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On farm

Immunocastration in male cattle

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Abstract

Immunological castration (immunocastration) of bulls later in life would take advantage of the beneficial anabolic effects of testosterone early in life and then suppress testosterone at an appropriate time to control body composition and behaviour. Crossbred bulls (2-year-old) immunocastrated with Improvac® underwent a decline in testis size and testosterone secretion and a progressive shift in body characteristics (eye muscle area; P8 and rib fat) to those typical of steers ($P<0.05$). Immunocastrates showed a slower rate of live weight gain ($P<0.05$) and at slaughter had a lighter carcase, smaller eye muscle area, but improved fat colour score, compared with bulls ($P<0.05$). A second study examined whether immunocastration of bulls might produce an acute decline in aggressive and sexual behaviour that would have application in live export. Brahman bulls (3-year-old) immunocastrated with a prototype vaccine thought to be superior to Improvac® showed a relatively modest immunological response with no apparent change in testosterone secretion. Bulls appeared to become accustomed to the experimental behaviour tests and it is recommended that future research in this area may need to be conducted under industry conditions. Immunocastration has potential application as a new technology in value-based markets and possible application in the live export of bulls warrants further investigation.

Executive Summary

The aim in this project was to obtain preliminary information on whether immunological castration (immunocastation) of bulls later in life had potential as a new technology that would provide an additional management strategy to control carcase yield, carcase type and meat quality in male cattle. The project was based on the understanding that, from the time of immunocastration, body (and carcase) type would progressively change from that of an entire bull to that typical of a steer. These changes in body type could be monitored using the technology of ultrasound body scanning and this information would be used to make informed decisions on when to market animals into value-based markets. This strategy would have application in both feedlot-finished and pasture-finished male cattle.

The initial design of the project included comparisons amongst relatively large numbers of entire bulls, steers and late immunocastrates that would be both feedlot-finished and pasture-finished. Changes in the availability of animals, personnel and resources necessitated a smaller-scale project that compared growth, body and carcase features in small groups of bulls and immunocastrates. The underlying objective of the project was however maintained. A second modification to the project was the inclusion of a study to examine whether short-term changes in aggressive and sexual behaviour occurred in bulls after immunocastration. If acute changes in behaviour occur after immunocastration then this would have application in the live export of bulls.

Immunocastration involves the administration of a vaccine that induces animals to raise antibodies against a key reproductive hormone, gonadotrophin releasing hormone (GnRH). GnRH is released from the brain and initiates the reproductive hormone cascade that is required for growth and function of the testes. Immunoneutralisation of GnRH blocks the reproductive hormone cascade and the testes become inactive (immunocastrated). The immunocastration response can be maintained provided that anti-GnRH antibodies in blood remain above the threshold necessary to neutralise GnRH as it is released from the brain. GnRH has the same structure in all mammals and a GnRH vaccine can therefore theoretically be used across species.

The GnRH vaccine Improvac® that is commercialised for use in male pigs was used in the first study. Crossbred bulls (2-year-old) were immunised using Improvac® and monitored for longitudinal changes in testis function, live weight and body features (eye muscle area, P8 fat and 13th rib fat). Carcase characteristics were determined at slaughter. Immunocastrated bulls tended to show a slower rate of live weight gain than entire bulls and the body features of immunocastrates gradually changed from those typical of bulls to those characteristic of steers. At slaughter, immunocastrates had a lighter carcase, smaller eye muscle area, but improved meat and fat colour scores compared with bulls ($P<0.05$). Immunocastration was effective in inducing a progressive transition in body and carcase characteristics from those of bulls to features typical of steers. However, the lighter carcases of immunocastrates resulted in a reduced value as price was based solely on a carcase weight differential (higher per kg price for heavier carcases). Any future use of immunocastration would therefore need to be in association with value a value-based marketing system.

In the above study with Improvac®, it was found that anti-GnRH antibody levels in blood declined relatively quickly after vaccination. It was necessary, therefore, to give repeat vaccinations in order to maintain anti-GnRH antibody titres in blood above a threshold considered necessary for an immunocastration response. The second study utilised a prototype GnRH vaccine under development by CSL Limited for specific use in cattle. Groups of Brahman bulls (3-year-old) were immunised and then monitored for changes in aggressive and sexual behaviour. One group of bulls received two vaccinations with a 1-weekly interval and a second group of bulls received two vaccinations with a 2-weekly interval. The latter vaccination interval has been used previously in cattle but a 1-week vaccination interval, if successful, was considered to have particular application in the live export of bulls where the opportunity to market animals can be relatively short.

The prototype vaccine induced relatively low anti-GnRH antibody titres and there was no apparent decline in testosterone secretion in immunised bulls. Immunised bulls were therefore not considered to have been effectively immunocastrated and a comparison of aggressive and sexual behaviour between control bulls and truly immunocastrated bulls was not possible. This study did, however, provide important information that is relevant to future research in this area. Bulls appeared to become accustomed to the behaviour tests during the course of the study and this was thought to influence their behaviour. It is recommended that future research on the effects of immunocastration on behaviour may need to be conducted with industry. Groups of normal bulls and immunocastrated bulls should ideally be introduced for the first time when behaviour is evaluated.

This project has demonstrated the potential of immunocastration as an additional management strategy to control the body and carcase characteristics of male cattle. The ability to take advantage of the anabolic effects of testosterone in young bulls followed by immunocastration at a time suitable to management and production goals would add a further strategy to value-based beef production. If proven to be efficient and practical, immunocastration would have advantages compared with surgical castration at later ages in bulls.

However, there will need to be major advances in immunocastration technology before it could be adopted by industry. The present technology requires two vaccinations, the immunological response (anti-GnRH antibody titres) is relatively low in the majority of animals, and the response is not sustained for the length of time typically required by industry.

Study 1

Longitudinal changes in reproductive status, live weight, and body characteristics after immunisation of postpubertal bulls against GnRH

Summary

Longitudinal changes in reproductive status, live weight and body characteristics were monitored after active immunisation of mature bulls against GnRH using the commercial vaccine Improvac®. Bulls were observed for anti-GnRH antibody titres, plasma testosterone, testis size and live weight at 4-weekly intervals. Eye muscle area and P8 and 13th rib fat were measured using ultrasonography at around 2-monthly intervals. Immunisation against GnRH caused a reduction in testis size and decline in circulating concentrations of testosterone. Immunocastrated bulls tended to show a reduced rate of live weight gain and higher deposition of fat at the P8 and 13th rib sites. At slaughter, immunocastrates had a lighter carcase, smaller eye muscle area, but improved meat and fat colour scores compared with normal bulls. Immunocastration was effective in inducing a progressive transition in body and carcase characteristics from those of bulls to features typical of steers. However, the lighter carcases of immunocastrates resulted in a reduced value as price was based solely on a carcase weight differential, with heavier carcases having a greater per unit kilogram value. It was necessary in this study to give repeat vaccinations against GnRH as anti-GnRH antibodies in circulation declined relatively quickly after each vaccination. It was concluded that immunocastration has potential to control body and carcase features in bulls but further research is required on the GnRH vaccine technology and immunocastrated bulls would need to be sold into value-based markets.

Introduction

Reproductive function in bulls relies on hormonal communication between the brain, anterior pituitary gland and testes (Figure 1.1; D'Occhio 1993). The brain releases gonadotrophin releasing hormone (GnRH) that stimulates the release of the two gonadotrophic hormones luteinising hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland. Luteinising hormone stimulates steroidogenic function in the Leydig cells of the testes and FSH maintains the Sertoli cells which support spermatogenesis. Androgenic and oestrogenic steroids produced by the testes have critical roles in growth and behaviour in males (Figure 1.1). Basic studies on reproductive function in laboratory animals demonstrated that it was possible to immunise animals against GnRH. Immunisation against GnRH results in neutralising anti-GnRH antibodies in blood that block the actions of GnRH at the pituitary gland (D'Occhio 1993). In the absence of GnRH stimulation the anterior pituitary ceases the production and release of LH and FSH which, in turn, causes atrophy of the testes. Entire males effectively immunised against GnRH are therefore essentially similar to surgical castrates.

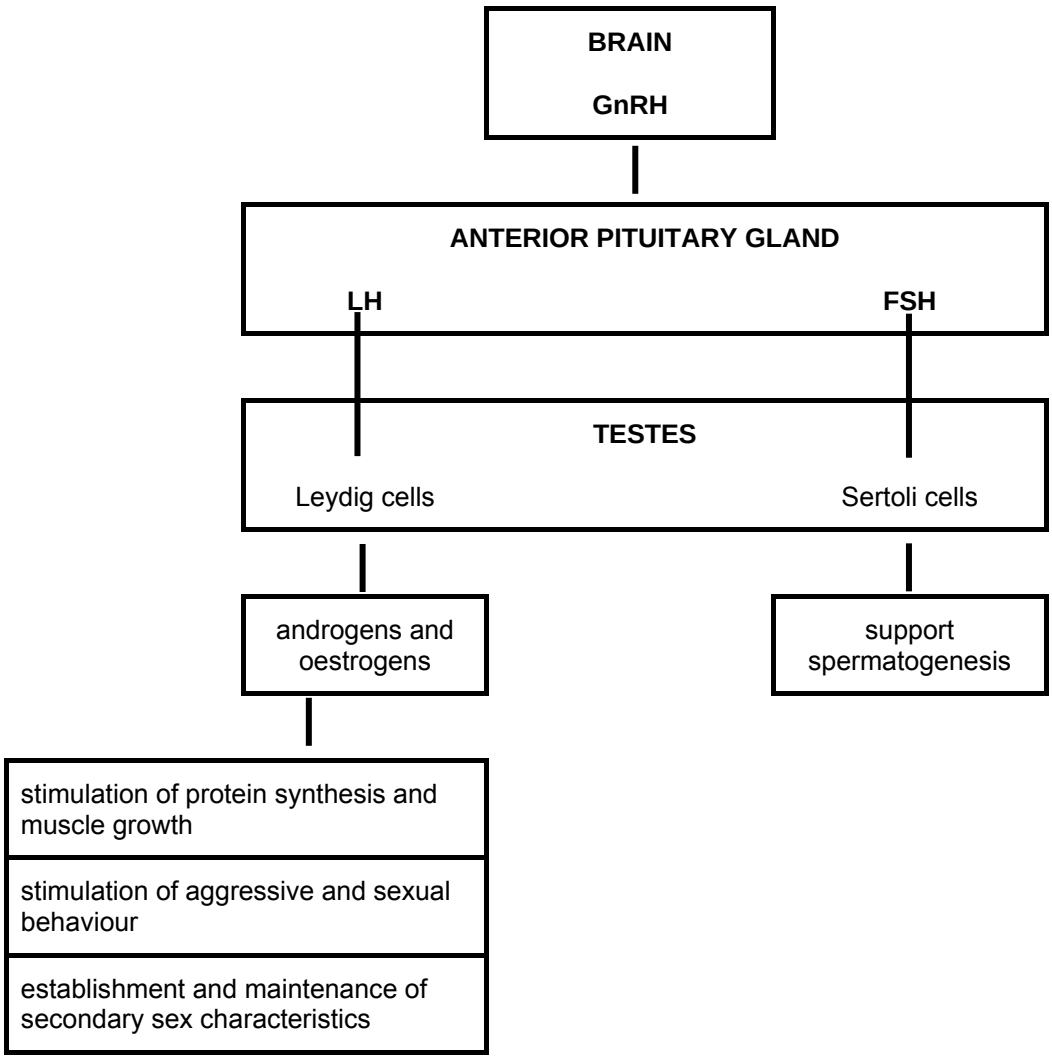


Figure 1.1. Hormonal interrelationships between the brain, anterior pituitary gland and testes in males and the role of testicular steroids (androgens and oestrogens) in growth and in the maintenance of behaviour and secondary sex characteristics.

Immunisation against GnRH reduced circulating concentrations of testosterone in bulls (Robertson et al 1979, 1984; Flint 1990) and suppressed reproductive function (D'Occhio 1993, 1994; D'Occhio et al 2001). In a series of studies, immunocastrated bulls had a greater rate of live weight gain than steers and carcase and meat quality characteristics were intermediate between steers and bulls (Robertson et al 1982, 1984; Jones et al 1983; Moore et al 1989; Lobley et al 1992; D'Occhio et al 2001). These studies demonstrated the potential of immunocastration as a strategy to control body and carcase features of bulls.

Bulls have a greater efficiency of live weight gain than steers and this is evident even before bulls reach full sexual maturity. The greater efficiency of live weight gain in bulls is attributable to the anabolic actions of testicular steroids (Figure 1.1). The observation that bulls show improved growth performance before puberty led to the suggestion that castration should be delayed until the time when bulls start to show levels of aggressive and sexual behaviour that have a detrimental effect on their performance (Gregory and Ford 1983; Vanderwert et al 1985; Jago et al 1999). In one study, bulls immunocastrated after puberty has superior meat quality attributes, for a particular market, than bulls surgically castrated either before or after puberty (Jago at al 1999).

Bulls effectively immunocastrated after puberty would be expected to undergo a progressive transition in body and carcase features from those characteristic of bulls to features typical of steers. This could theoretically provide an additional strategy to more precisely control the carcase attributes of male cattle that are destined for value-based markets.

The recent advent of ultrasonography to measure muscle and fat characteristics on live animals provides a technology to monitor longitudinal changes in body type in cattle. Ultrasonography could therefore be used to inform management decisions on when to sell animals into targeted markets.

The main aim in the present study was to integrate immunocastration of mature bulls with ultrasonography to monitor longitudinal changes in body features. The carcase characteristics of immunocastrated bulls were compared with those of normal bulls at the end of the study.

Materials and Methods

Animal experimentation ethics approval

The study was conducted in accordance with the guidelines set out in the joint publication of the National Health and Medical Research Council, the Commonwealth Scientific and Industrial Research Organisation and the Australian Agricultural Council entitled Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 6th Edition (Australian Government Publishing Service, Canberra, 1997). The Animal Experimentation Ethics Panel of Central Queensland University approved the study (01/11-117). The anti-GnRH vaccine Improvac® (Commonwealth Serum Laboratories, Victoria) is registered for use in pigs and was used in the present study with approval from the Therapeutic Goods Administration (PER5017).

Animals and management

A group of 24-month-old Hereford-Shorthorn x Africander bulls were block randomised on live weight to a control group (n=7) and a GnRH immunised group (n=8). Animals were maintained on natural pasture under standard management practices for the duration of the study.

GnRH immunisation

Immunisation against GnRH involved subcutaneous administration of 5ml Improvac® (1000μg GnRH-protein conjugate) in the dorsal neck region. The volume of Improvac® was based on the recommended dosage for pigs and adjusted for the greater live weight of cattle. A 5ml volume of GnRH vaccine has been used previously in cattle (Hoskinson et al 1990; Jago et al 1997, 1999; D'Occhio et al 2001). The two-week interval between primary, secondary and tertiary immunisations was based on previous experience with this protocol in cattle (D'Occhio et al 2001). Bulls received a primary immunisation at week 0 and booster immunisations at weeks 2, 4, 15, and 19. Immunisations were administered on the right side of the neck on weeks 0, 4, and 19 and the left side on week 15.

Animal sampling

Activities undertaken during the study are summarised in Table 1.1.

Table 1.1 Activities undertaken during Study 1.

Week of Study	Activity
0	Live weight, scrotal circumference, blood sample, ultrasound, primary vaccination
2	Blood sample and secondary vaccination
4	Live weight, scrotal circumference, testicular diameter, blood sample, third vaccination
5	Blood sample
6	Blood sample
8	Live weight, scrotal circumference, testicular diameter, blood sample, ultrasonography
15	Live weight, scrotal circumference, testicular diameter, blood sample, ultrasonography, fourth vaccination
16	Blood sample
17	Blood sample
19	Live weight, scrotal circumference, testicular diameter, blood sample, fifth vaccination
20	Ultrasonography, blood sample
21	Blood sample
23	Live weight, scrotal circumference, testicular diameter, blood sample
25	Blood sample
28	Ultrasonography
33	Live weight, scrotal circumference, testicular diameter, blood sample
33	Slaughter

Blood sampling

Blood samples for the measurement of testosterone and anti-GnRH antibody titres were collected at weeks 0, 2, 4, 5, 6, 8, 15, 16, 17, 19, 20, 21, 23, 25 and 33 by coccygeal venipuncture. Blood samples were stored on ice prior to centrifugation at 800 x g to recover plasmas. Plasmas were stored at -20°C until required for testosterone or antibody titre analysis.

Anti-GnRH antibody titres

Anti-GnRH antibody titres were determined using validated ELISA methodology developed in our laboratory.

Plasma testosterone

Plasma concentrations of testosterone were determined using a validated radioimmunoassay (D'Occhio and Setchell 1984).

Testis size

Testis size was determined by two methods. Scrotal circumference was measured using an aluminium measuring tape and the individual diameter of the left and right testes was measured with callipers. To avoid differences in measuring technique the same person carried out all scrotal and testicular measurements.

Live weight

Animals were weighed at weeks 0, 4, 8, 15, 19, 23, and 33 directly off pasture.

Ultrasonography

Real time ultrasonography scanning was used to measure eye muscle area and fat depth at the P8 rump site and between the 13^{th} and 14^{th} ribs (13^{th} rib site). Scanning was carried out at weeks 0, 8, 15, 20, and 28. Measurements of fat depth to the nearest millimetre at the specific rib and rump sites of the bulls were recorded directly. A cross sectional image of the longissimus dorsi muscle was taken for the calculation of eye muscle area. The data was stored using a CX100 frame grabbing board and the image was then traced using Ultrasound Image Analysis V1.4 software (copyright Weststock/Systems Intellect, Perth, 1996). This software calculates the traced eye muscle area in square centimetres.

Post-slaughter measurements

Testis weight

To record testis weight the epididymis was removed and weighed separately.

Carcase assessment

Hot carcase weight was recorded by the abattoir as standard procedure. After conventional storage at 10°C overnight carcasses were assessed for fat colour and

meat colour by trained assessors using standard AusMeat colour chips. Eye muscle area was determined at the same time using a standard rib eye analysis grid.

Statistical analyses

Data at individual time points were analysed by ANOVA using the General Linear Models (GLM) procedure of SAS/STAT (SAS 1990). Data analyses over time were undertaken by repeated measures analysis using SAS/SATA procedure MIXED with REML estimation (autoregressive - 1) and the model being y = treatment, time, treatment x time, with animal as the repeated measure (SAS 1992). Results are reported as untransformed arithmetic means $\pm$ SEM.

For anti-GnRH antibody titre determination, the mean absorbance of control samples (representing non-specific binding) at each date was subtracted from the absorbance on the same date for individual samples of immunocastrated bulls. The repeated measures model for analysis of antibody titres was y = time with animal as the repeated measure. The correlation coefficient for the relationship between carcase weight and carcase value was determined using Minitab correlation.

Results

Anti-GnRH antibody response

All bulls immunised against GnRH developed anti-GnRH antibodies with the relative antibody titres showing a degree of variability between bulls. The initial period of vaccination at 0, 2 and 4 weeks was followed by a rise ($P < 0.05$) in antibody titres (Figure 1.2). Tires had declined ($P < 0.01$) at week 15 and a booster immunisation was followed by a rise in antibody titres to levels similar to those observed after the initial period of immunisation between weeks 0 and 6. A similar rise in antibody titres was observed after booster immunisation at week 19.

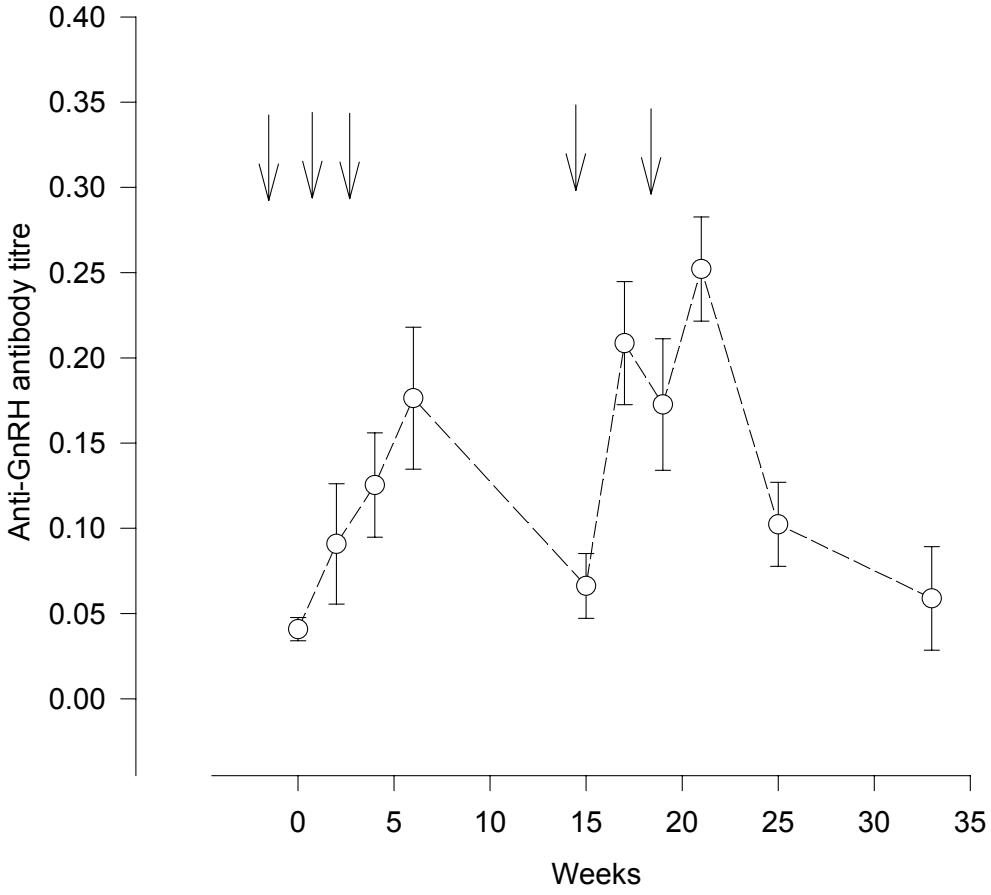


Figure 1.2. Anti-GnRH antibody titre for bulls immunised against GnRH in Study 1. Arrows indicate immunisation and results are mean ± SEM (n = 8).

Testosterone

Plasma concentrations of testosterone declined in response to immunisation against GnRH and at 4 weeks were lower ($P<0.05$) in immunised bulls Figure 1.3; Table 1.2). The variability in plasma testosterone for control bulls meant that the difference in plasma testosterone between immunised and control bulls was not significant at all times during the study. Plasma testosterone showed evidence of increasing in immunocastrated bulls towards the end of the study.

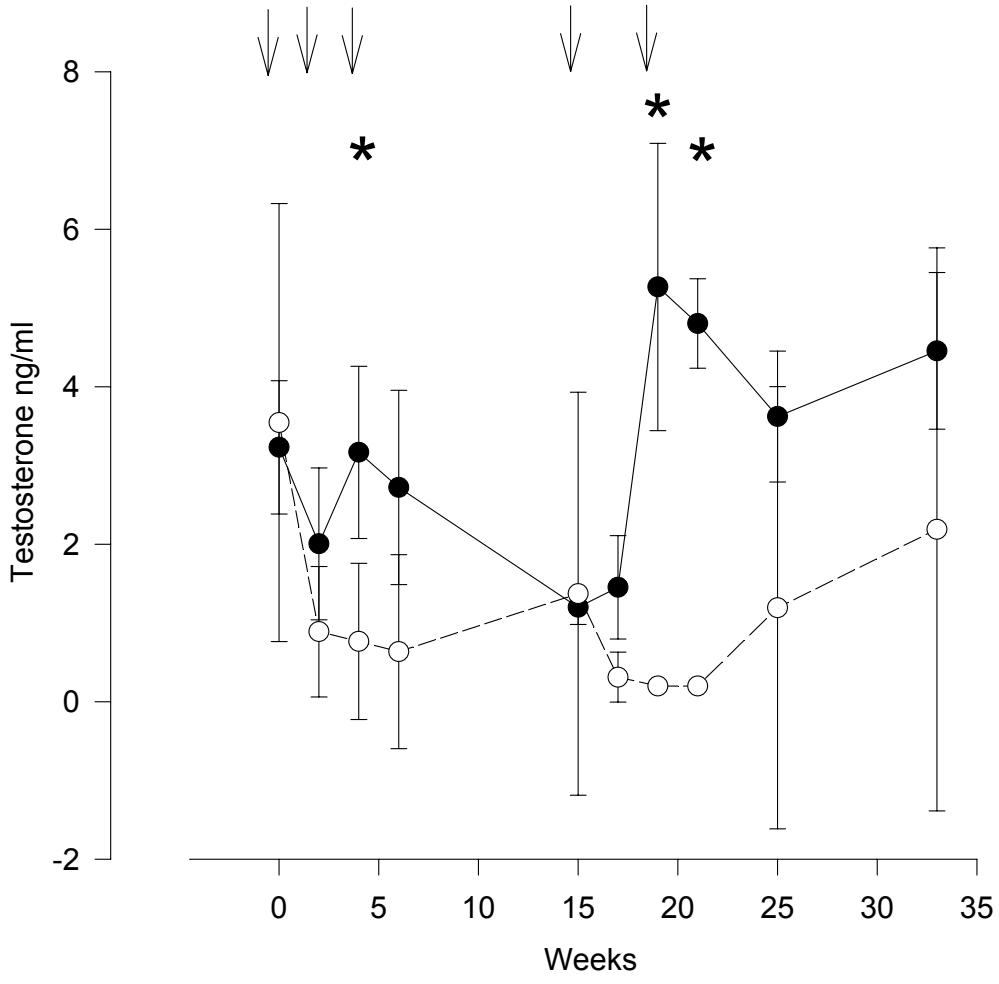


Figure 1.2. Plasma concentrations of testosterone for control bulls (●) and bulls immunised against GnRH (o) in Study 1. Arrows indicate immunisation. Results are means ± SEM (* P<0.05 at weeks 4 and 19 and P<0.01 at week 21).

Table 1.2 Plasma testosterone concentrations in control bulls and bulls immunised against GnRH in Study 1.

Week of Study	Control (n = 7)	GnRH immunised (n = 8)
0	3.2 ± 0.8^{a}	3.5 ± 0.9^{a}
4	3.1 ± 1.0^{a}	0.7 ± 0.3^{b}
6	2.7 ± 1.2^{a}	0.6 ± 0.4^{a}
19	5.2 ± 1.8^{a}	0.2 ± 0.0^{c}
21	4.8 ± 0.5^{a}	0.2 ± 0.0^{c}
25	3.6 ± 0.8^{a}	1.1 ± 0.9^{a}
33	4.4 ± 0.9^{a}	2.1 ± 1.2^{a}

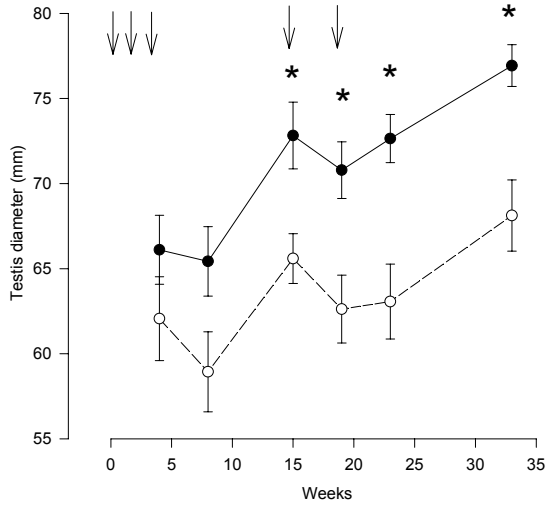
Means within rows without a common superscript differ ($^{a\,b}$ P<0.05; $^{a\,c}$ P<0.01)

Testis size

The testis diameter of bulls immunised against GnRH was smaller (P<0.05) than that of control bulls at 14 weeks after primary immunisation (Figure 1.4; Table 1.3). There was evidence that testis diameter in immunocastrated bulls had started to increase towards the end of the study although there was still a substantive (P<0.01) difference between immunocastrated and control bulls (Figure 1.4). The scrotal circumference of bulls immunised against GnRH was smaller (P<0.05) than that of control bulls at weeks 19 and 23 and, similar to testis diameter, was increasing in the former bulls towards the end of the study (Figure 1.4; Table 1.3).

Live weight

Longitudinal changes in live weight are shown in Figure 1.5 and the results are summarised in Table 1.4. Immunocastrated bulls tended to show a reduced rate of live weight gain and the treatment x time interaction approached significance (P = 0.08). Live weight gain from week 0 to week 33 was greater (P<0.05) for control bulls and at the end of the study immunocastrated bulls had a lower (P<0.05) live weight than control bulls (Table 1.4).



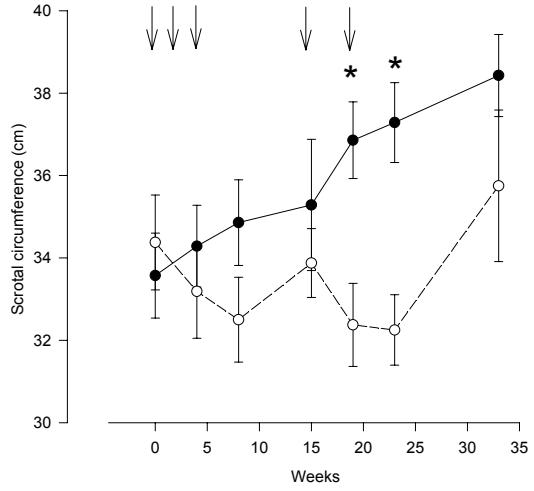


Figure 1.4. Longitudinal changes in testis diameter (top panel) and scrotal circumference (bottom panel) for control bulls (●) and bulls immunised against GnRH (o) in Study 1. Arrows indicate immunisation. Results are means ± SEM (* $P < 0.05$).

Table 1.3. Testis diameter and scrotal circumference for control bulls (n = 7) and bulls immunised against GnRH (n = 8) in Study 1. Results are means ± SEM.

Week of Study	Testis diameter (mm)		Scrotal circumference (cm)	
	Control	GnRH immunised	Control	GnRH immunised
0			33.5 ± 1.0[a]	34.3 ± 1.1[a]
4	66.1 ± 2.0[a]	62.0 ± 2.4[a]	34.2 ± 0.9[a]	33.1 ± 1.1[a]
8	65.4 ± 2.0[a]	58.9 ± 2.3[a]	34.8 ± 1.0[a]	32.5 ± 1.0[a]
15	72.8 ± 1.9[a]	65.5 ± 1.4[b]	35.2 ± 1.5[a]	33.8 ± 0.8[a]
19	70.7 ± 1.6[a]	62.6 ± 1.9[b]	36.8 ± 0.9[a]	32.3 ± 1.0[b]
23	72.6 ± 1.4[a]	63.0 ± 2.1[b]	37.2 ± 0.9[a]	32.2 ± 0.8[b]
33	76.9 ± 1.2[a]	68.1 ± 2.0[b]	38.4 ± 0.9[a]	35.7 ± 1.8[a]

[a, b] Means within rows, and within the respective parameter, without a common superscript differ (P < 0.05).

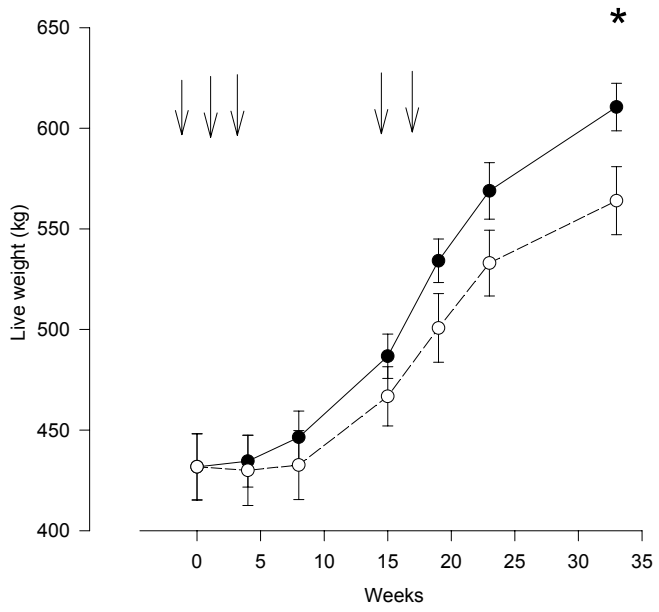


Figure 1.5. Longitudinal changes in live weight for control bulls (●) and bulls immunised against GnRH (o) in Study 1. Arrows indicate immunisation. Results are means ± SEM (* P<0.05)

Table 1.4. Live weight and live weight gain (Δ live weight) for control bulls and bulls immunised against GnRH in Study 1. Results are means ± SEM.

Week of Study	Control (n = 7)	GnRH immunised (n = 8)
0	431 ± 16 [a]	432 ± 16 [a]
15	486 ± 11 [a]	466 ± 16 [a]
23	569 ± 14 [a]	533 ± 16 [a]
33	610 ± 11 [a]	564 ± 16 [b]
Δ live weight	178 ± 7 [a]	132 ± 6 [b]

Means within rows without a common superscript differ (P < 0.05)

Ultrasonography of body characteristics

Results for ultrasonography scanning of eye muscle area, P8 rump fat, and 13th rib fat are shown in Figure 1.6. Control bulls showed a constant linear increase in eye muscle area. Immunocastrated bulls tended to show a reduction in eye muscle area growth from around week 8 and whilst there was a significant treatment x day interaction (P<0.05) the difference in eye muscle area between immunocastrated and control bulls only approached significance (P<0.1) at the end of the study (week 33).

The increase in P8 rump fat was similar for immunocastrated and control bulls to week 15 (Figure 1.6). After week 15, immunocastrated bulls tended to show a greater increase in P8 fat, although the treatment x time interaction was not significant (P>0.05). At the end of the study (week 33) immunocastrated bulls tended (P<0.1) to have greater P8 fat.

Immunocastrated bulls showed evidence of a greater rate of fat deposition at the 13t^{h} rib site between week 15 and week 33 although this was not significant (P>0.05). At the end of the study (week 33) there was no significant (P>0.05) difference in 13th rib fat between immunocastrated and control bulls.

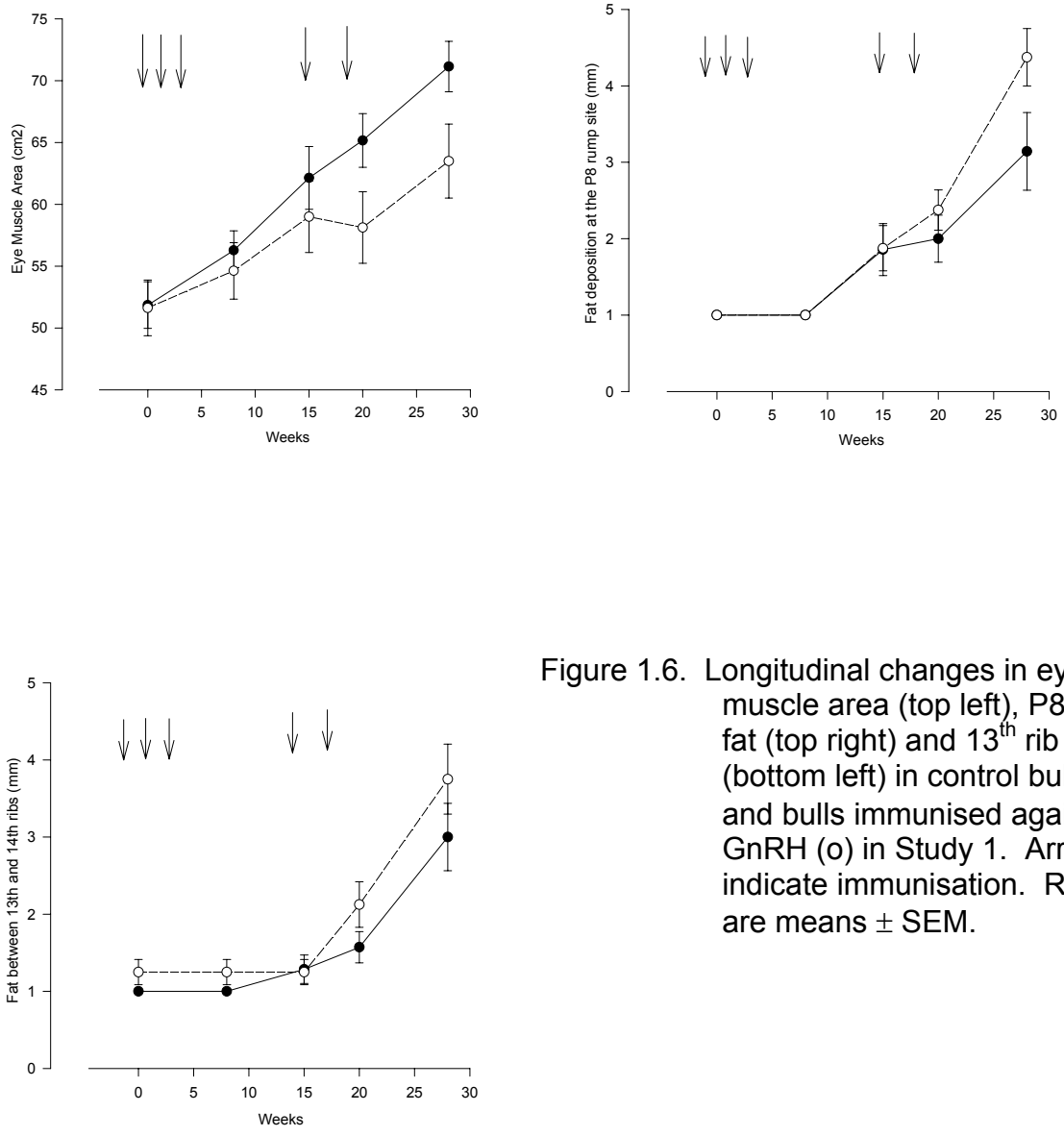


Figure 1.6. Longitudinal changes in eye muscle area (top left), P8 rump fat (top right) and 13th rib fat (bottom left) in control bulls (●) and bulls immunised against GnRH (o) in Study 1. Arrows indicate immunisation. Results are means ± SEM.

Post-slaughter measurements

Testis weight

At slaughter, the testes and epididymides of immunocastrated bulls were substantially smaller (P<0.01) than those of control bulls (Table 1.5). This finding demonstrated that immunisation against GnRH had been effective in inducing atrophy of the testes.

Table 1.5. Weight of the right testis and right epididymis for control bulls (n = 7) and bulls immunised against GnRH (n = 8) in Study 1. Results are means ± SEM.

	Testis (g)		Epididymis (g)	
	Left	**Right**	**Left**	**Right**
Control	259 ± 15 [a]	252 ± 15 [a]	25.7 ± 1.7 [a]	25.4 ± 1.3 [a]
GnRH immunised	98 ± 14 [b]	97 ± 11 [b]	14.7 ± 1.8 [b]	14.5 ± 1.2 [b]

Means within columns without a common superscript differ (P < 0.01)

Carcase weight

Control bulls had a greater (P<0.05) carcase weight than immunocastrated bulls (Table 1.6). In determining carcase value, heavier carcases attracted a greater per kilogram price than lighter carcases (r^2 = 0.93) (Figure 1.7). This meant that the carcases of immunocastrated bulls had a lower (P<0.05) value than the carcases of control bulls (Table 1.6).

Table 1.6. Carcase weight and carcase value control bulls (n = 7) and bulls immunised against GnRH (n = 8) in Study 1. Results are means ± SEM.

	Carcase weight (kg)	Carcase value ($)
Control	304 ± 6 [a]	680 ± 18 [a]
GnRH immunised	273 ± 9 [b]	587 ± 28 [b]

Means within columns without a common superscript differ (P < 0.05)

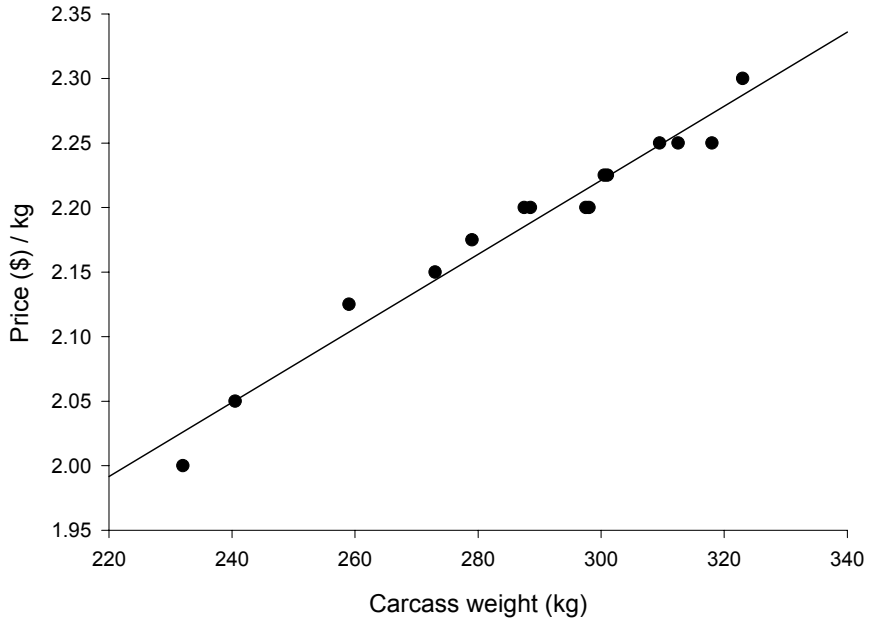


Figure 1.7. Relationship between carcase weight and per kilogram unit value.

Eye muscle area and meat and fat colour

The eye muscle area of immunocastrated bulls at slaughter was smaller (P<0.05) than that of control bulls (Table 1.7). Immunocastrated bulls tended to receive improved grading for meat colour and fat colour although the difference between immunocastrated and control bulls was only significant (P<0.05) for fat colour (Table 1.7).

Table 1.7. Carcase characteristics of control bulls (n = 7) and bulls immunised against GnRH (n = 6) in Study 1. Results are means ± SEM.

	Meat colour	**Fat colour**	**Eye muscle area (cm^2)**
Control	2.8 ± 0.5^a	1.2 ± 0.2^a	85.8 ± 2.4^a
GnRH immunised	2.1 ± 0.4^a	0.7 ± 0.2^b	75.5 ± 4.2^b

Means within column without a common superscript differ (P < 0.05)

Discussion

The objective in the present study was to monitor the progressive changes in body characteristics in mature bulls subsequent to the induction of an immunocastration condition by active immunisation against GnRH. Immunisation against GnRH was expected to lead to a progressive shift in body features (e.g. eye muscle, P8 fat, 13th rib fat) from those typical of bulls to those of steers. This shift could be monitored longitudinally by body ultrasonography.

Immunisation of bulls against GnRH using Improvac® induced anti-GnRH antibodies in all animals. The apparent anti-GnRH antibody titres varied considerably between bulls and peak titres were maintained for relatively short periods after immunisations. Improvac® is commercialised for use in male pigs and is therefore formulated to induce optimal responses in this species. As noted earlier, the structure of GnRH has been conserved in mammals and theoretically a vaccine developed for one species should be effective in other mammals. Whilst Improvac® was effective to a degree in bulls, a vaccine formulation that was tailored for cattle would need to be developed for practical application of immunocastration technology in bulls.

Bulls immunised with Improvac® had reduced plasma concentrations of testosterone and the testes of immunised bulls showed a reduced rate of growth compared with control bulls. These physical and endocrinological responses of the testes of immunocastrated bulls indicated that endogenous GnRH had been neutralised by anti-GnRH antibodies. However, the decline in antibody titres between vaccinations against GnRH would suggest that the degree of GnRH immuno-neutralisation fluctuated during the study. This suggestion was supported by apparent changes in the extent of testosterone suppression in immunocastrated bulls and the rise in testosterone and testis size towards the end of the study.

Notwithstanding the variability in the endocrine response to immunisation with Improvac®, immunocastrated bulls tended to show a reduced rate of live weight gain which was consistent with previous observations in bulls (Robertson 1984; Finnerty et al 1994; Jago 1996, 1999). At slaughter immunocastrated bulls had a lighter carcase than control bulls and this has also been previously observed (Adams and Adams 1992; Adams et al 1993, 1996; Jago 1996). The general conclusion from the literature is that the carcases of immunocastrated bulls are lighter than entire bulls but heavier than steers.

Absolute weight is one measure of carcase value but increasingly carcases will be judged on quality attributes for value-based markets. In this regard, the carcases of immunocastrated bulls received more favourable scores for meat colour and fat colour. However, this study involved only a small number of animals and observations on larger numbers of immunocastrates will be required before it can be concluded that immunocastration can be used to achieve the carcase attributes required for particular targeted markets.

A novel feature of the present study was the use of ultrasonography to monitor progressive changes in body characteristics after immunocastration. It was found that body features (eye muscle area, P8 fat, 13th rib fat) recorded by ultrasonography were

related to the carcase attributes at slaughter. Based on this finding, it is proposed that ultrasonography can be used to track the transition in body type from that of a bull to steer after immunocastration, and the information can be used to determine when animals are sold into a value-based marketing system.

Conclusion

Immunocastration has potential as an additional strategy to control carcase characteristics in male cattle. However, the adoption of immunocastration cannot occur without major advances in GnRH immunisation technology.

Study 2

Short-term changes in behaviour in bulls after immunisation against GnRH

Summary

Short-term changes in reproductive status and behaviour were monitored in mature bulls after active immunisation against GnRH using a prototype vaccine under development for use in cattle. Groups of 3-year-old Brahman bulls received 2 vaccinations with the prototype vaccine at an interval of either 1-week or 2-weeks. The study comprised three groups of bulls that included control bulls, 1-week vaccination interval and 2-week vaccination interval. Behaviour was recorded during (i) group behaviour trials when the three groups of bulls were placed together as a single group and (ii) competition trials when 3 bulls, with an animal from each group, were placed in a yard in competition for food. The prototype vaccine induced only modest anti-GnRH antibody responses and there was no apparent decline in plasma concentrations of testosterone after immunisation against GnRH. Bulls immunised using a 2-week interval showed fewer bunts during the group behaviour trials whilst bulls immunised using a 1-week interval had fewer bunts and head pushes during the competition trials. As there were no apparent differences in plasma testosterone between the treatment groups it could not be concluded whether differences in behaviour were a direct consequence of immunisation against GnRH. An important outcome of the behaviour trials was that bulls appeared to become accustomed to the conditions of the study. This observation was particularly significant and future studies on short-term changes in behaviour in mature bulls after immunisation against GnRH may need to be conducted in isolated groups of bulls that are introduced for the first time to evaluate behaviour.

Introduction

Aggressive and sexual behaviour in males is dependent on a functional brain-pituitary-testicular axis and in particular the secretion of testosterone by the testes (D'Occhio 1993, 1994). Accordingly, castration to remove testosterone is often practiced to suppress these behaviours in males. Testosterone secretion in males can also be suppressed by immunisation against GnRH (see Figure 1.1) (D'Occhio 1993, 1994) and bulls immunised against GnRH (immunocastrated) showed reduced aggressive and sexual behaviour (Jego et al 1999; Price et al 2003). In the latter studies, bulls were immunocastrated either before (Price et al 2003) or after (Jego et al 1999) puberty. Bull immunocastrated after puberty were reported to show reduced aggression 6 weeks after immunisation against GnRH (Jego et al 1999).

A sector of the live cattle export industry requires entire bulls for markets in Asia and the Middle East. The confinement of bulls during transport can be associated with significant and undesirable aggressive and sexual interactions. This is compounded by the introduction of bulls from different environments and the tendency of bulls to establish dominance hierarchies. The ability to suppress aggressive and sexual behaviour in bulls during handling and transport associated with live export would be beneficial to this sector of the live cattle export industry.

The aims in the present study were to evaluate the potential of a prototype GnRH vaccine to induce an effective immunocastration response in mature bulls and to ascertain whether this would be associated with acute changes in aggressive and sexual behaviour in bulls. Two groups of Brahman bulls were immunised against GnRH using either a 1-week interval or 2-week interval between primary and secondary vaccination. The two intervals were tested as it is not uncommon for opportunities to supply entire bulls for live export to arise at short notice.

Materials and Methods

Animal experimentation and ethics approval

The study was conducted in accordance with the guidelines set out in the joint publication of the National Health and Medical Research Council, the Commonwealth Scientific and Industrial Research Organisation and the Australian Agricultural Council entitled Australian Code of Practice for the Care and Use of Animals for Scientific Purposes 6th Edition (Australian Government Publishing Service, Canberra, 1997). The Animal Welfare Unit of the University of Queensland approved the study (SAS/318/03/MLA). The prototype anti-GnRH vaccine (Commonwealth Serum Laboratories, Victoria) was approved for use by the Australian Pesticides and Veterinary Medicines Authority (Permit 6719).

Animals and management

Twenty four mature Brahman bulls (3-year-old) were randomly assigned based on live weight to one of three groups and were maintained on pasture as distinct but adjoining groups

GnRH vaccine

The GnRH vaccine used in this study was provided by CSL Limited. The vaccine consisted of a prototype formulation and was being developed specifically for use in cattle. It was anticipated that this vaccine would induce a stronger immunological response than Improvac® that was used in Study 1. In Study 1 it had been necessary to administer repeat vaccinations with Improvac® in order to maintain anti-GnRH antibody titres.

Experimental design

As noted above, 24 mature Brahman bulls were randomly allocated to one of three equal groups. Bulls in the control group received no treatment and bulls in the other two groups were immunised against GnRH on 2 occasions with an interval between primary and secondary vaccination of either 1-week or 2-weeks. Bulls were vaccinated as described in Study 1.

Group behaviour trials

To conduct the group behaviour trials, bulls of all three groups were placed together and observed for aggressive and sexual behaviour over a period of 2 hours. The behaviours recorded included bunt, head push, head rub, spar, pawing, flehmen, attempted mounts and mounts.

Competition trials

After the group behaviour trials bulls were deprived of food overnight but had access to water. The following morning, bulls were introduced in groups of 3 to an enclosure that had food but with restricted access. Each group of 3 bulls included an individual from each of the treatment groups. Bulls were observed for 30 minutes for display of the behaviours listed above for group behaviour trials.

Anti-GnRH antibody titres

Anti-GnRH antibody titres were determined at weeks 2, 3, 4 and 6 using validated ELISA methodology developed in our laboratory.

Plasma testosterone

Plasma concentrations of testosterone were determined at weeks 1, 2, 3, 4, 5 and 6 using a validated radioimmunoassay (D'Occhio and Setchell 1984).

Statistical analyses

Data at individual time points were analysed by ANOVA using the General Linear Models (GLM) procedure of SAS/STAT (SAS 1990). Data analyses over time were undertaken by repeated measures analysis using SAS/SATA procedure MIXED with REML estimation (autoregressive - 1) and the model being y = treatment, time, treatment x time, with animal as the repeated measure (SAS 1992). Results are reported as untransformed arithmetic means $\pm$ SEM.

For anti-GnRH antibody titre determination, the mean absorbance of control samples (representing non-specific binding) at each date was subtracted from the absorbance on the same date for individual samples of immunocastrated bulls. The repeated measures model for analysis of antibody titres was y = time with animal as the repeated measure.

The behaviour data did not have a normal distribution and were analysed using the Kruskal-Wallis non-parametric test.

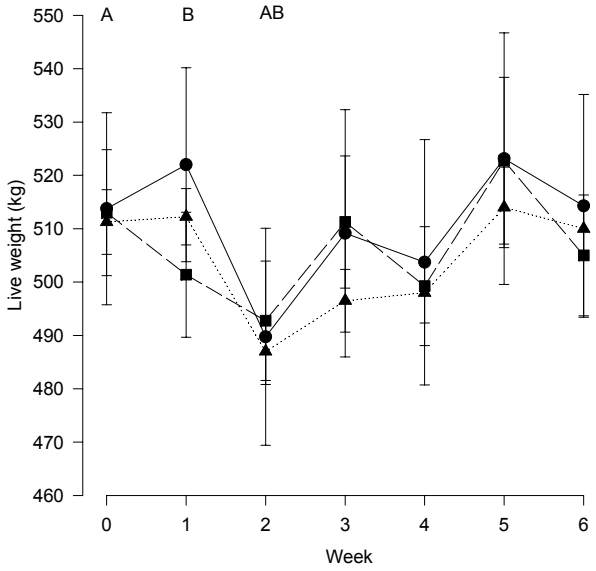
Results

Live weight

The live weight of bulls showed some fluctuation during the trial and there were no apparent differences ($P>0.05$) between control bulls and bulls immunised against GnRH (Figure 2.1).

Anti-GnRH antibody response

Immunisation against GnRH using the prototype vaccine induced relatively low anti-GnRH antibody titres in bulls (Figure 2.2). Bulls immunised with a 2-week interval tended to have higher antibody titres than bulls immunised with a 1-week interval, although overall titres did not differ (P>0.05) between the two groups.



FFigure 2.1. Changes in live weight for control bulls (●) and bulls immunised against GnRH at a 1-week (▲) or 2-week (■) interval. Results are means ± SEM (n=8) (A, primary vaccination of 2-week interval group; B, primary vaccination of 1-week interval group; AB, secondary vaccination of both groups).

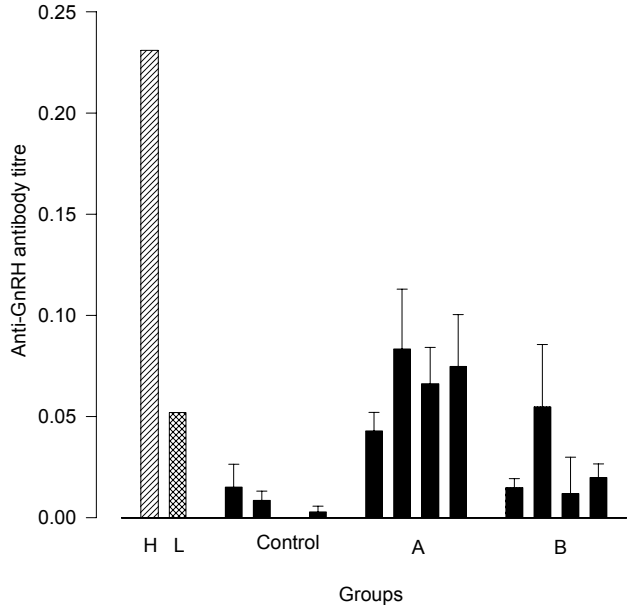


Figure 2.2. Anti-GnRH antibody titres in bulls immunised against GnRH using a prototype cattle vaccine. Bulls were immunised against GnRH using a 1-week interval (Group B) or 2-week interval (Group A) between primary and secondary vaccination. Representative high (H) and low (L) titres from Study 1 are also shown. Results for group data are means + SEM (n=8).

Testosterone

Plasma concentrations of testosterone did not differ (P>0.05) between control bulls and bulls immunised against GnRH (Figure 2.3).

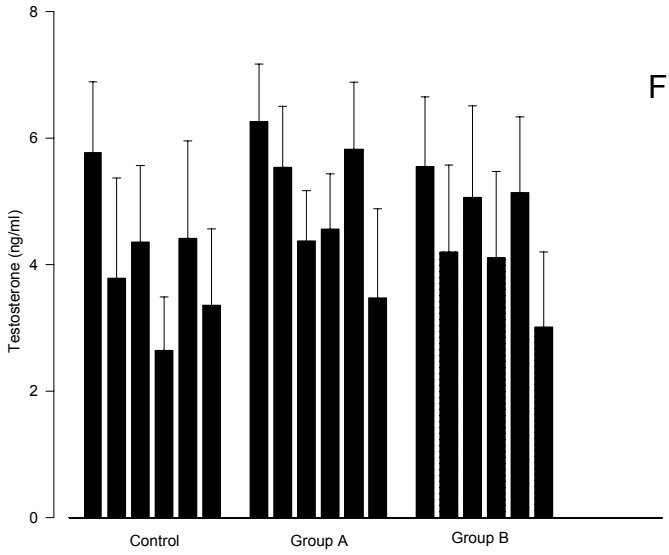


Figure 2.3. Plasma concentrations of testosterone for control bulls and bulls immunised against GnRH using a 1-week (Group B) and 2-week (Group A) interval between primary and secondary vaccination. Results are means + SEM (n=8).

Group behaviour trials

Results for the group behaviour trials are shown in Figure 2.4. The number of bunts displayed in the trials decreased during the course of the study and this was interpreted to suggest that the bulls were becoming accustomed to the conditions of the trial. Bulls immunised against GnRH using a 2-week interval (Figure 2.4, Group A) showed fewer bunts (P<0.01) and flehmen responses (P <0.05) than control bulls. The number of head pushes declined during the study and this was regarded as further evidence of familiarisation by bulls to the conditions of the trial. Sparing (P = 0.14), head pushes (P = 0.16) and mounts (P = 0.10) did not differ (P>0.05) between control and immunised bulls and these behaviours did not show any outstanding trends during the study.

Competition trials

Results for the competition trials are shown in Figure 2.5. Bulls immunised against GnRH using a 1-week interval showed fewer bunts (P = 0.01) and head pushes (P<0.01) over the course of the study. There were no differences between the groups in sparing (P = 0.34), flehmen responses (P = 0.47) and time spent in the control of food (P = 0.71). Overall, the bulls tended to engage in low-level interaction for access to food and typically took turns feeding. The order of feeding was fairly predictable for individual bulls irrespective of other bulls in their competitive trial group. Some bulls would consistently feed first whilst others would wait until the other two bulls in a group had eaten before approaching the feed. As noted above for the group behaviour trials, the bulls appeared to anticipate the circumstances of the competition trials as the study progressed and individual bulls showed predictable behaviour.

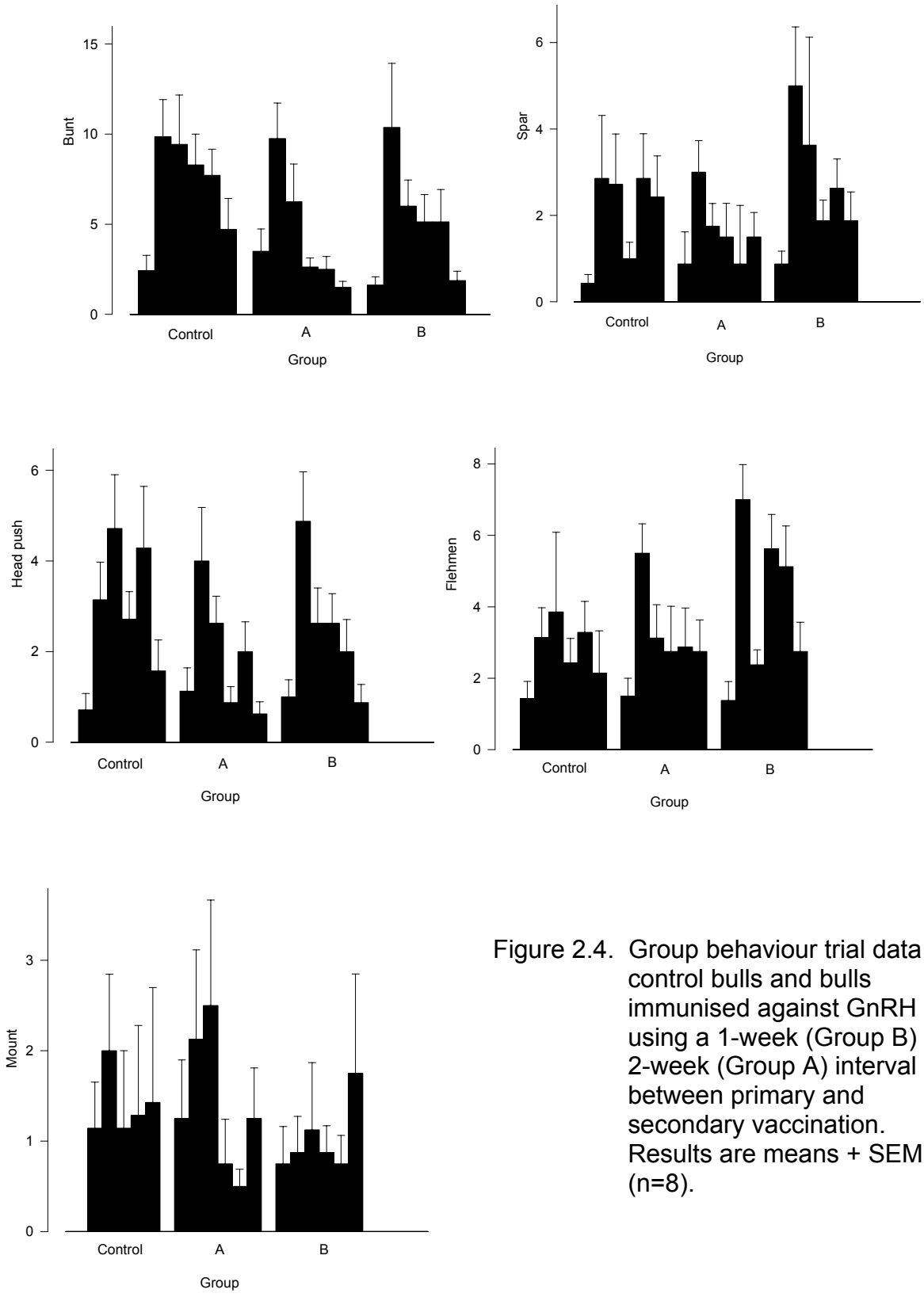


Figure 2.4. Group behaviour trial data for control bulls and bulls immunised against GnRH using a 1-week (Group B) and 2-week (Group A) interval between primary and secondary vaccination. Results are means + SEM (n=8).

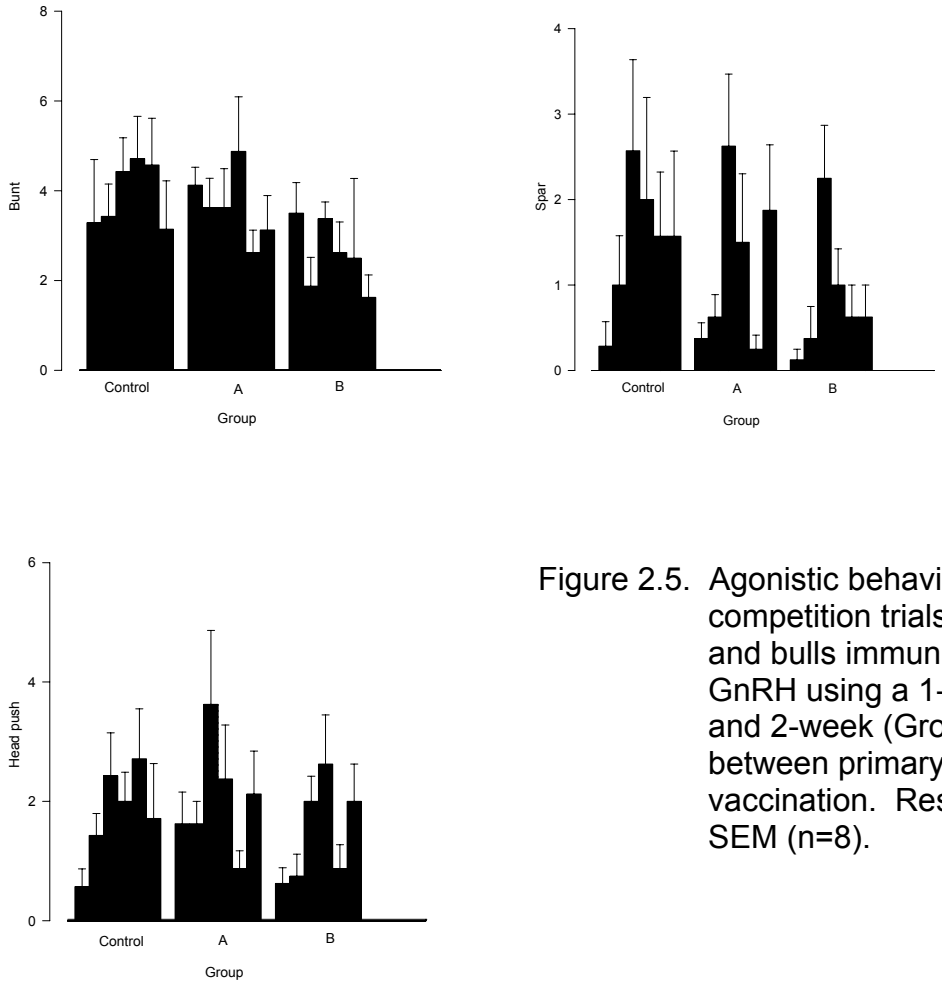


Figure 2.5. Agonistic behaviour during the competition trials for control bulls and bulls immunised against GnRH using a 1-week (Group B) and 2-week (Group A) interval between primary and secondary vaccination. Results are means + SEM (n=8).

Discussion

The aim in the present study was to determine whether immunocastration of sexually mature bulls by immunisation against GnRH would induce acute changes in the display of aggressive and sexual behaviour. If it was demonstrated that aggressive and sexual behaviour declined rapidly after immunisation then this would have application in the management of behaviour in entire bulls during transport and shipping in live export. Immunisation against GnRH presently requires a primary and secondary vaccination and significant anti-GnRH antibody titres are typically observed after the secondary vaccination. A 2-week interval between primary and secondary vaccination has previously been shown to induce anti-GnRH antibodies in cattle. A 1-week interval was evaluated in the present study as opportunities to supply entire bulls for live export can occur at short notice.

The prototype GnRH vaccine used in the present study was anticipated to induce greater anti-GnRH antibody titres than Improvac® which was used in Study 1. Improvac® is commercialised for use in male pigs and the prototype vaccine had been

formulated for cattle (personal communication, CSL Limited). However, the prototype vaccine induced a relatively low anti-GnRH antibody response that was lower than had been observed with Improvac® in Study 1. The reasons for the low immunogenic response to the prototype vaccine are not known at this time but may be related to cattle genotype. Previous trials with the prototype vaccine had been undertaken in British and European genotype cattle (*Bos taurus*) and the present study was the first time that the vaccine had been evaluated in a Zebu genotype (Brahman, *Bos indicus*). The present findings underline the need for further research on GnRH vaccine formulation in cattle. This research is warranted as a GnRH vaccine that was efficient and practical would have applications in cattle to control fertility, behaviour and meat quality.

Plasma concentrations of testosterone showed no apparent suppression in bulls immunised with the prototype GnRH vaccine. This was an unexpected finding notwithstanding the relatively low anti-GnRH antibody titres induced by the prototype vaccine. A proportion of bulls that received GnRH immunisation at a 2-week interval showed modest antibody titres but there was no apparent relationship between antibody response and plasma concentrations of testosterone. It is unlikely that the blood sampling schedule used to evaluate testosterone explains the apparent lack of testosterone suppression in immunised bulls, as effectively immunised bulls have constant basal concentrations of testosterone. It could be speculated that the anti-GnRH antibodies induced by the prototype vaccine had a relatively low affinity for GnRH and that stimulation of gonadotrophin secretion was maintained in immunised bulls. The failure to demonstrate a decline in plasma concentrations of testosterone in immunised bulls precluded any conclusions on whether changes in behaviour could be attributed to GnRH immunisation.

The group behaviour trials were intended to mimic the situation where groups of bulls are brought together during transport and shipping in live export. The introduction of bulls is typically associated with agonistic interactions as bulls attempt to establish dominance relationships. Interactions can be particularly pronounced amongst sexually mature bulls that are introduced for the first time. There was some evidence in the present study that bulls immunised using a 2-week interval between primary and secondary vaccination had a reduced incidence of bunts and general flehmen responses. This observation should be considered as preliminary based on the above discussion concerning the lack of apparent changes in testosterone after immunisation against GnRH. Also, there was evidence to suggest that bulls became familiar to the group behaviour trials during the course of the study and there was a general tendency for bunts and head pushes to decline during the study. The bulls in the present study were contemporaries from a single location and had remained together to three years of age. They were in separate but adjoining paddocks during the study. It is considered that the history and familiarity of the bulls may have influenced, in part, the behaviour interactions amongst bulls during the study. Future research of this type may need to be conducted in collaboration with industry and should involve groups of bulls at different locations that are introduced for the first time after immunocastration.

The competition trials were conducted to determine whether immunisation against GnRH reduced the agonistic tendency of bulls. Food was withheld overnight and was available from a limited source during the trials which was intended to induce aggressive interactions amongst the bulls. Evidence was obtained that bulls immunised using a 1-week interval between primary and secondary vaccination engaged in fewer bunts and head pushes during the competition trials. It was not possible to conclude whether this observation was related to immunisation against GnRH as discussed above. Overall, the bulls showed a lower level of interaction during the competition trials than had been anticipated. This may have been due to familiarity amongst the bulls and their apparent adaptation to the competition trials.

Conclusion

The prototype GnRH vaccine used in the present study induced relatively low anti-GnRH antibody titres and immunised bulls did not show a decline in plasma concentrations of testosterone after immunisation. These findings were unexpected and precluded conclusions on any short-term changes in aggressive and sexual behaviour in bulls after immunisation against GnRH. Evidence was obtained that bulls immunised against GnRH may have engaged in fewer bunts but this should be considered preliminary. Future studies on acute behavioural changes in sexually mature bulls after vaccination against GnRH should be conducted using bulls at different locations that are introduced for the first time when behaviour is assessed.

Success in achieving objectives

Objective

By June 2003, to identify the effects of late immunocastration on carcase yield, carcase type and meat quality in male cattle.

Groups of contemporary steers (n=30-50), late immunocastrates (n=30-50) and entire bulls (n = 30-50) were to be feedlot-finished or pasture-finished and compared for carcase yield, carcase type and meat quality. The design of the project was based on the availability of animals, resources and personnel in the Queensland Beef Industry Institute and Brigalow Research Station. A member of the Queensland Beef Industry Institute located at Brigalow Research Station was to undertake a Master of Applied Science candidature at Central Queensland University as part of the project. Subsequent to approval of the project, the personnel, animals and resources at Brigalow Research Station could not be made available. This required a revision of the project that was undertaken in consultation with MLA.

Notwithstanding the change in project design, the potential to combine the technologies of GnRH immunisation (immunocastration) and ultrasonography to control the body and carcase features of entire bulls destined for value-based markets has been demonstrated.

The project suffered from the inability to induce relatively high and sustained anti-GnRH antibody responses in bulls. Improvac® was used in Study 1 as it was available commercially but it produced modest antibody responses to GnRH in bulls and repeat immunisations were required to maintain antibody titres. Based on the relatively poor responses of bulls to Improvac® in Study 1, a prototype GnRH vaccine under development by CSL Limited for use in cattle was used in Study 2. The prototype vaccine induced lower antibody responses than Improvac®. The results with Improvac® and the prototype vaccine emphasised the need for additional research on GnRH immunisation technology in cattle.

Impact on the Meat and Livestock industry

There continues to be a requirement for new technology to manage growth, meat quality attributes and behaviour in entire male cattle. The present project has shown the potential of GnRH immunisation technology but unfortunately this technology remains pre-mature for adoption by industry. For example, this project was to have included a trial with the Australian Agriculture Company with commercial feedlot bulls but the trial did not go ahead as the findings in Study 1 demonstrated that the immunocastration technology was not sufficiently developed or robust for an industry trial. The future impact of immunocastration on the Meat and Livestock industry will be dependent on whether GnRH immunisation is viewed as an important technology and investment is made into the required research and development. Vaxstrate® was commercialised as a immunospaying vaccine in cattle but was unsuccessful because the technology was not sufficiently reliable or robust. CSL Limited has since commercialised Improvac® for use in male pigs and are conducting research on GnRH immunisation technology in cattle.

Conclusions and recommendations

This project has shown that immunocastration of sexually mature bulls results in a progressive shift in body and carcase characteristics from those of entire bulls to those of steers. It has also been demonstrated that body ultrasonography can be used to track the changes in body composition after immunocastration in order to determine when animals should be sold into value-based markets. The combination of immunocastration and ultrasonography therefore provides an additional potential management strategy for targeted marketing of entire bulls.

The utilisation of immunocastration technology will not be realised by the Meat and Livestock industry unless an investment is made in research and development.

It is recommended that a review is undertaken on the international status of GnRH immunisation technology. Meat and Livestock Australia could use the review to determine whether it would be justifiable to invest in this area of research, possibly in partnership with overseas researchers. The potential application of GnRH immunisation technology in both males and females provides a further reason for undertaking a thorough review of this field.

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