH i.v. in the various ages (age classes or days). Mean±S.E. for the six bulls.

AGE-DEPENDENT TESTOSTERONE-RELATED GROWTH IN BULLS

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Experiments with guinea pigs (Kochakian, Endocrinology 1956) and rats (Scow & Hagan, Endocrinology 1965) have indicated a responsiveness to testosterone by muscles important for sexual activity. Michal et al. (J. of endocr. 1975) and Krieg (Steroids 1976) reported identification of a testosterone and dihydrotestosterone receptor in the rat skeletal muscle, which might imply direct action of androgens also on the skeletal muscle in the rat.

In a study comprising 130 sheep, Schaubacher and Ford (J. anim. Science 1976) concluded that normal growth in the male was dependent on a threshold level of testosterone. Different testosterone concentration seemd to have no further effect on growth as the correlation coefficient between plasma testosterone level and gain was -0.05 to 0.02 in this species. In the bull, Secchiari et al. (J. anim. Science 1976) found a positive but not significant correlation coefficient of r = 0.16 between plasma testosterone and rate of gain during development in 6 animals.

On the meeting in Uppsala two years ago we presented similar data concerning the correlation between growth rate and plasma testosterone from bulls studies in 1975 and 1976. Based on multiple regression analysis the correlation coefficient was 0.21 (N.S.) in 1975 (59 bulls measured at two different points of time. The intra age group correlation was 0.24; $P < 0,01$ n = 210 in 1976. However, a variation in the correlation coefficient within the different age groups was found in these studies and also in a similar investigation in 1978 with 189 bulls 5 - 12 months of age. The highest values occurred at 5 months (r = 0.5) and secondly at 8 and 11 months of age.

Plasma testosterone in bulls vary spontaneously between 0.3 and 6 - 15 ng/ml during the day. In a study with 4 bulls we have showed that in hourly sampling in 12-hour periods an injection of 6000 i.u. HCG reduced the variance coefficient in plasma testosterone from 36 - 69 % to 5 - 14 %.

A dose response study of HCG and plasma testosterone in bulls showed that the response in plasma testosterone the following 9

hours after injection of 375, 750, 1500, 3000 and 6000 i.u. HCG was independent of dose, while a dose dependent further increase and duration of elevated plasma testosterone level was observed.

In 1975, 80 5 - 8 months old bulls were sampled 7 time with 2-hours intervals, and then 5 and 7 hours following an i.m. injection of 750 i.u. HCG.

This same sampling scheme was performed in 1978 on 27 5 months old bulls and 10 bulls in each of the age groups 6, 8, 9, 10 and 11 months. The plasma testosterone measured 5 and 7 hours after HCG injection was quite equal to the prestimulated maximal concentration. The coefficient being 0.98 ± 0.2.

The correlation coefficient between the average and maximal plasma testosterone concentration before stimulation and the testosterone levels measured 5 and 7 hours after the HCG injection was 0.7 or 0.8. The variation coefficient between the values measured 5 and 7 hours after HCG was 11 %. These results should justify comparing plasma testosterone between bulls by measuring plasma testosterone concentration in a single sample taken for instance 7 hours after an HCG injection.

In 1976, 222 bulls 6 - 13 months of age were sampled 7 hours after HCG injection. Similar experiments were performed in 1978 with 189 bulls 5 - 12 months of age. The correlation coefficients between plasma testosterone and growth rate in the month of sampling and in the total growth rate during the nine months testing periods were highest for the 5 months old animals ($r = 0.5$) and then secondly for the 8 and 11 months old group.

In a study where HCG was given to pairs of 3, 4, 5 and 7 months old animals, the 3, 4 and one of the 5 months old animals did not respond to HCG while one of the 5 months old bulls showed response similar to the 7 months old animals. Thus a variation in the maturation of the capacity to produce testosterone might be present in 5 months old bulls. In several reports the peak in monthly growth in bulls is found at 8 months of age. Average and maximal plasma testosterone level measured in 1975 and 1978 did also have a peak concentration in the 8 months old animals. Thus the initial increase in the capacity of the Leydig cells to produce testosterone at 5 months of age and the pubertal testosterone peak at 8 months of age might have influence on the growth rate in young bulls.

A SIMPLIFIED RIA FOR ANDROSTENONE IN BOAR FAT

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Androstenone (5α-androst-16-ene-3-one) is held to be responsible for the objectionable odour commonly referred to as boar taint or sex odour, which can be detected from heated fat of some boars (Patterson 1968). Radioimmunological methods for the quantification of androsterone have been published (Andresen 1974, Claus 1974, Andresen 1975). These methods are, however, time consuming and do not lend themselves easily for routine use when a large number of samples are to be evaluated per day. The aim of the work reported upon was to develop a simple and rapid method which could be practicable for instance in the slaughterhouse for routine evaluation of androstenone content in boar carcasses.

A general outline of the simplified RIA is given in Fig. 1. Androstenone is measured directly in the fatty tissue and time consuming steps as weighing of fat, extraction of steroid from the fat, purification and evaporation of organic solvents have been omitted.

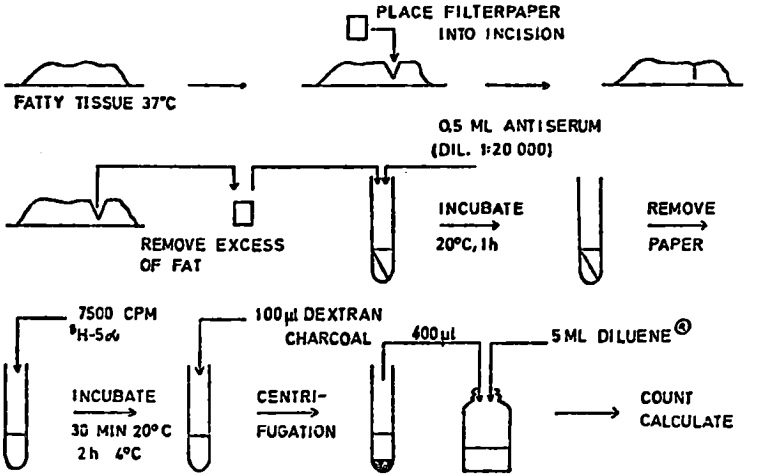

Fig. 1. General outline of the simplified RIA for androstenone in boar fat.

In short fat from boars is absorbed on to a piece of filter paper measuring 10 x 7 mm. Excess of fat is removed by blotting between to other larger filter papers, whereupon the piece of filter paper is placed in a glas tube. A buffer containing antibodies against androstenone in a dilution of 1:20 000 is added, and it turns out that the binding between the antibodies and the steroid takes place under the present conditions. Following incubation at 20°C for 1 hour the filter paper is removed, and by measurement of the residual binding capacity for androstenone in the buffer a relative value for the androstenone content of the fat can be obtained. The binding reaction has not been studied in detail and it is therefore not known whether androstenone is dissolved from the fat into the buffer or if the antibodies bind to the steroid when androstenone still is in contact with the fat.

The relationship between the results obtained by analyses of 28 samples of boar fat by the simplified RIA and by the ordinary RIA is illustrated in Fig. 2 and 3.

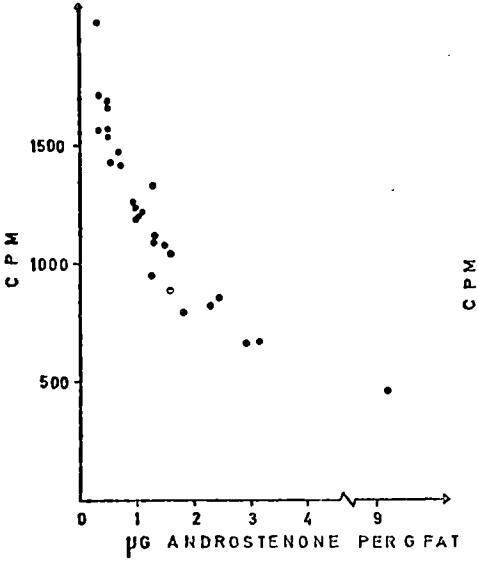

Fig. 2. Relationship between the results of analyses of 28 samples of boar fat by ordinary radioimmunoassay (abscissa) and by the simplified method (ordinate).

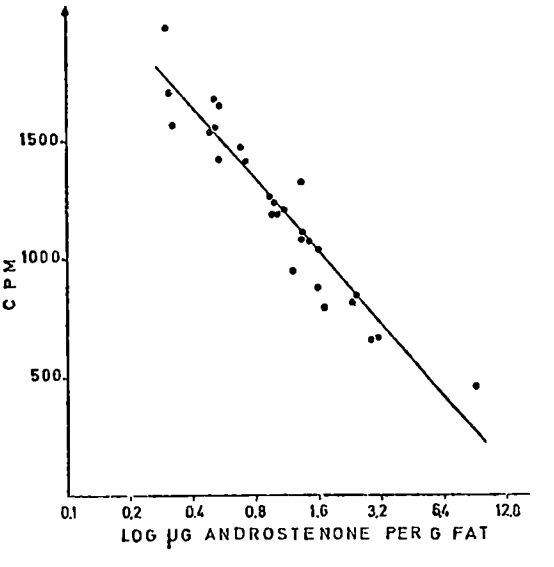

Fig. 3. Relationship between the results of analyses of 28 samples of boar fat by ordinary radioimmunoassay (abscissa) and by the simplified method (ordinate). The results on the abscissa is given on a logaritmic scale.

Fig. 2 indicates a curvilinear relationship. Transforming the concentration of androstenone into common logs (Fig.-3) gives a linear relationship with a coefficient of correlation of -0.95. The regression equation was calculated to be

$$y = 1238 - 1035 \log x$$

Analyses of 10 samples of fat from gilts ans castrated males by the simplified method resulted in a mean CPM value of 2185 (SD= 295)

The SD of duplicate determinations was calculated to be 113 CPM, corresponding to a coefficient of variation of 8,9 %.

As to the capacity of the method one would assume that one technician could at least be able to analyse 100 samples in duplicate per day.

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Swedish J. agric. Res 8: 171–180, 1978

5α-Androstenone and Testosterone in Boars

Early testing with HCG, sexual stimulation and diurnal variation

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Abstract. The effect of HCG-treatment on the levels of 5α-androstenone and testosterone in peripheral plasma and 5α-androstenone in back fat was studied in 30 boars at both 30 and 85 kg live weight. All animals responded to the treatment with increased levels of the two steroids at both stages of development. The steroid levels at 30 kg were not (or only weakly) correlated to the levels at 85 kg, which indicates that the ranking of the animals was not the same at the two stages of development. Without HCG-stimulation the concentration of 5α-androstenone in plasma was not significantly correlated to the levels in back fat. After HCG-treatment high correlations between the plasma and backfat levels were obtained. Similar relationships existed between the levels of 5α-androstenone and testosterone in the same plasma sample.

A single mating did not increase the content of 5α-androstenone in the back fat of 4 boars. The response in plasma levels of 5α-androstenone and testosterone due to mating varied between boars. In a single boar, where repeated plasma samples were taken during 24 hours, wide fluctuations in the plasma levels of 5α-androstenone and testosterone were found. The correlation between the two steroids was low and non-significant.

By use of HCG-stimulation, non-genetic factors influencing the variation of the 5α-androstenone content in the back fat or plasma probably become of minor importance. HCG may therefore be used as a standardization tool if selection against boar taint and 5α-androstenone is performed. Steroid profiles at about 100 kg cannot, however, be accurately predicted by an early test with HCG at 30 kg live weight.

Swedish J. agric. Res. 8: 113–122, 1978

Repeatability and Genetic Variation of Cholesterol Concentration in Bovine Blood Plasma

Correlation with growth rate, carcass quality and milk production

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Abstract. Cholesterol concentration in blood plasma was analysed in 46 monozygous twin pairs of cows and their offspring, 247 calves. In the calves, samples were taken at three ages, 5, 10 and 16 months, and in the cows twice per lactation. One to three blood samples per calf were taken and for each cow several lactations were studied.

The cholesterol level was higher in the heifers than in the bulls, and rose with increasing age. At 5 months the concentration was 95.8 mg/100 ml in the bulls and 102.5 mg/100 ml in the heifers. At 16 months the concentration had risen to 128.9 mg/100 ml and 132.5 mg/100 ml in the bulls and heifers respectively. The cholesterol level in the cows was affected by lactation stage. Early on during lactation the concentration was 120.9 mg/100 ml and in the fifth gestation month it had risen to 133.7 mg/100 ml.

The repeatability of cholesterol concentration between age classes was 0.21±0.06 for the bulls and 0.41±0.07 for the heifers.

Between the two stages of lactation the repeatability of the cholesterol level was 0.59±0.09. The repeatability estimate between lactation numbers was 0.77±0.06 for the first lactation stage and was 0.67±0.13 for the second.

The estimate of heritability of cholesterol concentration was 0.78±0.28 for the growing calves when calculated from an average of the three age classes, while the heritability estimate was 0.22±0.17 for the cows. The genetic correlation between cholesterol concentration and growth rate was 0.80±0.28 when calculated from the average of the three age classes, while the phenotypic correlation was low, 0.23. No significant genetic correlations were found between cholesterol concentration and carcass quality in the calves or milk production in the cows.

I. THE GENETIC BACKGROUND OF CORTICOSTEROID AND ITS RELATION TO BASIC METABOLISM AND GROWTH

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A. To serve as a model investigation for pig selection research, a selection experiment was carried out in mice 1974-79 with three selection criteria:

a. Body weight at 42 days of age.

b. "Basic metabolism", i.e. CO_2 produced as measured as produced Na_2CO_3 in grams according to the relation

$$2\ NaOH + CO_2 \rightarrow Na_2CO_3 + H_2O$$

The mice were kept in air tight glass chambers in atmospherical CO_2-free air singly, but contemporarily per litter, during one preparing hour, followed by five hours of accumulation time.

c. The level of plasma corticosteroid, the blood taken from the eyeball vein with 75 mm micro-hematocrit heparinized glass tubes.

The results given in Table 1 are preliminary only and comprise three generations, as the whole selection period will be analyzed unbroken in a later report.

In Table 2 are given the heritabilities and the line and maternal effects of the three selection criteria together with the figure of accuracy in selection (r_{IG}, i.e. $\sqrt{h^2}$) for these three criteria. In Table 3 are given the three types of correlations computed within the three first generations, i.e. excluding the effect of selection.

In the period 1974-76, the traditional three lines were practised: upwards, control and downwards. In the period 1976 to 79, however, the control line was taken out. This was done because of space lack and to speed up the differentiation of these two selection lines. Thus, the ability of estimating the exact genetic gain was sacrificed.

A resonable trend in selection is demonstrated in Table 1 of this provisional material. The heritabilities and maternal effects in these three criteria are of normal magnitude. Interesting is the significant heritability in basic metabolism as measured in produced g Na_2CO_3.

B. The ability of mobilizing endurance and this phenotypic action's relation to growth and the level of corticosteroid.

At the Polytechnical University of Western Berlin, Technische Universität Berlin, Institut für Tierproduktion, Berlin-Dahlem, after twelve generations of two-directional selection for endurance ability, measured in minutes, from a randomly bred basis population a significant difference was obtained: B^+ (Belastbarkeit + ; line average 16.4 minutes for endurance and 28.4 g for 60 days' body weight as a correlated response in selection), and B^- (Belastbarkeit - ; average 11.3 minutes for endurance and 32.8 g for 60 days' body weight as a correlated response in selection) (Horst et al. 1977).

MATERIAL

A random sample of breeding animals from each of the two selection lines at their stage of selection was obtained from the Berlin institute, and from each line sample each of ten sires was mated to four to him and them between unrelated females. This yielded a structure for investigation of (all figures relate to first litters):

	B^+	B^-	Total
Number of sires	10	10	20
Number of dams	30	32	62
Number of youngs at 60 days of age	255	283	538
Number of youngs per sire	26	28	27
Number of youngs per dam	8.5	8.8	8.4

At the stage of selection at generation 12, Weniger et al. (1976) (Horst et al. 1977) had found significant difference be-

tween negative and positive lines in the two selection questions 1. total protein in carcass and 2. endurance mobilization ability (rate of survival under unfavourable conditions). They had also found significant positive correlated response for protein deposition to increased body weight, and they found significant negative correlated response for mobilized endurance ability to decreased body weight. They found also positive phenotypic correlations between body weight and total protein in the carcass of $r_{phenotype} = +.65$ and between body weight and endurance ability of $r_{phenotype} = -.11$, the latter being much less in magnitude but, obviously, able to force a biological trend in a selection program.

In the Danish part of this investigation, the purpose was to estimate phenotypic and genetic parameters and trends at this niche in selection. The difference between the lines B^+ and B^- at generation twelve was significant in respect to body weight, as the correlated criterion, and the two selection criteria also, in the one part of the selection project it was the total content of protein in the carcass and in the other part, that part of the project here in question, endurance ability as measured in minutes of survival.

In the Danish part of the experiment, the means in body weight at 60 days (Table 4) corresponded very well with those obtained in the selection niche by Horst et al. (1976). This means that the sample received from the basic niche population for the Danish part of the experiment was unbiased.

The autocorrelation in body development between the two ages at observation, day 42 and day 60 within the two lines, was approaching unity.

As seen in Table 4, as expected, the difference between the level in corticosteroid was significant between lines B^+ and B^-, the latter having a 0.2 ng per ml lower level at that niche in the development of selection at day 42. This difference was, however, tripled at day 60 to 0.61 ng per ml. A hypothesis forewarded here is that this should be the reason why the linear regression, within litters, of corticosteroid level on weight gain from days 21 to 60 is insignificant negative within the line B^+, however raised four times ($P \leq .01$) in magnitude in the B^- line. Both regressions are given

in Table 4. As seen, the difference between the two lines in the phenotypic correlation between the two variables here in question is only slight but must, however, be biological significant. The continuation of the hypothesis given above is that within a population of stressed individuals (B^-) the ability of building up the corticosteroid level is significantly lower and the body weight significantly higher because, as computed here, the body weight gain has a significantly negative function on the building up of the corticosteroid level, this corticosteroid level in the B^--line significantly genetical being lower than in the B^+-line, the individuals of which are able to mobilize greater resistance against strain (have larger endurance ability). Correspondingly to this argument it was observed that mice within the B^+-line were definitely more aggressive than in the B^--line, in which the mice were definitely timid. Individuals within the B^--line had definitely lower basic metabolism which corresponds with the findings in Tables 1, 2 and 3 of Section A. in the present report.

A very interesting feature was observed when estimating the heritability of the two criteria, body weight gain from days 21 to 60 and corticosteroid level at day 60, these estimates given in Table 5. Because of the relatively limited number of individuals in the sample in the present investigation, the statistical analysis was undertaken with and without the square root transformation of the variates.

As seen in Table 5, a drastical increase in the relative magnitude of the additive gene action is demonstrated in the selection criterion corticosteron level when estimated at day 42 compared to that when estimated at day 60, the former being insignificant

($h^2_{\text{corticosteron level at day 42}} = 0.34 \pm .20$), the latter being significant at the $P \leq 0.05$ level

($h^2_{\text{corticosteron level at day 60}} = 0.70 \pm .29$).

The body weight gain obtained a heritability estimate of 12 per cent, not significant. All heritability estimates are computed within the two lines.

The difference between the two estimates in additive gene action in corticosteroid level must be due to difference in maturity of the individual. This is perhaps demonstrated in the dramatic

negative genetic correlation in Table 4 between the corticosteroid level taken at day 42 and that taken at day 60 within the same mouse. The phenotypic correlation of the corticosteroid level between these two stages of age development was estimated to $r_p = 0.099$ (535 d.f.; $P \leq 0.05$). The correlation of additive gene action, however, is also significant on the $P \leq 0.05$ level, but has changed sign. These two significant correlations could be caused by the fact that the immaturity of the individual at day 42 does not express the entire hormone action genetically (see h^2 estimates in question in Table 5); the entire number of loci in question have not reached expressivity. This stage of expressivity is reached at day 60.

No interaction in means between, on the one side, the lines B^+ and B^- and, on the other side, the two stages of body development were demonstrated within the two selection criteria.

C. The reasoning behind to chose corticosteroid as a criterion for stress susceptability and its relation to meat quality is given by Topel (1975) and Topel et al. (1967). The present report given is preliminary. An enlarged report on the complex discussed in this present report, covering the research undertaken during the period 1974-1979, will be published in the year 1979.

Table 1. Trends in selecting for plasma corticosteroid level, basic metabolism and body weight gain.

Generation	Year	N	Body-weight, g	g Na_2CO_3 produced in 5 hrs., g	Plasma-corticosteroid, ng per ml
0	1976	946	23.0	0.118	0.818
3	1977	996			
Line, selected upwards			24.7	0.465	2.52
Line, selected downwards			22.2	0.092	0.204

Table 2. The partitioning of the phenotypic variation of the three selection criteria.

Per cent.

	Line effect	Herita-bility	r_{IG}	Maternal effect	Residue
Degrees of freedom:	23	155		319	3329
Body weight	4.0±4	22.6±13	47	4.4±5	69
Basic metabolism	39.8±17	35.3±17	59	3.0±4	22
Plasma corticosteroid	24.5±13	24.0±13	49	7.3±5	44

Line size : 9 males; 3 females per male

Table 3. Covariances between pairs of selection criteria.

Correlation:	Additive genetic	Intra litter	Phenotypic
Body weight x basic metabolism	+.56±.25	+0.56	+0.25
Basic metabolism x corticosteroid	-0.09±.37	-0.24	-0.07
Body weight x corticosteroid	+.41±.33	-0.37	-0.12

Table 4. Relationship between endurance ability and body weight gain and corticosteroid level, respectively.

Line of selection	B^+	B^-
Number of individuals	255	283
Body weight at age 42 days, g	25.6±.23	26.6±.29
-"- -"- - " - - " - , s_p, g	3.7	4.8
Weight gain from 21. to 42. day, g	6.0	10.4
Body weight at age 60 days, g	27.9±.26	31.8±.36
-"- -"- - " - - " - , s_p, g	4.1	6.1
Weight gain from 21. to 60. day, g	8.4	15.6
$r_{phenotype}$ (body weight:42.day x 60.day)	+.94	+.90
Corticosteroid level, ng per ml:		
at 42 days of age	1.19	0.99
at 60 days of age	1.84	1.24
Linear regression within litters of corticosteroid level on weight gain from days 21 to 60	-0.03 not sig.	-.13 $P \leq .05$
$r_{phenotype}$ (within lines) (same variables)	-.20	-.25

Correlation within each line between the corticosteroid level at day 42 and that at day 60:

$r_{add.\ genetic} = -.52 \pm 26$; (18 degrees of freedom)

$r_{phenotype} = +.099$ (538-3 degrees of freedom)

Table 5. Heritabilities within the two lines, B^+ and B^-, of body weight gain and corticosteroid level and the phenotypic and genetic relations between these two criteria.

		$\sqrt{}$ trans-formation	No $\sqrt{}$ trans-formation
a.	Body weight gain from days 21 to 42	0.12±.19	0.12±.19
		not sign.	not sign.
	body weight gain from days 21 to 60	0.12±.15	0.12±.13
		not sign.	not sign.
b.	Corticosteroid level at day 42 of age	0.39±.20	0.34±.20
		not sign.	not sign.
	corticosteroid level at day 60 of age	0.67±.28	0.70±.29
		both estimates significant at the level $P \leq 0.05$	

c.	Correlation:	phenotypic	add. genetic
	Body weight gain from days 21 to 42 with corticosteriod at day 42	-0.24	+0.26±.64
	Body weight gain from days 21 to 60 with corticosteroid at day 60	-0.32	-1.3 standard error not possible to estimate
	Corticosteriod level at day 42 with that at day 60	+0.099	-0.52±.26
			$P \leq 0.05$

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PLASMA CORTICOSTEROID LEVELS IN LAYING HENS. EFFECT OF TWO DIFFERENT BLOOD SAMPLING TECHNIQUES AND OF ROUGH HANDLING OF THE ANIMALS

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Corticosteroids were measured in blood samples collected from 10 hens in two series with a time interval of 9 days. In the first series blood was collected by venipuncture (wing vein), in the second by cardiac-puncture. In each series, sampling took place immediately before (control), and 5 min. as well as 18 hrs. after intentional rough handling. Only samples obtained by cardiac puncture 5 min. after rough handling showed markedly elevated hormone levels.

Corticosteroids were also measured in blood collected from two other groups of hens, each including 10 birds. Samples were taken at 0 (control), 1, 5 and 40 min., one group being sampled by venipuncture, the other by cardiac puncture. None of the groups were subjected to rough handling. The hormone levels in samples obtained by cardiac puncture at 5 and 40 min. after the control samples were significantly higher ($p < 0.005$) than the levels in the corresponding samples obtained by venipuncture.

INTRODUCTION

As a criterium in judging bird welfare in modern intensive poultry production, plasma corticosterone has been used and is considered to be a sensitive method (Newcomber 1962, Nagra et al. 1963, Lei et al. 1971, Eskeland 1978).

The importance of the technique of blood sampling is often not emphasized when plasma corticosteroid levels are measured. Cardiac puncture is widely used because it takes short time and gives blood in sufficient quantities. Cardiac puncture may, however, have a deleterious effect on performance, and in broilers

Key words: Blood sampling techniques, rough handling, plasma corticosteroids, laying hens.

and replacement pullets Buckland et al.(1974), found reduced weight gain of bled chicks compared to non-bled chicks.

The objective of this study was to compare the effect of different blood sampling techniques on plasma corticosteroid levels, and to examine the effects of rough handling and of taking several blood samples.

MATERIALS AND METHODS

This study was carried out in order to compare the effect of venipuncture and cardiac puncture on plasma corticosteroid levels in one year old laying White leghorn hens. Precautions were taken not to disturb the hens before blood sampling. The sampling took place immediately after the hens were taken out of their cages. The hens used in this study were exposed to the same environment and were caged individually in cages of 40 cm x 38 cm. They were fed ad libitum of a standardized ration.

In the first part of the study 10 hens were bled by regular venipuncture in the right wing. After 9 days and at the same hour of the day, the same 10 hens were bled by cardiac puncture using 5 ml heparinized syringe with 50 mm long needle (diam. 0,8 mm).

After the control sample had been obtained either by cardiac or venipuncture, each hen was subjected to rough handling about 30 sec. The birds were then put into their respective cages whereupon they were rebled after 5 min. and 18 hrs.

In the second part of the study, series of samples were taken from two groups each of ten hens. One group was bled by cardiac puncture, the other by venipuncture. In both groups the control sample for each hen was followed by additional sampling, 1, 5 and 40 min. later, without preceeding rough handling.

Corticosteroids in blood plasma were determined by competetive protein binding (Murphy 1967) mainly as described by Kolanowski & Pizarro (1969). Blood plasma from pregnant women in the last trimester was used as a source of steroidbinding protein. Free and protein-bound steroids were separated with Florisil (60-100 mesh, Sigma). All measurements were performed in duplicate and coefficients of variation obtained were: 3.0 (mean: 3.29

ng/ml) and 3.9 (mean: 7.51 ng/ml).

RESULTS AND DISCUSSION

The results from the first part of the study are given in Table 1. The hens were bled first by venipuncture and 9 days later by cardiac puncture (Table 1). Blood sampling by each of the methods took place at the same time of the day to avoid effects of possible diurnal variations. Blood sampling resulted in a mean plasma corticosteroid level of 4.5 ± 0.5 ng/ml (SEM) and 3.9 ± 0.8 ng/ml for cardiac puncture. Out of the 10 hens bled, 9 had lower plasma corticosteroid level in blood obtained by cardiac puncture than in blood taken from the vein. This may be due to the longer bleeding time necessary using venipuncture, about 3 min., versus 10 sec. using cardiac puncture.

Sudden release of corticosteroids caused by the handling and sampling could thus represent a strain superimposed on the environmental factors to be studied. Therefore the blood samples should be collected within as short time as possible.

When frequent sampling was carried out, cardiac puncture gave a lower mean control plasma corticosteroid level (4.2 ng/ml) than venipuncture (4.6 ng/ml) (Table 2). The difference was, however, not statistically significant ($p > 0.05$). The plasma corticosteroid level of hens bled by cardiac puncture was not significantly increased 1 min. after the first blood sampling (Table 2), but was significantly increased ($p < 0.005$) 5 and 40 min. after the first blood sampling.

In peripherally bled hens, a significant increase ($p < 0.01$) in plasma corticosteroid level was observed at 5 min. after the first blood sampling, but at 40 min. the plasma corticosteroid level had returned almost to the initial values.

The increase in plasma corticosteroid levels from 1 to 5 to 40 min. after the control sampling, using cardiac puncture, may be due to the trauma of repeated bleedings.

Rough handling of the animals followed by blood sampling 5 min. later, resulted in a mean increase in plasma corticosteroids of 49 and 287 per cent, using venipuncture and cardiac puncture,

respectively (Table 1). When no rough handling was applied, the mean percentage increases after 5 min. were 56 and 324 for the two blood sampling techniques, respectively.

CONCLUSIONS

1. Cardiac puncture is a far more rapid method of blood sampling than venipuncture, the time used being 10 sec. and 3 min., respectively. To minimize effects of the sampling procedure on the plasma corticosteroid level, cardiac puncture is therefore recommended when only one blood sample per animal is to be taken. On the other hand, if many samples are to be taken during a rather short period of time, venous sampling seems to be the method of choice.

2. The present data also indicate that the rise in plasma corticosteroid level brought about by the sampling procedure is not further accentuated by rough handling.

SAMMANDRAG

Kortikosteroider ble målt i blodprøver fra 10 høner, tatt i to serier med 9 dagers intervall. I den første serien ble blod tatt med vingevene-punktur, i den andre serien med hjertepunktur. I begge seriene ble blodprøvene tatt like før (kontroll), 5 min. og 18 timer etter hardhendt behandling. Bare blodprøven som ble tatt med hjertepunktur 5 min. etter hardhendt behandling viste markert forsøket hormonnivå.

Kortikosteroidnivået ble målt i blod tatt fra to andre grupper av høner, hver gruppe inneholdt 10 dyr. Prøvene ble her tatt ved tid 0 (kontroll), 1, 5 og 40 min., hvorav den ene gruppen ble tappet med venepunktur og den andre med hjertepunktur. Ingen av gruppene her ble utsatt for hardhendt behandling. Prøver tatt 5 og 40 min. etter kontroll med hjertepunktur viste signifikant ($p < 0,005$) høyere hormon-nivå enn tilsvarende prøver tatt med venepunktur.

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Table 1. Plasma concentrations of corticosteroids (mean ± SEM, n = 10) in two groups of laying hens bled by venipuncture or cardiac puncture.

	Time after first blood sampling (min.)	Plasma corticosteroids (ng/ml) Venipuncture	Cardiac puncture
First blood sampling (control)		4.6±0.3 (a)*	4.2±0.5 (e)*
2nd blood sampling	1	5.6±0.6 (b)*	4.4±0.9 (f)*
3rd blood sampling	5	7.2±0.8 (c)	17.8±3.1 (g)
4th blood sampling	40	4.9±0.9 (d)*	25.5±2.9 (h)

*No significant difference between corresponding means.

(g-h), (c-d), $p < 0.05$.

(a-c), $p < 0.01$.

(b-g), (c-g), $p < 0.005$.

(b-h), $p < 0.001$.

Table 2. Effect of rough handling on plasma corticosteroid concentrations (ng/ml) of blood collected by venipuncture and by cardiac puncture.

Hen No	Controls (Before rough handling)		Time after rough handling			
			5 min.		18 hrs.	
	Venip.	Cardiacp.	Venip.	Cardiacp.	Venip.	Cardiacp.
1	2.9	2.6	5.6	3.4	3.6	3.9
2	3.7	2.6	4.2	26.8	4.2	3.1
3	2.8	2.5	3.8	5.7	3.6	2.9
4	5.6	3.2	9.2	19.6	4.9	4.6
5	3.9	2.9	6.9	20.5	3.9	3.6
6	4.0	2.1	8.3	20.9	3.6	1.8
7	8.6	10.9	10.2	18.6	9.4	10.0
8	4.9	4.5	5.8	8.2	4.6	4.8
9	4.0	3.4	6.8	8.3	4.2	4.0
10	4.6	4.5	6.3	18.9	5.1	5.6
Mean	4.5	3.9	6.7x	15.1	4.7	4.4
Standard error of the mean	0.5	0.8	0.6	2.5	0.5	0.7

xSignificantly different from control

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THE RELEASE OF PROSTAGLANDIN $F_{2\alpha}$ AS REFLECTED BY 15-KETO-13,14-DIHYDROPROSTAGLANDIN $F_{2\alpha}$ IN THE PERIPHERAL CIRCULATION DURING NORMAL LUTEOLYSIS IN HEIFERS.

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ABSTRACT

Progesterone and the main plasma metabolite of $PGF_{2\alpha}$, 15-keto-13,14-dihydro-$PGF_{2\alpha}$, were determined at hourly intervals in the peripheral circulation during luteolysis in two heifers. The prostaglandin release was found to occur during 2-3 days as rapid pulses with a duration of 1-5 hours prior to and during luteolysis, which was indicated by decreasing levels of progesterone.

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RELEASE OF PROSTAGLANDIN $F_{2\alpha}$ DURING THE BOVINE PERIPARTAL PERIOD

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ABSTRACT

Progesterone, estrone and 15-keto-13,14-dihydro-$PGF_{2\alpha}$ levels were determined in the peripheral blood circulation during the peripartal period in 12 cows. Plasma concentrations of progesterone showed a gradual and continuous decrease during the last 60 days before parturition. This gradual decrease was followed by an abrupt decline in the progesterone concentration occurring 24-48 hours before delivery. The plasma levels of estrone started to increase about 30 days prior to parturition with high concentrations attained during the last days of pregnancy. After delivery the estrone content decreased to baseline levels. Increased levels of the $PGF_{2\alpha}$ metabolite were recorded 24-48 hours before parturition. These increased $PGF_{2\alpha}$ metabolite levels occurred before or in conjunction with prepartum luteolysis. Prostaglandin metabolite levels remained high during parturition and returned to baseline 10-20 days after delivery.

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ØESTRUS SYNCHRONIZATION IN SHEEP USING ORAL MAP-TREATMENT FOR 10 DAYS ONLY.

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The practical value of oestrus synchronization in farm animals is undisputable. It facilitates the use of artificial insemination, and shortens the period of parturition in a given herd. This in turn improves the care of the newborn, reducing perinatal mortality, and at the same time forms the basis for rational feeding programmes. In research based on the use of flocks of animals, the design of experiments is in many instances improved when animals of approximately the same age can be used.

In our school, experiments on oestrus synchronization in ruminants, based on oral progestagen treatment, was initiated in the early sixties. Experiments have comprized goats, sheep and cattle.

Initially, several preparations were tried. The best results were obtained with MAP (medroxyprogesterone acetate) and only these results will be reported here.
In the first trials, animals were treated for the duration of a normal oestrous cycle or longer. However, it was found that periods of treatment of only 10 days also gave satisfactory results. The shorter period simplifies the procedure and reduces cost.

Regarding mode of administration, in most of the experiments MAP powder and saccarum lactis in the form of a premix has been mixed into concentrate and given by group feeding in the morning ration.

Trials with sheep.

Our most extensive experiments have been with sheep in connection with natural breeding. The animals belonging to the herd of the college farm were first treated for oestrus synchronization in 1964 using 50 mg. MAP per animal per day for 10 days. The preliminary trial in 1964 comprised 33 animals, of which 30 showed heat within 5 days after the end of treatment. 25 conceived in the first oestrus, and 7 after repeated breeding. In the previous year the record for the flock showed the breeding dates to be scattered very evenly over a period of 34 days.

In a similar trial in 1965, carried out in a flock of 39 sheep belonging to a farmer, 37 ewes came into heat within 3 - 5 days after treatment, the dates of lambing in 1966 being concentrated to two periods, 30 lambings taking place between April 15th and 20th, and 5 between april 30th and May 5th. In the previous year the breeding and lambing dates were scattered very evenly over periods of 31 and 35 days respectively.
So far as management is concerned, great advantages were achieved by treatment and the fertility was good, even following breeding at the first post treatment oestrus.

The data for the College flock, now covering the 12 year period, comprize 378 individual cycles. 89,3 per cent of the animals came into heat within the first 6 days after the last day of treatment. The average duration of the oestrus was 50 hrs. The conception rate at the first oestrus was 74,4 per cent against a national average of about 90 per cent for untreated animals. Interestingly, conception rates were significantly lower for ewes coming into oestrus on the first and second day, compared with those showing heat on day 3-8. This may be due to a too strong progestagen dominance of the uterus and tubes in the early phase after treatment.

Many of the ewes were subjected to oestrus synchronization for several concecutive years, without any observable deleterious effects. For the ewes not subjected to subsequent treatment during same seas the average number of offspring resulting from 282 matings was 1,86 per ewe, higher than the national average. The lambing dates were in the main concentrated to two periods, each of about one week's duration, about one week apart, corresponding to conceptions taking place at first or second oestrus after treatment. As many as 82,6 per cent of the lambings took place within these two periods, greatly facilitating management.

Trials using MAP protected in fatty acid pellets were also carried out. In one group of 33 ewes the daily dosage was 35 mg. 28 animals (84,8 per cent) came into heat within 6 days, showing that the effective dose can be considerably reduced when the preparation is protected against rumen fermentation. A daily dose of 20 mg. MAP was, however, shown to be too low.

The adequacy of a treatment period of only 10 days needs comments. As our records show, in a flock of untreated ewes the onset of oestrus is rather evenly distributed over a period of more than one month.
The duration of the oestrus cycle in sheep is about 16 days. Let us assume that we have a flock of 16 ewes with the onset of oestrus being evenly distributed over a 16 day period, one animal showing heat each day. If treatment is started on day 17 and continued until day 26, we see that even without treatment, 6 of the ewes would have come into heat within the 6 day period comprizing days 27 - 32. Assuming complete effectiveness of the preparation, the other 10 ewes which without treatment would have come into oestrus on days 17 - 26, will have their oestrus portponed until after the end of treatment. Altogether, in this theoretical model, 100% of the animals would show heat within the 6 day period following treatment. The 6 animals represent 37,5 per cent of the total of 16 in this model.

Referring now to the results of our trials over the 12 year period, one may assume that 37,5 per cent of the animals would have come into heat within a 6 day period even without treatment. The total number of animals coming into heat within a 6 day period after treatment in our trials with sheep was 89,4 per cent. Thus 89,4 - 37,5 = 51,9 per cent of the animals will have had their oestrus effectively postponed by the treatment. In the theoretical model 89,4 per cent corresponds to 14 of 16 animals. Thus for animals having cycle length of 16 days even as short a period of treatment as 10 days seems to give near maximum effect. In fact, a scanning of the litterature shows that no other method based on oral treatment with MAP gives better synchronization, and all other methods seem to give poorer conception rates than those obtained in our experiments using oral MAP treatment for 10 days.

THYROID MORPHOLOGY AND GROWTH PERFORMANCE OF YOUNG PROGENY GROUPS OF AI BULLS

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Introduction

Prof. M. Varo, together with Uudenmaan-Kymen Keinosiemennysyhdistys, carried out several growth performance experiments with progeny groups of AI-bulls. Animals were grown in Westerkulla's bull station, which is located few kilometers West from Helsinki. Feeding of animals and their growth performance has been reported earlier (KOSKULL 1967, 1968a, 1968b, 1971). Thyroid material was available from these experiments for closer evaluation.

Thyroid function is known to be important in growth, reproduction and lactation. Animals with optimal thyroid function are likely to be better producers than those with unbalanced thyroid function. In this study, thyroid morphology has been used as an indicator of thyroid activity and also as an indicator of goiter. Purpose of the study was to investigate whether and to what degree thyroid characteristics are related with growth performance of young male calves, and to what extent thyroid characteristics differ from each other in different progeny groups of AI bulls.

Material and methods

Thyroids and other data were collected from five subsequent experiments in 1967-71 (Table 1). Thyroids were collected at slaughter and handled as earlier described (KOSSILA 1967).

Preparations were made at the Department of Pathology, College of Veterinary Medicine, Helsinki. Thyroids were investigated in the same manner than in the previous study (KOSSILA et al. 1970). Statistical calculation of the data was done by Computing Service of Agricultural Research Centre.

The calves were about ten days old and their live weight was about 36-38 kg on the average in the beginning of the experiments. They received skim milk powder containing starter feeds, abundant amount of concentrates (oats, barley, sugar beet pulp) and mineral mixture. Also hay was given at maximum rate of 1 kg/day/calf. All calves were of Ayrshire breed and males. In 1967 they were slaughtered at the age of 181 days, in 1968-70 at the age of about 200 days, and in 1971 at the age of 217 days.

Results and discussion

Effect of year

Average values obtained in different years are summarized in Table 1. Rate of live weight gain was 965 g, 1000 g, 995 g, 1047 g and 928 g/day in 1967, 1968, 1969, 1970 and 1971 respectively. Average live weight, carcass weight and rate of gain were highest in 1970 but lowest in 1971.

Thyroid weight was lower in 1967 and 1971 than in 1968-1970 (Table 1). Percent distribution of the thyroid weight in different years is presented in Fig.1. In 1967 and 1971, peak frequency occurred at weight range of 10-14 g and in 1970 at weight range of 16-18 g. In 1969, tree peaks appeared, one at 14-16 g, second at 18-20 g, and third at 22-24 g. These peaks demonstrate occunrence and degree of goitre in this particular data.
Thyroid weight correlated poorly with live weight, carcass weight and age at slaughter (Table 3, entire data 1967-71).

Histological activity of the thyroid was higher in 1968-70 than in 1967 and 1971 and this higher activity seemed to be related with higher thyroid weight (Table 1). However, in the entire material thyroid weight was negatively correlated with histological

activity of the gland (Table 4). Histological activity correlated positively with carcass weight, +.178 in years 1968-70, +.129 in years 1967-71 (Table 3).

Follicle number (FN) was highest in 1967 and lowest in 1971 (Table 1). This means that follicular size become larger with increasing age of the animals. Correlation between FN and age was -.426 in 1967-71 (Table 3).

Epithelial cell height (Eu) was lowest in 1967 and highest in 1970 (Table 1). In the data of the years 1967-71, age has significant effect on Eu (+.344). Eu correlated significally also with live weight (+.21) and carcass weight (+.20) when data of several years was combined (Table 3). Eu was negatively correlated with thyroid weight and positively correlated with histological activity (Table 4).

Amount of epithelial tissue in the thyroid (E g) can be calculated when thyroid weight and percentage of epithelial tissue in the gland are known. If in young cattle E g is higher than 5 g, the thyroid is likely goitrous (KOSSILA et al. 1970). Such is case in the data obtained in 1968-70 (Table 1). E g was not much influenced by age (Table 3) which finding is in agreement with an earlier observation (KOSSILA 1967 p. 79, 1969a). Positive correlations were found between E g and carcass weight (+.152) as well as with live weight (+.183) in the entire data (Table 3).

Percentage of epithelial tissue in the thyroid (E %) was higher in years 1968-70 than in 1967 and 1971 (Table 1). Age had hardly any influence on the E %, but positive correlations were found between E % and live weight as well as carcass weight (Table 3). E % correlated negatively with thyroid weight (Table 4) indicating that large thyroids were hypoepithelial goiters. In previous study (KOSSILA et al. 1970), E % correlated positively with thyroid weight indicating that large thyroids were hyperepithelial goiters.

Percentage of colloid in the thyroid (C %) tended to increase with age but decrease with increasing live weight or carcass weight of the animals (Table 3). C % correlated positively with thyroid weight indicating that large thyroids were hypoepithelial colloid goiters.

Percentage of stroma (S %) in the thyroid was found to decrease (-.416) significantly with age of the animals in the entire data (Table 3), while correlations with live weight and carcass weight were nonsignificant.

Effect of sire

Data obtained from progeny groups of 50 different sires, as averages, have been summarized in Tables 2a-2e. Average live weight and carcass weight of the progeny groups of different sires varied considerably. Differences in the thyroid characteristics were also noted in different progeny groups. Average thyroid characteristics of different progeny groups in relation to their carcass weight are depicked in figs. 2-7. Each sire can be identified by its number within the circle (see also Tables 2a-2e).
Histological activity of the thyroid correlated positively with carcass weight among sires (Fig. 3). Similar tendencies are observable in case of E µ (Fig.5) and E % (Fig. 7). Positive tendency is observable in case of E g (Fig. 6) while FN and thyroid weight cannot be shown to be correlated with carcass weight by this way (Figs 2 and 4).

It has been shown in previous studies that thyroid characteristics may pass from parent to offspring and that some animals are more resistant towards goiter than others. Also, productional capasity of animals having enlarged (abnormal) thyroid is smaller compared to animals who have normal thyroids (KOSSILA 1969a, 1969b).

Conclusions

Thyroid characteristics of young bull calves were significantly influenced by the year and by the sire. Certain characteristics of the thyroid (histological activity, E µ, E g, E %) correlated significantly with live weight and carcass weight of the animals. FN, E µ and S % were significantly influenced by age of the animals. Part of the thyroid material in the present study could be classified as goitrous. Thyroid gland is morphologically very sensitive for iodine deficiency and for certain other nutritional defects. Therefore one has to be careful in predicting extent of thyroxine secretion in an animal, on the basis of thyroid morphology.

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Table 1 . Age and live weight at start and end, carcass weight and thyroid characteristics of calves in different and in combined years

	1967 N = 73 $\bar{x}$ ± sd	1968 N = 52 $\bar{x}$ ± sd	1969 N =106 $\bar{x}$ ± sd	1970 N =107 $\bar{x}$ ± sd	1971 N =116 $\bar{x}$ ± sd	1967-71 N =454 $\bar{x}$ ± sd
1. Age, days at start	8.0 ± 2.71	-	11.0 ± 4.16	10.0 ± 4.18	10.53	-
2. " " at end	181.0 ± 1.56	200.8 ± 1.73	200.2 ± 2.00	200.1 ± 3.47	217.7 ± 4.34	201.61±12.02
3. Live wt. at start	36.8 ± 3.86	38.2 ± 4.89	36.0 ± 4.59	37.5 ± 5.72	37.97± 4.82	37.25± 4.91
4. " " at end	204.3 ±16.22	228.4 ±18.29	224.0 ±21.15	237.0 ±25.57	229.9 ±25.50	225.91±24.72
5. Carcass wt.kg	98.7 ± 8.82	106.8 ± 9.64	105.4 ±11.35	113.5 ±13.47	109.2 ±14.03	107.36±12.96
6. Live wt. of sire	817.0 ±74.45	804.9 ±56.81	874.3 ±75.37	935.0 ±124.23	-	-
7. Thyroid wt., g	16.77 ± 8.45	22.52 ±13.54	23.42±12.84	22.44±17.55	16.26± 6.39	20.19±12.76
8. Hist. act.	2.32 ± 0,62	2.78 ± 0.67	2.79± 0.59	2.87± 0.66	2.40± 0.80	2.63± 0.71
9. FN	20.4 ± 7.32	18.33 ± 7.72	18.64± 6.24	19.91± 6.80	11.42± 2.51	17.34± 7.01
10. Ep	5.73 ± 1.49	6.78 ± 1.99	6.61± 1.26	8.68± 2.87	8.35± 2.07	7.42± 2.33
11. Eg	3.50 ± 1.52	5.43 ± 1.66	6.11± 2.17	5.68± 2.72	3.70± 1.15	4.90± 2.24
12. E%	21.75 ± 6.18	27.25 ± 8.86	28.54± 7.80	29.27± 7.95	24.19± 7.32	26.36± 8.08
13. C%	69.00 ± 7.59	62.24 ±10.91	60.51± 9.26	60.52± 9.69	70.61± 7.24	64.66± 9.93
14. S%	9.26 ± 2.19	10.48 ± 3.03	10.94± 3.11	10.22± 2.82	5.19± 2.96	8.98± 3.65

$\bar{x}$ = mean, sd = standard deviation, N = number of cases, Eg = amount of epithelial tissue in the thyroid gland, C% = percentage of colloid in the thyroid, S% = percentage of stroma in the thyroid.

FN = no. of follicles, Ep = heigh of epithelial cells, E% = percentage of epithelial tissue in the thyroid,

Table 2a. Number of calves, mean live weight and age at start and at end, carcass weight and thyroid characteristics in the progeny groups of different bulls

No.	Sire	N	Age days at start	Age days at end	Live wt kg at start	Live wt kg at end	Carcass wt kg	Thyroid characteristics: wt g	hist. act.	FN	Ep	Eg	E%	C%	S%
1967															
8	Pākas Kīppa 725 kg	8	9 ±2.43	181 ±0.76	37 ±4.22	213 ±16.6	103 ±9.54	13.4 ±3.04	2.4 ±0.32	19.3 ±7.34	5.7 ±1.03	2.81 ±0.59	21.17 ±2.90	68.83 ±5.04	10.00 ±2.50
5	Monaalan Kalpo 755 kg	7	9 ±1.86	181 ±3.21	37 ±4.86	202 ±21.3	97 ±11.55	13.9 ±3.42	2.0 ±0.29	16.6 ±3.60	4.7 ±0.49	2.41 ±0.41	17.97 ±3.60	74.41 ±4.27	7.62 ±0.90
9	Sipilän Este 815 kg	8	7 ±3.07	181 ±1.46	39 ±4.02	218 ±14.6	105 ±6.04	14.7 ±6.23	2.3 ±0.59	23.2 ±8.16	6.2 ±1.50	3.09 ±0.94	21.80 ±3.13	68.25 ±5.16	9.94 ±2.34
3	Jaguar 755 kg	8	11 ±1.51	181 ±1.41	37 ±3.65	194 ±7.8	94 ±5.59	14.8 ±3.40	2.3 ±0.46	17.1 ±3.30	5.8 ±1.23	3.11 ±1.03	21.05 ±5.02	70.00 ±6.45	8.95 ±2.20
4	Lukas 950 kg	8	8 ±2.93	181 ±1.25	35 ±4.02	196 ±16.0	94 ±7.44	15.3 ±3.69	2.6 ±1.09	25.8 ±11.28	6.1 ±2.15	3.69 ±1.43	27.14 ±13.31	63.75 ±14.65	9.11 ±2.05
2	Gustöle Viktor 815 kg	8	9 ±1.92	181 ±1.51	35 ±3.25	195 ±19.1	94 ±10.03	15.6 ±4.17	1.9 ±0.50	15.6 ±4.37	5.0 ±1.22	2.74 ±0.68	18.89 ±4.75	72.72 ± 5.99	8.39 ±1.56
6	Nykulla Ymär 715 kg	7	7 ±2.98	181 ±1.35	35 ±2.51	204 ±10.5	97 ±5.67	16.3 ±7.18	2.6 ±0.67	24.9 ±8.35	6.4 ±1.97	3.86 ±1.39	24.48 ±4.79	65.87 ± 6.69	9.65 ±2.08
1	Alikoikan Isku 840 kg	8	8 ±2.93	181 ±1.60	38 ±4.94	204 ±16.7	97 ±9.61	17.2 ±3.98	2.6 ±0.74	19.1 ±4.66	6.4 ±1.81	3.82 ±0.88	22.75 ±5.15	66.50 ±7.66	10.75 ±3.02
7	Pukin Parta 901 kg	11	8 ±2.84	181 ±1.44	38 ±2.64	209 ±9.6	104 ±6.09	25.9 ±16.93	2.2 ±0.40	21.3 ±6.40	5.3 ±1.14	5.04 ±2.57	20.73 ±4.10	70.40 ± 5.08	8.87 ±1.85

Table 2b.

No.	Sire	N	Age days at start	Age days at end	Live wt kg at start	Live wt kg at end	Carcass wt kg	Thyroid characteristics: wt g	hist. act.	FN	Ep	Eg	E%	C%	S%
1968															
12	Karvialsten Itara	8	-	201	39	237	112	15.4	3.1	24.9	7.6	4.93	32.72	56.67	10.61
	760 kg			±2.36	±4.64	±24.0	±11.97	±2.50	± 0.58	±10.86	±2.67	±1.00	± 7.82	±8.76	±2.27
16	Puotin Jyry	4	-	199	34	219	104	15.6	3.1	25.9	6.6	5.32	34.50	55.05	10.44
	755 kg			±1.89	±5.00	±14.6	± 7.35	±1.87	±0.63	±9.54	±0.76	±1.28	± 9.74	±11.02	±1.38
10	Ahtialan Jumo	5	-	201	38	240	112	19.6	2.6	17.3	6.0	4.98	24.67	63.60	11.73
	720 kg			±0.55	±3.11	±20.3	±8.88	±4.71	±0.65	±7.45	±0.71	±2.24	±6.86	±9.52	±3.41
14	Korpis Igor	5	-	201	36	222	107	21.2	2.5	16.6	6.1	4.40	21.69	65.98	11.33
	730 kg			±1.82	±2.77	±10.1	±4.98	±7.07	±0.35	±2.68	±1.19	±0.56	±3.94	±6.54	±3.06
11	Hiistan Innokas	4	-	201	38	236	110	21.6	3.1	21.4	7.7	6.63	33.66	54.11	12.22
	865 kg			±2.63	±3.70	±13.0	±8.70	±12.50	±0.85	± 5.17	±3.36	±2.28	±1.04	±1.30	±3.75
17	Rajalan Juupe	4	-	200	38	217	100	21.9	2.6	14.2	6.3	5.39	26.67	64.39	8.95
	830 kg			±1.63	±5.32	±15.1	±10.08	±12.43	±0.63	±2.49	±1.28	±2.05	±9.16	±11.53	±2.66
15	Niemis Irre	7	-	201	35	224	102	24.3	3.1	20.6	8.0	6.43	30.86	57.11	12.03
	830 kg			± 1.46	±3.68	±18.0	±10.52	±16.44	±0.80	±3.53	±2.57	±1.49	± 9.38	±12.21	±3.01
18	Toras Jarl	8	-	201	38	230	109	266	2.6	14.9	6.5	5.46	24.30	66.22	9.47
	875 kg			±1.31	±6.42	±23.0	±11.31	±16.94	±0.64	±5.41	±1.92	±1.52	±7.58	±9.97	±2.75
13	Keltin Heitto	7	-	201	44	225	102	32.1	2.2	10.7	5.9	5.42	19.07	72.32	8.32
	850 kg			±1.80	±1.70	±10.0	±4.28	±22.30	±0.49	±3.63	±1.07	±2.18	±4.18	±6.50	±3.68

Table 2c.

No.	Sire	N	Age days at start	Age days at end	Live wt kg at start	Live wt kg at end	Carcass wt kg	Thyroid characteristics: wt g	hist. act.	FH	Eμ	Eg	E%	C%	S%
1969															
28	Räbacka Isak	9	11	200	39	223	104	16.1	3.1	21.5	6.6	4.81	30.91	56.89	12.20
	870 kg		± 3.38	±1.20	±2.77	±17.50	±10.76	±3.59	±0.53	±5.44	±0.66	±0.66	±6.65	±7.44	±1.86
27	Päkärin Lykky	10	12	200	37	220	104	18.6	2.9	18.7	6.5	5.05	28.45	60.44	11.11
	1000 kg		±3.64	±2.67	±2.99	±22.94	±14.18	±5.51	±0.52	±5.05	±1.37	±0.98	±7.04	±7.01	±2.79
24	Pirkkulan Lalli	8	13	200	42	240	112	20.4	2.9	19.3	7.1	5.58	31.75	56.08	12.17
	910 kg		±4.96	±1.51	±3.91	±17.84	±8.65	±14.82	±0.50	±4.21	±0.97	±1.55	±7.68	±10.19	±3.66
22	Kopralan Hukava	10	7	200	33	207	97	22.1	2.6	18.6	6.4	5.32	25.42	64.00	10.58
	850 kg		±2.40	±1.47	±7.04	±18.47	±8.59	±6.31	±0.61	±6.87	±0.94	±0.73	±5.84	±7.06	±2.43
19	Alhan Maine	11	11	200	34	227	107	22.9	2.7	19.4	6.6	6.20	28.29	59.93	11.78
	850 kg		±3.77	±1.67	±3.45	±13.05	±5.64	±10.63	±0.46	±5.67	±1.15	±1.62	±7.63	±9.93	±4.35
29	Ylikosken Naila	10	10	200	36	222	104	23.0	2.6	18.2	6.2	6.39	29.84	61.37	8.79
	860 kg		±3.39	±1.40	±2.86	±20.58	±11.98	±12.19	±0.44	±3.89	±1.46	±1.85	±5.54	±6.19	±1.82
20	Alhan Opa	10	11	200	37	232	109	24.0	2.8	14.7	7.0	5.63	26.36	62.67	10.98
	875 kg		±5.68	±1.29	±3.61	±29.11	±16.04	±12.11	±0.59	±5.09	±1.36	±1.35	±7.29	±10.33	±3.93
26	Pukin Kirta	8	10	201	34	217	104	26.2	2.6	18.5	6.3	6.68	26.81	63.39	9.80
	690 kg		±2.93	±4.22	±4.41	±20.01	±11.54	±11.33	±0.35	±4.41	±1.16	±1.99	±4.36	±6.52	±3.00
25	Pirkkulan Orri	11	9	200	34	226	106	26.3	3.1	18.2	7.3	7.57	30.59	57.55	11.86
	825 kg		±2.34	±1.66	±3.20	±17.21	±10.92	±11.44	±0.63	±3.99	±1.70	±2.36	±7.57	±7.95	±2.85
21	Kirran Marski	7	10	200	37	244	115	28.0	3.3	26.2	6.7	8.58	37.75	51.84	10.41
	1000 kg		±5.91	±3.36	±5.77	±21.44	±8.78	±24.12	±0.91	±13.46	±1.00	±3.18	±12.82	±14.55	±2.31
23	Marttilan Lukko	11	12	200	34	215	100	29.8	2.4	14.7	5.9	5.92	21.51	67.74	10.75
	900 kg		±4.46	±1.19	±4.07	±14.73	±8.37	±19.61	±0.50	±4.35	±1.30	±3.62	±5.50	±8.28	±3.54

Table 2d.

No.	Sire	N	Age days at start	Age days at end	Live wt kg at start	Live wt kg at end	Carcass wt kg	Thyroid characteristics: wt g	hist. act.	FW	Ep	Eg	E%	C%	S%
1970															
34	Lotilan Loordi	11	13	201	42	243	115	16.0	2.9	20.7	9.9	5.51	34.72	55.54	9.74
	1230 kg		±5.00	±2.47	±5.71	±24.41	±12.41	±2.20	±0.61	±6.74	±3.49	±0.94	±5.21	±6.42	±1.90
31	Jaakkolan Linssi	10	10	201	38	242	112	16.6	2.8	18.7	7.9	4.35	27.31	63.95	8.73
	1030 kg		±5.03	±1.91	±5.32	±36.54	±12.82	±6.65	±0.63	±6.68	±2.29	±1.58	±8.00	±9.29	±2.69
33	Livius	12	11	201	40	253	122	17.2	3.2	21.2	9.6	5.55	31.94	55.11	12.94
	1010 kg		±4.37	±1.99	±4.53	±21.97	±13.76	±4.17	±0.75	±7.28	±2.20	±2.03	±8.30	±10.18	±3.09
35	Punanlenen Maksu	10	9	200	38	238	114	19.1	3.0	23.3	10.3	5.05	31.00	56.27	12.73
	900 kg		±2.84	±1.45	±3.45	±14.89	±9.78	±14.00	±0.82	±10.58	±3.48	±1.54	±9.45	±11.21	±3.32
32	Kestitalon Mister	12	13	201	37	248	118	20.4	3.3	18.7	10.5	6.59	33.30	55.76	10.94
	920 kg		±4.42	±2.33	±5.89	±26.79	±10.31	±5.34	±0.69	±4.96	±2.87	±1.35	±6.48	±8.14	±2.66
38	Tuomelan Mahti	11	7	200	38	239	117	21.6	2.7	23.5	7.1	5.00	29.90	60.34	9.76
	900 kg		±3.40	±1.58	±5.13	±15.62	±10.84	±16.66	±0.34	±5.18	±1.44	±4.55	±4.20	±4.49	±1.61
39	Äijänsillan Mortis	11	8	200	35	236	115	23.5	2.6	17.9	7.5	5.59	25.53	65.66	8.81
	850 kg		±2.91	±1.33	±5.30	±22.21	±11.72	±12.84	±0.54	±3.09	±2.23	±2.17	±5.78	±6.08	±1.54
36	Pakas Myklos	10	9	200	32	209	99	25.4	3.0	20.1	9.2	6.16	29.27	60.47	10.27
	750 kg		±3.11	±1.20	±5.07	±17.44	±10.70	±21.96	±0.58	±5.32	±3.05	±3.14	±8.93	±10.29	±2.24
30	Brönberga Marius	11	8	200	38	230	111	28.5	2.6	19.7	7.5	5.86	26.02	64.28	9.70
	850 kg		±2.41	±1.78	±4.55	±23.63	±14.68	±29.81	±0.55	±7.46	±2.55	±3.57	±7.44	±9.08	±2.20
37	Päivärinnan Lempo	9	8	197	35	226	108	39.2	2.4	14.4	6.8	7.13	21.66	70.44	7.90
	850 kg		±3.44	±10.17	±7.46	±27.33	±17.52	±31.53	±0.78	±6.67	±2.11	±4.10	±8.42	±10.56	±2.66

Table 2e.

No.	Sire	N	Age days at start	Age days at end	Live wt kg at start	Live wt kg at end	Carcass wt kg	Thyroid characteristics: wt g	hist. act.	FN	Ep	Eg	E%	C%	S%
1971															
40	Pajulan Nosto 900 kg	8	14,0	220,5 ±3,16	39,0 ±5,07	225,8 ±15,15	109,6 ±9,47	13,73 ±3,16	2,5 ±0,60	11,30	8,75	3,37	25,51	69,36	5,10
41	Saaren Nero 1005 kg	4	10,8	222,3 ±1,71	44,3 ±5,38	252,8 ±20,26	121,3 ±7,09	14,00 ±4,75	2,9 ±0,10	11,75	8,75	4,06	29,73	66,70	3,58
42	Fiskars Nunner 880 kg	9	7,2	214,5 ±2,74	36,2 ±3,63	220,6 ±15,63	102,6 ±8,65	14,26 ±3,90	2,3 ±0,75	10,57	8,24	3,25	23,19	71,6	5,20
43	Metsä-Paavolan Nekka 860 kg	12	9,6	216,8 ±2,86	39,7 ±4,08	232,8 ±26,12	107,2 ±15,93	15,08 ±3,95	2,3 ±0,81	10,30	8,35	3,51	24,43	71,43	4,15
44	Råbacka Niklas 820 kg	12	8,8	215,2 ±4,41	38,0 ±3,02	220,8 ±17,75	104,0 ±10,77	15,14 ±4,34	2,7 ±0,92	10,29	8,68	3,52	24,10	69,93	5,96
45	Högnäs Nalliman 890 kg	15	10,3	215,5 ±3,11	38,0 ±5,18	221,3 ±26,69	105,7 ±15,34	15,49 ±5,75	2,4 ±0,92	12,43	7,95	3,57	24,40	69,68	5,47
46	Pajulan Naskali	11	14,2	221,4 ±3,93	40,6 ±4,52	248,3 ±23,41	122,7 ±13,73	15,68 ±3,91	2,9 ±0,52	13,59	9,60	4,25	26,82	66,15	6,97
47	Suomiehen Nosteri 941 kg	12	13,3	220,3 ±2,38	37,9 ±5,16	238,3 ±18,30	114,2 ±10,02	16,28 ±3,59	2,3 ±0,58	11,42	8,33	3,69	23,33	71,44	5,13
48	Sjöholms Nasser 780 kg	8	9,6	216,0 ±2,56	35,8 ±5,55	212,3 ±20,53	97,1 ±9,94	17,76 ±10,77	2,2 ±1,03	10,14	8,53	3,52	22,93	73,28	3,8
49	Ainontapon Olli 921 kg	12	10,3	221,5 ±4,38	36,6 ±4,78	244,3 ±30,15	115,3 ±15,26	18,59 ±6,28	2,1 ±0,80	11,33	7,53	4,02	23,16	72,53	4,3
50	Uusitalon Nafta 820 kg	13	8,4	214,2 ±3,34	35,5 ±4,29	221,9 ±30,79	106,3 ±13,20	20,29 ±11,57	2,1 ±0,89	10,29	7,85	3,97	22,80	72,38	5,35
	$\bar{x}$	116	10,53	217,7 ±4,34	37,97 ±4,82	229,9 ±25,50	109,2 ±14,03	16,256 ±6,39	2,4 ±0,80	11,29	8,897	3,701	24,19	70,60	5,13

Table 3. Simple correlations between age, live weight, carcass weight and thyroid characteristics at different years

		Live weight start	Live weight end	Carcass wt	Live wt of sire	Thyroid wt	Hist. act.	PH	Eμ	Eg	E%	C%	S%
1967	Age at end	-.178	.044	.034	-.049	-.038	-.100	-.037	-.115	-.103	-.107	.084	.009
	Live wt at start		.538	.551	.046	.097	-.091	.122	.020	.030	-.147	.095	.085
	" " at end			.944	-.078	.097	-.129	.071	-.064	.002	-.181	.112	.120
	Carcass wt				-.003	.098	-.143	.059	-.060	.022	-.159	.109	.071
	Live wt of sire					.253	.045	.166	.013	.336	.160	-.124	-.023
1968	Age at end	.156	.330	.239	.011	-.064	-.010	-.042	-.034	.063	-.031	-.019	-.002
	Live wt at start		.583	.508	.204	.449	-.362	-.230	-.282	.216	-.375	.415	-.411
	" " at end			.954	-.065	.205	-.093	.045	-.065	.224	-.047	.054	.053
	Carcass wt				-.146	.173	-.108	.060	-.065	.183	-.062	.069	-.063
	Live wt of sire					.280	-.079	-.286	.042	.255	-.094	.125	-.187
1969	Age at end	.091	.104	.127	-.097	.019	-.033	-.139	.033	.020	-.045	.055	-.050
	Live wt at start		.582	.502	.187	-.233	.227	.081	.086	-.191	.192	-.230	.202
	" " at end			.956	.162	-.034	.295	.163	.145	.154	.271	-.303	.222
	Carcass wt				.108	-.032	.268	.147	.138	.140	.236	-.261	.183
	Live wt of sire					-.058	.144	.118	.016	-.046	.143	-.139	.055
1970	Age at end	.194	.169	.156	.152	.002	-.007	.045	-.032	-.013	-.049	.024	.055
	Live wt at start		.616	.592	.399	-.170	.103	.143	.126	-.045	.176	-.186	.143
	" " at end			.885	.282	-.086	.257	.074	.147	.081	.190	-.188	.109
	Carcass wt				.223	-.059	.194	.099	.109	.098	.174	-.170	.094
	Live wt of sire					-.213	.099	.055	.160	-.090	.257	-.233	.041
1971	Age at end	.139	.246	.247	-	-.046	.148	.027	.199	.176	.125	-.053	-.176
	Live wt at start		.414	.359	-	.040	.055	-.152	.007	.194	.070	-.043	-.073
	" " at end			.937	-	.055	-.002	-.090	-.066	.118	.017	.016	-.085
1967-71	Age at end	.107	.328	.267	-	-.046	.003	-.426	.344	-.030	.051	.111	-.416
1968-70		.164	.154	.135	-	.013	-.018	-.014	-.034	-.004	-.043	.033	.010
	Live wt at start		.509	.476	-	-.065	.022	-.046	.073	-.045	.031	-.012	-.038
			.598	.541	-	-.090	.055	.047	.073	-.078	.063	-.066	.043
	Live wt at end			.934	-	.046	.153	-.017	.209	.183	.177	-.145	.002
				.924	-	-.034	.217	.119	.201	.100	.183	-.180	.094
	Carcass wt				-	.031	.129	.010	.189	.152	.156	-.127	.001
					-	-.024	.178	.132	.201	.098	.166	-.157	.067

Table 4. Simple correlations within thyroid characteristics

	Thyroid wt	Hist. act.	FN	Eμ	Eg	E%	C%
Hist. act.	-.364						
FN	-.158	.466					
Eμ	-.384	.616	-.014				
Eg	.772	.124	.191	-.045			
E%	-.394	.824	.540	.604	.197		
C%	.371	-.836	-.634	-.527	-.202	-.938	
S%	-.136	.449	.530	.096	.115	.339	-.643

N=454 (1967-71)

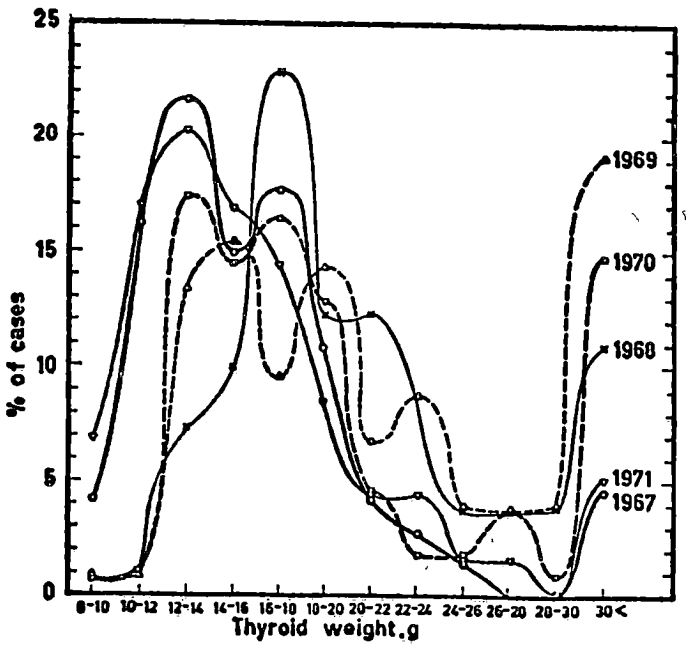
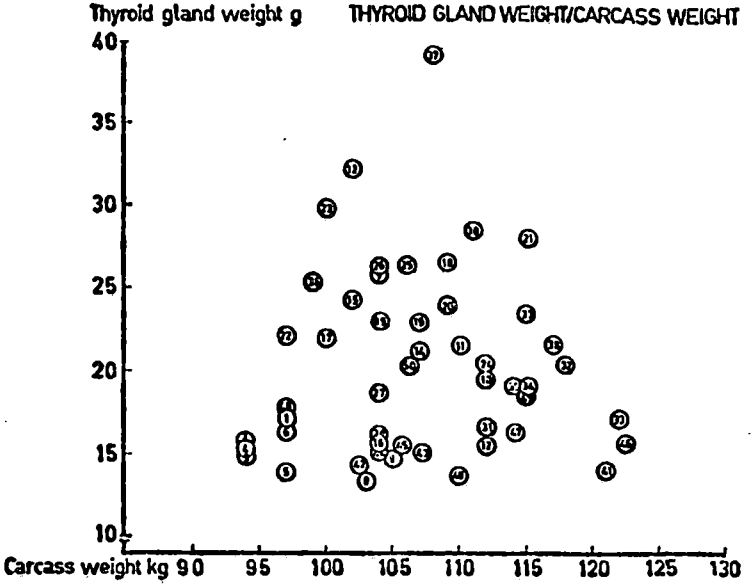


Fig. 1. Frequency distribution of thyroid weight in different years

Fig. 2.


Thyroid gland weight g
THYROID GLAND WEIGHT/CARCASS WEIGHT
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35
30
25
20
15
10
Carcass weight kg 90
95
100
105
110
115
120
125
130

Fig. 3.

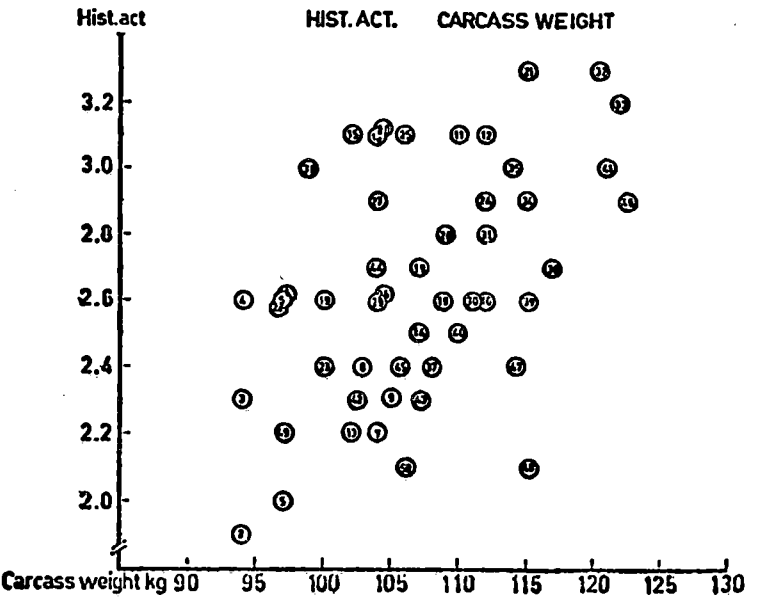

Hist.act
HIST. ACT. CARCASS WEIGHT
3.2
3.0
2.8
2.6
2.4
2.2
2.0
Carcass weight kg 90
95
100
105
110
115
120
125
130

Fig. 4.

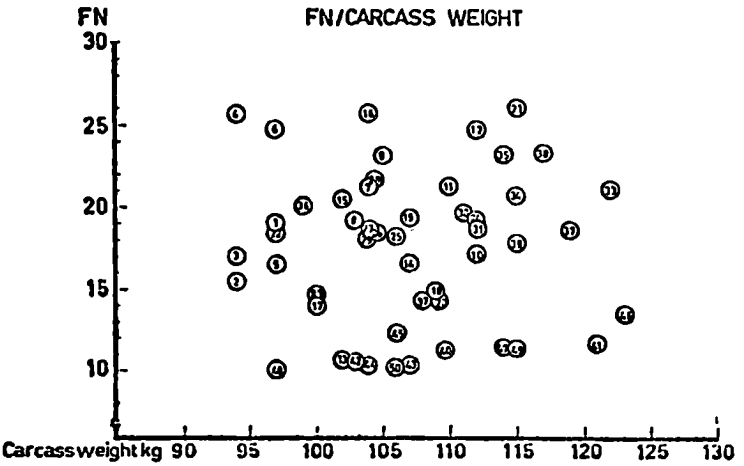

FN
FN/CARCASS WEIGHT
30
25
20
15
10
Carcass weight kg 90
95
100
105
110
115
120
125
130

Fig. 5.

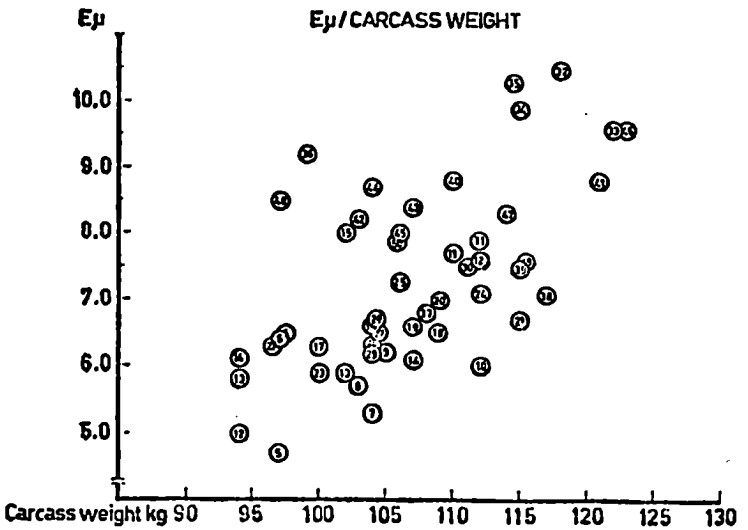

Eμ
Eμ/CARCASS WEIGHT
10.0
9.0
0.0
7.0
6.0
5.0
Carcass weight kg 90
95
100
105
110
115
120
125
130

Fig. 6.

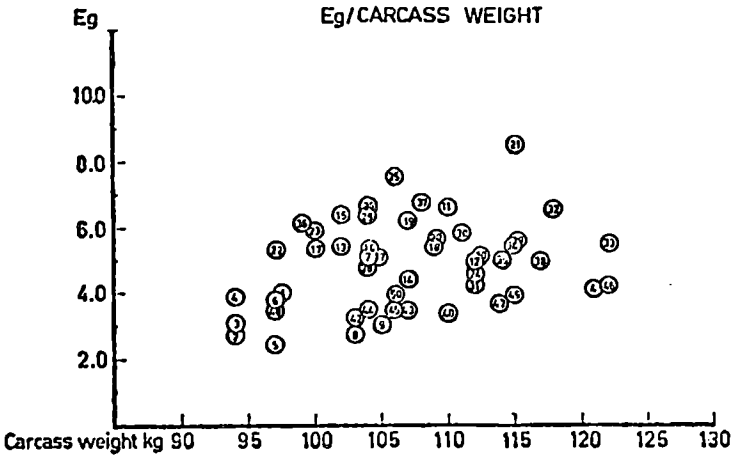
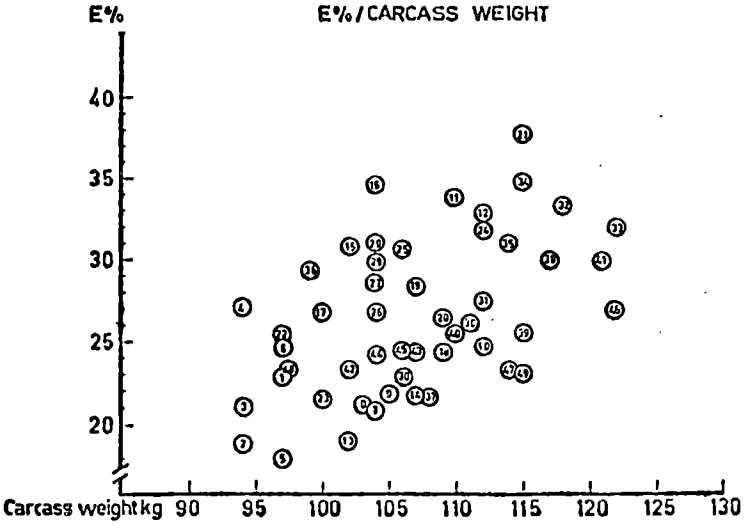

Eg
Eg/CARCASS WEIGHT
10.0
8.0
6.0
4.0
2.0
Carcass weight kg 90
95
100
105
110
115
120
125
130

Fig. 7.


E%
E%/CARCASS WEIGHT
40
35
30
25
20
Carcass weight kg 90
95
100
105
110
115
120
125
130

THYROXINE DEGRADATION RATE AND PLASMA LEVELS OF THYROXINE, FREE THYROXINE AND THYRO-TROPHIN MEASURED IN TEN YOUNG BULLS DURING FEEDING CONDITIONS AND 48 HOURS OF STARVATION.

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Introduction

High yielding lactating cows will be in a state of energy deficit in early lactation. If the energy deficit is severe, or the animals are disposed for it, this will result in ketosis. From a genetic point of view, it is of interest to study the response to energy deficit in bulls. The response can be measured by measuring as many hormone responses and metabolites as possible. Thyroidea hormones are main regulators of metabolic rate, and this paper describes the effect of 48 hours starvation on thyroidea hormones. In another paper, the variation in plasma glucose, acteoacetate, non-esterified fatty acids, insulin and growth hormone in the same animals are described.

Materials and Methods.

Ten bulls of Norwegian Red Breed (NRF) were used in the experiment The age of the bulls varied from 394 to 442 days as the experiments started, and the average weight of the animals was 354 kg (S.D. 16,25). Thyroxine degradation, plasma levels of thyroxine, free thyroxine and thyrotrophin were investigated during periods of feeding and fasting conditions. The animals were fasted for a period of 48 hours.

The bulls were separated into two groups, A and B, of approximately equal average body weights. On day zero all animals were injected 200 µCi of I-131 labeled L-thyroxine dissolved in 50% propylene glycol into the jugular vein. In the first experiment, group A was deprived food on day 1. and 2. (water ad lib) and re-fed on day 3. Group B was fed in the usual manner. Blood samples were taken 22, 34, 46, 58 and 70 hours after the I-131-injection from all animals. Three weeks

later a similar experiment was done where group B was deprived food one day 1. and 2. the experimental period, group A was fed in the usual manner.

Analyses of the thyroxine degradation was made according to the method described by Yousef and Johnson (1967)and modified by Joachimsen et al. (1971).

Plasma levels of thyroxine, free thyroxine and thyrotrophin were measured on most of the samples, and the feeding levels are means of 4 samples of the same animals, taken at 6 a.m.

The hormones were measured by radioimmunoassay, and the results were handled according to Feldman & Rodbard (1971) with a computer programme for logit B/B0 against log dose. In each series, the linearity of the standard curve, the values of 5 control samples and the standard deviation of independent duplicates were checked. The results are means of duplicates.

Thyroxine: The analyses were mainly carried out as described by Larsen et al. (1973). Antiserum was kindly provided by Kruse (1976).

Standard deviations between duplicates varied somewhat in series: 5-12%. Between series: mean of 5 controls was held within a limit of about 5% of expected mean if the series were accepted.

Free thyroxine analyses: 1.5 ml plasma were dialyzed against 1.5 ml buffer. The buffer used was 0.01 M HEPES buffer (N-2-hydroxyethylpiperazine-N'-2'ethane sulfonic acid, Calbiochem) pH 7.4, made isotonic by 0.62% NaCl and with 0.001 M sodium azide as a bacteriostate. This buffer has been shown by Spaulding & Gregerman (1972) not to have any appreciable effect on the dialyzable fraction of thyroxine. The dialysis was performed in teflon chambers with dialyzing membrane between buffer and plasma, 16-18 hours at 37°C, in a shaking water bath. When the dialysis was terminated, 0.5 ml in duplicate was removed from the buffer chamber, and it was assumed that the concentration of thyroxine in the buffer was equal to the concentration of free thyroxine in the plasma, that is 50% of the endogenous concentration. Because of the low concentration, the radioimmunoassay had to be modified. The buffer contained 0.2% of gelatine instead of albumin to prevent adsorption. This because the reaction between antigen and antibody in the low concentration was slower in precence of albumin. Antigen and antibody was incubated 2 hours at 37°C, then the isotope

was added, and the tubes left at 4°C overnight. Dialysate of serum stripped from thyroxine by charcoal was added to the standards. Blanks of standards and controls were included. Antiserum was used in a final dilution of 1:250 000, and about 0.02 ng isotope was added to each tube, with total volume 1.0 ml. As isotope was used I-125-Thyroxine from the Radiochemical Centre, Amersham, with spesific activity >200 mCi/mg.

The antibody-bound antigen was separated from unbound by adding 1 ml Charcoal 0.5% (Norit A) suspended in buffer containing thyroxine free serum 2%, sodium salicylate 2% and dextran T-70 0.1%. The suspension was centrifugated after ½ hour, and the bound fraction counted. The calculating was made as for total thyroxine. Standard deviations between duplicates within series: 10-15%. Menan of 5 controls between series: Within about 5% of expected mean.

Thyrotrophin. The immunoassay was mainly carried out as described by Pekary & al. (1975) and Torjesen & al. (1973). The immunistation procedure was carried out as described by Pekary & al. (1975). In the initial immunizations were used the commercial available bovine thyrotrophin called Actyron (Ferring). The potency was 1 U/mg. In the last booster, we used bTSH supplied by NIAMDD, Maryland School of Medicine. The potency of this was 1.70 USP units/mg. Our results are expressed as microunits/ml plasma, with NIAMDD bTSH as standard.

Iodination procedure: 5µg bTSH were iodinated with 1 mCi Na-J-125, with sodium hypochlorite as reducing agent as decribed by Redshaw & Lynch (1974). As we obtained a maximum binding of about only 50% with the bTSH from NIAMDD, we assumed that a higher degree of purity would be preferable. From UCB, Bioproducts, Peptide Dept., Bruxelles. we obtained a praparation of bTSH, and when this was iodinated in the same way, we got a maximum binding of about 90%. Prior to each assay, the iodinated hormone was purified on a coulomn of Ultrogel AcA 54, LKB.

Antiserum was used in final dilution 1:50 000. To avoid problems with aggregates in the antiserum, dilutions of 1:25 000 were stored 1-6 days in refrigerator prior to use. Standard tubes did not contain serum of plasma, as low thyrotrophin-blood was not available. Test-tubes containing 0.1 ml standards or unknown were incubated over night with antiserum in refrigerator. Blanks of standards, control serum and control plasma were included.

The next day, isotope was added, and the tubes incubated in the refrigerator two days. Separation of bound from unbound was performed by adding 1.0 ml 5% charcoal (Norit A) with 0.5% Dextran T-70. This was incubatet 1 hour. The standard tubes were added serum or plasma 0.1 ml immediately prior to charcoal.
Serum or plasma used for this purpose gave insignificant difference in the binding of isotope. But plasma incubated two days with isotope gave higher unspesific binding than serum.

This phenomenon has been described by others e.g. Freeland (1971) The only way we could correct for it, was to use as control serum and plasma from the same animal, and use the difference in counts between serum and plasma as a correction for plasma unknowns.

Test of cross-reaction: Bovine LH was kindly provided by Dr. Andresen, NVH. We found some cross-reaction with this preparation. But the bLH might contain bTSH. As no peaks of high thyrotrophin occured in the material, we assumed that the cross-reaction with LH was not a problem in this material. Standard deviation between duplicates: 10-15%.

Results.

The results of the analyses of plasma levels of total thyroxine, free thyroxine and thyrotrophin are presented in table 1 as means for the ten bulls. After 24 hours of starvation, all the three parameters were significantly decreased. From 24 hours to 48 hours, the values were more decreased, but for thyrotrophin the decrease was not significant at the 5% level. After 48 hours of starvation, total thyroxine plasma level was 75% of normal, free thyroxine was 54% of normal and thyrotrophin was 84% of normal.

The correlation coeffisients were below 0.5 for the following parameters: Total and free thyroxine at feeding and starvation, free thyroxine and thyrotrophin at feeding and starvation, and for total thyroxine and thyrotrpohin at feeding and starvation.

The results of the measurements of thyroxine degradation and distribution space are presented in Table 2. The difference of distribution space at feeding and starvation is not significant. Therefore the distribution space for each animal is calculated as mean of the two measurements for use in table 3. Thyroxine fractional turnover rate is significant lowered when measured during a period of 48 hours starvation. The mehtod allows not measurements each day.

Therefore the figures represent a mean during the starvation period. The connection between halflife and K: $t_{\frac{1}{2}}=0.693/K$. Mean half life for thyroxine for the ten bulls at feeding: 52.5 hours. At starvation: 71 hours.

The thyroxine degradation after 24 and 48 hours of starvation is calculated on the basis of the mean fractional turnover for the starvation period. Table 3 shows that total thyroxine/animal decreases during starvation, at the same time as thyroxine half life is increased.

In table 4, the thyroxine degradation/day is compared with the decrease in total thyroxine/day. Thyroxine secretion from thyroidea is calculated as the difference between thyroxine degradation and the change in total thyroxine content in the animal.

Discussion.

Levels of plasma thyroxine are lower during lactation than during dry period for cows (Heitzmann & Mallinson, 1972). The higher level in the late pregnancy compared to early lactation period , could be due to a higher level of thyroxine binding protein in plasma, as seen during human pregnancy. The plasma thyroxine is raised 50-100%, and falls in threatened abortion (Eastham, 1967).

But this seem not to be the case for cows. Heitzman & Mallinson (1972) have found only slightly higher plasma thyroxine in late pregnancy as compared to dry and non-pregnant cows. But the values are significant lowered in early lactation. Cows which became acetonaemic during early lactation, had a mean thyroxine plasma concentration of only 40% of the mean for cows which not became acetonaemic. Tey also starved some cows in early lactation for 6 days, and mean plasma thyroxine were reduced to 70% of normal cows in the same stage of lactation.

Hart et al. (1977) also found lower plasma thyroxin during early lactation than during dry period, and lower values in high yielding than low yielding cattle.

Change in plasma concentration of thyroxine can also be caused by change in thyroxin secretion and degredation, if these are not changed in the same manner.

In rats made hypothyroid by thyroid ectomy, Cull et al. (1973) have shown that total thyroxin clearance rate was decreased, but distribution space was unchanged. In animals made thyrotoxic by thyroxine administration,total thyroxine clearance was doubled, but

distribution speace only slightly increased. Astier & al. (1972) have shown that thyroxin degredation rate in ducks is lowered by iodine deficiant diet, undernutrition and by elevated environmental temperature. Yousef & Johnson (1975) have shown that low thyroxine degredation associated with reduced metabolic rate represents an adaption mechanism for survival of desert rodents. Burger (1978) has shown that plasma T_3 is decreased by starvation in rats. Increase after refeeding represents de novo production of T_3. This increase in T_3 production is neither induced by the increase in availability of calories, nor causally related to alternations in blood glucose or ketone bodies. But the data suggest a relationship between the cytoplasmic redox state and the control of T_3 production.

On the basis of the figures in table 4, it can be calucated that the thyroxine degradation per day is 62% of normal after one day of starvation and 55% of normal after 2 days in the ten bulls.

But the secretion of thyroxine from thyroidea is still more decreased: 52% of normal after one day and 30% after two days.

The decrease in thyroxine concentration in plasma can be explained as a greater decrease in thyroxine secretion from thyroidea than the decrease in thyroxine degradation.

The concentration of total thyroxine in plasma decreased to 75% of normal, but the concentration of free thyroxine decreased to 54% of the normal after 48 hours of starvation. In per cent of total, the free thyroxine decreased from 0.048 to 0.035. Therefore there is no indication of decrease in thyroxine-binding proteins.

The starvation induces a decrease in all thyroid activity parameters: plasma total thyroxine, plasma free thyroxine, thyroxine turnover, thyroxine pool in the body, and plasma thyrotrophin. Low plasma thyrotrophin is in accordance to Campbell et al. (1977), they have shown decrased release of 5 anterior pituitary hormones in starved rats, and this was shown to be due to reduced hypotalamic stimulation.

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Table 1. Comparison of mean consentrations of thyroidea hormones in plasma of 10 young bulls during feeding and starvation.

	Feeding		Starvation 24 h.		Starvation 48 h.	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Total Thyroxine nanomol/l	77.1	8.1	65.5	7.8	57.5	9.0
Free Thyroxine picomol/l	37.0	6.2	24.1	4.7	20.1	5.4
Thyrotrophin uU/ml	24.1	9.3	22.3	8.0	21.0	8.7

S.D.= Standard deviation of individual observation.
The mean of differences between thyrotrophin at 24 and 48 hours of starvation is not significantly different from zero. All the other means of differences are significantly different from zero with $p<0.05$.

Table 2. Comparison of means of thyroxine fractional turnover rate (K) and thyroxine distribution space (TDS) in 10 young bulls during feeding and 48 hours of starvation.

	Feeding		Starvation	
	Mean	S.D.	Mean	S.D.
K/day	0.317	0.061	0.234	0.064
TDS l/animal	36.0	5.1	36.8	6.0
TDS l/100 kg	10.2			

S.D.= Standard deviations of individual observations.
The mean of differences between K at feeding and starvation is significantly different from zero, $p<0.05$
The mean of differences for (TDS) at feeding and starvation is not significantly different from zero.

Tabel 3. Comparison of means of total thyroxine content and thyroxine degradation (TD) during feeding and starvation.

	Feeding	Starvation 24 hours	Starvation 48 hours
Thyroxine Distribution Space (l)	36.9	36.9	36.9
Thyroxine conc. nanomol/l plasma	77.1	65.5	57.5
Total Thyroxine nanomol/animal	2845	2417	2122
Total thyroxine nanomol/100 kg	803.7		
K/day	0.317	0.234	0.234
Thyroxine degradation/day	902	565	497
Thyroxine degradation/day/100 kg	254.8		

Table 4. Comparison of thyroxine degradation and total thyroxine during feeding and starvation in 10 young bulls

		Starvation		
	Feeding	0-24 hours	24-48 hours	48 hours
Thyroxine degradation nanomol/day	902	902	565	497
Decrease in total thyroxine/day	0	428	295	
Thyroxine secretion nanomol/day	902	474	270	

GENETIC VARIATION IN THYROXINE DEGRADATION RATE AND PLASMA CHOLESTEROL CONCENTRATION IN CATTLE

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INTRODUCTION

The role of cholesterol in animal metabolism is of great importance. It acts as a constituent of the cell membranes and as a precursor of the steroid hormones. Cholesterol levels in blood plasma of cattle are influenced by both genetic and environmental factors, as described earlier (Edfors-Lilja et al., 1978).

The thyroid gland is involved in regulating the rate of metabolism by means of the hormones thyroxine and tri-iodothyronine. Reviews of the thyroid's function in this respect have recently been published by Bernal & Refetoff (1977) and Schaar (1978). It has been demonstrated that variation in thyroid activity is to a certain extent genetically controlled (Joakimsen et al., 1971; Bard, 1973; Shire, 1976; Stewart et al., 1978). In cattle, this activity is additionally influenced by breed (Nyberg, 1970; Doornenbal, 1977), age (Mixner et al., 1966; Anderson et al., 1973; Kahl et al., 1977) and season (Yousef & Johnson, 1966; Vanjonack & Johnson, 1975). The thyroxine degradation rate is taken to be a measure of thyroid activity.

The present report is a summary of two investigations (Edfors-Lilja et al., 1978; Edfors-Lilja et al., 1979) whose common purpose was chiefly to estimate the genetic variation in cholesterol concentration and thyroid activity and in addition to establish their relation to growth rate.

MATERIAL AND METHODS

Animal and chemical methods

The animal material consisted of two parts. The first was comprised of 244 calves of both sexes, and of two breeds, Swedish Red and White (SRB) and Swedish Friesian (SLB). This material is described in detail by Edfors-Lilja et al. (1978). The other part comprised 247 young bulls, again of two breeds, Danish Red (RDM) and Black Pied Danish (SDM). These animals were completing their performance testing at the Danish testing station Egtved. The treatment of the bulls was described earlier in detail by Lykke et al. (1974), Lykke et al. (1975) and Andersen et al. (1977).

Data were collected from March 1971 to September 1976 for the Swedish calves, and from June 1972 to August 1975 for the Danish bulls. From the Swedish calves, blood samples for cholesterol determinations were collected in heparinized tubes, one to three times per animal, at 5, 10 and 16 months of age. The samples were taken between 8 and 10 a.m. From the Danish bulls, two blood samples per day were taken for the cholesterol determinations, one in the morning and the other in the evening. This sampling was repeated one to three times per animal, between 4 and 10 months of age. Cholesterol was determined by an enzymatic colorimetric test (cat.no. 15738, Boehringer, Mannheim, West Germany). The thyroxine parameters were those established by Kruse et al. (1976) with a modification of the labelled thyroxine turnover method by Ingbar & Freinkel (1955), whereby the thyroxine concentration is calculated as the average of the concentrations in four consecutive blood samples. The thyroxine parameters were determined in the calves at the same ages as the cholesterol determinations and were calculated both per animal and per 100 kg body weight.

The growth rates of the Swedish calves were registered between birth and blood sampling, and for the Danish bulls between 42 days of age and blood sampling, respectively between 42 and

336 days of age. The relative growth rate (growth rate divided by body weight) between 42 days of age and blood sampling was also calculated for the Danish bulls. The routine for performance testing was changed somewhat at the beginning of 1974 and the material was divided in two parts for that reason.

Statistical methods

The genetic parameters were calculated by the method of least-squares analysis of data, with unequal subclass numbers, using the LSMLMM program (Harvey, 1972). All other calculations were carried out by the Statistical Analysis System (Barr et al., 1976).

The statistical methods applied to the Swedish data are described in detail by Edfors-Lilja et al. (1978). Due to the complicated nesting it was not possible to include the effect of sex in the statistical model. All estimates were therefore calculated for each sex separately. The effects of sire, twin pair, animal, and age class were studied. The effects of twin pair and animal were regarded as random and the other effects as fixed. The repeatability of cholesterol concentration was estimated as the intraclass correlation (t) calculated from the components of variance in the following way:

$$t = (\sigma_t^2 + \sigma_a^2) / (\sigma_t^2 + \sigma_a^2 + \sigma_e^2)$$

where

σ_t^2 = component of variance for twin pair
σ_a^2 = component of variance for animal
σ_e^2 = residual variance

The statistical analysis of the Danish data is described in detail by Edfors-Lilja et al. (1979). The effects of breed, sire, animal, and regression on age, in both linear and quadratic form, were studied. The effects of sire and animal were regarded as random and the effect of breed as fixed. Again due to the complicated nesting, the calculations were done separately

for the two parts (1972-1974 and 1974-1975). The sums of squares were then pooled. The repeatabilities of cholesterol concentration and the thyroxine parameters were estimated as the intraclass correlation (t) calculated from the components of variance in the following way:

$$t = (\sigma_s^2 + \sigma_a^2) / (\sigma_s^2 + \sigma_a^2 + \sigma_e^2)$$

where the elements are defined as before, and in addition:

σ_s^2 = component of variance for sire.

In both animal materials, the genetic parameters were calculated from the separate observations at all ages and from the averages of the three ages. The Danish data were precorrected for the effect of age when the average of the ages was used.

RESULTS

The effects of sex, breed and age on plasma cholesterol concentration and the thyroxine parameters are shown in Table 1, expressed as overall means and standard deviations. Cholesterol concentrations were significantly influenced by sex, with higher levels in heifers than in bulls. Cholesterol concentration and the thyroxine parameters, when calculated per animal, increased significantly with increasing age. The thyroxine parameters decreased with increasing age, however, when calculated per 100 kg body weight. No significant difference was found between the Swedish breeds as regards cholesterol concentration, while RDM had significantly higher levels than SDM. There were significant breed differences in thyroxine concentration, distribution volume and degradation rate when estimated per animal, though these differences were less prominent when the parameters were calculated per 100 kg body weight.

The estimates of repeatability for cholesterol concentration were affected by sex (Table 2), where the heifers had a higher repeatability than the Swedish bulls. The repeatability estimates for cholesterol level were also higher for the Danish than

the Swedish bulls. There were some differences in repeatabilities between the two Danish breeds but none was significant. The repeatabilities were somewhat lower for thyroxine distribution volume and concentration calculated per 100 kg body weight compared with the overall values. These differences were not significant, however. The heritability estimates for cholesterol concentration were influenced by sex, with higher estimates for heifers than for bulls. They also varied between the two materials, with higher estimates for Swedish than for Danish bulls (Table 3). The heritabilities also varied somewhat for the Danish breeds. Thyroxine distribution volume and degradation rate calculated per 100 kg live weight had lower heritability estimates than total thyroxine distribution volume and degradation rate, while contrary results were obtained for thyroxine concentration and degradation coefficient. None of these differences in heritability were significant, however.

The differences between the sexes were also prominent for the genetic correlations between cholesterol concentration and growth rate, with higher estimates for bulls than for heifers (Table 4). The genetic correlations between growth rate and cholesterol concentration were higher for the Danish than the Swedish bulls (Tables 4 and 5). The correlations of the thyroxine parameters with growth rate and relative growth rate, respectively, are shown in Table 5. The correlation between the different parameters calculated per animal and per 100 kg body weight were high, between 0.76±0.45 and 1.24±0.37.

DISCUSSION

The two animal materials differ from each other, for example, as regards age structure. Consequently the results can thus not be compared without reservation. For the Swedish calves the effect of sire was regarded as fixed and the genetic parameters were therefore estimated from the twin pair component in which both dominance and epistatic variance are included. The additive genetic fraction is therefore overestimated,

which could explain the high heritability estimates. Because of the complicated nesting, the genetic parameters in the Danish material were estimated with two separate statistical models for all ages and for the average of ages. This is discussed elsewhere in greater detail (Edfors-Lilja et al., 1979).

The method used for determinating thyroxine degradation rate is based on the isotope dilution principle and estimation of labelled thyroxine turnover rates. This method is not irreproachable, as was discussed by Irvine (1975) and Ślebodziński (1977). The thyroxine degradation rate is assumed to reflect the thyroid activity. Irvine (1969) found, however, that about 60% of the thyroxine that leaves plasma in sheep is not degraded and can therefore be assumed to have a negligible metabolic effect.

The high genetic correlations between the thyroxine parameters estimated per animal and per 100 kg body weight, from 0.76 to 1.0, respectively, indicate that the Danish bulls were fairly homogeneous as regards body weight. The breed differences, with lower thyroxine distribution volume, concentration and degradation rate for RDM than for SDM, were also less prominent when the thyroxine parameters were estimated per 100 kg body weight. Higher values for metabolic activity are often found in Jersey cattle than in other dairy breeds (Nyberg, 1970; Doornenbal, 1977). However, less prominent differences can be expected between the breeds RDM and SDM which are rather similar in size and production traits.

There were breed differences in cholesterol concentration in the Danish calves. RDM had a higher cholesterol level than SDM during the first period studied (1972-74). In the Swedish material no differences between pure SRB and crosses (SRB x SLB) were found.

The cholesterol concentration increased with increasing age in both animal materials, thus agreeing with the results of Tumbleson & Hutcheson (1971), Mi et al. (1973) and Arave et al.

(1975). The age influence on the thyroxine parameters (higher total thyroxine concentration and degradation rate but lower thyroxine concentration and degradation rate per 100 kg body weight with increasing age) agrees with earlier findings by Falconer & Robertson (1961), Mixner et al. (1966), Anderson et al. (1973) and Kahl et al. (1977).

The diurnal variation in cholesterol concentration, i.e. the difference between morning and evening samples, was also studied. No significant differences were found, again agreeing with earlier results (Lennon & Mixner, 1957).

The blood parameters studied had rather high repeatability and heritability estimates, indicating that they are genetically controlled. The genetic correlations between the blood parameters and growth rate are of interest as the blood parameters are involved in the growth process in different ways.

The genetic correlations with growth rate were found to be high in the material studied. A genetic correlation does not, however, explain the underlying causes of the connection found. An indirect selection for growth rate by selecting for the blood parameters studied therefore seems unrealistic at present, even if it is theoretically possible.

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Table 1. *Overall means ($\bar{x}$) and standard deviations (S.D.) for the blood parameters studied, and for absolute and relative growth rates*

Trait		Sex	SRB and SRB x SLB, age in months (n = 247)						RDM (n = 138)		SDM (n = 109)	
			5		10		16					
			$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Cholesterol concentration, mg/100 ml		bulls	95.8	22.8	107.6	29.3	128.9	37.6	79.4	23.3	70.4	18.4
		heifers	102.5	24.6	115.0	27.2	132.5	29.7				
Thyroxine distribution volume, litres	Total	bulls							35.44	7.41	34.98	7.44
	Per 100 kg	bulls							13.18	1.79	13.16	1.61
Thyroxine concentration nmol/l	Total	bulls							86.86	12.33	94.80	13.87
	Per 100 kg	bulls							33.69	9.52	37.42	11.25
Thyroxine degradation coeff., day^{-1}	Total	bulls							0.35	0.05	0.34	0.04
	Per 100 kg	bulls							0.14	0.05	0.14	0.05
Thyroxine degradation rate, µmol/day	Total	bulls							1.06	0.29	1.12	0.30
	Per 100 kg	bulls							0.39	0.07	0.42	0.07
Growth rate[a], g/day		bulls	750	105	970	86	994	76	962	134	985	137
		heifers	648	87	721	73	735	75				
Relative growth rate[b], %		bulls							0.50	0.15	0.52	0.16

[a] SRB and SRB x SLB: from birth to blood sampling; RDM and SDM: from 42 days to blood sampling.

[b] Relative growth rate at blood sampling.

Table 2. *Repeatabilities estimated as intraclass correlations (± standard errors) for the blood parameters studied*

Trait	SRB and SRB x SLB		RDM	SDM
	Bulls	Heifers	Bulls	Bulls
Cholesterol concentration	0.21±0.06	0.41±0.07	0.32±0.09	0.51±0.08
Thyroxine distribution volume				
Total			0.10±0.10	0.21±0.10
Per 100 kg			0.00±0.10	0.00±0.12
Thyroxine concentration				
Total			0.61±0.06	0.62±0.06
Per 100 kg			0.55±0.07	0.62±0.06
Thyroxine degradation coefficient				
Total			0.29±0.09	0.41±0.09
Per 100 kg			0.45±0.08	0.32±0.10
Thyroxine degradation rate				
Total			0.33±0.09	0.34±0.10
Per 100 kg			0.27±0.09	0.37±0.09

Table 3. *Estimates of heritability (± standard errors) for the blood parameters studied*

Trait	Sex	SRB and SRB x SLB		RDM and SDM	
		All ages	Average of the ages	All ages	Average of the ages
Cholesterol concentration	bulls	0.55±0.24	0.78±0.28	0.37±0.27	0.33±0.28
	heifers	0.80±0.29			
Thyroxine distribution volume					
Total	bulls			0.53±0.28	0.65±0.28
Per 100 kg	bulls			0.15±0.26	0.39±0.28
Thyroxine concentration					
Total	bulls			0.59±0.28	0.85±0.29
Per 100 kg	bulls			0.65±0.28	0.85±0.29
Thyroxine degradation coefficient					
Total	bulls			0.49±0.28	0.68±0.29
Per 100 kg	bulls			0.53±0.28	0.67±0.29
Thyroxine degradation rate					
Total	bulls			0.24±0.27	0.57±0.28
Per 100 kg	bulls			0.17±0.26	0.52±0.28

Table 4. *Phenotypic (r_p) and genetic (r_g) correlations (± standard errors) between cholesterol concentration and growth rate in Swedish calves estimated from all ages (5, 10 and 16 months) and from the average of the age classes*

Trait	Growth rate	
	r_p	r_g±S.E.
Cholesterol concentration		
Bulls, all ages	-0.05 n.s.	0.94±0.16
Heifers, all ages	0.21 **	0.05±0.26
Bulls and heifers, average of the ages	0.23 **	0.80±0.28

Levels of significance: n.s. = not significant ($P > 0.05$); * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

Table 5. *Phenotypic (r_p) and genetic (r_g) correlations (± standard errors) between the blood parameters studied and growth rate or relative growth rate in Danish bulls*

Trait	Growth rate		Relative growth rate	
	r_p	r_g ±S.E.	r_p	r_g ±S.E.
Cholesterol concentration				
All ages	0.15 *	0.15±0.39	0.01 n.s.	0.19±0.37
Average of the ages	0.11 n.s.	0.42±0.48		
Total thyroxine distribution volume				
All ages	0.32 ***	0.69±0.18	-0.06 n.s.	0.07±0.32
Average of the ages	0.50 ***	1.08±0.23		
Thyroxine distribution volume/100 kg				
All ages	-0.22 **	1.03±0.03	0.18 *	0.14±0.58
Average of the ages	0.04 n.s.	0.94±0.51		
Total thyroxine concentration				
All ages	0.13 n.s.	-0.14±0.32	-0.07 n.s.	0.01±0.31
Average of the ages	0.09 n.s.	0.02±0.34		
Thyroxine concentration/100 kg				
All ages	-0.24 **	-0.52±0.22	0.16 *	0.45±0.24
Average of the ages	-0.16 *	-0.10±0.35		
Total thyroxine degradation coefficient				
All ages	0.19 **	-0.09±0.35	0.16 *	-0.06±0.34
Average of the ages	0.02 n.s.	0.17±0.37		
Thyroxine degradation coefficient/100 kg				
All ages	-0.45 ***	-0.51±0.25	0.39 **	0.44±0.26
Average of the ages	-0.26 **	-0.16±0.40		
Total thyroxine degradation rate				
All ages	0.20 **	0.34±0.43	0.02 n.s.	-0.12±0.21
Average of the ages	0.33 **	0.76±0.30		
Thyroxine degradation rate/100 kg				
All ages	-0.15 *	-0.30±0.53	0.17 *	0.43±0.46
Average of the ages	0.08 n.s.	0.63±0.40		

Levels of significance: n.s. = not significant ($P > 0.05$); * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

THYROXINE AND TRIIODOTHYRONINE DEGRADATION IN LINES OF PIGS SELECTED FOR RATE OF GAIN AND THICKNESS OF BACKFAT.

By
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INTRODUCTION

It is well documented that planned selection for decreased backfat and increased growth and vice versa in pigs may be very efficient (Hetzer and Harvey, 1967; Vangen, 1977). It has also been shown that such selection may lead to correlated response in other traits, e.g. feed conversion ratio (Vangen, 1977). Whether the improved feed conversion ratio (feed/kg gain) for lean, fast growing pigs is due only to less energy produced in the carcass and reduced growth period is not known. It has even been suggested that the leanness is due to a higher metabilic rate and higher heat production, and that such selection therefore may produce pigs with a higher maintenance requirement.

One effect of the thyroid hormones is to increase the rate of energy metabolism. A total lack of the hormones may reduce energy metabolism by 40 percent, while an excess may increase metabolic rate by 100 percent (Dale, 1977). Tata (1974) found evidence suggesting that the calorigenic action of thyroid hormones is secondary to a general stimulation of protein synthesis.

The purpose of the present experiment was to study whether the thyroid hormone activity differed for lines of pigs selected in opposite direction for growth rate and backfat thickness for eight generations.

MATERIAL AND METHODS

Animals

Pigs from the eighth generation of a selection experiment, in which the selection criterion was an index including rate of gain and thickness of backfat, were used. One line, (HP-line) was selected for high rate of gain and low backfat thickness and one line (LP-line) was selected in the opposite direction. A control line (CL-line) maintained without deliberate selection (Standal, 1967; Vangen,1978) was not included in the present experiment.

Two female pigs out of each of six litters from each of the two selection lines were represented in this investigation. The pigs were weaned at six weeks of age and were fed to appetite twice a day from weaning to slaughter at approximately 90 kg live weight.

The experimental pigs remained in the pen with their littermates during the experiment. The total number in the pen varied from 6 to 9 pigs. Feed recording was on a litter basis, and was therefore not included in the recorded traits. The ham, loin and shoulder part of the half carcass was dissected into fat, muscles and bones. The following information for pigs born in 1975, coinciding with the 8th generation, indicates the differences between the selection lines (Vangen 1978, personal communication).

	HP-line	LP-line
Daily gain, g	594	531
Backfat thickness, mm	21.8	42.2
Feed conversion FU/kg gain	3.05	3.47

Estimation of thyroid activity

The method described by Yosef & Johnson (1967), modified for use in pigs was used to determine the degration of triiodothyronine (T_3) and thyroxine (T_4).

About 40 μCi of ^{131}I-labelled T_3 dissolved in 50% propylene glycol was injected into one ear vein. Blood samples were obtained from the anterior vena cava 1, 4, 7, 12, 24, 29, 37 and 48 hours after injection. The ratioactivity in the tricloracetic acid precipitated protein of 5 ml plasma was measured by means of a well-type scinsilation counter. Counting standards were prepared from the injection solution using inactive plasma as diluting medium. The results of the activity measurements expressed as percentage of the dose administered pr. litre plasma, were plotted on semilogaritmic paper with hours after injection as the abscissa and the natural logarithm of the activity as the ordinate. The semilogaritmic plot was not linear within the period 1 to 48 hours, but appeared to be linear within the period 12 to 37 hours post-injection. The rate constant for the labelled T_3 degration (K_{T_3}) was therefore calculated using the activity results for this period.

The line used for estimation at K_{T_3} was extraplated to time zero to give the concentration of the labelled triiodothyronine at time zero. The T_3 distribution space (T_3DS) was calculated as T_3DS = 100/radioactivity per litre as percentage of dose at time zero.

The rate constant for T_4 degradation (K_{T_4}) and the T_4DS were estimated on the same pigs 14 days later using the same procedure. In this experiment ^{131}I-labelled L-thyroxine was used, and blood samples were obtained at the same hours after injection as for the T_3. The semilogaritmical plot appeared to be linear between 12 and 48 hours post injection and this period was used for calculation of K_{T_4}.

Triiodothyronine and thyroxine serum levels (LT_3 and LT_4) were measured by radioimmunoassay. Antiserum against T_3 was obtained mainly as described by Gharib et al. (1971), and antiserum against T_4 was kindly provided by Viggo Kruse.

The immunoassay procedures are described by Bakke and Tveit (1977).

The T_3 and T_4 degradation (TD) was calculated as:

$$T_3D = T_3DS \times LT_3 \times K_{T_3}$$

RESULTS AND DISCUSSION

Average live weight and live weight range when the thyroid activity parameters were measured together with the carcass weights and weight ranges are given in Table 1. There was some difference in average live weight between the selection lines for the pigs used in the experiment, and also a considerable live weight range. This made it necessary to take the live weight into consideration when analysing the difference between selection lines.

The linear regressions of the thyroid activity parameters on live weight at the time of the experiment are given in Table 2. The regression of T_3 and T_4 level in serum on live weight was not significant, and is not given in the table. The regression coefficients given are significantly different from zero for all the parameters except for the T_3 degradation constant (K_{T_3}). Average carcass composition, and the differences between the two selection lines are given in Table 3. There are clear and large differences with 9.4 percentage units more dissected lean tissue and 12.8 percentage units less fat tissue in the dissected parts of the carcass of the selection line selected for low backfat and high growth rate (HP-line).

Table 4 gives the mean and standard deviation, of the thyroid activity parameters, and the difference between the selection lines.

There is a slightly higher rate constant for T_3 degradation in the HP line and the difference is significant at the 5% level. The same was true for the T_3 level in serum. This last finding was also in agreement with the result found by Bakke and Tveit (1977) for pigs from the same selection lines. For the other parameters investigated there were no significant differences between the two selection lines.

Estimates of K_{T_3} or biological half life ($t_{\frac{1}{2}}T_3$) are not found in the literature. The present results give an average K_{T_3} of 4.58%/hour and $t_{\frac{1}{2}}$ = 15.1 hours. The average T_3 distribution space of 93.2 litre expresses the volume needed if the ^{131}I-labelled T_3 was distributed in the whole body with a concentration equal to that found in the serum fraction.

The average T_4 degradation constant K_{T_4} of 3.33%/hour is slightly lower than that found by Marple and Nachreimer (1975). The T_4DS of 6.68 litre is probably also lower, even when the higher weight at test in their experiment is taken into account. The serum level of T_4 is similar to that reported by Bakke and Tveit (1977), and somewhat lower than the 3.56 µg/100 ml reported by Marple and Nachreiner (1975). The biological half life of T_4 was estimated at $t_{\frac{1}{2}}$ = 27.7 hours.

The accuracy of the method used was not tested since only one estimate of T_3D and one estimate of T_4D was obtained on each pig. The correlation between T_3D and T_4D, with live weight kept constant was r = 0.28. The correlation between the serum levels of T_3 and T_4 was also estimated at r = 0.28. These estimates are not significantly different from zero. The correlations between the other estimates of the T_3 and T_4 parameters were small and not significant.

Joakimsen (1975), using the same procedure on young bulls, reported correlations between repeated measurements of T_4D on the same bull of about 0.5.

Johnston et al. (1959) measured thyroxine secretion rate and oxygen consumption in dairy cows in a metabolic chamber and found high correlations between the two parameters. The correlation between oxygen consumption and PBI was low and nonsignificant.

Yosef and Johnson (1975) found low thyroxine secretion rate in rodents with low metabolic rate and vice versa. Robinson and Tam (1974) found 3-4 times higher T_4 secretion rate in laying Japanese quail than in mature males.

The results of the present investigation give no clear indication of a higher thyroid activity in the line of pigs selected for low backfat thickness and high growth rate, compared to the line selected in the opposite direction. The selection has led to marked differences especially for fat/lean ratio. It has been feared that the selection for low backfat thickness might have led to a higher metabolic rate and higher heat production. This might also imply a higher maintenance requirement and a lower caloric efficiency.

The results of this investigation does not support the hypothesis of a higher maintenance requirement for the line of pigs selected for low backfat thickness and high growth rate.

SUMMARY

A selection experiment in two directions has produced lines of pigs that differ considerably in fat/lean ratio and also in growth rate. It has been hypothesized that the selection for thin backfat may have altered the metabolic rate of the pigs, and that this may have led to a lower caloric efficiency in the lean pigs.

In order to investigate this hypothesis, thyroid activity parameters were estimated in 12 pigs, from each of the two selection lines, at a live weight of about 40 kg.

There was a slight difference between the two selection lines in triiodothyronine degradation constant, and in T_3 serum level. For the other tested parameters, T_3 and T_4 distribution space, T_3 and T_4 degradation/day, T_4 degradation constant and T_4 serum level there was no significant difference between the two selection lines.

The pigs were slaughtered at ordinary slaughter weight (carcass weight 65-69 kg). The difference in percent dissected fat in the half carcass amounted to about 13 percentage points, and was very highly significant.

It was concluded that the thyroid activity measurements did not give clear evidence of a difference in metabolic rate between the two selection lines.

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Table 1. Average live weight and weight range when T_3 and T_4 degradation rate was measured, and carcass weight and weight range at slaughter.

	LP-Group		HP-Group	
	Average	Range	Average	Range
T_3 measurement	35.1 kg	21-55	38.7 kg	27-59
T_4 measurement	38.8 "	24-59	43.3 "	30-63
Carcass weight	64.4 "	64-68	65.5 "	61-69

Table 2. Regression coefficients (b) of the thyroidea activity parameters on live weight at test ± standard error of the regression coefficients.

	b±S.E.
K_{T_3}/day	0.003±0.35
T_3DS, litre	2.12 ±0.42
T_3D nanomol/day	1.40 ±0.57
K_{T_4}/day	-0.07 ±0.02
T_4DS, litre	0.14 ±0.02
T_4D, nanomol/day	4.6 ±1.5

Table 3. Average carcass composition, within line standard deviation (SD) and diffferences between the two selection lines (HP-LP).

	Mean	S.D.	HP-LP	Level of significance
Muscle tissue (%)	53.3	5.4	9.4	***
Fat tissue %	27.2	6.9	-12.8	***
Bones %	13.9	1.8	2.8	**
Skin %	5.0	0.6	0.32	N.S.

**0.001<P<0.01 ***P<0.001

Table 4. Average degradation constant (K), distribution space (TDS), serum level (LT) and daily degradation (TD) for thyroxine and triiodothyronine. Within line standard deviation (S.D.) and difference between the two selection lines.

	Mean	SD	HP-LP	Level of significance
K_{T_3} /day	1.1	1.8	0.18	*
T_3DS litre	93.2	26.3	-8.1	N.S.
LT_3 nanomol/litre	0.985	0.18	0.17	*
T_3D nanomol/day	100.1	33.1	14.9	N.S.
K_{T_4} /day	0.80	0.1	0.02	N.S.
T_4DS, litre	6.68	1.55	0.30	N.S.
LT_4 nanomol/litre	39.1	10.9	-4.52	N.S.
T_4D nanomol/day	209.4	75.3	14.1	N.S.

*$0.01<P<0.05$

HORMONALLY INDUCED CHANGES IN THE LACTATIONAL PERFORMANCE AND BLOOD COMPONENTS OF DAIRY COWS

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Milk secretion is controlled by many hormones which act either directly upon milk secreting cells in situ or indirectly by increasing the secretion of other hormones essential for milk synthesis, or by increasing the level and availability of milk precursors from blood to the mammary gland or by promoting the let down of milk.

Thyroxine was the first hormone demonstrated to stimulate milk secretion of cows. Later studies indicated that optimal doses of prolactin, oxytocin, growth hormone (GH), parathyroid hormone (PTH), corticosterone and insulin stimulated milk secretion of normal lactating animals while adrenaline had opposite effect (TURNER 1968, 1971); aldosterone had hardly any effect (HAHN & TURNER 1966). Prolactin is released from the anterior pituitary (AP) and oxitocin from the posterior pituitary (PP) gland as a result of suckling or milking stimulus (TURNER 1966). Recent evidence indicates that release of ACTH from AP is also promoted by suckling stimulus (SMITH et al. 1972).

Prolactin is essential for the synthesis of milk in the secretory cells of the mammary gland (TURNER 1966). Optimal doses of thyroxine, cortisol, corticosterone, estrogens and testosterone stimulate prolactin synthesis in the AP (MOON 1962, MEITES & NICOLL 1965). In hypophysectomized animals prolactin and cortisol maintained ribonuclei acid (RNA), deoxyribonucleic acid (DNA), casein and cytoplasmic protein synthesis in the milk secreting cells, both hormones being effective in maintaining the levels of several enzymes involved in the synthesis of milk in the mammary gland (BALDWIN & MARTIN 1968). In adrenalectomized animals corticosterone and prednisolone were highly effective in maintaining milk secretion while desoxycorticosterone (DOCA) had very little effect (ANDERSON & TURNER 1962). In thyroidectomized animals lactation was maintained with thyroxine injections, in parathyroidectomized animals either with PTH, vitamin D_2 or vitamin D_3 injections, and in alloxan treated animals with insulin injections.

Removal of ovaries had no effect on milk yield (TURNER 1971). Estrogen treatment, however, arrested the decline in milk yield during descending phase of lactation (TURNER et al. 1957) and increased thyroxine secretion rate (TSR) of lactating cows (PIPES et al. 1958, 1960). Estrogens have been used to induce lactation in virgin heifers, whose milk yield was further increased by administration of thyroxine (TURNER 1965). Estrogenic substances given to dairy cows elevate lactose, solids not fat (SNF), fat and globulin contents but decrease casein content of milk (FOLLEY & SCOTT WATSON 1938, SPIELMAN et al. 1941, FOLLEY & MALPRESS 1944). Large doses of estrogen + testosterone reduced markedly the milk yield of cows, goats and sheep (CARUOLO & MOCHRIE 1968).

Thyroxine elevates milk yield, fat and lactose contents of milk (SMITH 1959) and blood sugar (SMITH & DASTUR 1940). Thyroid hormones given in excess reduced milk yield (SCHMIDT & MOGER 1967), decreased prolactin content of AP and increased the size of the adrenals this increase being evidently due to elevated ACTH secretion rate (SINHA & SCHMIDT 1970). SCHRIEFERS (1967) found that hepatic inactivation of adrenal steroids is accelerated by hyperthyroidism.

Principal glucocorticoids secreted by bovine adrenals are cortisol and corticosterone (TURNER et al. 1961). Cortisol tends to depress while Meticorten (prednisone) tends to increase TSR (TURNER et al. 1961). ACTH injections elevated blood cortisol but depressed corticosterone levels (VENKATASESHU & ESTERGREEN 1965) and preferentially stimulated the 17-hydroxylating system of the bovine adrenal (VENKATASESHU & ESTERGREEN 1970). ACTH induced elevation of blood 17-hydroxycorticosteroid level was greater in high than low yielding cows; β-casein content of milk correlated closely (+0.7) with corticosteroid levels (EISNER & REZNICHENKO 1968).

ACTH injected at levels 200 IU or higher/cow/day depressed milk yield and increased fat and total solid contents of milk, the lactose content remaining unchanged (FLUX et al. 1954, JUTILA 1956, BRUSH 1960, CAMPBELL et al. 1964, RADLOFF & MIYAKE 1969). SAARINEN (1956) reported three fold rise in blood sugar of normal lactating cows within 24 hrs after injection of 100,200 or 300 IU ACTH/cow; 1.5 cortisone had practically no effect in normally fed cows but nearly three fold rise was found in fasted cows respectively. Single injection of 1.5 cortisone/cow or 150 - 200 mg DOCA/cow

had no effect on milk yield or fat content of milk (JUTILA 1956).

Number of synthetic glucocorticoids, e.g. dexamethasone (9-fluoro-16-methylprednisolone have been widely accepted for treating bovine hypoglycemia since they are more potent glucocorticoids than cortisol, corticosterone or cortisone. TUCKER & MEITES (1965) induced lactation in pregnant heifers with 9-fluoro-prednisolone acetate. 10 mg dexamethasone-21 pyridine-4-carboxylate decreased milk yield of cows by 7 and 22 % on days 1 and 2 after injection. Blood glucose increased by 97 and 56 % in lactating and by 42 and 20 % in dry non-pregnant cows respectively (BAIRD & HEITZMAN 1969a). There was a marked accumulation of glycogen in the liver (BAIRD & HEITZMAN 1969b); changes were noted in the activities of several liver enzymes (HEITZMAN & BAIRD 1969). Blood glucose of heifers with live weight 200 kg, rised from 39 to 93 mg% within 24 hrs when they were injected with 10 mg of dexamethasone (VÄHÄ-VAHE et al. 1970).

Physiological amounts of corticosteroids inhibit the depressing action of insulin on blood glucose by reducing uptake of glucose by muscle and adipose tissues (HALES 1967). Insulin, ACTH and GH failed to influence the lactose content of milk (RADLOFF & MIYAKE 1969). In goats, yield and lactose content of milk decreased after administration of alloxan; fat and protein contents of milk rised significantly; insulin substitution therapy reversed these signs of insulin deficiency (NOWAK & DZIALOSZYNSKI 1967). Short acting insulin decreased yield and lactose content of milk and blood glucose in Holstein cows while fat and protein contents of milk increased; infusion of glucose together with insulin restored milk yield near-to-normal levels while fat content of milk remained elevated (SCHMIDT 1966). According to KRONFELD et al. (1963), decrease in milk yield after injection of insulin is due to hypoglycemia rather than to insulin per se. Prolactin tends to elevate (WILLIAMS et al. 1966) and GH to decrease blood glucose level. Hormonal interactions and control of milk secretion are very complex in nature and many details remain as unsolved yet. According to TURNER (1966), some cows may be low producers due to limited secretion of one hormone and if it is supplied by injection or feeding it will cause a great increase in the cow's milk yield. Other cows may secrete low levels of a number of hormones and fail to respond to the administration of a single

hormone due to deficiency of others.

Unbalanced hormonal interactions may result in lactational disturbances (SAARINEN 1956). For example acetonemia (SHAW 1956) and parturient paresis (WESTERMARCK 1959) have been cured with ACTH and glucocorticoids. However, administration of thyroid hormones or GH into cows near parturition may even provoke of these disturbances (EMERY & WILLIAMS 1962, HIBBITT 1964, KENDALL et al. 1965, HIBBITT 1966). Estrogens and thyrocalcitonin (TCT) (STOTT 1968, BARLET 1967, YOUNG & CAPEN 1967, BARLET 1975) are probably involved with the calcium imbalance in dairy cows.

Majority of the research on the hormonal control of lactation has been carried out with laboratory animals or with small ruminants. Animals in this study were Ayrshire cows slected from the herd in which yearly production level has been high, 6000 kg milk and 280 kg butterfat on the average per cow.

The aim of this study was to find which hormone, hormone level or hormone combination would produce greatest response in the lactational performance of the cow. At the same time blood glucose, hemoglobin and hematocrit as well as serum calcium, inorganic phosphorus and magnesium levels were determined in order to get more information on the effects of the hormones upon the said blood components. The hormones used in this study were ACTH, DOCA, thyroxine, 9-fluoro-16-methylprednisolone, estradiol-17β and a long acting estrogen "Dimenformon prolongatum", which contains estradiol-17-phenylpropionate + estradiol benzoate.

Material and methods

Animals and experimental design

The present data was obtained from six separate experiments which were carried out with lactating Ayrshire cows during the stall feeding period. Experiment I as well as II included 3 controls and 3 + 3 hormone treated cows all of which were pregnant. Experiments III, IV, V and VI, each included 3 controls and 3 + 3 + 3 hormone treated cows two out of the three in each group being pregnant. The cows received concentrates (oats + wheat millings + mineral salt mixture) and brewer's grains according to the level of their milk yield and hay and grass silage ad lib.

twice daily. The cows were milked at 6 - 7 A.M. and 4 - 5 P.M. Jugular blood samples were taken and hormones were injected at 8 - 9 A.M. The subsequent evening and morning yields were added in order to estimate the first 24 hr posthormonal effects on the lactational indices of the experimental cows, etc. Preparatory period of each experiment lasted 6 days, i.e. 2 threeday periods (A and B). The first blood sample was taken during A. The second blood sample was taken in the end of B, i.e. less than an hour before administration of hormone. The third blood sample was taken 24 hours (C), the fourth 48 hrs (D), and the fifth 96 hrs (E) after the first hormone injection; in few instances additional blood samples were taken at 168 hrs (F) and 240 hrs (G).

Three-day averages of milk, FCM, fat and protein yields as well as fat and protein percentages of milk were calculated for the periods A, B, E, F and G, while daily lactational indices were used for the periods C and D.

Preparations, doses, combinations and dosing times of the hormones

The hormone preparations used in this study were:
- Desoxycorticosterone trimethylacetate, Ciba's "Percorten" (DOCA)
- Corticotropin A, Ferring's "Acortan Prolongatum" long acting. ACTH, which has been prepared from the pituitaries of pig and whale.
- Dexamethasone i.e. 9-fluoro-16α-methylprednisolone Lääke Oy's "Dexa-Korti", a potent synthetic glucocorticoid (PRED).
- Estriol -17β, a short acting estrogen, LEO's "Estradurin" (ESD).
- Oestradiol-17-phenylpropionate + oestradiol benzoate 4:1 in oil, long acting estrogen, Organon's "Dimenformon Prolongatum" (DIMP).
- L-thyroxine natrium ($L\text{-}T_4$), which was granted for this study by Orion Oy.

All hormones were administered intramuscularly. The dose levels are given in Table 1. At first injection day (0-hour), $L\text{-}T_4$ was given simultaneously with PRED in the treatment groups 6, 7 and 8, with ESD in the treatment group 13 and with DIMP in the treatment groups 15 and 16; group 17 received only $L\text{-}T_4$. Subsequent to 0-hour hormone treatment, only $L\text{-}T_4$ was injected.

Analytical methods

Milk yield of the cows was weighed at each milking. Fat content

of milk was determined by the Gerber method and protein content by the dye-binding (amido-black) method. Daily FCM-yield was calculated by using the following equation:

12.5 x Fat yield, kg/day + 0.5 x Milk yield, kg/day = FCM yield kg/day

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Blood hemoglobin (Hb) and hematocrit (Hc) were determined as previously described (KOSSILA et al. 1970). NELSONS(1944) modification from the method of SOMOGYI (1945) was used in the determination of blood glucose. Plasma inorganic phosphorus was estimated according to TAUSSKY & SHORR (1953). Plasma cationes were estimated with AA 1000-Varian Techtron atomic absorption spectrophotometer.

The statistical calculations were performed in the Computing Center of the University of Helsinki.

Results

Lactational indices

Mean daily milk, FCM, fat and protein yields as well as fat and protein contents (%) of the milk (± standard deviations) in each treatment group (controls combined) of the experimental period are given in Tables 2 and 3.

One way variance analysis was applied in testing the significance of the unequality of the mean daily lactational indices in each treatment group. However, the said mean daily values varied within each group to such an extent that the effects of various hormone treatments were mostly nonsignificant as indicated by low F-values in the Tables 2 - 3. Only the fat percentage of the milk was significantly rised by 400 IU ACTH (treat. 4), 10 mg PRED (treat. 5) and 5 mg PRED + L-T_4 (treat. 8).

Students T-test was somewhat more effective in detecting significant changes in the said lactational indices, i.e. milk yield was decreased by 10 mg PRED + L-T_4 (treat. 6) but increased by 4 mg DIMP + L-T_4 (treat. 16, Table 2); treatment 16 increased the FCM yield (Table 2); fat content of milk was increased by the treatments 4 (400 IU ACTH), 5 (10 mg PRED), 8 (5 mg PRED + L-T_4,

15 (8 mg DIMP + L-T_4) and 16 (4 mg DIMP + L-T_4) (Table 2); protein content of milk was significantly rised by 4 mg DIMP + L-T_4 (treat. 16, Table 3).

In order to eliminate individual variations in the lactational indices within each treatment group, also relative changes in the milk, FCM, protein and fat yields was well as fat and protein contents of milk were investigated. These relative changes have been illustrated in the Figs. 1 - 6. The first column at left of each treatment group (groups 1 to 17) represents the mean milk yield (Fig. 1), mean FCM yield (Fig. 2) etc. during the entire preparation period (A+B), which is taken as 100. The second, third and fourth columns from the left represent the relative changes in the lactational indices during the experimental periods C, D and E respectively. The fith column of the groups 15, 16 and 17 in the Figs 1 - 6, represents the values of the experimental period F. The relative changes which are 5 % or less can be considered of no significance.

Relative changes in the daily milk yield of each experimental group at various stages of the experimental period can be seen from the Fig. 1. ACTH (treat. 3 and 4) produced promt decrease (14 %) in the milk yield which however rapidly returned to the initial level. Treatment 5 (10 mg PRED) produced greater (21 to 24 %) and more prolonged depression in milk yield than ACTH. L-T_4 administered with PRED (treat. 6, 7 and 8) alleviated this effect of PRED.ESD at the levels of 4 and 8 mg (treat. 10 and 11) depressed slightly the milk yield while ESD at the levels of 2 and 16 mg (treat. 9 and 12) had no effect. L-T_4 administered with 16 mg ESD (treat. 13) caused a progressive increase (11 %) while 8 mg DIMP (treat. 14) a progressive decrease (-16 %) in the milk yield by the period E. L-T_4 administered with 8 or 4 mg DIMP (treat. 15 and 16) alleviated the milk yield depressing effect of DIMP. L-T_4 given alone (treat. 17) caused a progressive rise in the milk yield the maximum level (+19%) being reached by the period E.

Relative changes in the FCM yield are shown in Fig. 2. Treatments 5 and 6 caused greatest and most rapid depression whereas the treatments 13 and 17 produced most marked elevations in the FCM yield.

Relative changes in the fat content of milk, due to various hormone

treatments, are demonstrated in Fig. 3. Treatments 2 (DOCA), 9, 10, 11 and 12 (ESD) had hardly any effect; ACTH (treat. 3 and 4) caused an immediate rise of about 13 %. 10 mg PRED given alone caused greatest rise (33 %) by the second day (period D); similar pattern in the rise of the fat content was noted when PRED was administered with L-T_4 (treat. 6, 7 and 8), thyroxine however, alleviated this effect of PRED. ESD administered with L-T_4 (treat. 13) caused 14 % elevation in fat content by the period E; DIMP (treat. 14) alone had similar but more pronounced effect (21 % rise by the period E). L-T_4 administered with DIMP (treat. 15 and 16) alleviated this rise while L-T_4 given alone (treat. 17) rised the fat content of milk only sligtly (about 7 %) by the period E.

Relative changes in the fat yield due to various hormone treatments are shown in Fig. 4. DOCA (treat. 2) seemed to depress the fat yield by the period E; ACTH (treat. 3 and 4) caused an elevation by the second day (period D) followed then by a depression (period E). PRED. (treat. 5) caused an immediate depression in the fat yield; the depression was alleviated by L-T_4 (treat. 6, 7, 8) especially at the highest L-T_4 level. ESD (treat. 9, 10, 11 and 12 or DIMP (treat. 14) alone had hardly any effect on the fat yield. L-T_4 given either with ESD (treat. 13) or alone (treat. 17) caused a marked elevation in fat yield (28 %) by the period E. Tendencies towards elevated fat yields were observed also when DIM was administered with L-T_4 (treat. 15 and 16).

The relative changes in the protein content of milk (Fig. 5) during the various stages of experiment were much smaller compared to those noted in fat content. PRED (treat. 5), at first, depressed and then elevated protein content milk compared to the initial level. DIMP administered either alone (treat. 14) or with L-T_4 (treat. 15 and 16) rised progressively milk protein content.

Relative changes in the protein yield (Fig. 6) resembled mostly those noted in the milk yield (Fig. 1). ACTH (treat. 3 and 4) and PRED (treat. 5) depressed protein yield; L-T_4 administered with PRED (treat. 6, 7 and 8) alleviated this effect of PRED. L-T_4 given together with either ESD (treat. 13) or DIMP (treat. 15 and 16) or especially when given alone (treat. 17) rised the protein yield. ESD at 4 and 8 mg level (treat 10 and 11) and

DIMP given alone (treat. 14) tended to depress the protein yield.

Blood composition

Hematocrit (Hc) and hemoglobin (Hb) values were not significantly affected by any of the hormone treatments according to results obtained by one way variance analysis (Table 4). Student T-test revealed significant ($P < 0.01$) change in the Hc of the group 5 treated with 10 mg PRED. An elevation was noted in Hb of group 2 treated with DOCA (Table 4).

Blood glucose level (Table 4) was rised significantly by ACTH (treat. 3 and 4) as well as by PRED given alone (treat. 5) or with L-T_4 (treat. 6, 7 and 8). L-T_4 either given alone (treat. 17) or together with DIMP (treat. 15 and 16) exhibited also some glucogenic effect. F-value of treatment 14 (DIMP alone) was also signficant, however, in this group glucose values varied considerably already during the preparatory period (Table 4).

Serum calcium level (Table 5) was not significantly influenced by most of the hormone treatments. Significant F-value was obtained only for the group 3 (ACTH). Calcium levels fluctuated during the preparatory period to such a degree that it is not justified, on the basis of student T-tests, to draw any conclusions on the possible effects of the tested hormones. This was also the case with serum magnesium levels (Table 4).

Serum inorganic phosphorus levels (Table 4) were significantly decreased by PRED given alone (treat. 5) or in combination with L-T_4 (treat. 6, 7 and 8). It also, seems that treatments 16 and 17 finally depressed serum phosphorus level. Phosphorus, however, varied considerably already during the preparatory period.

Effect of thyroxine given alone or with DIMP on serum mineral and trace mineral contents

Potassium (K), sodium (Na), iron (Fe), copper (Cu) and zinc (Zn) levels in serum of cows were estimated only during the experiment VI.

Three cows in experiment VI were kept as controls, 3 cows received hormone treatment 15, 3 cows treatment 16 and 3 cows treatment 17.

Variations in serum levels of K, Na, Fe, Cu and Zn in each group at various stages of the experiment are given in Table 6. One way variance analysis revealed significant changes in the K-level of group 15 and in the Na-level of groups 1, 15, 16 and 17 at various stages of experiment VI. Students T-test revealed significant changes in the K-level of group 1 and in the Na-levels of groups 1 and 15 during the preparatory period. These observations indicated that these variations were due to some other factor than to the tested hormones.

Serum Fe was not significantly influenced by any of the hormone treatments. On the other hand, it seemed as if serum Cu-level was somewhat depressed by the tratment 17. Serum Zn was significantly elevated by the treatments 15 and 16 (ESD + $L\text{-}T_4$) while treat. 17 ($L\text{-}T_4$ alone) had no such effect (Table 6).

Summary and conclusions

Hormone treatments used in this study influenced significantly yield and composition of milk and composition of blood. Tested hormones were physiologically steroid-like acting and they were tested alone or in combination of $L\text{-}T_4$.

It was found that $L\text{-}T_4$ alleviated the decreases in milk yield and rate of synthesis of milk protein and fat caused by administration of 9α-fluoro-16α-methyl-prednisolone or 17β-estriol or estradiol -17-phenylpropionate + oestradiol benzoate. According to HARTMANN & KRONFELD (1971) Dexamethazone diminishes glucose uptake by mammary gland and decreases rate of milk synthesis of the mammary gland, in spite of the fact that plasma glucose is elevated. This rate of milk synthesis can be restored by simultaneous administration of thyroxine as the results of present study indicate.

Glucogenic effect of glucocorticoid was increased by simultaneous administration of $L\text{-}T_4$. Oestradiol-17-phenylpropionate + oestradiol benzoate seemed to have a glucogenic effect which was exaggerated by simultaneous administration of $L\text{-}T_4$.

Inorganic phosphorus in serum was significantly reduced after administration of glucocorticoid but not by ACTH.

Oestradiol-17-phenylpropionate+oestradiol benzoate in combination

with L-T_4 elevated significantly plasma zinc, while L-T_4 alone had no such effect.

Many important hormones and hormone combinations could be investigated yet from the point of view of their effect on the lactational performance of cows. Magnitude of the response of cows to L-T_4 administration suggests that these cows have had low thyroxine secretion capacity from the point of view of optimal milk secretion rate.

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Table 1. Hormone preparations, doses, dose combinations and dosing times used in the study.

Treatment groups		Dosing time	0 hr	24 hr	48 hr	72 hr	96 hr
No	Hormone	No of dose 1st		2nd	3rd	4th	5th
		Dose	L-thyroxine mg/100 kg bwt				
1	Control						
2	Desoxycorticosterone (DOCA)	150-200 mg/cow					
3	Corticotropin A (ACTH)	200 IU /cow					
4	- " -	400 IU /cow					
5	9α-fluoro-16β-methylprednisolone, Dexa-Korti, (PRED)	10 mg/cow					
6	- " -	10 " " +	1	1	1/2		
7	- " -	10 " " +	2	2	1		
8	- " -	5 " " +	1	1	1/2		
9	17β-estriol, Estradurin (ESD)	2 mg/100 kg bwt					
10	- " -	4 " "					
11	- " -	8 " "					
12	- " -	16 " "					
13	- " -	16 " " +	4	2	2		
14	Oestradiol-17-phenylpropionate+ oestradiol benzoate 4:1, Dimenformon Prolongatum (DIMP)	8 " "					
15	- " -	8 " " +	4	2	2	1	1
16	- " -	4 " " +	4	2	2	1	1
17	L-thyroxine ($L\text{-}T_4$)		4	2	2	1	1

Table 2. Variations of milk and FCM yields and fat content of milk in each treatment group at stages of experimental period

Treatment No	A	B	C	D	E	F	G	F value
	Milk yield kg/day							
1	16.35 ± 4.81	16.17 ± 5.00	15.73 ± 4.89	15.77 ± 4.94	15.61 ± 4.94			0.028
2	13.77 ± 5.16	13.40 ± 4.68	14.07 ± 4.73	13.63 ± 4.31	13.57 ± 4.10			0.040
3	10.60 ± 3.44	10.83 ± 3.44	9.23 ± 3.23	10.53 ± 3.22	10.17 ± 3.29	10.40 ± 3.50		0.077
4	14.00 ± 3.14	13.63 ± 3.79	11.83 ± 3.20	13.80 ± 3.31	13.17 ± 3.81			0.162
5	11.57 ± 5.35	12.07 ± 5.50	9.33 ± 6.41	9.00 ± 4.59	11.43 ± 6.07	11.53 ± 6.40		0.167
6	14.73 ± 3.06	15.90 ± 1.75[e]	12.80 ± 0.97[f]	13.60 ± 0.52	15.20 ± 2.40			1.365
7	17.53 ± 5.59	17.83 ± 5.42	15.83 ± 5.86	16.87 ± 6.46	18.97 ± 4.90			0.116
8	17.03 ± 6.27	16.50 ± 6.56	14.93 ± 5.78	15.10 ± 6.09	16.87 ± 6.34			0.076
9	19.17 ± 3.19	18.83 ± 3.61	19.13 ± 4.13	18.67 ± 4.37	18.90 ± 3.55			0.007
10	18.90 ± 6.24	18.37 ± 6.29	17.33 ± 6.11	17.23 ± 6.84	17.57 ± 6.19			0.035
11	15.30 ± 2.11	14.67 ± 1.75	14.57 ± 2.25	13.47 ± 1.90	13.90 ± 0.99			0.376
12	16.60 ± 4.40	16.50 ± 4.36	16.17 ± 5.09	15.97 ± 5.25	16.50 ± 4.09			0.009
13	17.60 ± 3.68	17.03 ± 2.75	16.80 ± 2.76	18.40 ± 3.01	18.23 ± 2.93			0.271
14	20.73 ± 3.86	19.83 ± 4.07	19.00 ± 4.06	17.93 ± 3.12	17.07 ± 5.16			0.321
15	17.20 ± 3.70	17.00 ± 3.32	17.30 ± 3.52	15.63 ± 2.57	16.57 ± 2.44	16.73 ± 3.75	16.43 ± 2.93	0.852
16	17.90 ± 1.02	18.53 ± 0.60[e]	17.80 ± 0.53	17.07 ± 0.49[f]	18.33 ± 0.30	19.63 ± 1.67	19.17 ± 0.76[g]	1.259
17	21.90 ± 6.15	21.87 ± 6.32	22.03 ± 5.93	23.00 ± 6.45	26.07 ± 5.35	25.07 ± 4.35	22.93 ± 4.21	0.227
	FCM yield kg/day							
1	17.41 ± 5.19	17.15 ± 5.39	16.89 ± 5.35	16.90 ± 5.44	16.71 ± 5.44			0.044
2	14.77 ± 4.89	14.03 ± 3.92	14.93 ± 4.54	14.10 ± 5.44	12.97 ± 3.41			0.044
3	10.70 ± 2.95	11.00 ± 3.10	10.00 ± 3.14	11.17 ± 3.07	10.30 ± 3.14	10.53 ± 3.36		0.048
4	14.20 ± 3.14	13.90 ± 3.96	12.73 ± 3.36	14.77 ± 3.50	13.00 ± 3.73			0.135
5	12.07 ± 5.35	12.57 ± 5.20	10.23 ± 6.74	10.90 ± 5.11	11.73 ± 5.54	11.93 ± 6.07		0.059
6	15.43 ± 3.85	16.00 ± 2.90	13.77 ± 2.24	16.07 ± 1.72	16.03 ± 3.41			0.494

7	18,97 ± 4,92	19,17 ± 5,01	17,70 ± 4,81	19,60 ± 5,96	20,07 ± 4,43					0,877
8	17,93 ± 6,59	17,53 ± 7,02	15,17 ± 6,28	19,13 ± 7,61	17,77 ± 5,60					0,032
9	20,17 ± 2,88	19,93 ± 3,29	20,00 ± 3,90	19,67 ± 4,02	19,90 ± 3,47					0,006
10	20,80 ± 6,75	20,40 ± 7,02	19,27 ± 6,25	19,07 ± 6,99	19,83 ± 6,79					0,034
11	16,67 ± 1,72	15,00 ± 1,15	16,13 ± 1,74	14,83 ± 1,37	15,37 ± 0,47					0,535
12	17,90 ± 4,00	17,77 ± 3,96	17,53 ± 4,26	17,43 ± 4,61	18,23 ± 4,30					0,015
13	18,90 ± 3,22	18,43 ± 2,53	18,07 ± 2,43	20,23 ± 2,75	22,17 ± 3,67					0,898
14	22,83 ± 2,17	21,87 ± 2,22	21,63 ± 1,70	21,47 ± 1,59	20,67 ± 3,52					0,299
15	17,87 ± 3,50	17,50 ± 2,98	18,57 ± 3,69	16,53 ± 2,42	18,20 ± 2,89	17,43 ± 3,71	17,17 ± 2,59			0,109
16	19,07 ± 0,49^{f}	20,20 ± 0,40^{e}	19,43 ± 0,55	18,63 ± 0,83^{f}	20,59 ± 3,50	21,14 ± 2,80	20,73 ± 1,51			0,966
17	23,83 ± 5,86	23,53 ± 6,15	23,60 ± 4,97	25,37 ± 6,67	29,37 ± 5,55	28,30 ± 4,10	25,70 ± 2,98			0,505
	F a t c o n t e n t o f m i l k, %									
1	4,51 ± 0,38	4,49 ± 0,41	4,59 ± 0,45	4,60 ± 0,53	4,53 ± 0,48					0,094
2	4,65 ± 0,35	4,50 ± 0,60	4,57 ± 0,31	4,37 ± 0,42	4,37 ± 0,55					0,191
3	4,15 ± 0,41	4,20 ± 0,30	4,73 ± 0,42	4,52 ± 0,42	4,15 ± 0,26	4,13 ± 0,32				1,476
4	4,12 ± 0,03^{de}	4,09 ± 0,17^{f}	4,53 ± 0,40^{e}	4,57 ± 0,15^{ce}	3,90 ± 0,10^{df}					6,437^{xx}
5	4,40 ± 0,17^{f}	4,43 ± 0,47^{f}	4,95 ± 0,41	5,08 ± 0,59^{e}	4,40 ± 0,69^{f}	4,33 ± 0,47^{f}				4,875^{xx}
6	4,33 ± 0,65	4,49 ± 0,68	4,57 ± 0,85	5,45 ± 0,94	4,39 ± 0,53					1,086
7	4,78 ± 0,71	4,67 ± 0,47	5,17 ± 0,96	5,53 ± 1,10	4,83 ± 0,42					0,715
8	4,43 ± 0,08^{f}	4,50 ± 0,10^{f}	4,67 ± 0,06^{ef}	5,43 ± 0,42^{e}	4,42 ± 0,13^{f}					12,523^{xxx}
9	4,43 ± 0,21	4,50 ± 0,23	4,38 ± 0,16	4,47 ± 0,24	4,42 ± 0,10					0,130
10	4,83 ± 0,47	4,91 ± 0,48	4,98 ± 0,60	4,93 ± 0,55	5,07 ± 0,91					0,054
11	4,73 ± 0,40	4,90 ± 0,56	4,90 ± 0,61	4,87 ± 0,64	4,92 ± 0,47					0,047
12	4,70 ± 0,57	4,70 ± 0,71	4,82 ± 0,04	4,88 ± 0,84	4,99 ± 0,79					0,060
13	4,60 ± 0,44	4,58 ± 0,25	4,53 ± 0,28	4,83 ± 0,49	5,29 ± 0,41					1,315
14	4,91 ± 0,89	4,93 ± 1,04	5,27 ± 1,19	5,58 ± 0,94	5,93 ± 1,29					0,438
15	4,33 ± 0,40	4,27 ± 0,31^{f}	4,60 ± 0,34	4,53 ± 0,21	4,78 ± 0,04^{ce}	4,30 ± 0,20^{d}	4,37 ± 0,21^{d}			1,413
16	4,53 ± 0,31	4,57 ± 0,23	4,73 ± 0,12^{f}	4,73 ± 0,15	4,95 ± 0,08^{e}	4,70 ± 0,43	4,63 ± 0,40			0,624
17	4,77 ± 0,39	4,67 ± 0,38	4,67 ± 0,57	4,97 ± 0,31	5,04 ± 0,15	5,07 ± 0,38	4,97 ± 0,40			0,450

Table 3. Variations of fat yield, protein content and protein yield in each treatment group at different stages of experimental period

Treatment No	A	B	C	D	E	F	G	F-value
		Fat yield g/day						
1	739 ± 226	726 ± 232	722 ± 232	772 ± 232	711 ± 243			0.067
2	629 ± 188	585 ± 126	634 ± 174	584 ± 130	534 ± 112			0.193
3	431 ± 102	449 ± 113	430 ± 120	470 ± 116	419 ± 119	426 ± 130		0.079
4	576 ± 126	559 ± 148	545 ± 145	629 ± 147	513 ± 146			0.218
5	504 ± 216	523 ± 200	445 ± 201	512 ± 224	479 ± 203	489 ± 229		0.037
6	646 ± 194	715 ± 121	590 ± 144	742 ± 124	674 ± 179			0.371
7	817 ± 172	917 ± 190	782 ± 153	891 ± 212	847 ± 156			0.129
8	753 ± 273	744 ± 302	696 ± 269	828 ± 347	744 ± 273			0.062
9	846 ± 103	842 ± 119	934 ± 147	927 ± 146	833 ± 136			0.009
10	918 ± 101	897 ± 316	949 ± 254	834 ± 292	881 ± 315			0.034
11	720 ± 59	695 ± 34	706 ± 59	649 ± 45	676 ± 23			0.871
12	767 ± 144	751 ± 159	754 ± 147	755 ± 161	799 ± 150			0.040
13	871 ± 114	794 ± 96	774 ± 84	884 ± 113	1019 ± 187			1.741
14	995 ± 79	953 ± 91	959 ± 58	1000 ± 21	968 ± 114			0.184
15	740 ± 141	720 ± 109	793 ± 159	705 ± 90	771 ± 125	724 ± 158	714 ± 97	0.209
16	809 ± 10	859 ± 27	843 ± 27	838 ± 45	910 ± 140	927 ± 160	889 ± 97	0.771
17	1030 ± 223	1007 ± 239	1006 ± 162	1111 ± 240	1307 ± 233	1262 ± 159	1105 ± 115	0.907
	Protein content of milk, %							
1	3.66 ± 0.26	3.64 ± 0.29	3.65 ± 0.25	3.67 ± 0.30	3.63 ± 0.24	3.64 ± 0.20		0.030
2	3.78 ± 0.42	3.83 ± 0.39	3.77 ± 0.38	3.71 ± 0.35	3.77 ± 0.41			0.030
3	3.76 ± 0.24	3.74 ± 0.25	3.80 ± 0.44	3.75 ± 0.40	3.89 ± 0.38	3.77 ± 0.33		0.099
4	3.58 ± 0.21	3.51 ± 0.24	3.72 ± 0.43	3.65 ± 0.29	3.63 ± 0.22			0.189
5	3.89 ± 0.55	3.90 ± 0.51	4.54 ± 1.34	4.59 ± 1.25	4.05 ± 0.45	4.01 ± 0.54		0.371
6	3.70 ± 0.21	3.63 ± 0.29	3.80 ± 0.37	3.84 ± 0.52	3.71 ± 0.13			0.080

7	3.78 ± 0.42	3.75 ± 0.47	3.92 ± 0.46	3.92 ± 0.50	3.76 ± 0.31		0,117
8	3.79 ± 0.12	3.74 ± 0.16	3.73 ± 0.17	3.78 ± 0.13	3.84 ± 0.08		0,264
9	3.54 ± 0.50	3.57 ± 0.49	3.52 ± 0.44	3.64 ± 0.44	3.59 ± 0.45		0,009
10	3.83 ± 0.35	3.77 ± 0.37	3.75 ± 0.45	3.91 ± 0.39	3.92 ± 0.35		0,014
11	3.92 ± 0.34	3.84 ± 0.31	3.85 ± 0.38	3.92 ± 0.43	3.97 ± 0.34		0,028
12	3.76 ± 0.30	3.77 ± 0.31	3.87 ± 0.27	3.92 ± 0.27	3.94 ± 0.32		0,161
13	3.67 ± 0.44	3.75 ± 0.37	3.64 ± 0.33	3.61 ± 0.39	3.59 ± 0.42		0,059
14	3.45 ± 0.57	3.45 ± 0.42	3.45 ± 0.44	3.61 ± 0.42	3.59 ± 0.39		0,436
15	3.79 ± 0.42	3.79 ± 0.43	3.77 ± 0.41	4.05 ± 0.44	4.17 ± 0.13	3.96 ± 0.10	3.95 ± 0.35 0,467
16	3.89 ± 0.14	3.93 ± 0.05	3.92 ± 0.09	4.05 ± 0.03	4.08 ± 0.09	3.93 ± 0.04	3.94 ± 0.11 2,011
17	3.75 ± 0.31	3.81 ± 0.34	3.83 ± 0.31	3.91 ± 0.30	3.83 ± 0.18	3.82 ± 0.19	3.85 ± 0.22 0,040
			Protein yield, g/day				
1	592 ± 112	595 ± 143	562 ± 141	570 ± 135	574 ± 129		0,088
2	506 ± 130	501 ± 122	518 ± 119	477 ± 112	463 ± 105		0,077
3	393 ± 107	401 ± 105	351 ± 97	399 ± 84	389 ± 92	356 ± 103	0,091
4	499 ± 87	474 ± 104	433 ± 95	398 ± 87	473 ± 112		0,195
5	430 ± 146	451 ± 158	359 ± 191	374 ± 121	445 ± 198	444 ± 195	0,154
6	549 ± 135	597 ± 96	489 ± 79	524 ± 85	566 ± 105		0,175
7	647 ± 137	654 ± 131	504 ± 158	661 ± 124	706 ± 149		0,149
8	645 ± 247	615 ± 247	555 ± 219	558 ± 225	650 ± 252		0,100
9	698 ± 42	693 ± 49	692 ± 59	668 ± 80	698 ± 45		0,044
10	704 ± 188	679 ± 184	634 ± 176	639 ± 207	657 ± 186		0,065
11	595 ± 49	561 ± 54	558 ± 73	524 ± 56	537 ± 27		0,593
12	617 ± 123	614 ± 121	618 ± 155	601 ± 163	642 ± 161		0,025
13	635 ± 53	622 ± 35	607 ± 57	657 ± 42	594 ± 15		1,040
14	701 ± 14	677 ± 30	643 ± 49	639 ± 31	651 ± 124		0,507
15	642 ± 80	635 ± 47	544 ± 77	627 ± 45	691 ± 107	651 ± 115	543 ± 43 0,188
16	696 ± 49	732 ± 31	697 ± 35	693 ± 21	747 ± 115	781 ± 59	754 ± 10 1,206
17	810 ± 157	820 ± 122	832 ± 154	774 ± 148	901 ± 93	953 ± 79	931 ± 113 0,656

Table 4. Variations of blood haematocrit, haemoglobin and glucose levels in each treatment group at different stages of experimental period

Treatment No	A	2 - 9 hr	24 hr	48 hr	96 hr	168 hr	240 hr	F-value
	Haematocrit, %							
1	34.61 ± 2.29	34.03 ± 2.76	34.17 ± 2.44	33.50 ± 2.31	33.67 ± 2.70	33.67 ± 1.53		0.216
2	33.67 ± 0.58	33.67 ± 1.53	33.00 ± 1.00	33.33 ± 1.53	33.67 ± 1.53			0.165
3	33.67 ± 1.53	33.00 ± 2.00	32.00 ± 2.65	32.00 ± 1.73	33.67 ± 4.04	35.00 ± 2.64		0.629
4	34.00 ± 3.00	34.00 ± 3.00	33.00 ± 3.61	33.33 ± 3.06	34.00 ± 2.65			0.058
5	33.00 ± 2.65	32.67 ± 0.58[c]	31.00 ± 0.00[d]	32.33 ± 2.31	33.33 ± 2.52[c]	34.00 ± 1.00[c]		1.406
6	34.67 ± 2.52	35.00 ± 3.46	33.00 ± 2.72	33.67 ± 4.16	33.67 ± 4.99			0.165
7	34.33 ± 3.06	34.00 ± 4.58	31.33 ± 3.00	32.33 ± 4.04	31.67 ± 4.25			0.248
8	35.33 ± 3.06	33.33 ± 1.76	34.17 ± 2.26	33.83 ± 2.93	33.33 ± 1.75			0.412
9	36.00 ± 2.65	34.33 ± 4.35	34.50 ± 2.78	34.67 ± 2.07	34.33 ± 2.94			0.110
10	34.83 ± 4.50	34.33 ± 4.93	33.00 ± 4.57	33.50 ± 5.22	33.33 ± 5.39			0.035
11	35.17 ± 5.25	35.00 ± 3.61	35.83 ± 4.37	35.33 ± 4.51	33.50 ± 4.09			0.927
12	33.67 ± 5.10	33.50 ± 3.50	32.33 ± 3.25	32.50 ± 3.44	33.00 ± 3.78			0.139
13	32.50 ± 4.55	32.17 ± 4.25	31.83 ± 4.41	31.50 ± 4.93	32.83 ± 4.16			0.195
14	32.97 ± 2.51	34.00 ± 3.61	34.00 ± 2.82	33.67 ± 2.75	34.83 ± 3.25			0.182
15	32.10 ± 1.61	31.20 ± 2.86	32.77 ± 2.45	32.00 ± 2.97	34.67 ± 2.56	33.43 ± 2.00	33.10 ± 2.39	2.432
16	33.70 ± 1.35	33.03 ± 1.58	34.03 ± 1.53	31.47 ± 1.22[f]	35.13 ± 1.53[g]	34.47 ± 1.24[g]	33.23 ± 1.89	1.773
17	30.40 ± 2.31	30.53 ± 2.66	30.63 ± 2.96	29.97 ± 2.54	31.11 ± 3.21	31.33 ± 3.87	28.73 ± 0.76	0.257
	Haemoglobin, g/100 ml							
1	10.98 ± 0.82	11.01 ± 1.09	11.01 ± 0.91	10.99 ± 1.16	11.07 ± 0.85	10.93 ± 0.95		0.036
2	10.43 ± 0.47[f]	10.37 ± 0.45[f]	10.30 ± 0.52[f]	11.43 ± 0.70[g]	10.90 ± 0.63	-		8.419
3	11.43 ± 0.95	10.73 ± 0.46	11.03 ± 1.16	10.73 ± 0.75	10.90 ± 1.06	11.10 ± 0.90		0.903
4	10.47 ± 0.65	10.57 ± 0.85	10.30 ± 0.72	10.93 ± 0.91	10.77 ± 0.65	-		0.272
5	11.63 ± 0.56[g]	10.70 ± 0.46	10.43 ± 0.21[f]	11.00 ± 0.53	10.97 ± 0.23[g]	10.77 ± 0.35		2.576
6	10.97 ± 0.25	11.03 ± 1.16	11.42 ± 0.75	11.00 ± 1.22	11.07 ± 1.21			0.280

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7	11.59 ± 1.13	11.73 ± 1.39	10.78 ± 1.15	10.52 ± 1.09	10.67 ± 1.40				0.272
8	10.92 ± 0.91	11.12 ± 0.99	11.50 ± 1.00	10.85 ± 0.89	11.15 ± 0.91				0.196
9	10.89 ± 1.01	11.37 ± 1.27	11.33 ± 0.88	11.53 ± 0.91	11.54 ± 0.89				0.324
10	10.77 ± 2.07	11.39 ± 1.82	10.75 ± 1.83	11.07 ± 2.00	11.03 ± 1.62				0.048
11	11.32 ± 1.63	12.06 ± 1.99	12.03 ± 1.61	11.69 ± 1.09	11.31 ± 1.03				0.185
12	11.23 ± 1.07	11.90 ± 1.04	10.45 ± 0.81	10.91 ± 1.31	10.95 ± 1.31				0.171
13	10.8[illegible] ± 1.12	10.60 ± 1.10	10.00 ± 1.21	10.22 ± 1.21	11.11 ± 1.16				0.437
14	10.97 ± 0.85	10.93 ± 1.19	11.42 ± 1.07	11.14 ± 0.99	11.57 ± 1.15				0.227
15	9.62 ± 0.59	9.42 ± 0.45	9.35 ± 1.14	10.20 ± 1.46	9.79 ± 1.72	10.33 ± 1.26	10.31 ± 1.39		0.191
16	9.43 ± 0.99	9.98 ± 0.71	9.53 ± 0.39[f]	9.66 ± 0.54	10.89 ± 0.99	10.36 ± 0.44[e]	10.43 ± 1.05		1.525
17	9.15 ± 0.92	8.84 ± 0.94	8.82 ± 0.44	8.89 ± 0.86	9.77 ± 0.81	8.65 ± 1.04	10.19 ± 1.58		0.849
	Biomass, mg								
1	43.84 ± 4.41	43.84 ± 4.29	44.27 ± 4.14	43.35 ± 4.88	44.49 ± 3.71	45.23 ± 4.52			0.357
2	44.01 ± 4.40	41.59 ± 1.41	41.13 ± 2.14	42.21 ± 4.39	45.43 ± 3.80				1.948
3	47.67 ± 4.84	42.90 ± 2.85[d]	55.33 ± 1.23[ce]	47.23 ± 4.09[f]	44.97 ± 3.03[d]	51.10 ± 2.01			5.677[xx]
4	39.67 ± 3.35[d]	41.23 ± 1.01[d]	51.10 ± 1.02[ce]	40.57 ± 4.01[f]	42.07 ± 1.80[d]				8.021[xx]
5	48.10 ± 4.66[d]	37.43 ± 4.45[df]	72.13 ± 2.72[ce]	51.23 ± 2.35[df]	45.47 ± 2.25[de]	50.33 ± 1.53[de]			19.213[xxx]
6	41.67 ± 4.07[bd]	47.57 ± 3.67[bf]	63.53 ± 1.11[a]	55.57 ± 1.50[bce]	47.93 ± 1.31[bf]				100.555[xxx]
7	37.40 ± 6.42[bf]	45.23 ± 1.36[bf]	51.13 ± 3.19[ac]	55.33 ± 3.29[de]	49.87 ± 3.00[bc]				55.150[xxx]
8	42.67 ± 2.71[f]	49.53 ± 2.04[f]	71.60 ± 14.13[a]	59.53 ± 6.54[f]	49.87 ± 3.37[f]				7.195[xx]
9	40.93 ± 3.05	42.37 ± 0.35	44.70 ± 2.29	39.91 ± 2.52	44.50 ± 3.65				1.695
10	43.13 ± 4.18	43.10 ± 3.22	45.97 ± 2.84	43.73 ± 1.12	45.77 ± 1.13				0.621
11	42.33 ± 2.09	42.17 ± 6.10	44.90 ± 4.15	42.00 ± 4.47	47.40 ± 6.58				0.604
12	40.93 ± 5.35	44.07 ± 4.09	44.90 ± 3.31	41.40 ± 1.18[f]	44.50 ± 0.66[b]				0.677
13	42.90 ± 4.28	50.30 ± 5.52	49.33 ± 2.09	48.57 ± 5.95	51.17 ± 5.49				1.014
14	39.93 ± 0.45[bd]	45.43 ± 2.38	49.73 ± 1.19[a]	47.03 ± 1.45	42.77 ± 5.70				5.[illegible][xx]
15	52.17 ± 1.44[ce]	43.97 ± 2.55[df]	42.70 ± 4.48[f]	55.47 ± 2.95	61.77 ± 8.03	60.47 ± 12.12	55.13 ± 6.59[a]		3.05[x]
16	44.23 ± 3.01[df]	42.57 ± 1.21[df]	44.00 ± 2.44[d]	54.33 ± 3.01[ce]	57.70 ± 3.61[a]	58.33 ± 3.71[c]	51.77 ± 3.91[a]		11.051[xxx]
17	45.27 ± 3.18[f]	46.80 ± 1.48[f]	43.73 ± 2.57[df]	50.21 ± 1.11[a]	57.83 ± 3.59[a]	55.93 ± 3.58[ce]	52.27 ± 1.81[a]		9.808[xxx]

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Table 5. Variations of serum calcium, phosphorus and magnesium levels in each treatment group at different stages of experimental period

Treatment no	A	0 hr	24 hr	48 hr	96 hr	168 hr	240 hr	F-value
		Calcium, mg%						
1	10.67 ± 0.93	10.84 ± 0.97	10.97 ± 0.93	10.88 ± 0.95	10.75 ± 0.86	9.75 ± 0.17		0.854
2	9.92 ± 0.49	10.02 ± 0.19	10.25 ± 0.26	10.29 ± 0.21	10.28 ± 0.27			0.897
3	9.41 ± 0.29[f]	9.71 ± 0.30[f]	10.67 ± 0.51[e]	10.05 ± 0.36	9.92 ± 0.21	9.99 ± 0.37		3.914[x]
4	9.58 ± 0.57	9.61 ± 0.36	9.81 ± 0.50	9.94 ± 0.57	9.99 ± 0.78			0.459
5	9.71 ± 0.46	10.03 ± 0.30	10.23 ± 0.25	9.88 ± 0.19	10.07 ± 0.23	9.95 ± 0.13		1.516
6	10.52 ± 0.72	10.69 ± 0.55	11.20 ± 0.64	10.89 ± 0.49	11.57 ± 1.36			0.703
7	10.44 ± 0.34	10.59 ± 0.51	11.09 ± 0.59	10.72 ± 0.65	10.29 ± 0.62			0.441
8	10.33 ± 0.67	10.77 ± 0.50	11.48 ± 0.47	11.04 ± 0.56	11.20 ± 0.20			1.459
9	11.01 ± 0.47	11.93 ± 1.46	10.87 ± 0.44	10.92 ± 0.63	11.63 ± 1.41			0.732
10	11.51 ± 0.26	11.56 ± 1.34	11.20 ± 1.77	11.09 ± 0.46	11.39 ± 1.61			0.077
11	10.68 ± 0.83	12.17 ± 0.82	11.28 ± 0.42	11.43 ± 1.16	10.53 ± 1.19			1.915
12	11.19 ± 0.28[e]	10.69 ± 0.34	10.79 ± 0.51	10.28 ± 0.48[f]	10.29 ± 0.43[f]			1.949
13	12.77 ± 1.16	14.01 ± 2.17	12.45 ± 1.84	12.44 ± 0.52[f]	13.37 ± 0.16[a]			0.725
14	12.37 ± 0.69[a]	12.75 ± 1.48	11.08 ± 0.12[f]	10.55 ± 1.40	12.15 ± 1.69			1.324
15	10.74 ± 0.39[e]	9.79 ± 0.49[ef]	10.47 ± 1.12	9.72 ± 0.08[bf]	10.70 ± 0.50[e]	11.18 ± 1.03	10.79 ± 0.19[abg]	1.976
16	10.39 ± 0.47	9.61 ± 1.42	10.85 ± 1.45	10.18 ± 3.61	11.00 ± 0.52	11.08 ± 0.81	10.65 ± 0.37	0.231
17	10.05 ± 0.75[f]	9.65 ± 0.93	9.48 ± 0.61[f]	11.64 ± 0.35	10.93 ± 0.45[e]	10.24 ± 1.34	10.27 ± 0.70	1.184
	Magnesium, mg%							
1	2.26 ± 0.35	2.47 ± 0.38	2.38 ± 0.39	2.24 ± 0.38	2.36 ± 0.31	2.54 ± 0.07		1.018
2	1.85 ± 0.12[d]	1.87 ± 0.08[d]	2.01 ± 0.11	2.07 ± 0.27	2.15 ± 0.06[c]			1.849
3	2.12 ± 0.05[df]	2.32 ± 0.05[c]	3.05 ± 0.51[e]	2.22 ± 0.51	2.14 ± 0.21[f]	2.43 ± 0.36		2.613
4	2.07 ± 0.24	1.99 ± 0.04[d]	2.01 ± 0.11[c]	1.95 ± 0.13	2.11 ± 0.16			1.017
5	2.14 ± 0.09[df]	2.01 ± 0.28[cde]	2.58 ± 0.10[bc]	2.04 ± 0.04[bdf]	2.49 ± 0.17[e]	2.80 ± 0.21[ac]		12.749[xxx]
6	2.47 ± 0.13	2.43 ± 0.11	2.48 ± 0.14	2.51 ± 0.02	2.44 ± 0.07			0.454

7	2.44 ± 0.28	2.46 ± 0.23	2.36 ± 0.17	2.48 ± 0.20	2.41 ± 0.25			0.920
8	2.59 ± 0.23	2.44 ± 0.14	2.49 ± 0.17	2.51 ± 0.09	2.43 ± 0.12			0.397
9	2.44 ± 0.03	2.53 ± 0.00	2.47 ± 0.09	2.41 ± 0.10	2.51 ± 0.02			0.944
10	2.49 ± 0.10	2.39 ± 0.06	2.39 ± 0.12	2.36 ± 0.02	2.35 ± 0.19			0.897
11	2.39 ± 0.14	2.44 ± 0.24	2.49 ± 0.21	2.40 ± 0.24	2.35 ± 0.14			0.160
12	2.33 ± 0.15	2.49 ± 0.23	2.51 ± 0.19	2.57 ± 0.31	2.64 ± 0.32			0.277
13	2.44 ± 0.28	2.56 ± 0.25	2.47 ± 0.21	2.51 ± 0.24	2.56 ± 0.18			0.133
14	2.28 ± 0.14	2.41 ± 0.12	2.47 ± 0.02	2.39 ± 0.29	2.44 ± 0.12			0.519
15	2.80 ± 0.05[e]	2.17 ± 0.07[bf]	2.69 ± 0.25[a]	2.71 ± 0.35[a]	2.34 ± 0.14[d]	2.29 ± 0.09[b]	2.22 ± 0.10[d]	4.349[zz]
16	2.71 ± 0.21[a]	2.34 ± 0.27	2.47 ± 0.04[ce]	2.79 ± 0.44	2.99 ± 0.10[df]	2.35 ± 0.13	2.34 ± 0.04[f]	3.167[z]
17	2.83 ± 0.59	2.49 ± 0.31	2.39 ± 0.30	2.45 ± 0.55	2.15 ± 0.21	2.49 ± 0.39	2.64 ± 0.51	0.651
	Inorg. phosphorus, mg%							
1	5.72 ± 0.95	5.98 ± 0.79	5.91 ± 1.21	5.96 ± 0.79	5.53 ± 0.76	5.72 ± 0.18		0.541
2	5.52 ± 0.75	5.65 ± 1.03	5.38 ± 0.86	5.24 ± 0.62	5.37 ± 0.59			0.115
3	6.24 ± 1.01	5.93 ± 0.38	5.59 ± 0.51	6.08 ± 0.54	6.25 ± 0.53	5.33 ± 0.59		0.951
4	4.99 ± 0.14	4.97 ± 0.41	4.99 ± 0.42	4.80 ± 0.85	4.92 ± 0.84			0.810
5	5.76 ± 0.23[a]	5.78 ± 1.25	4.11 ± 0.95[f]	5.57 ± 0.93	5.81 ± 0.09[b]	5.66 ± 0.51		1.950
6	6.47 ± 0.41[a]	7.51 ± 0.43[c]	4.94 ± 0.57[df]	6.31 ± 0.58	6.97 ± 0.76[a]			5.147[zz]
7	6.19 ± 0.85	6.39 ± 0.32[ce]	4.49 ± 0.11[bf]	5.59 ± 0.19[cf]	6.40 ± 0.35[ae]			8.197[zz]
8	7.13 ± 0.31[ce]	6.54 ± 0.22[a]	4.93 ± 0.76[df]	5.89 ± 0.49[f]	5.25 ± 0.19[af]			8.795[zz]
9	6.28 ± 0.09[ce]	6.16 ± 0.43	5.85 ± 0.03[d]	6.64 ± 0.94	5.99 ± 0.11[f]			0.935
10	6.54 ± 0.24	6.51 ± 0.65	5.76 ± 0.78	6.31 ± 0.46	5.62 ± 0.54			1.575
11	6.02 ± 0.54	5.96 ± 0.19[d]	6.00 ± 0.35[f]	6.74 ± 0.07[ce]	5.35 ± 0.53[f]			8.236[z]
12	6.55 ± 0.40	6.48 ± 1.27	6.91 ± 1.15	5.72 ± 0.54	5.94 ± 1.03			0.597
13	6.42 ± 1.01	5.62 ± 1.42	6.50 ± 1.14	5.89 ± 0.62	5.44 ± 0.47			0.492
14	6.28 ± 0.59	6.34 ± 0.16	5.72 ± 1.09	5.33 ± 0.80	5.22 ± 0.77			0.859
15	6.70 ± 0.78	7.00 ± 0.83	6.71 ± 0.58	8.36 ± 1.10	7.14 ± 0.45	5.92 ± 0.47	5.91 ± 0.77	0.935
16	6.23 ± 1.37	7.05 ± 0.85[a]	7.19 ± 0.11[a]	6.27 ± 0.78	6.22 ± 0.43[df]	6.06 ± 0.67	5.53 ± 0.11[bf]	1.935
17	6.70 ± 1.29	6.94 ± 2.09	7.44 ± 0.85[a]	7.81 ± 0.84[ce]	5.59 ± 1.50	5.34 ± 0.42[f]	4.85 ± 0.25[df]	2.549[z]

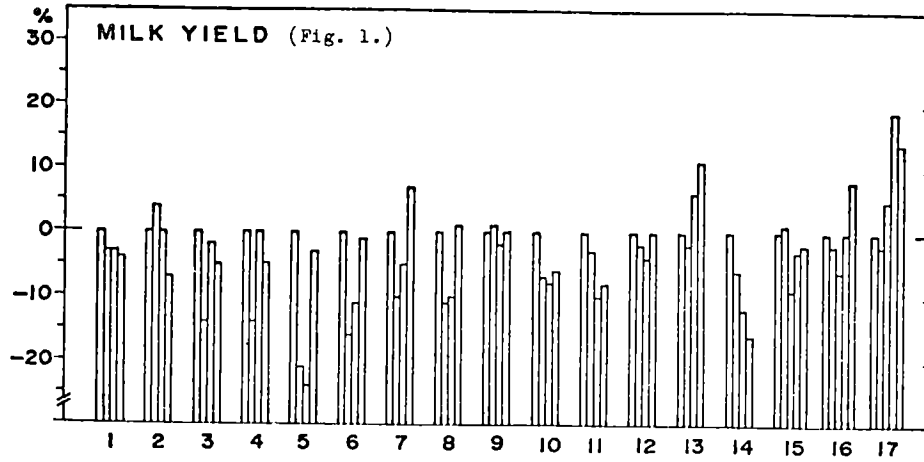
Table 6. Serum potassium, sodium, iron, copper and zinc levels of control

Treatment No x		A	B - 0 hr	24 hr	48 hr
K mg%	1	20.66 ± 0.41^{d}	22.61 ± 0.59^{c}	21.79 ± 2.78	19.49 ± 2.57
	15	19.84 ± 0.74	18.29 ± 1.47	20.60 ± 0.66^{e}	19.24 ± 0.40
	16	19.23 ± 0.99	19.51 ± 0.51	20.45 ± 2.11	20.55 ± 2.42
	17	20.70 ± 2.19	20.29 ± 0.48	19.90 ± 0.43	21.10 ± 1.04
Na mg%	1	318 ± 1.5^{d}	329 ± 2.3^{ce}	322 ± 3.0^{f}	326 ± 1.0^{c}
	15	315 ± 1.0^{df}	333 ± 4.0^{ce}	324 ± 3.1^{cf}	327 ± 4.6^{e}
	16	314 ± 0.6	327 ± 3.1	327 ± 1.5	321 ± 2.3
	17	314 ± 4.9	325 ± 1.7	327 ± 1.2	323 ± 4.4
Fe µg %	1	144 ± 24	161 ± 60	151 ± 33	138 ± 31
	15	149 ± 9	175 ± 23	166 ± 21	163 ± 21
	16	128 ± 14^{f}	159 ± 23	146 ± 17	149 ± 16
	17	124 ± 24	144 ± 33	159 ± 35	131 ± 20
Cu µg%	1	70.0 ± 3.61	81.0 ± 8.19	83.0 ± 9.64	75.0 ± 7.00
	15	73.3 ± 3.21	79.3 ± 7.09	75.7 ± 6.35	79.7 ± 7.23
	16	79.7 ± 8.74	82.3 ±10.79	83.7 ± 5.51	85.3 ±14.19
	17	82.7 ± 4.04	94.7 ± 8.08^{e}	87.7 ± 5.69^{e}	82.0 ± 3.46
Zn µg%	1	64.3 ± 11.02	61.3 ± 5.77	74.7 ± 8.08	70.3 ± 2.08
	15	68.7 ± 7.09^{f}	64.7 ±14.19^{f}	80.7 ± 4.93^{cf}	109.7 ±15.28^{e}
	16	75.7 ± 9.45	56.3 ± 2.52^{bd}	72.0 ± 10.15^{f}	92.7 ± 5.03^{ace}
	17	66.3 ± 13.50	60.3 ± 8.74	74.3 ± 14.80^{e}	76.7 ± 9.50^{e}

x 1 - controls; treatments 15, 16 and 17 are explained in Table 1.
a - effect of the stage of the experiment; b - effect of the experimental group;
ab - ab interaction
a<b - P< 0.001^{xxx}; c < d - P< 0.01^{xx}; e<f - P<0.05^{x}

and hormone treated cows at different stages of the experiment VI

96 hr	168 hr	240 hr	F-value 1-way	F-value 2-way
20.43 ± 1.46	20.69 ± 1.44	21.35 ± 1.36	0.767	ab 0.918
19.98 ± 1.13	19.36 ± 0.90	18.19 ± 0.75^{f}	2.844^{x}	a 4.242^{xx}
19.79 ± 0.39	21.07 ± 1.91	18.93 ± 2.50	0.508	b 0.783
20.33 ± 0.54	19.39 ± 0.86	18.83 ± 2.32	0.938	
319 ± 6.1	318 ± 2.7^{d}	316 ± 6.7^{f}	4.466^{xx}	ab 2.101^{x}
328 ± 5.1^{e}	324 ± 3.5^{ef}	318 ± 2.0^{d}	7.866^{xxx}	a 3.077^{x}
327 ± 2.1	327 ± 3.1	323 ± 5.9	6.765^{xxx}	b 14.176^{xxx}
325 ± 3.2	323 ± 3.1	320 ± 2.3	5.354^{xx}	
160 ± 33	147 ± 33	151 ± 57	0.150	ab 0.205
187 ± 78	207 ± 90	165 ± 12	0.478	a 1.929
173 ± 22^{e}	171 ± 17^{e}	177 ± 50	1.452	b 1.921
177 ± 53	177 ± 59	158 ± 63	0.655	
76.0 ± 7.21	74.3 ± 9.02	73.3 ± 4.16	1.018	ab 0.427
73.0 ± 9.54	78.0 ± 13.08	74.7 ± 13.01	0.723	a 7.427^{xxx}
81.0 ± 7.21	77.7 ± 10.50	76.7 ± 6.51	0.336	b 2.157^{x}
62.0 ± 3.61	78.3 ± 4.73^{f}	77.7 ± 2.52^{f}	3.915^{xx}	
56.3 ±12.10	60.3 ± 8.96	62.7 ± 5.03	2.035	ab 2.219^{x}
104.3 ±21.20^{e}	71.7 ± 7.09^{f}	60.0 ± 8.89^{df}	7.249^{xxx}	a 10.939^{xxx}
91.7 ± 5.51^{ace}	73.0 ± 5.20^{cf}	57.3 ± 4.93^{hd}	14.000^{xxx}	b 10.563^{xxx}
69.0 ±15.72	59.7 ± 15.31	49.7 ± 2.08^{df}	1.795	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MILK YIELD (Fig. 1.)
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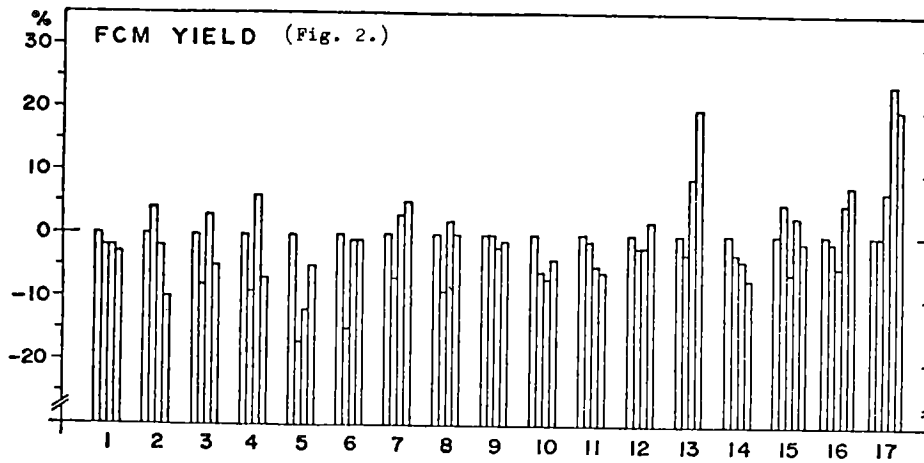
0
-10
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17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FCM YIELD (Fig. 2.)
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10
0
-10
-20
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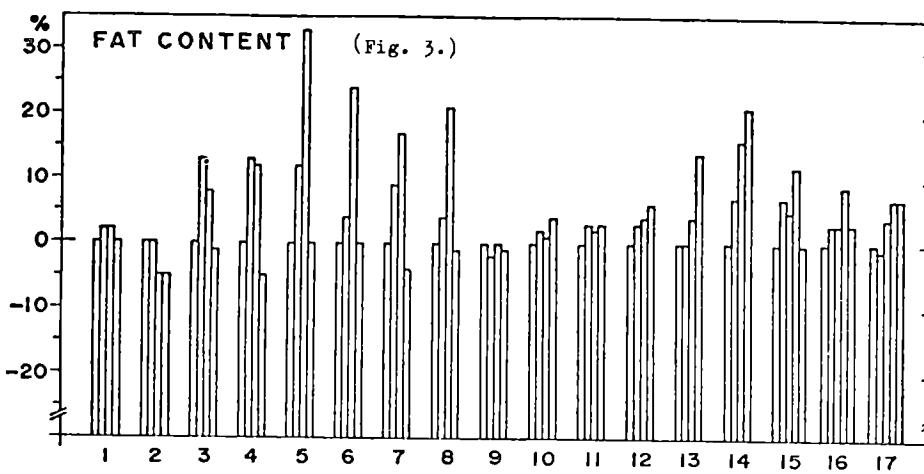
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FAT CONTENT (Fig. 3.)
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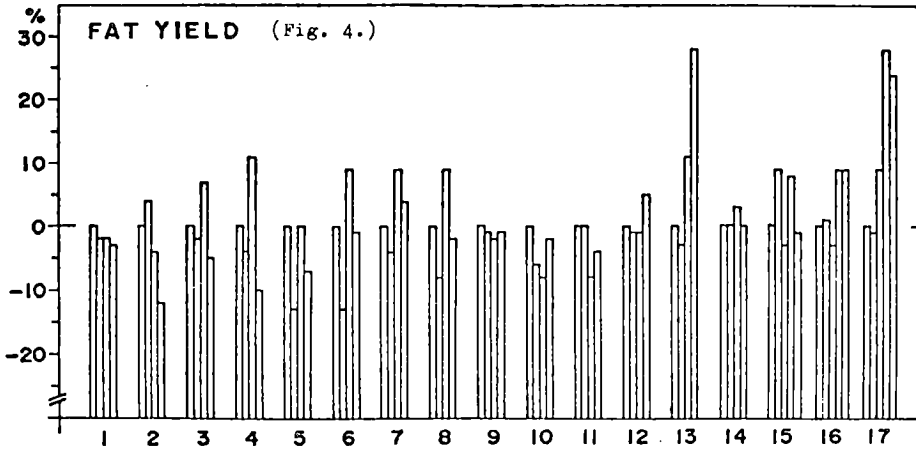
20
10
0
-10
-20
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Treatment no.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20
10
0
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FAT YIELD (Fig. 4.)
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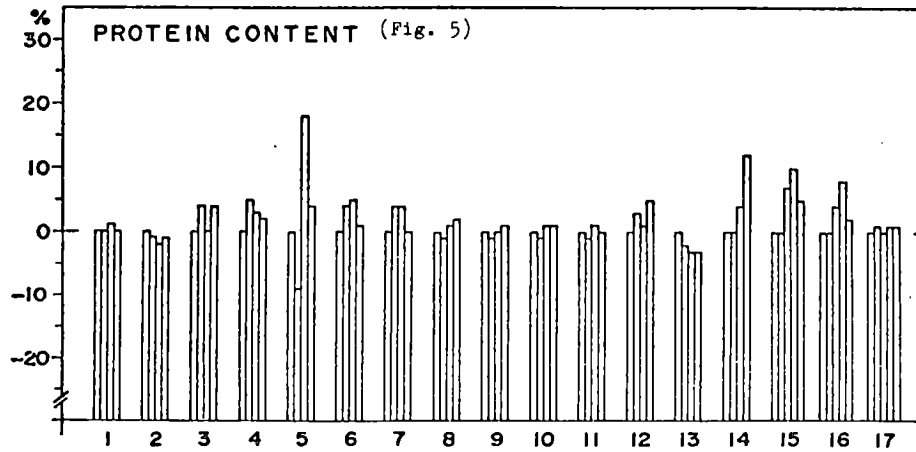
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-10
-20
PROTEIN CONTENT (Fig. 5)
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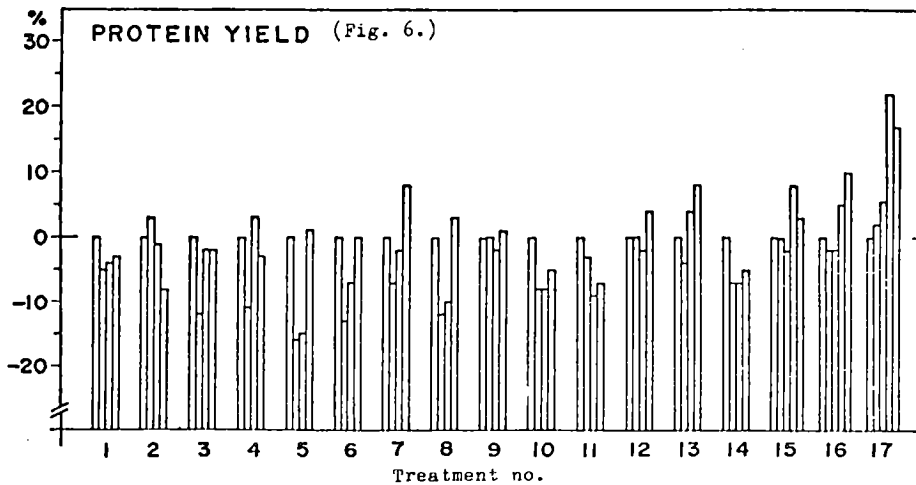
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20
10
0
-10
-20
PROTEIN YIELD (Fig. 6.)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Treatment no.

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