FOREWORD

High prices were paid for emu fat because emu oil was believed to have therapeutic activity. In Australia and many overseas countries it is an offence to make any therapeutic claims for a product until its efficacy has been demonstrated and the product registered by the relevant authority. Therefore to expand the therapeutic use of emu oil, registration as an active ingredient under the Therapeutic Goods Act is required. The first step towards registration is to demonstrate the efficacy of emu oil in a scientifically acceptable manner.

This report complements other RIRDC research projects investigating the potential therapeutic applications of emu oil. These are the anti-inflammatory properties of emu oil, the effects of emu oil on wound healing and cellular regeneration and the anti-viral/anti-bacterial activity of emu oil.

This publication describes an investigation into the anti-inflammatory properties of topically applied emu oil. The other reports are also available as RIRDC research publications.

The report, a new addition to RIRDC’s diverse range of almost 400 research publications, is part of our New Animal Industries Program which aims to accelerate the development of viable new animal product industries.

Most of our publications are available for viewing, downloading or purchasing online through our website:
• downloads at www.rirdc.gov.au/reports/Index.htm

Peter Core
Managing Director
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Contents

Foreword iii
Executive Summary v
Introduction 1
Objectives 3
Methodology 5
Results and Discussion 7
Anti-inflammatory Activity 7
(i) The Adjuvant Induced Polyarthritis Model 7
(ii) The Carrageenan Oedema Model 16
Correlation Between The Two Models 24
Carrier Studies 25
Carrageenan Induced Oedema 25
In Vivo Penetration Study 26
In Vitro Permeability Studies 27
Fatty Acid Profiles 28

Implications 29

Recommendations and Conclusions 31

References 37

RIRDC Publications
Executive Summary

In the past, the profitability of the emu industry relied largely on breeding and selling chicks into an expanding industry. However, it is apparent now that the future of the industry will have to depend on the sale of emu products. The products include meat, skins, fat (oil) and feathers. Based on the current and predicted prices for these products, it would seem likely that emu farming will only remain profitable if the very high price previously paid for the fat is maintained. Until recently, the price obtained for emu fat was between $20 and $25 per kilo, compared to around 20 cents per kilo for fat from other animal sources. However, this has drop rapidly, as increasing supplies become available, and will continue to do so unless high value end uses are established for the oil.

High prices are paid for emu fat because emu oil is believed by some to have therapeutic and cosmetic applications. It is probable that in the near future the amount of emu oil required for cosmetics production will be limited. Reasons for this include the small percentage of oil in the products, the cost of production (around $2 per kilo for rendering and around $5 per kilo for refining) and there is a lack of any documentary evidence that emu oil performs any better than a range of cheaper alternatives. Furthermore, particularly in Australia, there is a move away from cosmetics that contain animal products (personal communication, David Stacy, Orion Laboratories, WA).

In Australia and many overseas countries it is an offense to make any therapeutic claims about a product until its efficacy has been demonstrated and the product registered with the relevant authority. Therefore to expand the therapeutic uses of emu oil, registration as an active ingredient under the Therapeutic Goods Act is required. The first step towards registration is to demonstrate the efficacy of emu oil in a scientifically acceptable manner.

Prior to the commencement of this study, the evidence for the efficacy of emu oil as an anti-inflammatory agent has been largely anecdotal, such as ‘Australian aboriginals have used emu oil for centuries to treat inflamed joints’. The first accounts of the efficacy of this oil were published in the mid 1800's[1]. However recently, a wide range of therapeutic applications for the oil have been claimed in two United States patents [2,3]. Unfortunately, no statistical evaluation of the results was presented in these patents.

Also, it is often stated that emu oils from different sources and refined by differing processes differ widely in their therapeutic activity but no scientific studies have been published that confirm this.

The objectives of this study were:

1. By using an appropriate animal model(s), to determine if emu oil alone or in combination with other agents is efficacious in the treatment of inflammation.

2. Isolate the active agents in emu oil or develop a test to determine the level of efficacy of emu oil samples.

3. Conduct clinical studies on the efficacy of emu oil.
4. To investigate the effects of the following factors on the efficacy, yield and properties (physical and chemical) of emu oil:

4.1. fat handling and storage procedures
4.2. fat rendering and refining regimes
4.3. oil storage procedures.

5. To prepare specifications for the production of emu oil to be used in anti-inflammatory applications.

In this study, the anti-inflammatory activities of different preparations of emu oil have been examined using an adjuvant induced polyarthritis in rats and a carrageenan oedema in rats. Adjuvant induced polyarthritis results in a chronic systemic inflammation with multiple joint involvement. Carrageenan induces an acute localised inflammation. Both these rat models have been commonly used for the detection and development of clinically effective anti-inflammatory drugs.

Various emu oil preparations both from farm reared and wild birds have been shown to be highly efficacious in both models. For example in the polyarthritis model, emu oil preparations have shown to equal or greater anti-inflammatory activity to Ibuprofen, one of the most powerful non prescription anti-inflammatory available. Also in the carrageenan induced oedema model, emu oil preparations have been shown to have anti-inflammatory to prednisolone, a powerful steroid anti-inflammatory drug.

However a wide range in efficacy was observed. The reasons for this wide variability in efficacy remain to be established but some factors have been identified. These include diet and method of oil preparation. Also it was found that it is highly likely that two different components of the oils are the active ingredients in the two models. To date attempts to identify the active constituents have been unsuccessful.

None of the emu oil preparation have caused any adverse effects such as skin irritation.

Analytical studies showed that emu oil prepared from the fat of farm reared birds consisted of a relatively simple mixture of a limited number of compounds. Also that a single triglyceride constituted around 70% of the oil. The triglyceride is of a type not generally found in any quantity in animal fats in that it has the same unsaturated fatty acid (oleic) at each end and a saturated fatty acid (palmitic) in the middle. The high concentration of this triglyceride is probably responsible for the unusual properties of emu oil.

Whilst the data is consistent with this triglyceride being the major constituent of oils prepared from farm reared birds this does not appear to necessarily be the case for oils prepared from the fat of wild birds particularly those feeding on native flora. The variation in fatty acid profiles of oils prepared from the fat of wild birds is much greater than that seen with oils prepared from the farmed birds. This variation may reflect varying diets since birds shot close together have similar profiles. Oils have been prepared from the fat of wild birds that were 30% omega 3 fatty acid whereas with farmed birds this fatty acid constituted less than 1% of the oil.
Whilst only briefly addressed in this study it, would seem likely that emu oil may be useful as a trans dermal carrier of therapeutic agents, particularly of agents that are soluble in emu oil.

Objectives 1 and 4 were examined in detail but because of the nature of the results and reduction in funding it was not possible to meet the other objectives.

However following recommendations and conclusions are made related to Objectives 1 and 4:

1. Emu oils with potent anti-inflammatory activity can be prepared

2. Different components of the oil are active in the different rat models.

3. Factors affecting efficacy include diet and rendering procedure.

4. Marked differences were found between the oils prepared from the fat of wild birds and farmed birds.

5. Further investigation into the anti-inflammatory activity of emu oil should be undertaken. These studies should include human clinical trials at the earliest opportunity.
Introduction

In the past, the profitability of the emu industry relied largely on breeding and selling chicks into an expanding industry. However, it is apparent now that the future of the industry will have to depend on the sale of emu products. The products include meat, skins, fat (oil) and feathers. Based on the current and predicted prices for these products, it would seem likely that emu farming will only remain profitable if the very high price previously paid for the fat is maintained. Until recently, the price obtained for emu fat was between $20 and $25 per kilo, compared to around 20 cents per kilo for fat from other animal sources. However, this has dropped rapidly, as increasing supplies become available, and will continue to do so unless high value end uses are established for the oil.

High prices are paid for emu fat because emu oil is believed by some to have therapeutic and cosmetic applications. It is probable that in the near future the amount of emu oil required for cosmetics production will be limited. Reasons for this include the small percentage of oil in the products, the cost of production (around $2 per kilo for rendering and around $5 per kilo for refining) and there is a lack of any documentary evidence that emu oil performs any better than a range of cheaper alternatives. Furthermore, particularly in Australia, there is a move away from cosmetics that contain animal products (personal communication, David Stacy, Orion Laboratories, WA).

In Australia and many overseas countries it is an offense to make any therapeutic claims about a product until its efficacy has been demonstrated and the product registered with the relevant authority. Therefore to expand the therapeutic uses of emu oil, registration as an active ingredient under the Therapeutic Goods Act is required. The first step towards registration is to demonstrate the efficacy of emu oil in a scientifically acceptable manner.

Rural Industries Research and Development Corporation (RIRDC) invited Agriculture Western Australia to facilitate a national emu oil research and development project over the period 1996/97 to 1998/99. As part of the facilitation, Agriculture Western Australia organised a meeting, sponsored by RIRDC, of industry representatives and researchers with expertise and interest in emu oil research to develop a plan for a national emu oil research development program to be funded by RIRDC. The participants identified the following as areas requiring further research, the areas are listed in order of priority:
1. Anti-inflammatory properties of emu oil.
2. The effects of emu oil on wound healing and cellular regeneration.
3. Anti-viral/anti-bacterial activity.
Subsequently, RIRDC funded projects in the first three areas.

Prior to the commencement of this study, the evidence for the efficacy of emu oil as an anti-inflammatory agent has been largely anecdotal, such as ‘Australian aboriginals have used emu oil for centuries to treat inflamed joints’. The first accounts of the efficacy of this oil were published in the mid 1800’s[1]. However recently, a wide range of therapeutic applications for the oil have been claimed in two United States patents [2,3]. Unfortunately, no statistical evaluation of the results was presented in these patents.
Also, it is often stated that emu oils from different sources and refined by differing processes differ widely in their therapeutic activity but no scientific studies have been published that confirm this.

In this study, the anti-inflammatory activities of different preparations of emu oil have been examined using an adjuvant induced polyarthritis in rats and a carrageenan oedema in rats. Both these rat models have been commonly used for the detection and development of clinically effective anti-inflammatory drugs [4-6].
Objectives

1. By using an appropriate animal model(s), to determine if emu oil alone or in combination with other agents is efficacious in the treatment of inflammation.

2. Isolate the active agents in emu oil or develop a test to determine the level of efficacy of emu oil samples.

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4. To investigate the effects of the following factors on the efficacy, yield and properties (physical and chemical) of emu oil:
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   4.3. oil storage procedures.

5. To prepare specifications for the production of emu oil to be used in anti-inflammatory applications.
Methodology

Emu Oil Samples

The details of the oils used in a particular study are given in Results and Discussion.

Adjuvant Induced Polyarthritis

Adjuvant arthritis was induced by injecting a mixture of \textit{M. tuberculosis} (800 ug) in squalane (0.1mL) into the tail base of female Wistar rats (160-200g) on day 0. On day 10, the rats were shaved just behind the ears to expose approximately 6 cm$^2$ of dorsal skin. Mixtures of 85% v/v emu oil and 15% cineole, a penetration enhancer [7] were prepared. A mixture of 85% olive oil and 15% cineole was used as a negative control. Four to six animals per group were used. The mixtures were applied at the rate of 2mL/kg on days 10, 11, 12 and 13. The rear paw diameters were measured on days 10 and 14 using a micrometer.

Carrageenan Induced Oedema

The rats were shaved just behind the ears to expose approximately 6 cm$^2$ of dorsal skin the day before testing.

The animals were anaesthetised using Halothane/oxygen and 0.1mL of 1% lamda carrageenan dissolved in physiological saline was injected into the right hand rear foot pad. Then 1mL of oil or sorbolene (control) was applied to the animal, approximately 0.2 mL rubbed into the affected paw and 0.8 mL rubbed into the shaved area. Eight animals per group were used. The paw diameters were measured at 0, 4 and 6 hours.

The paws were also inspected at 24 hours and the results from any animals showing heamorrhage at the injection site were rejected.

In vivo Penetration Study

A commercial emu oil preparation (EO1) and oil prepared from the intra-dominal fat of birds raised by Agriculture WA (EO2) were used in this study.. Approximately 1 kg of fat was heated in a 650 watt microwave oven on high for about 20 minutes. The oil was then filtered through cotton wool, stored in a glass beaker sealed with plastic wrap.

 Fifteen rats were anaesthetised with ether and shaved just behind the ears to expose approximately 6 cm$^2$ of dorsal skin. Blood samples, approximately 0.5mL, were then obtained by heart puncture. 0.5mL samples of hydrocortisone (5mg/mL) dispersed in emu oil or a commercial preparation of hydrocortisone (5mg/mL) cream were then applied to the shaved area and rubbed in for 20 seconds. Further blood samples were taken at 3 and 5 hours. Prior to taking blood samples, the animals were anaesthetised. The blood was allowed to clot and the serum collected. The serum samples were kept frozen until required.
The hydrocortisone content of the serum was determined using a commercially available radio immune assay (Incast Corporation, Stillwater, Minnesota, U.S.A.). The measurements were performed by the Chemistry Centre (WA) on contract to Agriculture Western Australia.

**In vitro Permeability Studies**

These studies were performed by InterDerm Pty Ltd on contract to Agriculture WA.

Briefly, the test procedure used glass diffusion cells with horizontally mounted sections (approximately 1 cm²) of human epidermal membrane from a single donor. Test formulations composed of the radioactively labeled drug dispersed in the relevant oil were applied to the upper stratum corneum side and the penetration of the labeled drug into the underlying receptor medium was measured over a 48 hour period.

For the first experiment, H³-cortisone was the drug used. The cortisone was dispersed in olive oil (control), commercial emu oil (EO1) or emu oil prepared by Agriculture Western Australia (EO3). (EO4) was prepared by Dr C. Davis, Department of Primary Industries, Hamilton, Queensland. The oil was prepared from the subcutaneous fat of an emu raised at Cherbourg. The oil was obtained by low temperature (40°C) rendering and clarified by centrifugation at 12,000g for 10 minutes at 30°C. As tested, the oil was almost colourless with a small quantity of solids at room temperature. The tissue sample was obtained from a 82 year old female.

The second experiment was essentially the same as the first except the drug used was C¹⁴-diclofenac, a non steroid anti-inflammatory drug, and the tissue sample was obtained from an 78 year old female.

The third experiment was essentially the same as the first except the hydrocortisone was dispersed in EO2, and the tissue was obtained from an 25 year old female.

**Fatty Acid Analysis**

Routine fatty acid analyses were performed by the Animal Health Laboratories, Agriculture Western Australia using established techniques including Gas Chromatography (GC).

**GC/Mass Spectroscopy and Nuclear Magnetic Resonance Studies**

The CG/Mass Spectroscopy and Nuclear Magnetic Resonance Studies were performed by the Centre for Drug Design, University of Queensland using oil samples supplied by Agriculture Western Australia.
Results and Discussion

Anti-inflammatory Activity

(i)The Adjuvant Induced Polyarthritis Model.

In the initial study, the anti-inflammatory properties of four different preparations of emu oil were examined. These were sourced from birds raised in quite different habitats, three of these were from Western Australia (EO1, EO2 and EO3) and one from Queensland (EO4). Olive oil was used as the control.

It can seen (Figure 1) that dermal application of three of the four emu oil preparations significantly reduced the increase in paw diameter due to arthritic inflammation over the treatment period. Also, EO4 was significantly more effective at reducing swelling than EO2 or EO3, the negative value showing that it reduced incipient inflammation already pre-established on day 10. Anova of the mean paw measurements obtained in the oil study gave a P of < 0.001
Figure 1. The effects of the various emu oil preparations on adjuvant induced arthritis.

None of the rats showed any adverse skin reactions at the site of application of any of the oil preparations.

The administration of ibuprofen, one of the most effective anti-inflammatory drugs available over the counter, significantly reduced (P<0.001) the increase in paw diameter due to arthritic inflammation over the treatment period but on average it was less effective than EO2, EO3 or EO4 (8).
The four oil samples were then subjected to a variety of analytical procedures, including nuclear magnetic resonance (NMR) spectroscopy. Simplistically, the NMR spectroscopy can be considered to give a fingerprint of the type of carbon and hydrogen atoms in the material. An unexpected finding was the apparent simplicity of all the spectra (Figure 2). This indicates a relative simple mixture of a limited number of compounds.

![Figure 2. A typical NMR spectrum of emu oil.](image1)

Also, there were no significant differences between the four spectra which indicates that the active ingredient is only a (very) minor component of the oil. Using this and other data, the major component (around 70%) of all the emu oil samples was identified as the triglyceride shown in Figure 3.

![Figure 3. The structure of the major component of emu oil.](image2)

This type of triglyceride is unusual to be in present animal and may, at least in part, be responsible for the unusual qualities of emu oil in that there is a unsaturated fatty acid (oleic acid) each end and a saturated fatty acid (palmitic acid) in the middle.

EMU OIL 5 (EO5) WAS A COMMERCIALLY RENDERED PREPARATION PREPARED FROM THE FAT OF BIRDS RAISED AT CHERBOURG, QUEENSLAND. AS SUPPLIED THE OIL CONTAINED APPROXIMATELY 25%(V/V) SOLIDS. THE CRUDE OIL WAS FILTERED BEFORE USE. THE OIL USED WAS A PALE YELLOW VISCOUS LIQUID.
To examine the dose response, mixtures of EO5 and olive oil in the ratios of 1:1 (EO:OO 1:1) and 1:3 (EO:OO 1:3) were prepared. Adjuvant arthritis was induced in a further 16 female rats and on day 10 the rats were shaved and the rear paw diameters measured as described above. Mixtures of 85% EO5, EO:OO 1:1 or EO:OO 1:3 and 15% cineole were prepared and each applied to four rats (2mL/kg) on days 10, 11, 12 and 13. Again a mixture of 85% olive oil and 15% cineole was used as a negative control on four rats. The rear diameters were measured again on day 14.

The dose response obtained is shown in Figure 4, relative swelling is the ratio of the swelling in the emu oil treated animals to that in the olive oil treated animals.

![Figure 4. Dose response observed with EO5.](image)

The next four oils to be tested were prepared from the fat of two birds raised by Agriculture Western Australia feed on the standard heat based diet. Oil samples EOA, OEB, EOC and EOD were prepared by heating samples of diced fat, two thirds subcutaneous and one third gut, in covered glass beakers at 50, 60, 80 or 100° C for 3 hours respectively. Once again a range of efficacies was observed (Figure 5).
Figure 5. The effect of rendering temperature on efficacy. EOA was rendered at 50°C, EOB at 80°C, EOC at 80°C and EOD at 100°C.

No simple correlation between efficacy and rendering temperature was observed.

To examine the effects of diet, twelve diets were prepared consisting of a common basal diet to which was added different lipid (oils or fat) preparations. Each diet was fed to three birds for twelve weeks prior to slaughter. The fat was collected at slaughter and stored frozen until required.

In order to obtain larger quantities of oil an alternative rendering procedure was used. The fat was minced and the oil extracted by placing the fat on wire mesh in an oven at varying temperatures (60, 80 or 100°C) for varying times.

For the first study the oil extracted at 60°C from the subcutaneous fat of birds fed on of four different diets (A, B, C and D) were used. The diets were prepared by adding 10% of oil to a wheat based basal diet. Diet A had cod liver oil, Diet B had a hydrogenated mixture of canola and palm oils (40% trans 18:1), Diet C had tallow and Diet D had canola oil. The results of the study are shown in Figure 6.
Figure 6. The effects of diet emu oil efficacy.

The swelling of the animals treated with the emu oils from all diets was significantly less than the controls. Furthermore there were significant differences between diets.

The oils tested in this model have been subjected to chemical analysis, including fatty acid composition, and there appears to be a good correlation between efficacy and the concentration of an unidentified compound (Figure 7), presumably a polyunsaturated long chain fatty acid.
The highest concentration of this compound has been approximately 5mg/mL and so it is not surprising that we were unable to detect differences between the NMR spectra of efficacious and non-efficacious samples of oil. We have been unable to identify the compound using GC-Mass spectroscopy.

There is considerable interest in the properties of oil prepared from the fat of wild birds. Birds were shot in three distinct regions of Western Australia and oil samples were prepared by rendering the fat in a water bath at 60 °C.

The first three samples to be tested were to have been prepared from the fat of birds collected from 3 widely separated regions of Western Australia. The preparations were supposed to prepared from WE1 from the fat of wild bird 2 (WB2) shot in the South West forest region, WE2 from WB’s5&6 shot at Southern Cross, a eucalyptus and mallee region and WE3 from WB’s 11&12 shot at Paynes Find, a mulga region. However, it is apparent from subsequent fatty acid analysis that an error had been made and WE2 had been
prepared from the fat of WB1, shot in the South West region. It can be seen (Figure 8) that only one preparation (WE3) significantly reduced swelling.

Figure 8. The efficacy of emu oil prepared from the fat of wild birds.

WB1 and WB2 were both females shot in the Augusta region of Western Australia. They were grazing on pasture, mainly weeds, and both stomachs were packed with dandelion flowers. WB 11 was a male and WB 12 was a female both were shot outside the dog fence in the Mount Magnet area and their stomachs contained mulga and quandong seeds.
The fatty acid profiles obtained for these samples are very interesting for a number of reasons. Firstly, WE1 and WE2 have little any efficacy and have very similar profiles, which are markedly different from that obtained for the active WE3 (Table 1).

Furthermore, the fatty acid profiles obtained for the oils prepared from the wild birds are very different from the profiles obtained for oils prepared from farmed birds. These differences include:

1. The ratio of C181n9:C16. With all oils prepared from farm reared birds this ratio is close to 2:1 (Table 1) as it is with WE1 and WE2 the ratio is close to 2:1 but with WE3 the ratio is 6.6:1.
2. Twelve percent of WE3 is an unidentified fatty acid, running between C204n6 and C205n3.
3. Almost 30% of WE1 and WE3 is C183n3 whereas with the farmed birds this was generally less than 1%.
### Table 1. Fatty acid profiles of emu oils prepared from the fat of farm bred (FB) and wild (WE) emus.

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D1 was a standard diet with no added fat or oil. D2, D3, D4 and D5 were a basal diet to which 10% cod liver, hydrogenated canola/palm, tallow and canola oil was added respectively.
Additional oil samples were prepared from the fat of individual wild birds. Unfortunately there was some spillage during transport and to obtain sufficient material for testing the samples had to be diluted 1:1 with olive oil prior to testing. Even so all oil preparations were found to be highly efficacious (Figure 9).

![Figure 9](image_url)

Figure 9. The efficacy of oils prepared from the fat of individual wild birds.

No correlation has been found between the efficacy of the oils prepared from the fat of the wilds and the fatty acid profiles of the oils (Figures 8&9, Table 2).

It is interesting to note that the oils prepared from birds shot outside the verminfence, and therefore living on native fauna and flora, are very much more efficacious than the oils prepared from the wild birds feeding on improved pasture.
Table 2. The fatty acid profiles of oils prepared from the fat of wild emus.

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The second animal model used is referred to as the carrageenan induced rat paw oedema model. Whilst progress with using the carrageenan induced rat paw oedema model to demonstrate efficacy was slower than with the adjuvant model, the model is now better understood. It is now possible to distinguish between the two effects of carrageenan i.e. the rapidly induced oedema (0 to 6 hours) and the development of granuloma (1 to 5 days). Initially, considerable time and effort were spent examining the effects of emu oil on granuloma development with limited success.

With this model, a small quantity of carrageenan, a sulphated polysaccharide, is injected into one of the rear paws of rats. The diameters of the rear paws were measured and the oil preparations applied to a shaved area on the back of the rats. The paw diameters were measured at 2, 4 and 6 hours. The degree of swelling is taken as a measure of the severity of the disease.

For the initial study using the revised procedure four oils were prepared from the fat of two birds raised by Agriculture Western Australia. Oil samples EOA, OEB, EOC and EOD were prepared by heating samples of diced fat, two thirds subcutaneous and one third gut, in covered glass beakers at 50, 60, 80 and 100°C for 3 hours respectively. A range of efficacies was observed (Figure 10).

![Figure 10](image_url)  
**Figure 10.** The effects of emu oil preparations on carrageenan induced oedema.
At 2 hours, all treated animals showed significantly less swelling than the controls. The animals treated with EOA, EOC or EOD also showed significantly less swelling at 4 and 6 hours. The oils extracted at the higher temperatures (80 and 100°C) were found to be more efficacious than those prepared at the lower temperatures.

As described previously, to examine the effects of diet, ten diets were prepared consisting of a common basal diet to which was added different lipid (oils or fat) preparations. A sample of fat from a Tasmanian bird, feeding on forage, was also included in the study. The fat samples were rendered in an oven at varying temperatures (60, 80 or 100°C) for varying times.

The oils extracted at 60°C from the subcutaneous fat of birds fed on diets A, B, C and D were used. Diet A had cod liver oil as the added lipid, Diet B had hydrogenated fat, Diet C had tallow and Diet D had canola oil.

The results obtain when the efficacy of these oil samples were tested using the oedema model are show in Figure 11, eight animals per treatment were used.
At 2 and 4 hours there was no significant difference between the results obtained with the control (sorbolene treated) animals and those animals treated with the different emu oil preparations. However, at 6 hours there was a slight but significant difference ($P<0.02$) between the controls and the Diet B treated animals. This result was unusual in that in previous studies the efficacy had shown up earlier. Also, the average swelling of the Diet D treated animals was significantly greater than that of the Diet B treated animals at both 4 and 6 hours.

It was considered possible that the apparent lack of efficacy may have been a result of the low yields of oil obtained with the $60^\circ$C oven temperature, only approximately 10 percent. It was decided therefore to use the oil extracted from the same fat samples at $80^\circ$C where the yield was around 80 percent. Essentially the same results were obtained (Fig. 12).

![Figure 11. The effect of diet on the efficacy of emu oil.](image)

**Figure 11.** The effect of diet on the efficacy of emu oil.

At 2 and 4 hours there was no significant difference between the control and emu oil treated animals at 2 and 4 hours but a slight, but again significant ($P=0.03$), difference between the control and Diet B
treated animals at 6 hours. Also, again the average swelling of the Diet D treated animals was significantly greater than that of the Diet B treated animals at 2, 4 and 6 hours.

For the next study the oils prepared from the subcutaneous fat of birds fed on four different diets (E, F, G and H) were used. Diet E was the basal diet with no added lipid, Diet F had olive oil added, Diet G had linseed oil added and Diet H was the forage diet. The oils were extracted in the oven at 80°C. When tested using the rat oedema model, none of the oil preparations were found to be efficacious (Figure 13).

![Figure 13.](image)

Since efficacious oils had been prepared previously from the fat of birds on essentially the basal diet (Figure 10), it seemed likely that some other factor (factors) was responsible for the absence of, or low, activity of these oil preparations. Two possibilities were the lack of gut fat or the rendering procedure. The oils which had shown efficacy previously had been prepared from a mixture composed of two thirds subcutaneous fat and one third gut fat. In the latter preparations minced fat was placed on a stainless steel mesh and placed in an oven at the required temperature. The oil dripped through the mesh and was collected in a stainless steel container. Whilst the previous oils had been prepared by dicing the fat with a knife, placing it in a glass beaker which was covered with plastic wrap to exclude air and placed in a water bath at the required temperature.

For the next study, the four oil samples tested were two prepared using the oven from the gut fat of birds fed Diet B or D, a commercially rendered oil from birds fed on essentially
the basal diet and a sample where Vitamin A (~1mg/mL) was added to the commercially prepared oil. This last sample was included because it was claimed in a United States patent that bleaching the oil destroyed its anti-inflammatory activity and the addition of retinal acetate to the bleached oil restored its activity. The results of the study are shown in Figure 14.

![Figure 14](image)

There was no significant difference between the results obtained with the control animals and those obtained with the animals treated with Diet B and D emu oils. The animals with the commercially rendered oil had significantly (P=0.02) less swelling than the control animals at 2 and 4 hours but not 6 hours. However, the addition of the Vitamin A resulted in the swelling being the same as the control animals.

All oils described to this point have been chemically characterised but no correlation between chemical composition and efficacy has been established.

At this time it was decided to discontinue testing oils prepared by heating in the oven and go back to the original method of heating in sealed glass containers. Also, it was decided to use samples composed of two thirds subcutaneous and one third gut fat.

The next study was a combination study. The oil used was prepared from the fat of birds fed diet B (Diet B*) using the new rendering procedure. The other samples tested were this oil to which various long chain polyunsaturated fatty acids were added at the concentrations shown in Figure 15. The oil samples and sorbolene were only applied once, immediately after the injection of the carrageenan.
Figure 15. Emu oil as a trans-dermal carrier.

The results obtained with animals treated with the oil alone (Figure 15) appears to very different from that observed previously with the oil prepared from the same fat using the oven method. In this study there was a significant (P<0.01) reduction in swelling at 2 hours after which there appears to have been a ‘rebound’ reaction which could be expected if the level of the active ingredient had fallen below efficacious levels. At two hours there were no significant differences between the animals treated with oil alone and those animals that had been treated with oils that the various compounds had been added. At all times there was no significant difference between the oil alone and the oil plus substance A (cis 5,8,11,14,17 Eicosapentaenoic acid). However, the animals treated with oil plus substance B (Arachadonic acid) or C (cis 11,14, 17 Eicosatrienoic acid) had significantly less swelling than those treated with oil alone at 4 and 6 hours.
To test the rebound theory, the Diet B* oil was applied 0, 2 and 4 hours. The results show (Figure 16) that in this case the oil resulted in a significant reduction in swelling at all times and no rebound was observed. At the same time oils prepared from the fat of birds fed diet A (Diet A*) or diet D (Diet D*) were tested together with the oil prepared from a commercially bred emu (Prod.1). These oils were only applied once at time zero.

![Graph showing swelling over time](image)

**Figure 16.**

The Diet D* and Prod.1 oils produced no significant reduction in swelling (Figure 16). The animals treated with Diet A oil had significantly less swelling than the controls at 4 hours. The Diet B* was significantly more effective than the other oil preparations so diet certainly appears to influence efficacy.

It is interesting to compare the results of the effect of repeated application of the active oil (Figure 17).
There has been considerable interest in the properties of oil prepared from the fat of wild birds. Birds were shot in three distinct regions of Western Australia and oil samples were prepared by rendering the fat in a water bath at 60 or 80°C. The results of the first study using these oils at 0 and at 2 hours are shown in Figure 18.
Figure 18. The efficacy of emu oils prepared from the fat of wild birds.

At 2 hours the oil treated animals were the same and had markedly less swelling than the controls. At 4 hours animals treated any one of three oil preparations were the same and with considerable less swelling than the controls. At 6 hours three of the oil treatments have the same swelling as the controls and there appears that there may have been some rebound.
The first three samples to be tested were to have been prepared from the fat of birds collected from 3 widely separated regions of Western Australia. The preparations were supposed to be prepared from WE1 from the fat of wild bird 2 (WB2) shot in the South West forest region, WE2 from WB’s 5&6 shot at Southern Cross, an eucalyptus and mallee region and WE3 from WB’s 11&12 shot at Paynes Find, a mulga region. However, it is apparent from subsequent fatty acid analysis that an error had been made and WE2 had been prepared from the fat of WB1, shot in the South West region.

The rendering temperature (60 or 80°C) had no effect on the fatty acid composition, i.e. WE3(60) and WE3(80) had almost identical fatty acid profiles.

The results obtained with additional samples prepared from the fat of individual wild birds are shown in Figure 19.

![Figure 19](image-url)  
**Figure 19.** The efficacy of emu oils prepared from the fat of wild emus.

All these samples prepared from the fat of the wild birds had similar efficacies but had widely differing fatty acid profiles (Table 2).
The last two oils to be tested were prepared from the fats of WB’s 11 and 12. The results are shown in Figure 20.

![Graph showing swelling over time for different oils](image)

**Figure 20.** The efficacy of emu oils prepared from the fat of wild emus.

At all times the average swelling of the emu oil treated animals was less than the controls, the differences were not statistically different (P<0.05) from the controls.

**EVEN WITH THE CURRENT RENDERING TECHNIQUE CONSIDERABLE VARIATION IN THE EFFICACY IS OBSERVED. THE REASONS FOR THE VARIATION REMAIN TO BE ESTABLISHED. TO DATE NO CORRELATION BETWEEN CHEMICAL COMPOSITION AND EFFICACY HAS BEEN ESTABLISHED FOR THIS MODEL.**

The maximum reduction in swelling observed has been 40% of the paw thickness. This still may represent considerable potency since published studies show that even a very powerful anti-inflammatory drug such as hydrocortisone will not prevent swelling occurring and will only reduce the swelling by a comparable amount. To examine this the effects of topical application of an active emu oil (WB5) at 0 and 2 hours were compared to the effects of...
interperitonial injection of 1, 2 or 4 mg of prednisolone at 0 hours. The results of the study are shown Figure 21.

![Graph showing the comparison of the efficacies of prednisolone and emu oil.](image)

**Figure 21.** Comparison of the efficacies of prednisolone and emu oil.

At 2 hours, all 3 doses of prednisolone gave the same result indicating this is the maximum reduction in swelling that can be achieved with this drug which is around 40%. Since the emu oil used achieved a comparable reduction in swelling to prednisolone, it must be considered to have potent anti-inflammatory activity. The time course observed with the prednisolone is different to that observed with the oil which suggests they have different modes of action.

**Correlation Between The Two Models**

In Figure 22 the relative swellings (swelling in the treated animals/ swelling in the controls) measured in the adjuvant model is plotted against that measured in the carrageenan model when the same oil was tested in both models.
Figure 22. Correlation of emu oil efficacy.

It is apparent that there is little if any correlation between efficacy in the different models

**Carrier Studies**

Whilst not scientifically established, emu oil appears to penetrate the dermis much more readily and more completely than other oils. If this is correct then emu oil may be useful as a trans-dermal carrier of therapeutics agents.

**Carrageenan Induced Oedema**

Early attempts to use emu oil as a carrier for traditional anti-inflammatory drugs, aspirin, phenylbutazone and hydrocortisone, were unsuccessful but in hindsight this was to be expected, as they are not soluble in the oil. It was concluded that it would be more appropriate to test materials that were soluble in emu oil.

Previous studies (Figure 15) had shown that when two substances (B and C) where added to the emu oil (2.5 mg/mL) they appeared to significantly enhance the efficacy of the oil. So a dose response study using substance B was undertaken. The concentrations used were 5, 2.5 or 1.25mg/mL. In this test, the carrageenan produced a very severe inflammation in both the test and control animals. The inflammation was very much more severe than seen normally and none of the applied materials significantly effected the severity of the inflammation. The severity was such that it was considered it could be masking any efficacy the material may have. The concentration of the carrageenan solution was checked
and found to be correct and there was no obvious explanation for the severity of the oedema. The experiment was therefore essentially repeated but one group of animals was given a high dose (10mg) of Ibuprofen. Again, very severe inflammation occurred in both the test and control animals and none of the treatments, including the Ibuprofen, reduced oedema. On further investigation, it was found that the carrageenan had been microwaved, rather than, autoclaved into solution. A further study confirmed microwaved carrageenan produced a more severe oedema than the autoclaved carrageenan.

We have been unable to repeat this dose response study.

In the light of this, the results of previous studies (e.g. Figure 13) where very severe inflammation occurred will have to be re-assessed.

It was considered that because of its structure, Ibuprofen should have reason solubility in emu oil. For the final study in this series the efficacy of an oil prepared by commercially rendering fat from birds raised by Agriculture (Commercial) with that of the oil to which Ibuprofen (10mg/ml) had been added. For comparison the oil from WB12 was included. (Figure 23).

![Figure 23. Emu oil as a trans-dermal carrier of Ibuprofen](image)
At all times the average swelling of the oil treated animals was less than the controls, the only statistically significant difference was between the animals treated with the oil containing Ibuprofen and the controls at 2 hours.

**In Vivo Penetration Study**

The average hydrocortisone levels measured in the blood of animal treated with hydrocortisone dispersed in a commercially available emu oil (HCEO1), an oil prepared from intra-dominal fat (HCEO2) or a commercial hydrocortisone (HCCOM) cream are shown in Figure 24.

![Graph showing cortisol concentration over time for HCEO1, HCEO2, and HCCOM](image)

**Figure 24.**

The results showed that at two hours the level of hydrocortisone in the animal treated with HCEO2 was significantly higher (P<0.05) than that of the animals treated with HCEO1 but not significantly higher than those treated with the commercial cortisone preparation. There was no significant difference the animals treated with HCEO1 and those treated with the commercial cortisone.
At 5 hours there were no significant differences in the concentrations of hydrocortisone.

The apparently significant finding has to be viewed with caution since in all cases the levels of hydrocortisone are low, most at the limit of detection, and would represent only a very slight contamination with the applied material. For example the highest concentration in blood measured was a 100,000 fold lower than the applied concentration. Furthermore, the variance between replicates was very high.

It was decided to discontinue this form of study for a number of reasons including cost and the trauma caused to the animals.

**In Vitro Permeability Studies**

The results obtained showed there was no significant differences between the steady fluxes of hydrocortisone from the various preparations. The fluxes measured (fraction applied/hr/cm²±SEM) in the first experiment were for hydrocortisone in EO1 1.88x10⁻⁴±4.1x10⁻⁵, for hydrocortisone in EO3 2.36x10⁻⁴±4.7x10⁻⁵ and for hydrocortisone in olive oil 2.61x10⁻⁴±4.1x10⁻⁵. The fluxes measured (fraction applied/hr/cm²±SEM) in the third experiment were for hydrocortisone in EO2 10.0x10⁻⁴±10x10⁻⁵ and for hydrocortisone in olive oil 9.0x10⁻⁴±4x10⁻⁵.

The results also showed there was no significant differences between the steady fluxes of diclofenac from the various preparations. The fluxes measured (fraction applied/hr/cm²±SEM) in the first experiment were for diclofenac in EO1 1.91x10⁻⁵±2.2x10⁻⁶ for diclofenac in EO3 1.82x10⁻⁵±1.6x10⁻⁶ and for diclofenac in olive oil 1.64x10⁻⁵±2.6x10⁻⁶.

**Fatty Acid Profiles**

The fatty acids profiles of oil samples prepared from the fat of 8 wild birds are shown in Table 2.

The main features to note are:

1. The wide variation in the fatty acid profiles. As stated previously, the variation seen in fatty acid profiles of the oils prepared from the fat of wild birds vary much more than that seen with farmed birds feed a variety of diets. This variation may reflect varying diets because birds shot close together ((1&2), (5&6) and (11&12)) have similar profiles.

2. As discussed previously, the omega 3 fatty acid content

3. Also as discussed previously, the C18ln9:C16 ratio.

4. The actual C19ln9 content. In farmed birds the C18ln9 content varied little (50±5%) but with the wild birds this varies from 30 to 60%.

5. The variation in the amount of the unidentified fatty acid, running between C204n6 and C205n3. Samples containing this fatty acid were sent to the Centre for Drug Design and Development, University of Queensland for analysis by GC/Mass spectroscopy but the substance was not identified.
Fatty acid analysis of the same oil preparation immediately after preparation and again after storage at 5°C showed that changes in fatty acid profiles did occur during storage. In particular it was noted that the fatty acid that appeared to correlate with the efficacy of the oils prepared from farm reared birds (see Figure 7) appeared to decrease during storage,
Implications

The demonstration that oil preparations with potent anti-inflammatory active can be prepared and that there were no adverse effects of emu oil observed in this study nor in a previous study (9) should facilitate obtaining permission from the relevant Ethics Committees to perform human clinical trials on the anti-inflammatory activity of emu oil. Such trials are required before Therapeutic Goods Act registration can be obtained.
Recommendations and Conclusions

Objectives 1 and 4 were examined in detail but because of the nature of the results and reduction in funding it was not possible to meet the other objectives:

However the following conclusions and recommendations are given.

1. Emu oils with potent anti-inflammatory activity can be prepared
2. Different components of the oil are active in the different rat models.
3. Factors affecting efficacy include diet and rendering procedure.
4. Marked differences were found between the fatty acid profiles of oils prepared from the fat of wild birds and those prepared from the fat of farmed birds.
5. Further investigation into the anti-inflammatory activity of emu oil should be undertaken. These studies should include human clinical trials at the earliest opportunity.
References


