The effect of gestation and lactation on bone calcium, phosphorus and magnesium in dairy cows

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ABSTRACT

A study was conducted to monitor changes in cortical bone mineral in the dairy cow in response to demands of lactation and pregnancy using rib bone biopsies in serial sampling. Sixteen Friesian cows from the University dairy herd were used to collect 9 samples during the lactation period and 5 samples during the dry period. The data were analysed using a split-plot design analysis of variance. There were no significant ($P > 0.05$) differences in cortical bone phosphorus concentrations in rib bone during the lactation period, but calcium concentrations in cortical bone were significantly ($P < 0.05$) higher at parturition and during the first 30 days of lactation compared to the next 30 days and between 90 and 120 days. Results reported here indicate that the cow resorbs cortical bone during the middle of the lactation period and not during the periparturient period as previously thought. Magnesium concentrations were also significantly ($P < 0.05$) higher at the beginning of lactation compared to some of the other sampling times, but cortical bone was significantly ($P < 0.05$) thinner at the beginning of lactation compared to several of the other sampling times. There were no significant ($P > 0.05$) differences in cortical bone Ca or Mg concentrations during the gestation period. Cortical bone P concentrations significantly ($P < 0.05$) decreased during the first 180 days, but significantly ($P < 0.05$) increased at 181–230 days and significantly ($P < 0.05$) decreased again at 231days to term. Cortical bone thickness decreased significantly ($P < 0.05$) from the beginning of gestation to term. There were no significant ($P > 0.05$) differences in cortical bone thickness or Ca or Mg concentrations in cortical bone during the dry period, but cortical bone P concentrations were significantly ($P < 0.05$) greater at the end of the dry period compared to the first 30 days of the period. In general, cortical bone Ca and Mg values decreased as milk production increased up to 20 kg/day and cortical bone *P* values and bone thickness increased. In animals producing over 20 kg/day, however, cortical bone mineral values were greater and cortical bone thickness was lower compared to those animals producing less than 20 kg.

Key words: bone mineral, calcium, gestation, lactation, magnesium, milk fever, phosphorus.

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INTRODUCTION

Much has been written about the detrimental effects of milk fever and the attempts to prevent it in the *post partum* cow. Hypocalcaemia occurs because calcium leaves the extracellular fluid pool to enter the mammary gland faster than it can be replaced by intestinal Ca absorption or bone Ca absorption[8]. Approximately 2.5 g of Ca are extracted from blood for each kilogram of colostrum produced. This is roughly equal to the total amount of Ca present in the blood at any given time. Therefore, a dairy cow producing 25 kg of milk will have to replace her total blood Ca about every hour[9]. The intravenous administration of Ca has been used for many years to treat clinical cases of hypocalcaemia, but more recently mild cases have been successfully treated using oral Ca gel[7]. Attempts to prevent hypocalcaemia have had varying success, owing in part to the lack of knowledge of the bone mineral homeostatic mechanisms that supply Ca to the blood pool, especially during the periparturient period. Hove[11] reported that although bone is capable of maintaining normal plasma Ca concentrations at parturition, too little is known about the size of the bone pools of Ca and how they change near parturition. Before 1993 it was not possible to monitor bone mineral values in serial sampling, but it has been shown[1] that ribs 9–12 of the right and left sides in the bovine can be used to take 8 serial samples for comparison of mineral concentrations over time. In the present study, serial sampling of ribs was used to monitor changes in bone mineral related to gestation, lactation and milk production.

MATERIALS AND METHODS

Sixteen Friesian cows from the University dairy herd were used in a research project to determine the effects of pregnancy and lactation on cortical bone mineral. None of the cows were subclinically or clinically hypocalcaemic during the experiment. Sampling of each cow began at 2 weeks *pre partum* based on breeding dates. Thereafter, samples were collected on the day of parturition and 30, 60, 90, 120, 180 and 240 days *post partum*, day of drying off (300 days *post partum*), 4 times during the dry period and on the day of the next parturition.

Cortical bone samples were first collected from the middle of the ribs, beginning with rib 9 on the left side (L9M) and continuing with rib 10 (L10M), rib 11 (L11M) and rib 12 (L12M). Sampling was then performed on the right side and samples were collected from rib 9 (R9M), rib 10 (R10M), rib 11 (R11M), and rib 12 (R12M). Sample collection then returned to the left side where cortical bone was taken 12 cm dorsal to the first sample sites on the ribs and 12 cm ventral to the vertebral attachment of the rib. Because more than 6 months had elapsed since the ribs had been sampled the first time, they were completely healed and previous sampling did not effect the mineral content of the ribs[1]. Collection on the left side continued with sampling from L9D, L10D, L11D and L12D and finally on the right side from R9D and R10D.

Cortical bone samples were surgically collected from ribs using a trephine. To collect bone samples, the area over the rib was shaved and scrubbed for surgery and blocked using lignocaine hydrochloride (0.02 g/mℓ; Premier Pharmaceutical Company). A 2.5 cm incision was made in the skin and carried down through the muscle to the rib. The periostium over the rib was incised in a cross shape and reflected. A 12.7-mm trephine was used to remove a piece of cortical bone from the rib, absorbable suture was used to close the

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muscle and non-absorbable suture was used to close the skin. Sandpaper was used to completely remove trabecular bone that might have been taken out with the bone sample, leaving only cortical bone for use in analysis. Callipers were then used to measure the thickness of the bone in millimetres.

Trabecular bone was removed because it contains a variable number of red blood cells that contain P. Since the amount of trabecular bone is never consistent in a biopsy sample, and in addition the number of red blood cells varies in each sample, the trabecular bone was removed to eliminate inconsistent *P* values due to the P in the red blood cells of the trabecular bone. Previous research[2] has shown cortical bone to be very dynamic tissue, demonstrating acute changes in bone mineral content.

Cortical bone samples were weighed first in air, then in water and the specific gravity was determined by taking the mass of the bone samples first in air, then in water. The mass in air minus the mass in water gave the volume and the mass of bone in air divided by the volume gave the specific gravity. Specific gravity of fresh bone multiplied by mg/g P in fresh bone was used to calculate bone P per unit of volume (mg/mℓ)[12]. Cortical bone samples were then weighed as fresh, dry and ashed bone. Drying took place at 106 °C for 16 h and ashing at 600 °C for 16 h. Bone ash was dissolved in 5 N HCl and reconstituted in 100 mℓ volumetric flasks using distilled water. Thirty millilitres were stored at –20 °C for later analysis and all samples were analysed for P by the molybdenum blue method[5] using the autoanalyser (Autoanalyzer II, Technicon Instruments Corporation). Calcium and Mg were measured using atomic absorption spectrophotometry[13]. Data were analysed using a split-plot analysis of variance in which the cows were the blocks, the sides were the main plots and the ribs the split plots[15].

Animals were fed *ad libitum* a mixture of 50 % lucerne and 50 % blue buffalo grass hay milled through a 32 mm screen. Mineral content was estimated at 1.5 % Ca and 0.21 % P for the lucerne and 0.35 % Ca and 0.21 % P for the blue buffalo grass. They were fed a concentrate mixture during milking (Table 1). The amount of concentrate fed could not be measured, as the cows were fed the meal on a more or less demand basis during their time in the parlour.

Table 1: **Ingredients and chemical composition of concentrate fed to cows in milk.**

Ingredients		Protein (%)	Fibre (%)	Fat (%)	TDN (%)	Ca (%)	P (%)
Yellow maize meal	60.0 kg	10.1	4.0	5.6	88.0	0.03	0.31
Sunflower oil cake	15.0 kg	37.2	12.0	1.0	68.0	0.30	0.50
Wheat bran	13.5 kg	14.6	10.5	4.1	63.0	0.14	1.17
Crushed cotton seed	6.0 kg	20.0	20.7	17.5	80.9	0.14	0.68
Cotton seed oil cake	5.0 kg	43.0	11.0	5.1	72.6	0.23	1.07
Fine salt	0.25 kg						
Ruminant premix[1]	0.25 kg						

[1]Vit A 21million IU; Vit B3 1.5 million IU; Vit E 36,000 IU; Mn 480 g; Zn 600 g; Cu 90 g; MgO 0.4 %; I 6 g; Co 4.8 g; Fe 250 g; Se 1.8 g, included at 0.25 kg per 100 kg of feed.

Table 2: **Effect of stage of lactation on cortical bone calcium, phosphorus and magnesium and cortical bone thickness.**

Days in milk	Bone P (ash weight)		Bone Ca (ash weight)		Bone Mg (ash weight)		Bone thickness	
	mg/g	SEM	mg/g	SEM	mg/g	SEM	mm	SEM
0	157.3[a]	5.1	357.9[a]	12.9	7.67[a]	0.54	2.39[ac]	0.22
30	165.9[a]	4.2	361.6[a]	11.1	5.83[bc]	0.46	3.24[b]	0.18
60	171.5[a]	6.4	310.4[bc]	15.5	7.24[ac]	0.65	3.43[b]	0.28
90	169.9[a]	5.8	327.7[ac]	14.5	6.04[bc]	0.61	2.77[ab]	0.25
120	158.9[a]	5.8	315.7[bc]	15.6	5.52[bc]	0.65	3.12[b]	0.25
180	167.3[a]	5.1	338.9[ac]	13.0	6.87[ac]	0.54	3.01[b]	0.22
240	161.1[a]	4.9	339.0[ac]	12.4	5.91[bc]	0.52	3.03[b]	0.22
300	160.4[a]	5.1	331.3[ac]	12.5	6.84[ac]	0.52	1.90[c]	0.23

[a,b,c]Means with the same letter are not significantly different ($P > 0.05$) between sampling periods.

RESULTS AND DISCUSSION

There were no significant ($P > 0.05$) differences in P concentrations in cortical rib bone throughout the lactation period but the trend was to store cortical bone P during the first part of lactation (Table 2). Calcium concentration in cortical bone was significantly ($P < 0.05$) higher at parturition and 30 days lactation compared to bone sampled at 60 days and 120 days *post partum* (Table 2). Braithwaite[4] reported increased bone resorption at the onset of lactation to meet the need for additional Ca, and that the high demands for Ca are not met from the diet alone but that skeletal Ca must also be withdrawn. It has been accepted that cows use their stores of bone Ca for milk production during early lactation and replenish those stores during late lactation, but in this research the cows were storing cortical bone Ca at early lactation and only sometime between 30 and 60 days did the animals begin resorbing Ca from the bones (Table 2), in agreement with Ramberg *et al.*[14], who reported that it was not until several weeks after parturition that increases were observed in removal of Ca from bone. In this study cortical bone was significantly ($P < 0.05$) thinner at parturition and at the end of lactation compared to the rest of the period, indicating that cortical bone mass was being depleted during these times. Cortical bone samples taken at parturition and when the cows had been lactating for more than 240 days were significantly ($P < 0.05$) thinner than at other sampling periods (Table 2). The Mg concentration in rib cortical bone was significantly ($P < 0.05$) higher at parturition compared to samples taken at 30, 90, 120 and 240 days of lactation.

There were no significant ($P > 0.05$) differences in Ca and Mg concentrations in rib cortical bone throughout the gestation period, but cortical bone P was significantly ($P < 0.05$) higher in those animals sampled between 180 and 230 days of gestation than the rest of the gestation period, and significantly ($P < 0.05$) lower in those animals sampled near term at 230 or more days of gestation compared to 0–60 days and 181–230 days (Table 3, Fig. 1). Despite the common belief that the cow is normally drawing on bone stores of Ca in the periparturient period, this study showed that these animals were maintaining cortical bone stores of Ca with 418.8 mg/mℓ during the last 50 days of gestation and 446.5 mg/mℓ at the time of parturition and 443.0 mg/mℓ during the first 30 days of lactation compared to 414.0 mg/mℓ at 60 days lactation. Cortical bone thickness decreased significantly ($P < 0.05$) as the gestation period progressed, from a high of 3.33 mm at the beginning of gestation to a low of 2.41 mm

in the final days of pregnancy (Table 3).

There was a significant ($P < 0.05$) change in cortical bone P concentrations during the last trimester (Fig. 1). Between 180 and 230 days the bone P was at the highest level of the entire gestation period, but after 230 days, bone P values were at their lowest, indicating a drain on bone P by the advancing pregnancy (Table 3). In addition, when bone P was measured in relation to days prelactation, cows demonstrated significantly ($P < 0.05$) more bone P on the day of parturition, 103.3 mg/g fresh weight compared to 93.6 mg/g fresh weight at 1–15 days *pre partum*, indicating movement of P into bone during the last 2 weeks of gestation. This would have decreased serum P, and is in agreement with Gardner and Park[6], who reported depressed serum P values associated with parturient paresis. Further research is required to determine bone P concentrations in cows with parturient paresis to access the role of P in Ca homeostasis related to periparturient hypocalcaemia.

It has been suggested[6] that a restricted Ca intake *pre partum* may increase *peri partum* blood Ca by increasing bone resorption due to stimulation of the parathyroid glands, and Block[3] has shown that a diet high in anions can be effective in preventing milk fever, although the physiological response was unclear. This research has shown that serial sampling of cortical bone can be an effective monitor of loss or gain of bone Ca during the periparturent period. Animals in the present study were grazing during the dry period with *ad libitum* roughage available during the dry season, so it was not possible to monitor the Ca intake during the *pre partum* period, but they were not receiving a Ca supplement. The animals in this study were storing bone P just before parturition, and the trend was toward storage of Ca and Mg, although it was not significant (Fig. 2). Cortical bone Ca concentrations were highest during the first 30 days of lactation (Table 2). This research would indicate that the tendency was for these cows to store Ca during the periparturient period rather than resorb Ca from the bone, as there was no significant ($P > 0.05$) decrease in cortical bone Ca during the dry period (Fig. 2) or during gestation (Table 3). The significantly ($P < 0.05$) higher bone Ca at parturition (Table 2) might explain why those stores of Ca could have been available for resorption from the bone and incorporation in the serum in Block's research[3]. Further research is needed to determine to what extent Ca restriction during the dry period can increase bone stores of Ca for later resorption, to make Ca available to the blood to compensate for reduced intestinal Ca absorption during the periparturient period. This method of serial sampling of bone tissue could be used to determine the extent of these stores of bone Ca.

During the lactation period animals were storing cortical bone Ca up to 30 days, but for the rest of the lactation period cows were resorbing Ca from the bone (Table 2). This resorption of Ca must have been for milk production, as there was significantly ($P < 0.05$) less cortical bone Ca as milk production increased up

Table 3: **Effect of stage of gestation on cortical bone calcium, phosphorus and magnesium and cortical bone thickness.**

Days gestation	Bone P		Bone Ca		Bone Mg		Bone thickness	
	mg/mℓ	SEM	mg/mℓ	SEM	mg/mℓ	SEM	mm	SEM
0–60	206.4[b]	3.8	415.7[a]	8.3	7.48[a]	0.27	3.33[a]	0.08
61–120	199.0[bc]	9.0	393.4[a]	22.6	8.53[a]	0.74	2.93[ab]	0.25
121–180	197.2[bc]	10.3	415.6[a]	24.1	7.26[a]	0.79	2.80[ab]	0.29
181–230	224.9[a]	7.4	445.5[a]	17.8	8.46[a]	0.58	2.53[b]	0.21
230–term	186.7[c]	5.5	418.8[a]	13.0	8.29[a]	0.43	2.41[b]	0.15

[a,b,c]Means with the same letter are not significantly different ($P > 0.05$) between sampling periods.

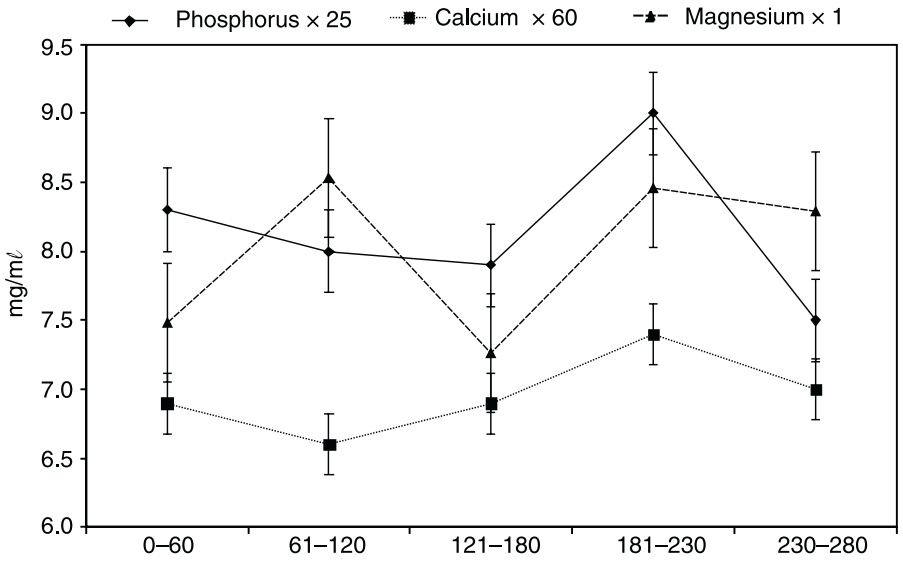

Fig. 1: **Bone mineral during gestation.**

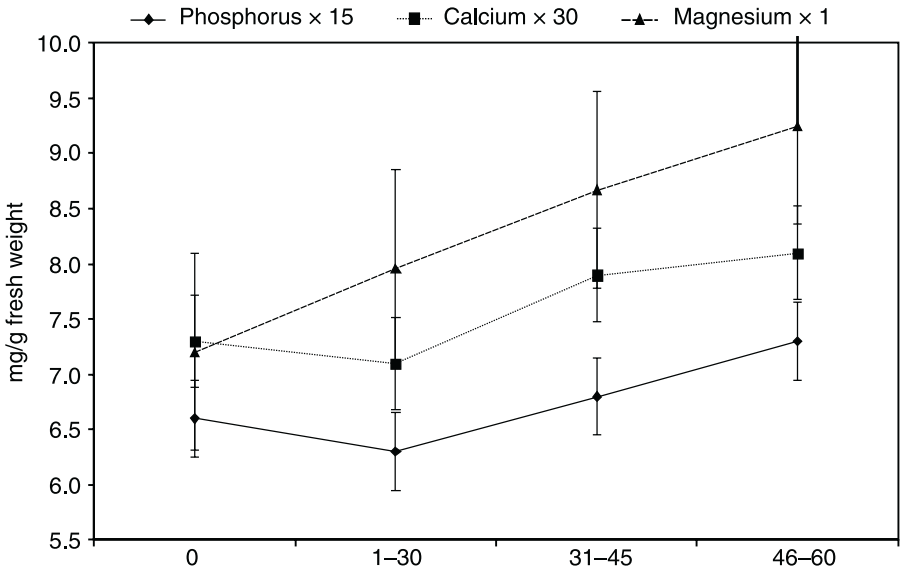

Fig. 2: **Bone mineral during dry period.**

Table 4: **Effect of milk production on cortical bone calcium, phosphorus and magnesium and cortical bone thickness.**

Daily milk production	Bone P		Bone Ca		Bone Mg		Bone thickness	
(kg)	mg/mℓ	SEM	mg/mℓ	SEM	mg/mℓ	SEM	mm	SEM
5–10	196.4[a]	3.5	426.4[a]	7.7	4.10[a]	0.13	2.50[a]	0.10
10.1–15	216.4[b]	5.3	406.0[ab]	11.6	3.59[b]	0.20	2.95[b]	0.14
15.1–20	196.8[ac]	7.7	385.4[b]	15.5	3.88[ab]	0.27	3.13[b]	0.21
20.1–27	223.8[bc]	12.3	536.1[c]	35.7	5.39[c]	0.61	2.83[ab]	0.34

[a,b,c]Means with the same letter are not significantly different ($P > 0.05$) between sampling periods.

Table 5: **Effect of the dry period on cortical bone calcium, phosphorus and magnesium and cortical bone thickness.**

Days dry	Bone P (fresh weight)		Bone Ca (fresh weight)		Bone Mg (fresh weight)		Bone thickness	
	mg/g	SEM	mg/g	SEM	mg/g	SEM	mm	SEM
0	99.6[ab]	3.8	219.9[a]	8.1	7.20[a]	0.57	2.31[a]	0.24
1–30	94.2[a]	3.6	213.8[a]	8.3	7.96[a]	0.60	2.26[a]	0.23
31–45	101.3[ab]	4.6	237.5[a]	10.1	8.67[a]	0.72	2.64[a]	0.29
46–60	110.1[b]	5.2	242.7[a]	12.5	9.25[a]	0.89	3.10[a]	0.45

[a,b,c]Means with the same letter are not significantly different ($P > 0.05$) between sampling periods.

to 20 kg a day (Table 4), and there were no significant ($P > 0.05$) differences in cortical bone Ca values related to the gestation period. The trend was for storage of cortical bone Ca as the gestation period progressed, in contrast to a significant ($P < 0.05$) reduction in cortical bone P during the last 2 months of gestation (Fig. 1), with an increase in the Ca:P ratio to 2.25 compared to 1.98 for the previous sampling period. There was a significant ($P < 0.05$) reduction in the thickness of cortical bone in the last trimester, which, with the reduction of cortical bone P, it is assumed, allowed for the production of the foetal skeleton. The absence of significant losses of Ca from cortical bone at this time would indicate intestinal absorption as the source for foetal Ca.

During the periparturient period when the cows should have been resorbing Ca from cortical bone to make it available for colostrum production, the cortical bone was absorbing Ca. During the first 30 days of the dry period the cows had a mean of 213.8 mg/g Ca fresh weight compared to 242 mg/g Ca fresh weight during the last 15 days of the dry period (Fig. 2), although these are not significantly ($P > 0.05$) different. Table 2 shows significantly ($P < 0.05$) more cortical bone Ca in samples taken at parturition and during the first 30 days of lactation compared to those samples taken after 30 days. In Table 3 the absence of any significant differences in Ca concentration during the gestation period, especially near term, further implies an absence of Ca resorption by bone. These results indicate that the animals were drawing on the bone stores of Ca midway in the lactation period but not during the periparturient period. Since this is the critical time for the cow to resorb Ca from bone to make it available to the blood, this tendency of the cow to store Ca at this time is detrimental in the prevention of milk fever. This could explain why Block[3] was successful in preventing milk fever using a anionic diet to cause resorption of bone Ca during the periparturient period. Further research is needed to determine why Ca is not being resorbed at this time.

Cortical bone P increased significantly ($P < 0.05$) during the dry period as the cows moved closer to parturition, increasing significantly ($P < 0.05$) from 94.4 mg/g P fresh weight during the first 30 days of the dry period to 110.1 mg/g P fresh weight at during the last 15 days of the dry period. (Fig. 2). There were no significant ($P > 0.05$) differences seen during the dry period in cortical bone thickness or in Ca or Mg content of cortical rib bone, but the tendency was for cortical bone to store those minerals with no significant ($P > 0.05$) decreases in Ca or Mg during the dry period.

Cows producing 5–10 kg milk and those producing 15.1–20 kg milk had significantly ($P < 0.05$) less cortical bone P than those producing 10.1–15 kg milk daily. Phosphorus, Ca and Mg content of cortical rib bone was greatest in those cows producing more than 20 kg daily. Cortical bone Ca and Mg, however, decreased significantly ($P < 0.05$) as milk production increased up to 20 kg per day. Cortical bone thickness increased significantly ($P < 0.05$) as the cows produced more milk up to 20 kg (Table 4).

It was unexpected that cortical bone Ca, P and Mg concentrations were all significantly ($P < 0.05$) higher in animals producing more than 20 kg of milk. This could have been due to the fact that most cows in the herd were producing less than 20 kg per day, so only a few samples were taken from animals producing more than 20 kg of milk per day. In addition, those cows producing more than 20 kg per day would have been in the milking parlour longer and would have consumed more concentrates during the time of milking, as cows were fed more or less *ad libitum* in the parlour. Those animals producing more than 15 kg of milk had significantly ($P < 0.05$) less bone Ca, indicating that they were drawing on their bone reserves of Ca. In addition, cows producing 10–15 kg of milk had significantly more cortical bone P than those producing less milk, indicating the independent resorption and absorption of cortical bone P and Ca.

Independent absorption and resorption of Ca and P in bone as the result of an anionic diet[2] and reduced Ca retention with no change in P retention in ruminants in response to ammonium chloride[10] have been reported. In this study there was independent movement of Ca and P from cortical bone tissue without the addition of anions or cations to the diet (Table 2). The results presented here show that Ca and P homeostasis is a dynamic process in which the animal has the ability to absorb and resorb bone Ca and P at a rapid pace and independently of each other. Further research is needed into this aspect of Ca and P homeostasis.

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Book review – Boekresensie

Fish diseases and disorders

Volume 1: Protozoan and metazoan infections

Edited by P T K Woo

1995. CABI Publishing, CAB International, Wallingford and New York, 768 pp., hard cover. £99.96(US$185). ISBN 0 85198 8237.

Volume 2: Noninfectious disorders

Edited by J F Leatherland and P T K Woo

1998. CABI Publishing, CAB International, Wallingford and New York, 768 pp., hard cover. £75.00 (US$140). ISBN 0 85199 1262.

Volume 3: Viral, bacterial and fungal infections

Edited by P T K Woo and D W Bruno

1998. CABI Publishing, CAB International, Wallingford and New York, 768 pp., hard cover. £99.50 (US$185). ISBN 0 85199 1947.

The growth in both aquaculture and the ornamental fish trade worldwide during recent years has led to an increased interest in fish diseases. *Fish diseases and disorders* is a work in three volumes dealing with both infectious and non-infectious diseases of fish and shellfish. The 1st volume contains chapters on protozoan and metazoan parasites. Chapters on zoonoses associated with fish, the immune system of fish and immunological techniques are also included. The 2nd volume deals with non-infectious diseases ranging from genetic conditions to nutritional disorders. It also contains chapters on neoplasia, various toxins, stress physiology and problems associated with intensive aquaculture systems. The 3rd volume is on bacterial, viral and fungal diseases and also contains a chapter on applying epidemiology to infectious conditions of fish.

A consistent feature of this work is a lack of illustrations. This is especially apparent in the volume dealing with parasites, where good quality photographs or light micrographs, together with line-drawings, should be considered indispensable. Many conditions have no relevant illustrations. For example, the chapter on *Saprolegnia* contains only electron micrographs of the fungus, together with one line-drawing of poor quality, whereas the diagnostician is most likely to observe this organism under the light microscope. The quality of the text varies and some chapters are decidedly incomplete. However, other sections contain useful and interesting information that would not be included in most general works on fish diseases. *Fish diseases and disorders* is primarily a reference work and worth adding to a specialist collection, but better books are available for the private practitioner seeking help with day-to-day diagnostics.

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