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改良並建立我國保健食品補鐵機能評估的檢測方法

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目　　錄

表　次

頁數

圖　　次

中文摘要

富裕的社會仍有鐵營養不足的高風險族群，食品工業因而研發各式補充鐵營養之新式食品。食品鐵質之吸收率受鐵之化學形式與食品組成份之影響，若形式不當或配方中有不利於鐵吸收之因素，則干擾其揮營養功效。為了保證產品之功效，保障消費者的權益，檢驗食品鐵利用率的標準方法有其必要。本研究為建立適用於我國保健食品補鐵機能評估的檢測方法與標準程序以供利用，乃採用 AOAC 大鼠血紅素再生法，針對基礎飼料配方，動物規格，飼養時間，鐵利用之生物指標與統計方法逐項評估，以為標準化之依據。結果發現，AOAC 配方所含鈣與磷過高而降低鐵之利用效率，所添加硫酸亞鐵之相對生物價僅達 AIN-76 配方之80%，故不適用。再生期 10 天或 14 天，AIN-76 配方添加鐵量為 6 至 24 ppm 時，再生後血紅素濃度、再生期血紅素濃度增加量、或再生期血紅素鐵增加量與飼料鐵濃度或鐵攝取量之間有良好線性迴歸關係；添加鐵量為 35 ppm 時，線性關係反而減弱。比較 AIN-76 與 AOAC 兩配方對硫酸亞鐵利用率之影響時，以再生後血紅素濃度、再生期血紅素濃度增加量、或再生期血紅素鐵增加量作為鐵利用之生物指標計算而得之相對生物價相近，表示三種指標均適用。

根據本研究結果所建議之評估方法概述如下：大鼠血紅素再生法之原理與基本執行步驟沿用 AOAC 法（1995），但改變其飼料配方，並調整再生期天數。實驗分為耗鐵期與再生期，耗鐵期以大鼠血紅素值低於 6 g/dl

為標準，採用不加鐵之基礎配方。實驗動物採用 Wistar 雄性大鼠，鼠齡限定為離乳，約四週大，體重不超過 70g，每一組用大鼠 6-7 隻。基礎飼料配方採不加鐵之 AIN-76 配方，標準飼料採 AIN-76 配方，添加鐵的形式採硫酸亞鐵，添加鐵量可採 6、12、18、24 ppm 一系列濃度。試驗飼料之鐵以測試樣品提供，添加鐵量相當於 6、12、18、24、35 ppm 或以上。再生期開始時各組大鼠之血紅素與體重平均值應無顯著差異。再生期除了試驗組之外，必須有標準組。飼料所添加鐵量，若採一系列濃度，標準飼料添加鐵量為 12、18、24 ppm，試驗飼料添加鐵量為 12、18、24、35 ppm；若採單一濃度則標準飼料與試驗飼料均添加 24 ppm。再生期天數至少 10 天，最多 14 天。再生期間應紀錄飼料攝取量，再生期結束時應測量體重與血紅素值。鐵利用之生物指標可採用再生後血紅素濃度、再生期血紅素濃度增加量、或再生期血紅素鐵增加量。採系列鐵濃度時，將鐵利用指標對應飼料添加鐵量或再生期鐵攝取量進行迴歸分析，檢驗線性關係，以回歸係數計算試驗飼料組對標準飼料組之比例，作為測試樣品之鐵相對生物價。採單一鐵濃度時，以再生期血紅素鐵增加量對鐵攝取量計算血紅素再生效率 HRE，計算試驗飼料組對標準飼料組之比例，作為測試樣品之鐵相對生物價；或直接以再生期血紅素鐵增加量計算比例亦可。

關鍵詞：鐵質生體可用率，鐵質相對生物價，大鼠血紅素再生法，
AIN-76 配方，AOAC 配方，鈣，磷

英文摘要

The AOAC Official Method presents a rat hemoglobin regeneration bioassay for assessing bioavailability of iron. The concentrations of calcium and phosphorous in the AOAC basal diet were 1.3 times those in the AIN-76 diets. When ferrous sulfate was added to these two diets, bioavailability of iron in the AOAC diet was only 70% that in the AIN-76 diet. For the AIN-76 diets containing a series of added iron ranging from 6 ppm to 24 ppm, significant linear regression relationship existed between replete hemoglobin concentration, hemoglobin gain or hemoglobin iron gain and dietary iron concentrations as well as intake of dietary iron. Relative biological values calculated from these three biomarkers of iron nutrition were similar. Therefore, AIN-76 formula is a more appropriate basal diet for rat hemoglobin regeneration bioassay. Therefore, we propose some modifications of the AOAC method.

In the modified assay, male weanling Wistar rats aged less than 4-week and weighed less than 70 g are used; in iron depletion period, an iron-free AIN-76 diet is used and hemoglobin of rats are render < 6 g/dL; in iron repletion period, test diets contains 12, 18 , 24 and 35 ppm of Fe, reference diets contains ferrous sulfate at 12, 18 and 24 ppm of Fe, 6-7 anemic rats are included in each group, repletion time ranged from 10 to 14 days, dietary intakes are recorded, body weight and hemoglobin concentration are measured at the end of the repletion period. Dose-response relationship between iron biomarkers and dietary iron intake or iron concentration is evaluated by regression analysis. The ratio of regression coefficient between the test diets and the reference diets can be taken as the relative biological value. If a single dietary Fe concentration should be used in the bioassay, 24 ppm of Fe is suggested, and a ratio of hemoglobin iron gain or hemoglobin regeneration efficiency between the test diets and the reference diets is taken as the relative biological value.

Key words: Iron bioavailbility, relative biological value, rat hemoglobin regeneration bioassay, AIN-76formula, AOAC modified formula, calcium

前　言

一、建立檢驗方法之重要意義與應用價值

鐵是人體必須的營養素，富裕社會仍存有缺鐵之營養問題，美國最新的健康調查 NHANES III 指出生育年齡女性為主要缺鐵族群（1），我國亦不例外。衛生署執行之「民國 81-86 年國民營養健康狀況變遷調查」結果指出，女性鐵攝取量有偏低的現象，13-34 歲女性平均鐵攝取量不超過建議量的 75%，35-54 歲女性則可達建議量的 84%；以血液生化指標評估鐵營養狀況的結果可見，女性的鐵營養狀況顯著較男性為低落，四歲以上國人之總缺鐵率，男性有 2.1%，女性則高達 10.7%（2）。此外，孕婦與素食者也是缺鐵發生率較高的族群（3，4）。可見鐵營養為經濟富裕國家共同面臨之保健問題。

根據消費者的常識，缺鐵與貧血有關；因此，許多保健食品以補充鐵質為營養訴求，或是以補血為健康訴求，例如高鈣高鐵奶粉。市面上許多針對兒童、青少年與孕婦的營養補充品都以鐵為添加成分之一，例如成長奶粉、兒童奶粉等。加鐵強化之食品其鐵質之生體可用率（bioavailability）必須加以考慮。根據衛生署委託之研究計劃，以大鼠血紅素再生法，評估目前市售高鈣高鐵奶粉之鐵質生體可用率之結果可見，某些知名品牌的鐵質無法為嚴重缺鐵貧血之大鼠所利用，其鐵質生體

可用率明顯偏低，以硫酸亞鐵利用率為100，高鈣高鐵奶粉的鐵相對生物價（RBV，relative biological value）只有26（5）。可見加鐵強化之食品的營養功能可能與其健康訴求不符。

鐵是預防缺鐵性貧血不可或缺的成分，然而，人體鐵營養狀況充足與否，除了與鐵攝取量有關之外，還取決於飲食鐵質的生體可用率，因為鐵的吸收受個人之鐵營養狀況、鐵的化學形式、與食品中其他成分等三類因素的影響（6）。食物中鐵的化學形式依照吸收機制分為血原素鐵（heme-iron）與非血原素鐵（non-heme-iron）兩大類。 血原素鐵吸收率較高，約為20-30% 不受飲食成分的影響；非血原素鐵吸收率偏低，約1-8%，其吸收受飲食成分的影響，已知促進鐵吸收的成分有肉類蛋白質與維生素C，抑制吸收的成分有膳食纖維、植酸、草酸、大量鈣鹽、單寧、牛奶與蛋等。鐵營養強化食品的加工製造必須重視鐵的化學形式與食品的組成份，因為營養強化用的各種鐵化合物屬於吸收率較低的非血原素鐵。

成功有效的食品加鐵強化應該選擇合宜的攜載食品（food vehicles）與鐵化合物（7，8）。合宜的攜載食品是鐵吸收抑制物含量少，而且不會有攝食過量的危險者。常用的鐵化合物為各類無機或有機的鐵鹽，具有不同的生體可用率；生體可用率高的鐵化合物如硫酸亞鐵、葡萄糖酸亞鐵、檸檬酸銨鐵、硫酸銨亞鐵等，水溶性高，多屬於亞鐵鹽，化學性質非常活

發，會催化產品中脂肪酸的氧化酸敗，對食品的顏色與風味等官能性質會有不良的影響（9）。化性穩定的鐵化合物對產品較為有利，例如：正磷酸鐵、焦磷酸鐵、元素態鐵等。但是這類化合物不溶於水，在稀酸中的溶解度也很低，其生體可用率偏低（10）。食品加工為了兼顧營養與品質，不斷研發新式的鐵劑或是加工程序，例如 SFE-171 為含硫酸亞鐵的磷脂質微膠囊（11）。食品廠商應對產品之訴求負責，故採用新式鐵劑或加工方法，推展新式保健食品時，應依需要對其產品進行鐵可用率的評估。

針對保健食品或健康訴求的管理，借鏡於先進國家，美國對健康訴求或營養支持聲明的要求是「具有科學界公認的證據」，日本對「特定用保健食品」之審核，也需要「有充分之臨床科學依據」(12)；其共通之處為持守科學原理，以保障消費者的安全和權益。目前我國食品衛生主管機關尚無管理的法令與規範，但根據衛生署保健食品管理專家會議之決議，保健食品之功能項目應明定，並需定出功能評估方法，指出未來的趨勢也必力求建立科學性的評估檢驗準則，讓廠商在研發產品時有所依循，以昭公信。

初擬之保健食品的功能項目中有改善貧血一項，市面上相關的產品主要是加鐵強化食品或鐵補充劑。鐵可用率的觀念奠基於營養科學原理，可供參考利用之相關研究與文獻很多，並且有合乎學理之評估方法可供利

用。在衛生署嘗試規劃保健食品訴求之功能評估方法之際，建立我國適用之補鐵機能生物分析方法是可行的起步，本研究之目的即為建立適用於我國之保健食品補鐵機能評估的檢測方法與標準程序，編寫成手冊。所獲取之經驗亦可供其他功能項目之參考。

二、AOAC 大鼠血紅素再生法之改良

鐵可用率的生物分析方法以貧血大鼠血紅素再生法為主。此方法於 1974 建立(13)，1980 年正式登錄於第 14 版 AOAC Official Methods of Analysis (14)，一直沿用至今 (15)。其原理是利用成長中的貧血大鼠對食物鐵質有最大的吸收能力，並且吸收的鐵質優先用於合成血紅素；故以含鐵食物或化合物添加於不含鐵之基礎飼料中餵養，追蹤其血紅素濃度之增加，可以反映食物鐵質被動物吸收利用的程度，據以區別不同鐵源的利用效率 (13)。國際營養性貧血顧問小組(INACG，the International Nutritional Anemia Consultative Group) 曾經比較動物法與人體吸收實驗，指出 AOAC 法的結果與人體吸收率有良好的相關性 (16)。

AOAC 法自 1980 年建立以來並無任何修訂版本，然而隨者動物實驗技術之進步，以及鐵吸收機制之新知，有些建議使用之實驗條件已不適用。1989 年 INACG 的研究中已經略有修改，基礎飼料配方之 degermed yellow

corn meal 不再使用，全部改用純化的原料與 AIN-76 維生素配方，但是礦物質配方仍然維持不變（16）。目前，礦物質配方特別需要注意，因為 AOAC 配方的礦物質種類與含量和美國營養學會（AIN，American Institute of Nutrition）所建議的嚙齒類實驗動物配方有許多不同之處：AOAC 配方不含微量礦物質硒與鉻，其鈣與磷的含量均高達 8000 mg/kg（15，16），鈣的形式為碳酸鈣；而 AIN-76 則添加微量元素，而且鈣與磷量均較低，分別為 5000 mg/kg 與 4000 mg/kg，採用磷酸氫鈣（17）。根據近年的研究，鈣的差異對鐵的利用可能有所影響。

動物實驗指出鈣具有抑制鐵吸收利用的效應。給大鼠氯化鈣溶液會抑制放射性鐵進入小腸黏膜的細胞，鈣的濃度越高，放射性鐵的吸收率越低（18）。當鈣的劑量都必須比正常的 0.5%為多時就會產生抑制效應；鈣量從 0.75% 到 1.25% 都抑制大鼠對鐵的利用（19, 20），也減少懷孕母鼠多種組織的鐵含量，更進一步使胚胎組織的含鐵量降低（20）。成長中的大鼠餵食三倍於正常量的鈣時，飼料 20 ppm 的鐵濃度不足以維持正常的血漿鐵濃度，必須提供四倍的鐵量才能達到正常（18）。鈣的作用還受其鹽類型式的影響，除了飼料的正常鈣量之外，另以碳酸鈣額外提供 0.25% 鈣時，足以抑制貧血大鼠的鐵利用效率，若是以硫酸鈣形式則需要額外提供 0.75%才有抑制效應（19）。AOAC 配方採用碳酸鈣，用量為 0.8%，達到足

以抑制大鼠鐵利用的程度，是不利於鐵吸收的條件，有低估鐵利用率之疑慮。故基礎飼料的鈣量與形式有修訂之必要。

檢驗方法必須講求時效快速，而且要考慮經濟成本。AOAC 法的步驟中，動物需先經耗鐵期，餵以缺鐵飼料以誘發缺鐵貧血（血紅素值 <6g/dL），然後進入再生期，分組餵以含標準鐵或待測食品之飼料。再生期為 14 天，耗鐵期可能長達 3 週以上。檢驗時需要以利用率高之硫酸亞鐵作為對照標準，為了建立劑量反應（dose-response）的迴歸直線，標準鐵與待測試之含鐵食品都需要配製三種不同的鐵濃度，故每一種食品之檢測至少需要 6 組動物。如果能夠合理縮短所需天數，減少動物組數，都有助於提昇效率並減少負擔。

AOAC 法中主要的測量項目是再生期結束時之血紅素濃度，數據分析是對血紅素濃度與飼料鐵濃度的對數進行迴歸分析。但是再生期開始補充鐵後，大鼠的生長和體重變化量受鐵量的影響，血液體積也會隨之增加，血紅素濃度則相對受到稀釋，可能低估了血紅素的總量，同時低估了鐵的吸收利用率。如果計入體重的變化，換算出血紅素鐵變化量，替代血紅素濃度，可以校正低估的誤差（21，22）。就理論而言，鐵的攝取指標可用飼料鐵濃度或是再生期鐵攝取總量，血紅素再生指標可用血紅素濃度、血紅素濃度變化量或是血紅素鐵變化量，總共有六種關係模式可供選用，彼此

之間的優點與限制值得加以比較釐清，以為選用指標之標準。

鐵質相對生物價的計算方法也有多種選擇。針對血紅素指標與鐵量的劑量反應直線，如果食物與標準鐵之直線呈互相平行的關係，則採用 parallel lines type assay，以血紅素濃度達到相同水準所需要的鐵量作為計算 RBV 的依據；如果直線之間不是平行關係，則採用 slope-ratio assay，以直線的斜率作為計算依據（21, 22）。血紅素再生效率（HRE，hemoglobin regeneration efficiency）則是以再生期血紅素鐵增加量佔鐵攝取量的比例來計算 RBV（23）。

建立檢驗方法時，這些實驗條件，包括；基礎飼料配方、動物規格、測試食物之準備與供應方式、飼養時間、分析項目與統計方法等，都必須標準化。本研究擬依序進行三個動物實驗來界定檢驗所需要的實驗條件，以建立標準化之實驗步驟。所獲之成果不僅適用於建立我國的檢驗方法，也可提供 AOAC 作為修訂其方法之參考。

本研究首先探討 AIN 與 AOAC 基礎飼料配方對標準鐵劑 $FeSO_4$ 之利用率的影響，比較兩種配方之差異以及飼養天數的影響，利用統計方法比較六種迴歸模式之線性關係與迴歸係數，以鑑定最適用之鐵攝取指標與血紅素再生指標，最後據以撰寫檢驗手冊草案。

材料與方法

實驗設計

大鼠血紅素再生法的標準實驗步驟，根據 AOAC (1995)Official Method of Analysis (15)，飼養期分為兩階段：第一段是耗鐵期，目的在使動物缺鐵貧血；第二段是血紅素再生期，測試食物中鐵質被動物利用的程度。耗鐵期餵食不加鐵的基礎飼料，定期經尾巴採血獲取血樣，追蹤血紅素濃度，以< 6 g/dL 為理想貧血程度；此時依血紅素濃度將動物適當分組，每組 7 隻，進行第二段實驗。第二段實驗開始時，先紀錄大鼠體重與血紅素濃度；實驗組別依飼料性質分為含標準鐵劑 ($FeSO_4.7H2O$) 之標準對照組與含測試樣品之試驗組；飼養期間經尾巴採血獲取血樣，測定追蹤血紅素濃度變化狀況；定時紀錄體重與飼料攝取量，配合血崇素值以供數據分析。

本實驗共用大鼠 85 隻，耗鐵期中有 77 隻大鼠飼以不加鐵之 AIN-76，另以 8 隻大鼠飼以不加鐵之 AOAC 配方 (AOd 組)。飼養期間追蹤測量血紅素值的變化，直到血紅素降至 60 g/l 以下。　進入再生期時，首先將 77 隻大鼠分為 11 組，各組織起始之血紅素值與體重平均值均沒有顯著差異，其中六組飼以一系列加鐵量為 0、6、12、18、24、35 ppm 之 AIN-76 配方，組名分別稱為 AI0、AI6、AI12、AI18、AI24、AI35；其餘五組則飼以一系

列加鐵量 6、12、18、24、35 ppm 之 AOAC 配方，組名分別稱為 A06、A012、A018、A024、A035。至於 Aod 組則於再生期飼以含鐵 18 ppm 之 AOAC 配方。再生期亦定時紀錄體重與飼料攝取量，同時於第 5、10、14 天從尾巴採血，測量血紅素值以觀察其變化。

實驗動物

實驗動物採用 Wistar 雄性大鼠，購自或台灣大學醫學院附設醫院之動物中心。鼠齡限定為離乳，約四週大，同時限定體重不超過 70 公克。實驗動物之飼養遵循基本程序，動物室控制溫度與光暗週期。實驗期間供給去離子水。水與飼料均採自由攝食。

飼料配方

基礎飼料配方根據 AIN76 與 AOAC 兩種而加以調整，以黃豆沙拉油取代玉米油，用量為 7%以供應充足的必須脂肪酸，其組成列於表一，兩種配方之礦物質組成列於表二，其中不加鐵。飼料以購入之原料自行配製。再生期用含鐵飼料則於基礎飼料中添加 $FeSO_4.7H2O$，使鐵的含量分別為 6、12、18、24、35 ppm。

化學分析與計算

各組實驗飼料之鈣、磷、鐵均定量檢驗，飼料以微波爐（MLS-1200 MEGA, Milestone）消化後，經適當稀釋，以原子吸光法定量鈣與鐵，以比色法定磷（24）。血紅素定量採用 cyano-methemoglobin 法，以 Drabkin's 試劑呈色，測量波長 540 nm 的吸光值，經乘以 36.8 而得血紅素濃度（25）。

大鼠的血液體積以每公克體重有 0.067 mL 估計，血紅素的含鐵量以 0.335%計算，配合血紅素濃度可以計算血紅素鐵總量；再生期前後血紅素濃度差即為血紅素變化量，血紅素鐵總量的差額即為血紅素鐵增加量；鐵攝取量為攝食量與飼料鐵濃度乘績；血紅素再生效率（HRE, hemoglobin regeneration efficiency）等於血紅素鐵增加量對攝取鐵量的百分比例。各項計算公式如下：

血紅素增加量（mmol/l）＝再生期之血紅素值 －再生期起始之血紅素值；

血紅素鐵總量（μmol/rat） = 體重 x 0.067 x 血紅素值 x 0.335%；

血紅素鐵增加量（hemoglobin Fe gain, μmol/rat） = 再生期之血紅素鐵總量 －再生期始之血紅素鐵總量；

HRE = 血紅素鐵增加量÷ 再生期鐵攝取量× 100%。

統計分析

耗鐵期 AIN-76 與 AOAC 飼料的差異以 student's t test 檢定。再生期間 AIN-76 與 AOAC 各組之間的差異以 Duncan's multiple range test 檢定。飼料配方與鐵含量的主效應分析以雙因子變異數分析（two-way ANOVA）檢定，涵蓋的組別是 AI6 ~ AI35 與 AO6 ~ AO35 共十組。除了 AI0 與 AO18d 兩組之外，針對 AIN-76 與 AOAC modified 飼料分別以迴歸模式檢定鐵攝取指標與血紅素再生指標之間的線性關係；可用的鐵攝取量指標有：飼料添加鐵濃度與再生期鐵攝食量，；可用的血紅素再生指標有：再生期結束時之血紅素值、血紅素增加量與血紅素鐵增加量；總共可有六種迴歸模式，並且比較包括 AI35 與否兩種條件。鐵相對生物價（RBV，relative biological value）之計算有兩種方法：其一利用直線迴歸係數，以 AIN 飼料之值為 100，計算 AOAC 飼料之相對比例而得；其二利用 HRE 值，以 AIN 組 HRE 值為 100，計算 AOAC 組 HRE 值之相對百分比例而得。各項統計分析均採 SAS 程式（SAS 6.12, Cary, CN）以電腦執行。

結果與討論

攝食量與體重

血紅素再生期中大鼠的飼料攝取量列於表三，雙因子變異數分析顯示飼料配方主效應之影響沒有統計顯著性，但是飼料添加鐵量主效應對 10 與 14 天之飼料攝取量則有顯著性的影響，隨著鐵含量之升高而攝取增多，

未加鐵之基礎飼料組 AI0 攝取的飼料最少。

經過 28 天耗鐵期後，再生期開始時大鼠的平均體重為 158 公克，各組之間沒有顯著差異。再生期之生長狀況列於表四。再生期之體重不受飼料配方主效應之影響；飼料鐵含量主效應對 5 或 10 天之體重沒有影響，但對 14 天之體重則效應顯著，隨著鐵含量之增高，體重亦呈升高之趨勢。再生期 5 天時，AI35 組之體重已經顯著高於其他各組。再生期 10 與 14 天時，AI0 組之體種則顯著低於其他各組。

鐵利用生物指標之變化

再生期 5 天時大鼠的血紅素與血紅素鐵的變化列於表五。變方分析顯示血紅素濃度、血紅素增加量與血紅素鐵增加量受飼料鐵濃度與配方主效應之影響（$P = 0.0001$），各項指標均隨著鐵量之升高而增高，並且以 AIN-76 配方的增加量高於 AOAC 配方。此時之血紅素值、血紅素增加量與血紅素鐵增加量均以 AI0 顯著最低；血紅素值與血紅素增加量顯著最高的是 AI24 與 AI35 兩組，血紅素鐵增加量則以 AI35 顯著最高。

再生期 10 天時大鼠的血紅素與血紅素鐵的變化列於表六。變方分析顯示血紅素濃度、血紅素增加量與血紅素鐵增加量受飼料鐵濃度與配方主效應之影響（$P = 0.0001$），各項指標均隨著鐵量之升高而逐漸增高，並且以 AIN-76 配方的增加量高於 AOAC 配方。此時之血紅素值、血紅素增加

量與血紅素鐵增加量均以 AI0 顯著最低，而以 AI35 顯著最高。

再生期 14 天時大鼠的血紅素與血紅素鐵的變化列於表七。變方分析顯示血紅素濃度、血紅素增加量與血紅素鐵增加量受飼料鐵濃度與配方主效應之影響（$P = 0.0001$），各項指標均隨著鐵量之升高而增高，並且以 AIN-76 配方的增加量高於 AOAC 配方。此時之血紅素值、血紅素增加量與血紅素鐵增加量仍以 AI0 顯著最低，而顯著高於其他各組的有 AI24、AI35 與 AO35 三組。

迴歸分析

再生期血紅素濃度對飼料鐵含量之迴歸分析結果如圖 1.1 所示，無論是 AIN-76 或是 AOAC modified 配方，均呈現顯著的線性關係，相關係數 R^2 的範圍分別是 AIN-76 配方為 0.9057 ~ 0.9626，AOAC 配方為 0.9788 ~ 0.9962；迴歸係數值（即斜率）隨著再生期天數之增長而增大，以 AIN-76 配方大於 AOAC 配方。再生期血紅素增加量對飼料鐵含量之迴歸分析結果如圖 1.2 所示，兩種配方均呈現顯著的線性關係，相關係數 R^2 的範圍分別是 AIN-76 配方為 0.9121 ~ 0.9611，AOAC 配方為 0.9798 ~ 0.9966；迴歸係數值亦隨著再生期天數之增長而增大，仍以 AIN-76 配方大於 AOAC 配方。再生期血紅素鐵增加量對飼料鐵含量之迴歸分析結果如圖 1.3 所示，兩種配方均呈現顯著的線性關係，相關係數 R^2 的範圍分別是 AIN-76 配方為

0.927 ~ 0.9483，AOAC 配方為 0.9832 ~ 0.9967；迴歸係數值（即斜率）隨著再生期天數之增長而增大，均以 AIN-76 配方大於 AOAC 配方。三種迴歸模式中，AIN-76 配方各組於再生期 14 天時之相關係數 R^2 值均為最小，表示線性關係減弱，主要原因是攝取加鐵 35 ppm 之 AIN-76 配方兩週，動物的血紅素反應已經接近生理恆定的水準，上升趨勢呈現緩和的現象。

迴歸分析時不包括 AI35 組時，再生期血紅素濃度對飼料鐵含量之迴歸分析結果如圖 1.4 所示， AIN-76 配方的相關係數 R^2 的範圍是 0.9692 ~ 0.998。血紅素增加量對飼料鐵含量之迴歸分析結果如圖 1.5 所示，AIN-76 配方的相關係數 R^2 的範圍分別是為 0.964 ~ 0.9929。血紅素鐵增加量對飼料鐵含量之迴歸分析結果如圖 1.6 所示，AIN-76 配方的相關係數 R^2 的範圍為 0.9561 ~ 0.9802。三種迴歸模式中，AIN-76 與 AOAC modified 配方均呈現顯著的線性關係，比較包含 AI35 組與否之結果，不包含時，AIN-76 配方的相關係數 R^2 較大，表示此時之線性關係較強；迴歸係數值（即斜率）均隨著再生期天數之增長而增大，亦較包含 AI35 組時為高，仍以 AIN-76 配方大於 AOAC 配方。

迴歸模式中獨立變數改用鐵攝取量，以再生期血紅素濃度為依變數之迴歸分析如圖 2.1 所示，兩種配方均呈現顯著的線性關係，相關係數 R^2 的範圍分別是 AIN-76 配方為 0.8941 ~ 0.9664，AOAC 配方為 0.9649 ~

0.9977。依變數為血紅素增加量的分析結果如圖 2.2 所示，線性關係顯著，相關係數 R^2 的範圍分別是 AIN-76 配方為 0.9029 ~ 0.9665，AOAC 配方為 0.9645 ~ 0.991。依變數為血紅素鐵增加量之迴歸分析結果如圖 2.3 所示，相關係數 R^2 的範圍分別是 AIN-76 配方為 0.922 ~ 0.9814，AOAC 配方為 0.9709 ~ 0.9921。迴歸係數值（即斜率）隨著再生期天數之增長而增大，以 AIN-76 配方大於 AOAC 配方。三種迴歸模式中，AIN-76 配方各組於再生期 14 天時之相關係數 R^2 值均為最小，表示線性關係減弱，主要原因是攝取加鐵 35 ppm 之 AIN-76 配方兩週，動物的血紅素反應已經接近生理恆定的水準，上升趨勢呈現緩和的現象。

迴歸模式中獨立變數改用鐵攝取量，而且不包括 AI35 組時，依變數為血紅素濃度之結果如圖 2.4 所示， AIN-76 配方的相關係數 R^2 的範圍是 0.9816 ~ 0.9996；依變數為血紅素增加量之結果如圖 2.5 所示，AIN-76 配方的相關係數 R^2 的範圍分別是為 0.978 ~ 0.9986；依變數為血紅素鐵增加量之結果如圖 2.6 所示，AIN-76 配方的相關係數 R^2 的範圍為 0.9716 ~ 0.9918。三種迴歸模式中，均呈現顯著的線性關係，不包含 AI35 組時，AIN-76 配方的相關係數 R^2 較大，即線性關係較強；迴歸係數值（即斜率）均隨著再生期天數之增長而增大，亦較包含 AI35 組時為高，且以 AIN-76 配方大於 AOAC 配方。

AOAC modified 配方之鐵相對生物價

根據六種迴歸分析可見，AOAC modified 配方之迴歸係數較 AIN-76 配方為小，表示其中硫酸亞鐵的利用率較 AIN-76 配方為低。六種迴歸分析下計算而得之鐵相對生物價列表八，當迴歸模式中包含 AI35 組時，所得 RBV 值均大於不含 AI35 組時，並且以 14 天再生期之值遠高於其他兩個時段，表示有高估之慮，乃因此時 AI35 組之鐵生物指標已經偏離線性反應。迴歸模式不包含 AI35 組時，六種迴歸模式計得之 RBV 值均隨著再生天數增長而增大，以 14 天時最大，數值範圍是 63～74，兩種自變數之結果相似，三種依變數中以血紅素鐵增加量計得之數值較高。

檢測鐵相對生物價適用之單一鐵濃度

根據 student's t test，耗鐵期中血紅素濃度不受飼料配方的影響，不加鐵之 AOAC modified 配方與 AIN-76 配方所達成的耗鐵效果一樣。再生期中，AO18 與 AO18d 兩組之血紅素濃度、血紅素增加量、血紅素鐵增加量等均無顯著差異，此兩組於耗鐵期所用飼料不同，但再生期均用 AOAC 配方，表示再生期的反應不受耗鐵期飼料之影響。AI18 與 AO18 兩組之間，血紅素濃度、血紅素增加量、血紅素鐵增加量僅於再生期 5 天呈顯著差異，另於再生期 10 天有血紅素增加量顯著不同，於 14 天時各項鐵生物指標均

無顯著差異。因此，添加鐵量 18 ppm 者無法有效區分不同利用效率之鐵源，而不適用於單一濃度的檢測實驗。

以血紅素鐵增加量計算而得之 RBV 值列於表九。飼料添加鐵量為 24 ppm 者，於再生期三個時段，兩種飼料配方之間呈顯著性差異，再生期 10 天與 14 天所得 RBV 值 68 與 73，再生期長者數值較大，與迴歸分析所得數值範圍 63～74 一致。以添加鐵量 24 ppm 之 AI24 與 AO24 組計算血紅素再生效率與 RBV 之結果列於表十。再生期三個時段之 HRE 值均以 AIN-76 配方大於 AOAC 配方，利用 HRE 計算之 RBV 值與利用血紅素鐵增加量計得之值相同，隨時間變化之趨勢亦同。因此，鐵利用率之檢測若添加鐵量採用單一濃度時，添加鐵濃度可採用 24ppm，生物指標可用血紅素鐵增加量，RBV 之計算可根據血紅素鐵增加量或 HRE 均可。

富含鐵質食物之鐵質利用率評估

以大鼠血紅素再生法評估含鐵濃度高達 0.9 mg/g 乾重之紫菜，可見紫菜鐵質之相對生物價為 26，與磷酸鐵鹽相近（附錄）

結論與建議

大鼠血紅素再生法適用於評估食物鐵源之生體可用率，AOAC 配方所含鈣與磷過高而降低鐵之利用效率，所添加硫酸亞鐵之相對生物價僅達

AIN-76 配方之 70%。再生期 10 天或 14 天，AIN-76 配方添加鐵量為 6 至 24 ppm 時，再生後血紅素濃度、再生期血紅素濃度增加量、或再生期血紅素鐵增加量與飼料鐵濃度或鐵攝取量之間有良好線性迴歸關係；添加鐵量為 35 ppm 時，線性關係反而減弱。比較 AIN-76 與 AOAC 兩配方對硫酸亞鐵利用率之影響時，以再生後血紅素濃度、再生期血紅素濃度增加量、或再生期血紅素鐵增加量作為鐵利用之生物指標計算而得之相對生物價相近，表示三種指標均適用。

根據本研究結果所建議之補鐵功效評估方法概述如下：大鼠血紅素再生法之原理與基本執行步驟沿用 AOAC 法（1995），但改變其飼料配方，並調整再生期天數。實驗分為耗鐵期與再生期，耗鐵期以大鼠血紅素值低於 6 g/dl 為標準，採用不加鐵之基礎配方。實驗動物採用 Wistar 雄性大鼠，鼠齡限定為離乳，約四週大，體重不超過 70g，每一組用大鼠 6-7 隻。基礎飼料配方採不加鐵之 AIN-76 配方，標準飼料採 AIN-76 配方，添加鐵的形式採硫酸亞鐵，添加鐵量可採 6、12、18、24 ppm 一系列濃度。試驗飼料之鐵以測試樣品提供，添加鐵量相當於 6、12、18、24、35 ppm 或以上。再生期開始時各組大鼠之血紅素與體重平均值應無顯著差異。再生期除了試驗組之外，必須有標準組。飼料所添加鐵量，若採一系列濃度，標準飼料添加鐵量為 12、18、24 ppm，試驗飼料添加鐵量為 12、18、24、35 ppm；若採單一濃度則標準飼料與試驗飼料均添加 24 ppm。再生期天數至少 10

天，最多 14 天。再生期間應紀錄飼料攝取量，再生期結束時應測量體重與血紅素值。鐵利用之生物指標可採用再生後血紅素濃度、再生期血紅素濃度增加量、或再生期血紅素鐵增加量。採系列鐵濃度時，將鐵利用指標對應飼料添加鐵量或再生期鐵攝取量進行迴歸分析，檢驗線性關係，以回歸係數計算試驗飼料組對標準飼料組之比例，作為測試樣品之鐵相對生物價。採單一鐵濃度時，以再生期血紅素鐵增加量對鐵攝取量計算血紅素再生效率 HRE，計算試驗飼料組對標準飼料組之比例，作為測試樣品之鐵相對生物價；或直接以再生期血紅素鐵增加量計算比例亦可。

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表一、大鼠血紅素再生法用基礎飼料 AIN-76 與 AOAC modified 配方的組成

AIN-76[1] (g/kg)		AOAC modified[2] (g/kg)	
Casein	200	Casein	200.0
Cornstarch	630	Cornstarch	614.8
Soybean oil	70	Soybean oil	70.0
Fiber	50	Fiber	50.0
Vitamin mixture AIN76[3]	10	Vitamin mix AIN76[3]	10.0
Mineral mix AIN76[4],	35	Trace-element	2.7
(Fe free, but including		premix[5]	
		(Fe free)	
salts of Ca, P, Na,		NaH_2PO_4	20.0
K, Cl)		$CaCO_3$	20.0
		KCl	5.0
		NaCl	5.0
DL-methionine	3	DL-methionine	1.0
Choline bitartrate	2	Choline chloride	1.5
Total	1000	Total	1000.0

1 J. Nutr. 1977; 107:1340-1348

2 Am. J. Clin. Nutr. 1989; 49:225-238

3 Vitamin mix AIN 76 contains the following components in g per kg feed: thiamin.HCl, 0.6; riboflavin, 0.6; pyridoxine.HCl, 0.7; nicotinic acid, 3.0; D-calcium pantothenate, 1.6; folic acid, 0.2; D-biotin, 0.02; cyanocobalamin, 0.001; retinyl palmitate, 400,000 IU; (all-rac-α-tocopheryl acetate, 5000 IU; cholecalciferol, 100,000 IU; menadione, 0.005; powdered sucrose, 980.

4 Iron-free mineral mix AIN-76 contains the following components in g per kg

mixture: calcium phosphate, dibasic, 500; tri-potassium citrate, monohydrate, 220; NaCl, 74; K_2SO_4, 52; MgO, 24; zinc carbonate, 1.6; manganous carbonate, 3.5; cupric carbonate, 0.3; KIO_3, 0.01; sodium selenite, 0.01; chronium potassium sulfate, 12 hydrate, 0.55; and powdered sucrose 118.

5 Iron-free trace mineral premix contains the following components in g per kg mixture: 738.2 MgSO4, anhudrous; 196.6 $ZnSO_4.7H_2O$; 57.3 $MnSO_4.7H_2O$; 7.3 $CuSO_4.5H_2O$; and 0.6 KIO_3.

6 NA: not added.

表二、大鼠血紅素再生法用基礎飼料 AIN-76 與 AOAC modified 配方之礦物質化學形式與含量

Element	AIN-76[1]		AOAC modified [2,3]	
	化學形式	飼料含量 (mg/ kg)	化學形式	飼料含量 (mg/kg)
Ca	$CaHPO_4$	5200	$CaCO_3$	8000
P	$CaHPO_4$	4000	NaH_2PO_4	5200
K	Tri-potassium citrate, monohydrate	3600	KCl	2617
Na	NaCl	1020	NaCl	1965
Mg	MgO	500	$MgSO_4$	400
Zn	Zinc carbonate	30	$ZnSO_4.7H_2O$	120
Mn	Manganous carbonate	54	$MnSO_4.7H_2O$	30.5
Cu	Cupric carbonate	6	$CuSO_4.5H_2O$	5
I	KIO_3	0.2	KIO_3	0.96
Se	Sodium selenite	0.1		NA[3]
Cr	Chromium potassium sulfate, 12 hydrate	2.0		NA[3]
Cl	NaCl	1560	NaCl, KCl	5418
Sulfate	K_2SO_4	1000	$MgSO_4$, $ZnSO_4.7H_2O$, $MnSO_4.7H_2O$, $CuSO_4$	1833

1 J. Nutr. 1977; 107:1340-1348 ; 2 Am. J. Clin. Nutr. 1989; 49:225-238

3 NA: not added.

表三、血紅素再生期間大鼠對一系列含鐵之 AIN-76 與 AOAC modified 飼料的攝取量

Dietary Groups	N	Repletion diet	Dietary Fe added (ppm)	5-day intake (g/day/rat)[1]	10-day intake (g/day/rat)[1]	14-day intake (g/day/rat)[1]
AI0	7	AIN-76	0	63.2 ± 3.2	117 ± 8	166 ± 11
AI6	7	AIN-76	6	64.1 ± 4.7	132 ± 12	194 ± 18
AI12	7	AIN-76	12	66.7 ± 5.6	141 ± 12	213 ± 11
AI18	7	AIN-76	18	67.8 ± 6.2	140 ± 17	209 ± 21
AI24	7	AIN-76	24	71.5 ± 8.2	152 ± 18	227 ± 9
AI35	7	AIN-76	35	70.1 ± 6.9	151 ± 16	232 ± 6
AO6	7	AOAC	6	67.0 ± 5.0	135 ± 19	197 ± 20
AO12	7	AOAC	12	63.3 ± 13	139 ± 18	206 ± 19
AO18	7	AOAC	18	69.4 ± 4.6	139 ± 16	205 ± 20
AO24	7	AOAC	24	70.3 ± 7.5	153 ± 18	225 ± 11
AO35	7	AOAC	35	72.9 ± 7.5	153 ± 18	231 ± 9
AO18d	8	AOAC	18	67.1 ± 4.5	136 ± 16	207 ± 14
P-value from two-way ANOVA[2]						
Feed formula				0.7542	0.8547	0.5690
Dietary Fe concentration				0.0605	0.0071	0.0001

1 Mean ± s.d..

2 AI0 and AO18d are not included in two-way ANOVA analysis.

表四、血紅素再生期間大鼠攝取一系列含鐵之 AIN-76 與 AOAC modified 飼料之體重變化

Dietary Groups	n.	Repletion diet	Dietary Fe Added (ppm)	Depletion Initial weight[1] (g/rat)	Body weight during Fe repletion (g/rat)[1] Initial	5days	10 days	14 days
AI6	7	AIN-76	6	53.5 ± 7.2	158 ± 19	177 ± 17^{b}	205 ± 22^{ab}	209 ± 21^{cb}
AI12	7	AIN-76	12	53.8 ± 3.6	157 ± 12	180 ± 12^{b}	219 ± 13^{a}	224 ± 14^{ab}
AI18	7	AIN-76	18	54.8 ± 7.5	158 ± 14	176 ± 11^{b}	208 ± 16^{ab}	215 ± 22^{abc}
AI24	7	AIN-76	24	55.6 ± 3.6	158 ± 12	180 ± 15^{b}	217 ± 14^{a}	225 ± 11^{ab}
AI35	7	AIN-76	25	54.0 ± 3.6	159 ± 13	194 ± 15^{a}	214 ± 18^{a}	233 ± 15^{a}
AO6	7	AOAC	6	55.5 ± 6.3	158 ± 14	175 ± 14^{b}	200 ± 20^{ab}	201 ± 20^{cd}
AO12	7	AOAC	12	53.7 ± 4.1	158 ± 15	176 ± 14^{b}	211 ± 12^{a}	216 ± 13^{abc}
AO18	7	AOAC	18	54.2 ± 7.0	159 ± 12	179 ± 10^{b}	212 ± 17^{a}	220 ± 17^{abc}
AO24	7	AOAC	24	52.8 ± 7.0	158 ± 17	175 ± 11^{b}	218 ± 17^{a}	225 ± 16^{ab}
AO35	7	AOAC	35	54.5 ± 3.9	159 ± 13	178 ± 8.3^{b}	220 ± 1.0^{a}	232 ± 9^{a}
AI0	7	AIN-76	0	54.4 ± 3.7	159 ± 11	175 ± 11^{b}	190 ± 15^{b}	184 ± 2^{d}
AO18d	7	AOAC	18	55.7 ± 5.7	152 ± 18	173 ± 13^{b}	206 ± 20^{ab}	216 ± 18^{abc}
P-value from two-way ANOVA[2]								
Feed formula				0.8754	0.9999	0.1136	0.8843	0.5077
Dietary Fe concentration				0.9957	0.9484	0.2953	0.0765	0.0005

1 Mean ± s.d.. Values in each column sharing the same superscript letters are not significantly different by Duncan'c multiple range test at $p < 0.05$.

2 AI0 and AO18d are not included in two-way ANOVA analysis.

表五、血紅素再生期第 5 天一系列含鐵之 AIN-76 與 AOAC modified 飼料對大鼠體重、血紅素濃度與血紅素鐵量之影響

Dietary groups	N	Repletion Diet	Dietary Fe added (ppm)	Hemoglobin (mmol/l)[1]			Hemoglobin Fe (umol/rat)[1]		
				Initial	5-day repletion	gain	Initial	5-day repletion	gain
AI6	7	AIN-76	6	0.72 ± 0.05	0.79 ± 0.07 ^{ghi}	0.08 ± 0.06 ^{gh}	30.4 ± 4.2	37.9 ± 5.7 ^{fgh}	7.51 ± 2.85 ^{g}
AI12	7	AIN-76	12	0.71 ± 0.10	0.91 ± 0.12 ^{efg}	0.20 ± 0.15 ^{ef}	30.0 ± 4.8	43.9 ± 7.2 ^{defg}	13.9 ± 7.2 ^{def}
AI18	7	AIN-76	18	0.76 ± 0.13	1.09 ± 0.16 ^{bc}	0.34 ± 0.09 ^{c}	32.3 ± 7.1	52.2 ± 10.9 ^{cd}	19.9 ± 4.4 ^{d}
AI24	7	AIN-76	24	0.75 ± 0.09	1.36 ± 0.13 ^{a}	0.60 ± 0.15 ^{a}	32.0 ± 3.4	65.2 ± 7.7 ^{b}	33.5 ± 8.5 ^{b}
AI35	7	AIN-76	35	0.75 ± 0.10	1.42 ± 0.16 ^{a}	0.67 ± 0.09 ^{a}	32.1 ± 4.5	74.2 ± 9.4 ^{a}	42.1 ± 6.0 ^{a}
AO6	7	AOAC	6	0.75 ± 0.10	0.76 ± 0.13 ^{hi}	0.01 ± 0.11 ^{hi}	31.7 ± 4.7	35.8 ± 6.5 ^{gh}	4.1 ± 4.9 ^{gh}
AO12	7	AOAC	12	0.71 ± 0.08	0.84 ± 0.11 ^{fgh}	0.13 ± 0.08 ^{fg}	30.2 ± 5.0	40.0 ± 7.4 ^{efg}	9.8 ± 5.0 ^{fg}
AO18	7	AOAC	18	0.73 ± 0.09	0.93 ± 0.10 ^{def}	0.20 ± 0.07 ^{def}	31.2 ± 4.9	44.8 ± 4.6 ^{def}	13.6 ± 2.6 ^{ef}
AO24	7	AOAC	24	0.73 ± 0.06	1.04 ± 0.05 ^{cd}	0.31 ± 0.04 ^{cd}	31.3 ± 5.5	49.1 ± 4.5 ^{d}	17.7 ± 2.3 ^{de}
AO35	7	AOAC	35	0.72 ± 0.04	1.20 ± 0.15 ^{b}	0.48 ± 0.16 ^{b}	30.6 ± 3.6	57.3 ± 7.8 ^{c}	26.6 ± 8.8 ^{c}
AI0	7	AIN-76	0	0.74 ± 0.09	0.67 ± 0.08 ^{i}	-0.07± 0.05 ^{i}	31.7 ± 4.1	31.5 ± 4.8 ^{h}	-.23 ± 2.73 ^{h}
AO18d	8	AOAC	18	0.75 ± 0.06	1.01 ± 0.08 ^{cde}	0.25 ± 0.06 ^{cde}	30.8 ± 4.8	47.0 ± 6.2 ^{de}	16.2 ± 2.2 ^{de}

P-value from two-way ANOVA[2]

Feed formula	0.6034	0.0001	0.0001	0.7618	0.0001	0.0001
Dietary Fe concentration	0.8186	0.0001	0.0001	0.9034	0.0001	0.0001

1 Mean ± s.d.. Values in each column sharing the same superscript letters are not significantly different by Duncan'c multiple range test at $p < 0.05$.

2 AI0 and AO18d are not included in two-way ANOVA analysis.

表六、血紅素再生期第 10 天一系列含鐵之 AIN-76 與 AOAC modified 飼料對大鼠體重、血紅素濃度與血紅素鐵量之影響

Dietary groups	N	Repletion Diet	Dietary Fe added (ppm)	Hemoglobin (mmol/l)1			Hemoglobin Fe (umol/rat)1		
				Initial	10-day repletion	10-d gain	initial	10-day repletion	10-d gain
AI6	7	AIN-76	6	0.72 ± 0.05	0.84 ± 0.05 ^{fg}	0.12 ± 0.05^{f}	30.4 ± 4.2	46.2 ± 5.8 ^{ef}	15.8 ± 3.0^{g}
AI12	7	AIN-76	12	0.71 ± 0.10	1.10 ± 0.10 ^{e}	0.39 ± 0.10^{d}	30.0 ± 4.8	64.9 ± 8.5 ^{d}	34.8 ± 9.1^{e}
AI18	7	AIN-76	18	0.76 ± 0.13	1.35 ± 0.16 ^{d}	0.59 ± 0.05^{c}	32.3 ± 7.1	75.8 ± 13.8 ^{cd}	43.5 ± 8.2^{cd}
AI24	7	AIN-76	24	0.75 ± 0.09	1.72 ± 0.10 ^{b}	0.97 ± 0.11^{b}	32.0 ± 3.4	100.4 ± 6.4 ^{ab}	68.4 ± 6.6^{b}
AI35	7	AIN-76	35	0.75 ± 0.10	1.92 ± 0.18 ^{a}	1.16 ± 0.11^{a}	32.1 ± 4.5	110.5 ± 15.6 ^{a}	78.5 ± 12.3^{a}
AO6	7	AOAC	6	0.75 ± 0.10	0.77 ± 0.06 ^{gh}	0.03 ± 0.06^{f}	31.7 ± 4.7	41.3 ± 4.1 ^{fg}	9.6 ± 1.8^{g}
AO12	7	AOAC	12	0.71 ± 0.08	0.96 ± 0.07 ^{f}	0.25 ± 0.04^{e}	30.2 ± 5.0	54.3 ± 6.1 ^{e}	24.1 ± 3.2^{f}
AO18	7	AOAC	18	0.73 ± 0.09	1.22 ± 0.16 ^{de}	0.49 ± 0.10^{cd}	31.2 ± 4.9	69.1 ± 9.1 ^{cd}	37.9 ± 7.1^{de}
AO24	7	AOAC	24	0.73 ± 0.06	1.33 ± 0.12 ^{d}	0.59 ± 0.12^{c}	31.3 ± 5.5	77.8 ± 11.9 ^{c}	46.5 ± 8.9^{c}
AO35	7	AOAC	35	0.72 ± 0.04	1.59 ± 0.20 ^{c}	0.87 ± 0.20^{b}	30.6 ± 3.6	93.7 ± 11.4 ^{b}	63.0 ± 11.5^{b}
AI0	7	AIN-76	0	0.74 ± 0.09	0.65 ± 0.08 ^{h}	-0.09± 0.02^{g}	31.7 ± 4.1	33.1 ± 5.0 ^{g}	1.5 ± 2.4^{h}
AO18d	8	AOAC	18	0.75 ± 0.06	1.25 ± 0.08 ^{d}	0.49 ± 0.08^{cd}	30.8 ± 4.8	69.5 ± 9.9 ^{cd}	38.7 ± 6.1^{cde}
P-value from two-way ANOVA[2]									
Feed formula				0.6034	0.0001	0.0001	0.7618	0.0001	0.0001
Dietary Fe concentration				0.8186	0.0001	0.0001	0.9034	0.0001	0.0001

1 Mean ± s.d.. Values in each column sharing the same superscript letters are not significantly different by Duncan'c multiple range test at $p < 0.05$.

2 AI0 and AO18d are not included in two-way ANOVA analysis.

表七、血紅素再生期第 14 天一系列含鐵之 AIN-76 與 AOAC modified 飼料對大鼠體重、血紅素濃度與血紅素鐵量之影響

Dietary groups	N	Repletion Diet	Dietary Fe added (ppm)	Hemoglobin (mmol/l)[1]			Hemoglobin Fe (umol/rat)[1]		
				Initial	14-d repletion	14-d gain	initial	14-d repletion	14-d gain
AI6	7	AIN-76	6	0.72 ± 0.05	0.88 ± 0.07^{e}	0.16 ± 0.07^{f}	30.4 ± 4.2	49.3 ± 7.1^{f}	18.9 ± 4.6^{f}
AI12	7	AIN-76	12	0.71 ± 0.10	1.19 ± 0.19^{cd}	0.49 ± 0.21^{de}	30.0 ± 4.8	71.7 ± 12.0^{d}	41.7 ± 15.0^{d}
AI18	7	AIN-76	18	0.76 ± 0.13	1.48 ± 0.06^{b}	0.72 ± 0.12^{bc}	32.3 ± 7.1	85.4 ± 10.1^{bc}	53.1 ± 8.3^{bc}
AI24	7	AIN-76	24	0.75 ± 0.09	1.84 ± 0.10^{a}	1.09 ± 0.08^{a}	32.0 ± 3.4	111.4 ± 4.5^{a}	79.4 ± 3.1^{a}
AI35	7	AIN-76	35	0.75 ± 0.10	1.91 ± 0.11^{a}	1.17 ± 0.12^{a}	32.1 ± 4.5	118.8 ± 7.9^{a}	87.0 ± 7.4^{a}
AO6	7	AOAC	6	0.75 ± 0.10	0.84 ± 0.12^{e}	0.09 ± 0.11^{f}	31.7 ± 4.7	45.2 ± 7.6^{f}	13.4 ± 6.0^{f}
AO12	7	AOAC	12	0.71 ± 0.08	1.06 ± 0.12^{cd}	0.36 ± 0.08^{e}	30.2 ± 5.0	61.9 ± 9.0^{e}	31.7 ± 5.0^{e}
AO18	7	AOAC	18	0.73 ± 0.09	1.31 ± 0.07^{c}	0.58 ± 0.05^{cd}	31.2 ± 4.9	77.1 ± 7.8^{cd}	46.0 ± 6.1^{cd}
AO24	7	AOAC	24	0.73 ± 0.06	1.49 ± 0.09^{b}	0.75 ± 0.13^{b}	31.3 ± 5.5	89.5 ± 4.5^{b}	58.2 ± 4.4^{b}
AO35	7	AOAC	35	0.72 ± 0.04	1.84 ± 0.14^{a}	1.12 ± 0.14^{a}	30.6 ± 3.6	114.5 ± 11.1^{a}	83.9 ± 10.1^{a}
AI0	7	AIN-76	0	0.74 ± 0.09	0.59 ± 0.08^{f}	-0.15 ± 0.06^{g}	31.7 ± 4.1	29.1 ± 3.8^{g}	-2.6 ± 2.6^{g}
AO18d	8	AOAC	18	0.75 ± 0.06	1.33 ± 0.27^{c}	0.58 ± 0.26^{cd}	30.8 ± 4.8	76.6 ± 15.3^{cd}	45.8 ± 15.4^{cd}
P-value from two-way ANOVA[2]									
Feed formula				0.6034	0.0001	0.0001	0.7618	0.0002	0.0001
Dietary Fe concentration				0.8186	0.0001	0.0001	0.9034	0.0001	0.0001

1 Mean ± s.d.. Values in each column sharing the same superscript letters are not significantly different by Duncan'c multiple range test at $p < 0.05$.

2 AI0 and AO18d are not included in two-way ANOVA analysis.

表八、六種迴歸分析模式獲得相對生物價（Relative biological values）之比對

Repletion days	Dependent variable	AI35 included[1]		AI35 excluded[1]	
		Vs. dietary Fe concentration	Vs. Fe intake	Vs. dietary Fe concentration	Vs. Fe intake
5	Repleted hemoglobin concentration	65	62	49	48
10	Repleted hemoglobin concentration	73	72	59	58
14	Repleted hemoglobin concentration	93	94	65	63
5	Hemoglobin gain	73	70	57	55
10	Hemoglobin gain	77	74	62	60
14	Hemoglobin gain	97	98	69	67
5	Hemoglobin Fe gain	60	58	54	52
10	Hemoglobin Fe gain	82	80	43	64
14	Hemoglobin Fe gain	99	100	74	72

1 AI35: AIN-76 diet containing 35 ppm of Fe added as ferrous sulfate.

表九、血紅素再生期飼料鐵濃度與飼養時間對相對生物價（Relative biological values）之影響

Parameters	Repletion days	Hemoglobin Fe gains (umol/rat)				
Dietary Fe levels		6 ppm	12 ppm	18 ppm	24 ppm	35 ppm
AOAC modified diet	5	4.1	9.8	13.6	17.7	26.6
AIN-76 diet	5	7.5	13.9	19.9	33.5	42.1
RBV (%)[1]	5	54.6	70.5	68.3	52.8	63.2
AOAC modified diet	10	9.6	24.1	37.9	46.5	63
AIN-76 diet	10	15.8	34.8	43.5	68.4	78.5
RBV (%)[1]	10	60.8	69.3	87.1	68.0	80.3
AOAC modified diet	14	13.4	31.7	46	58.2	83.9
AIN-76 diet	14	18.9	41.7	53.1	79.4	87
RBV (%)[1]	14	70.9	76.0	86.6	73.3	96.4

1 RBV: relative biological value, calculated as a ratio of hemoglobin Fe gain of AOAC diet to that of AIN-76 diet.

表十、血紅素再生期飼料採用單一加鐵濃度 24 ppm 而得之鐵質相對生物價

Repletion days	Parameters	Hemoglobin Fe Gain (umol/rat)	Dietary Fe Intake (umol/rat)	HRE (%) [1]
5	AOAC modified diets	17.7	30.1	59
5	AIN-76 diet	33.5	30.6	109
5	RBV (%)2	53	98	54
10	AOAC modified diets	46.5	65.6	71
10	AIN-76 diet	68.4	65.1	105
10	RBV (%)[2]	68	101	67
14	AOAC modified diets	58.2	96.5	60
14	AIN-76 diet	79.4	97.1	82
14	RBV (%)[2]	73	99	73

1 Hemoglobin regeneration efficiency, percentage of hemoglobin Fe gain to dietary Fe intake

2 For each parameter, RBV represents a ratio of the AOAC group to the AIN-76 group.

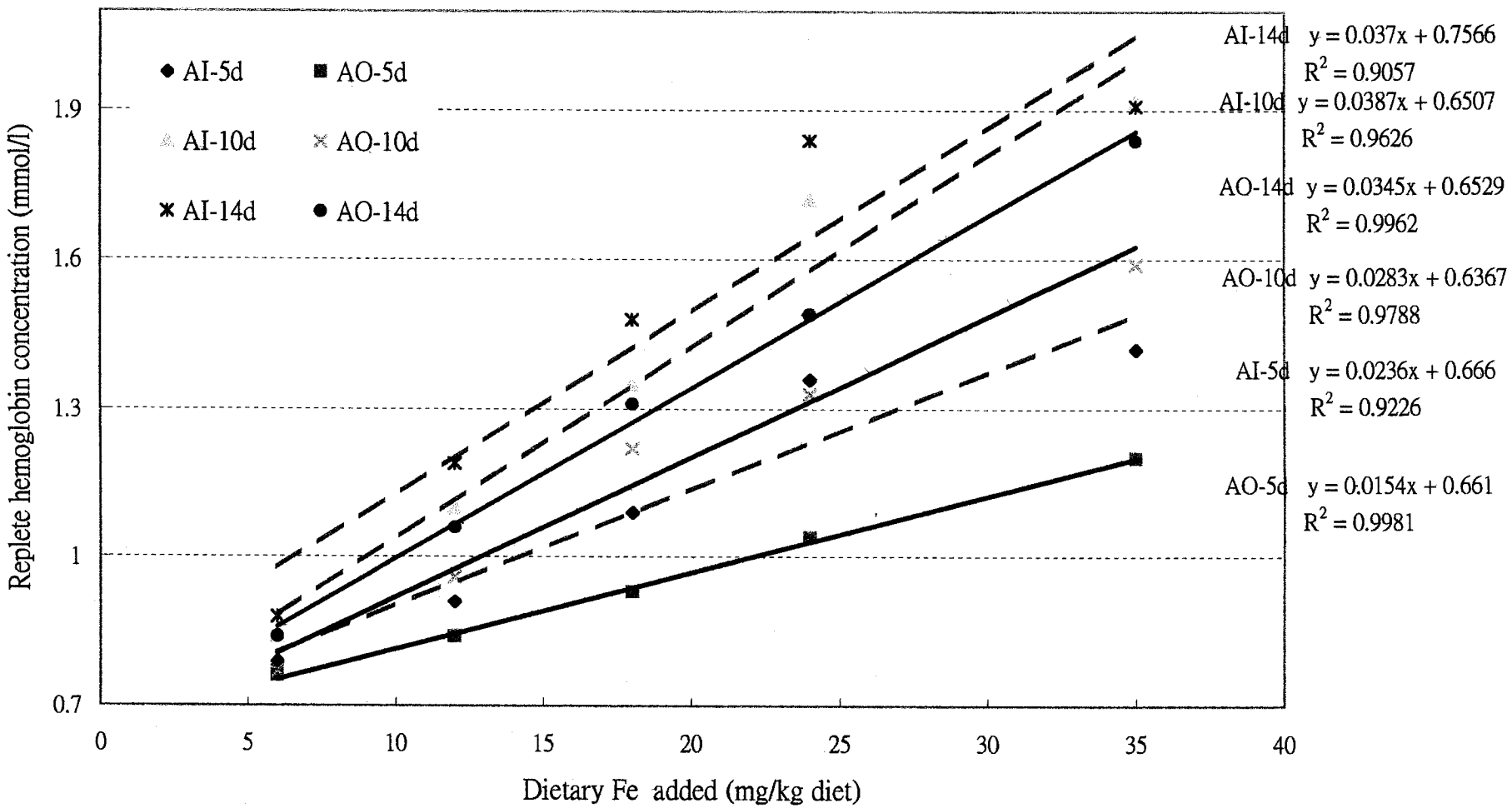

Fig. 1.1 Regression analysis of replete hemoglobin concentration on concentration of added iron in AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 included in analysis)

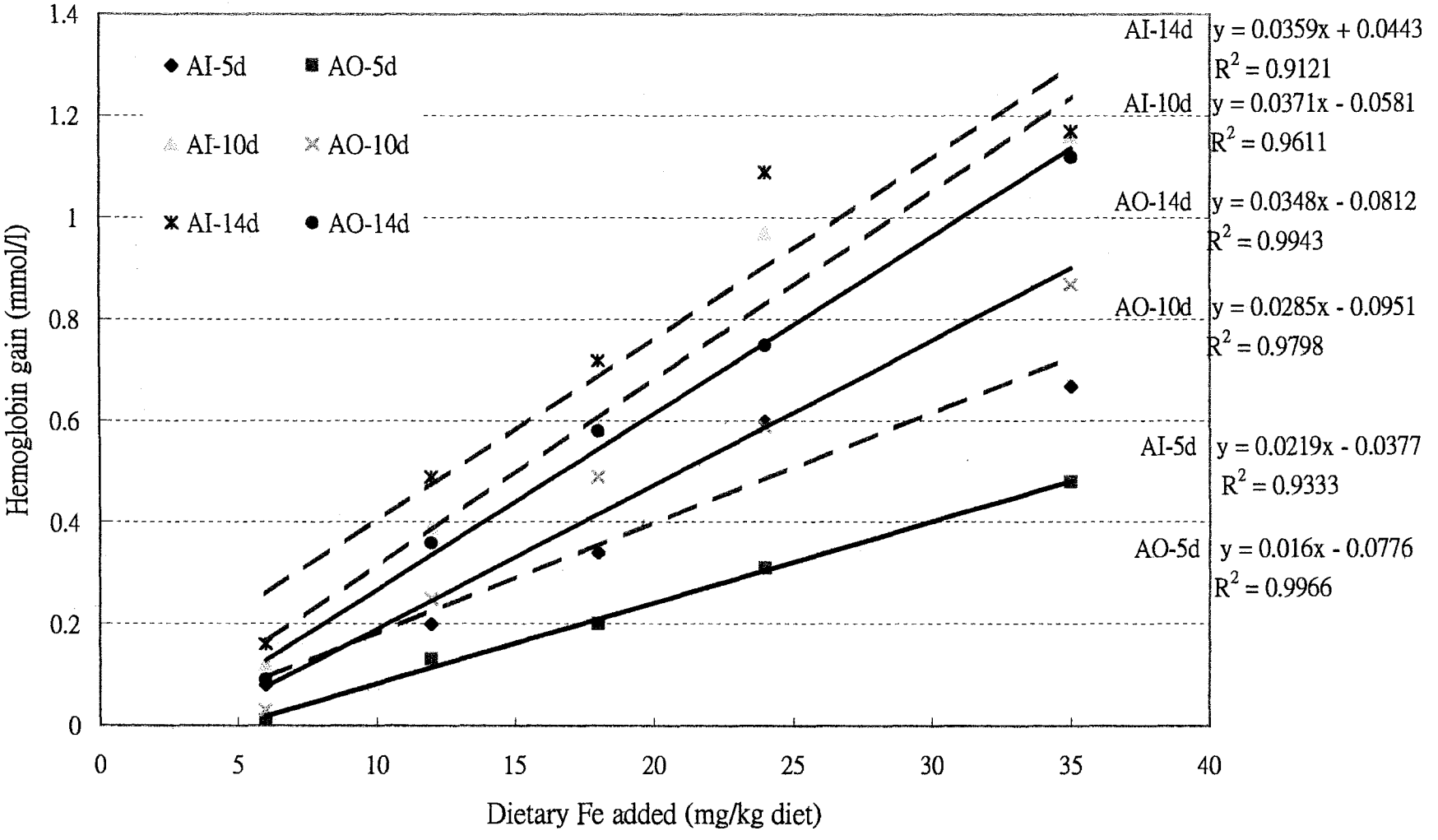

Fig. 1.2 Regression analysis of hemoglobin gain on concentration of added iron in AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 included in analysis)

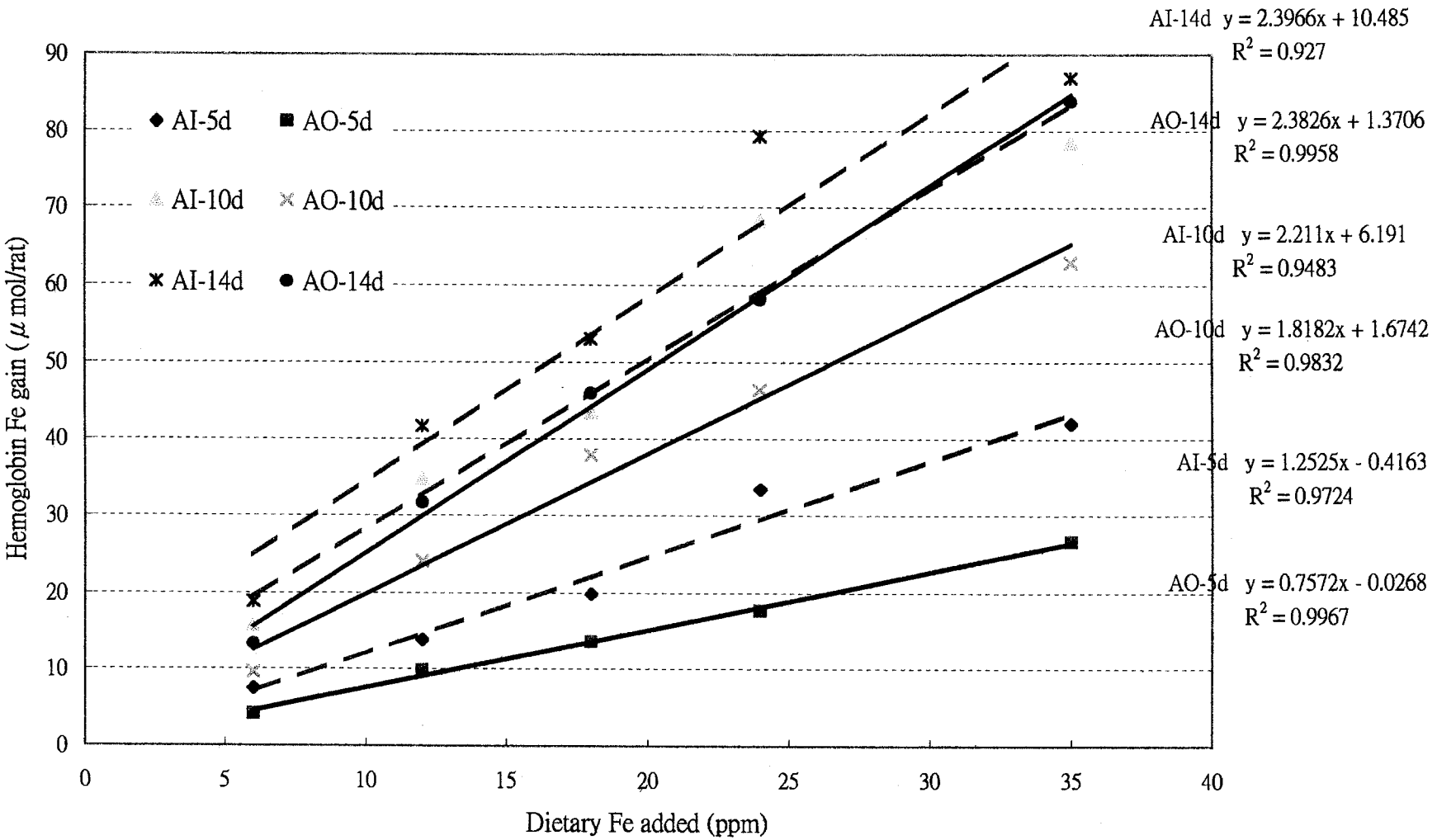

Fig. 1.3 Regression analysis of hemoglobin Fe gain on concentration of added iron in AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 included in analysis)

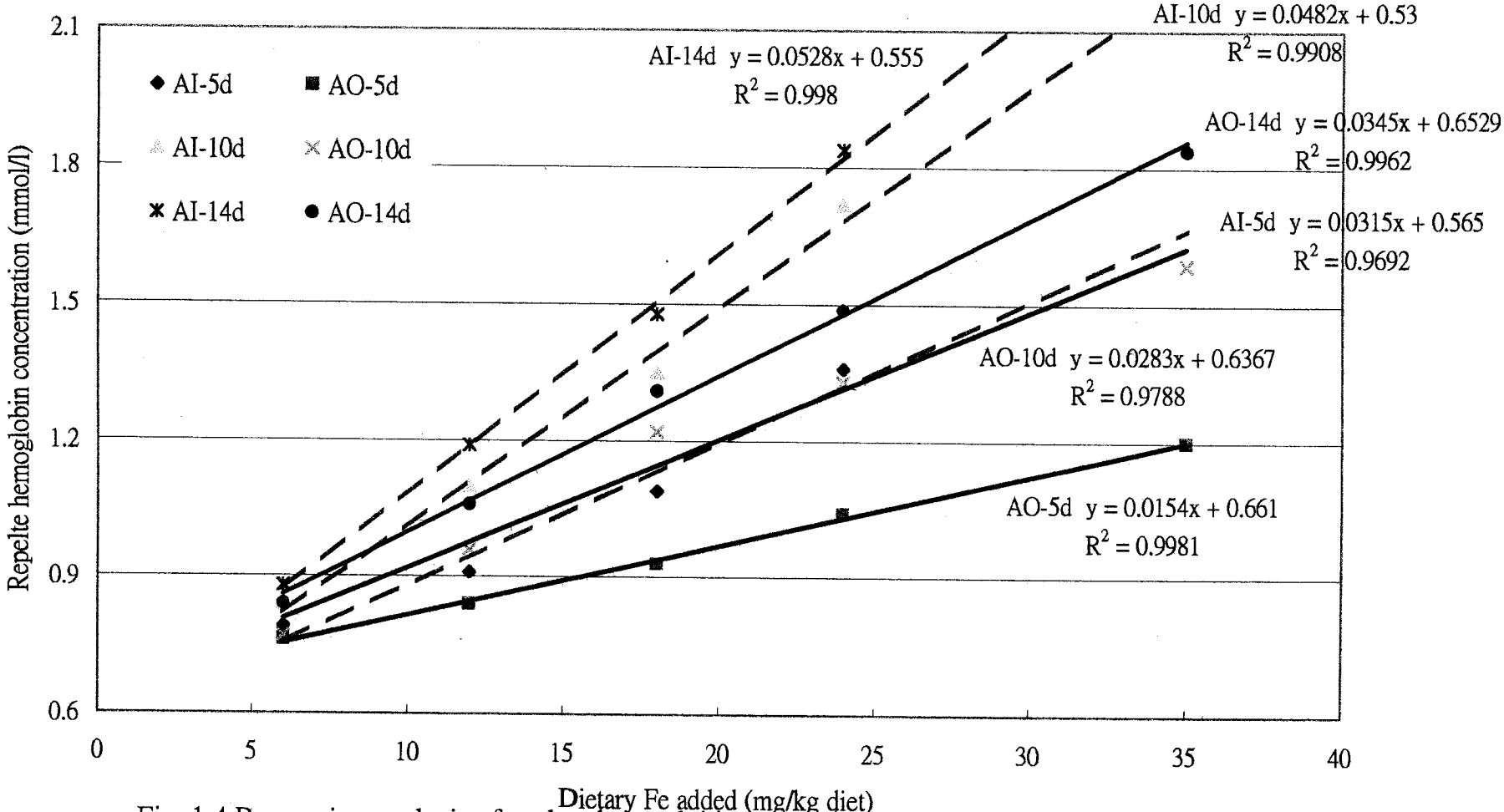

Fig. 1.4 Regression analysis of replete hemoglobin concentration on concentration of added iron in AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 not included in analysis)

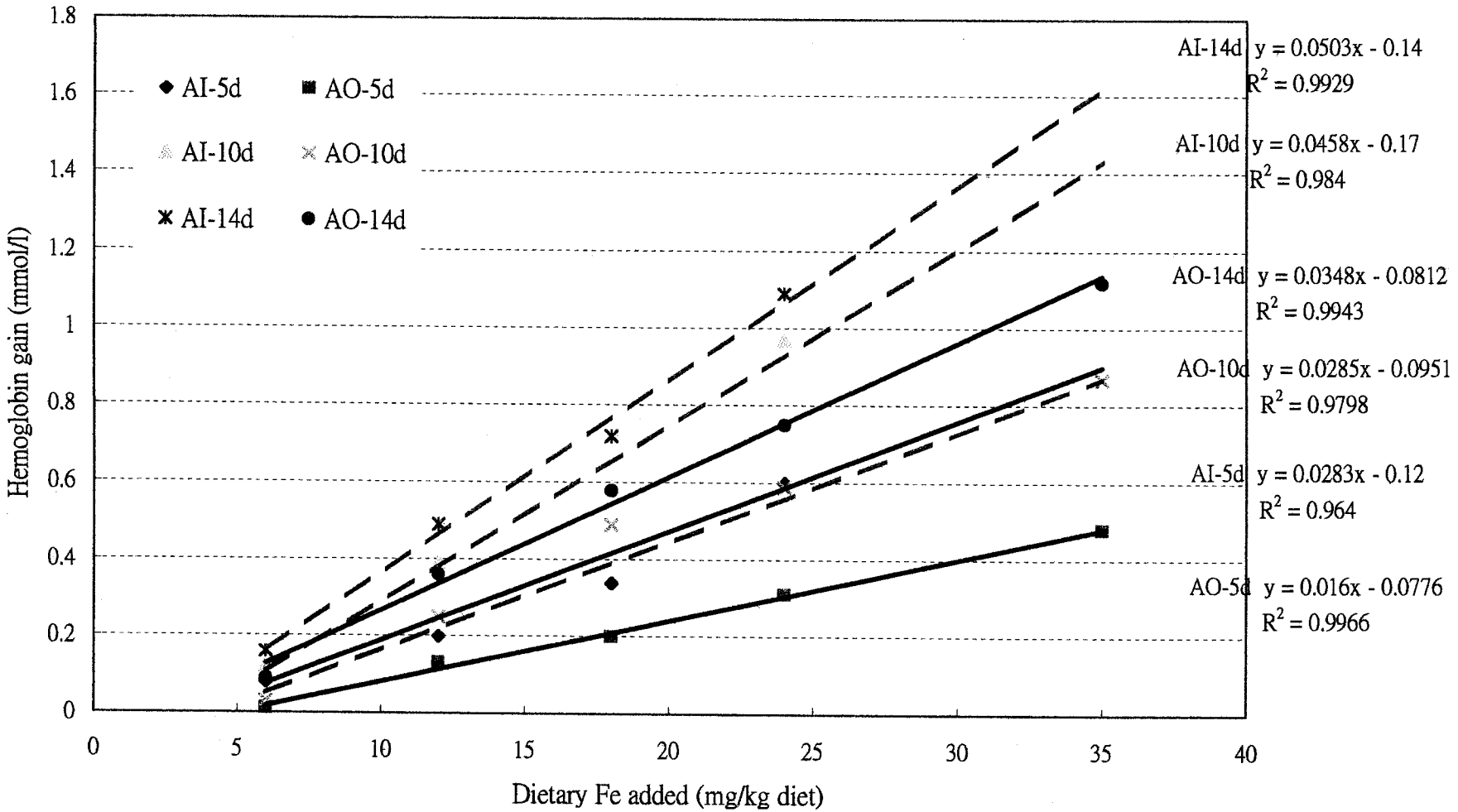

Fig.1.5 Regression analysis of hemoglobin gain on concentration of added iron in AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 not included in analysis)

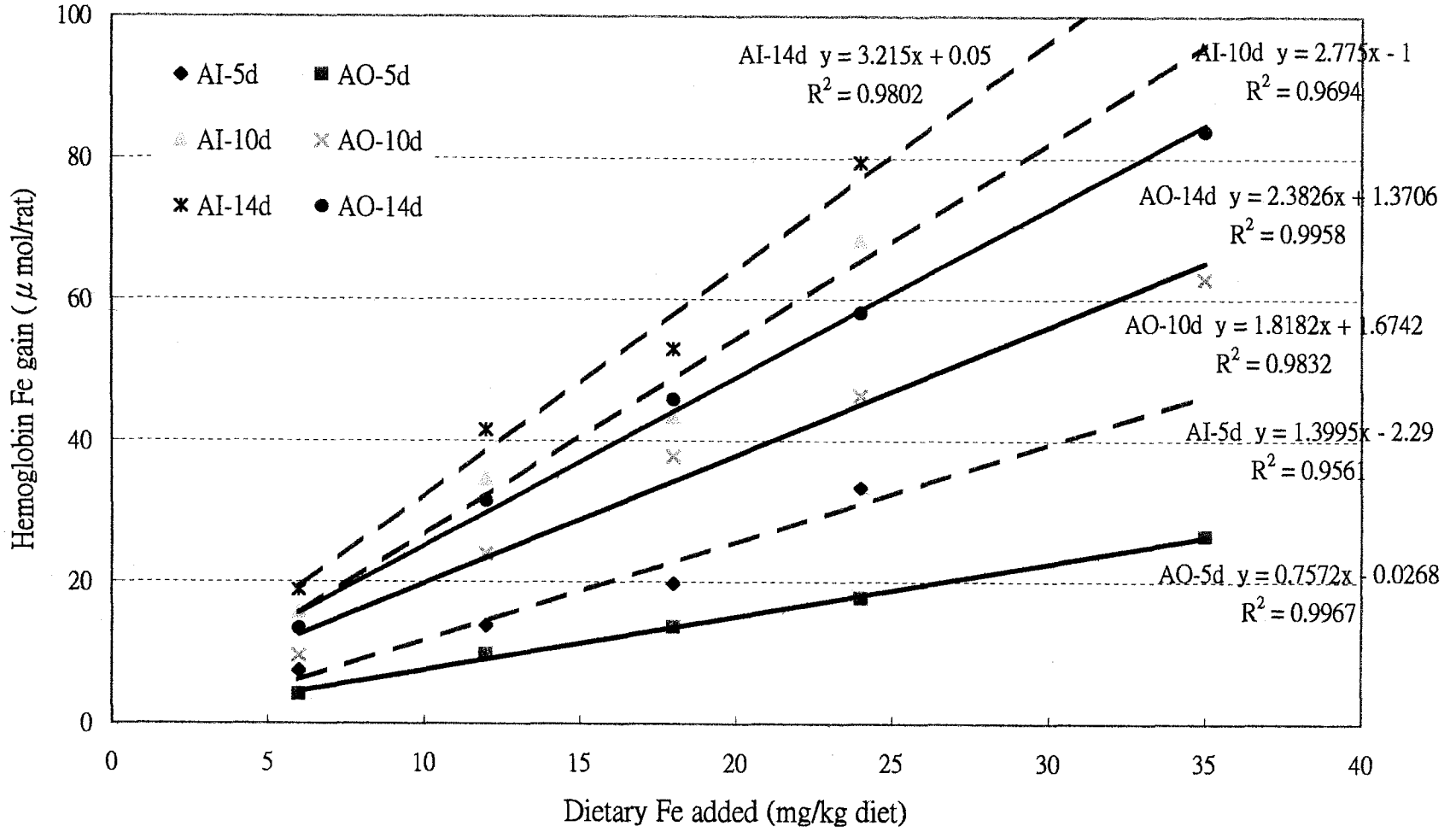

Fig. 1.6 Regression analysis of hemoglobin Fe gain on concentration of added iron in AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 not included in analysis)

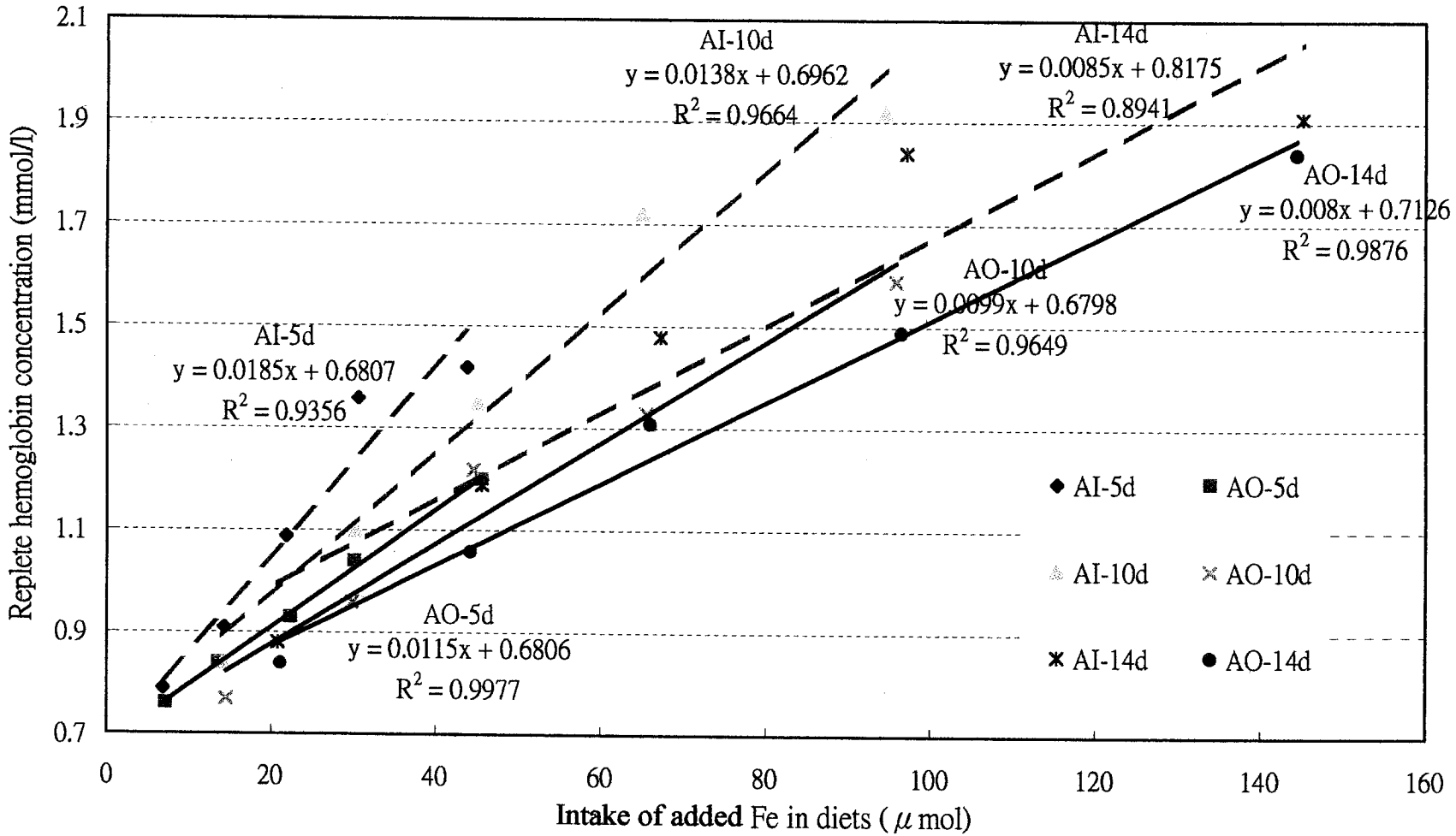

Fig.2.1 Regression analysis of replete hemoglobin cioncentration on intakes of added iron from AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 included in analysis)

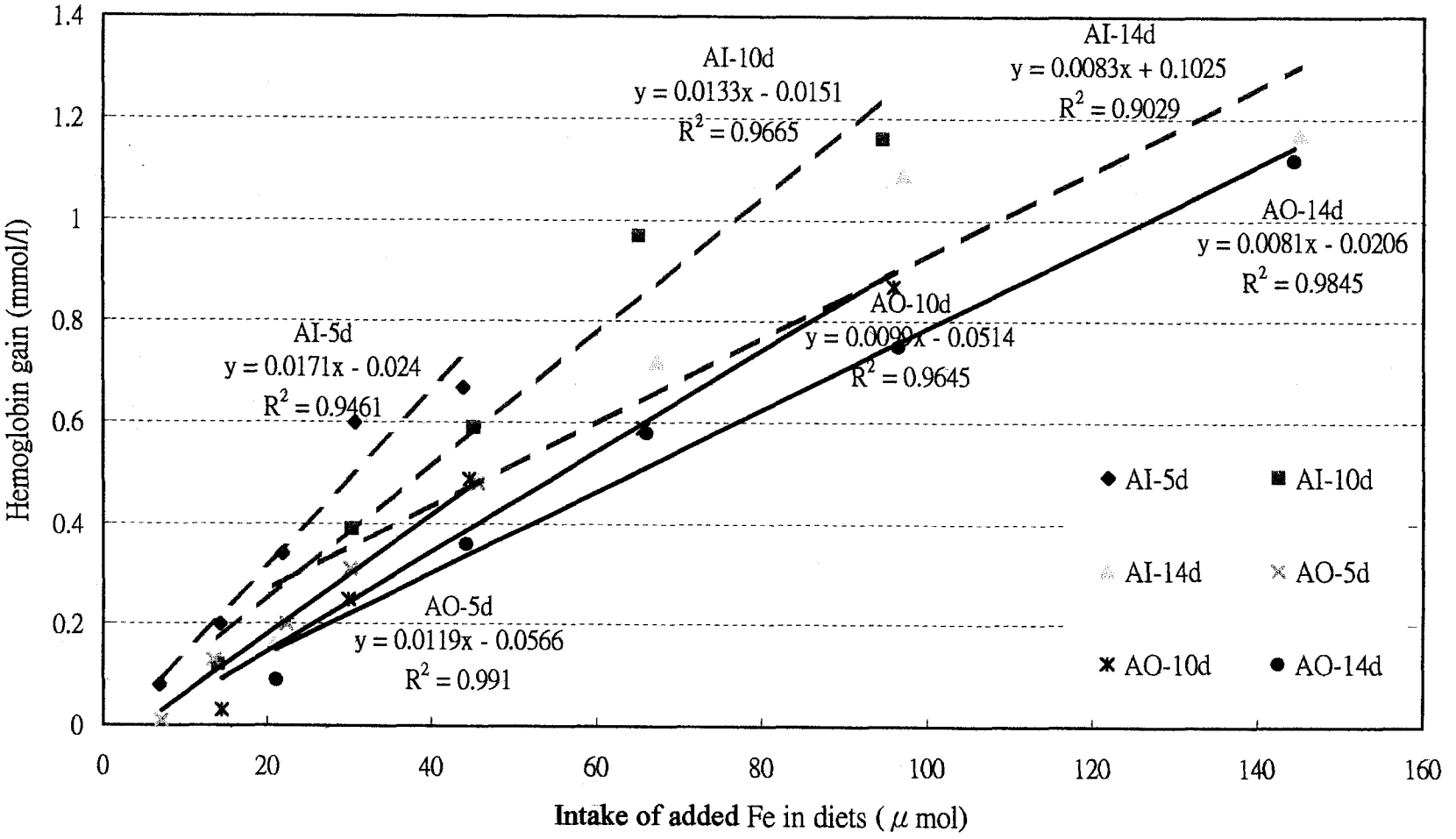

Fig.2.2 Regression analysis of hemoglobin gain on intakes of added iron from AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 included in analysis)

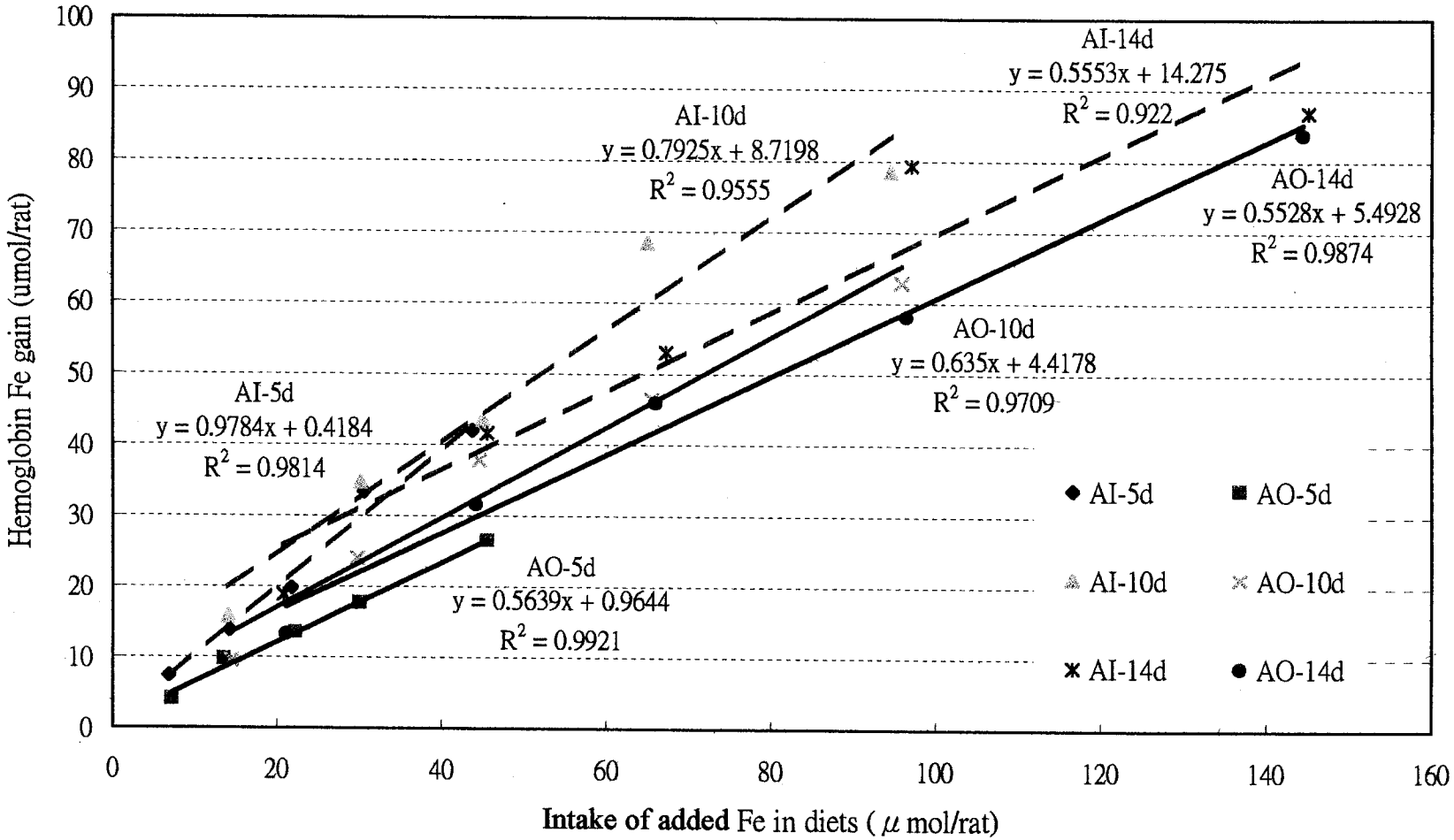

Fig.2.3 Regression analysis of hemoglobin Fe gain on intakes of added iron from AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 included in analysis)

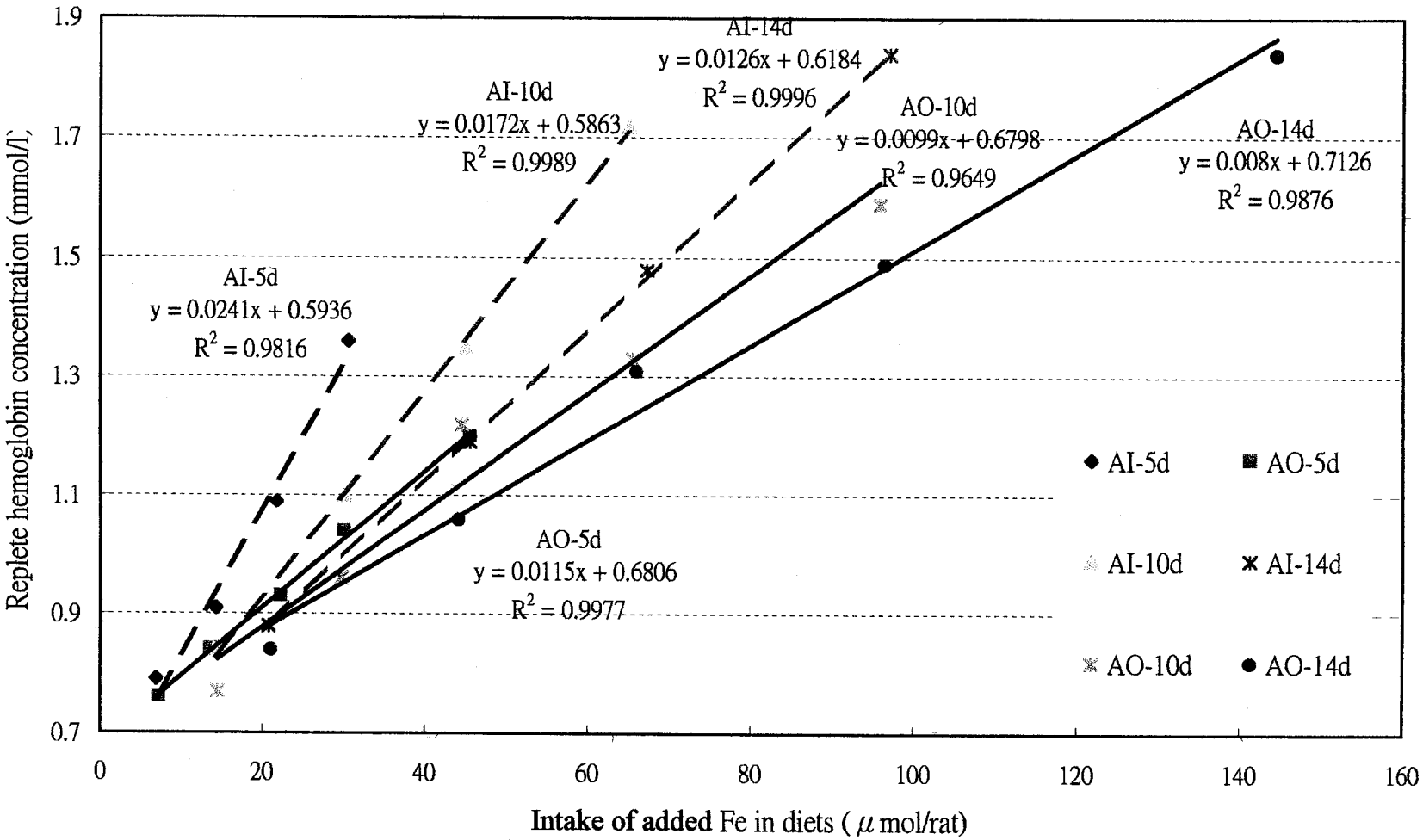

Fig.2.4 Regression analysis of replete hemoglobin concentration on intakes of added iron from AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 not included in analysis)

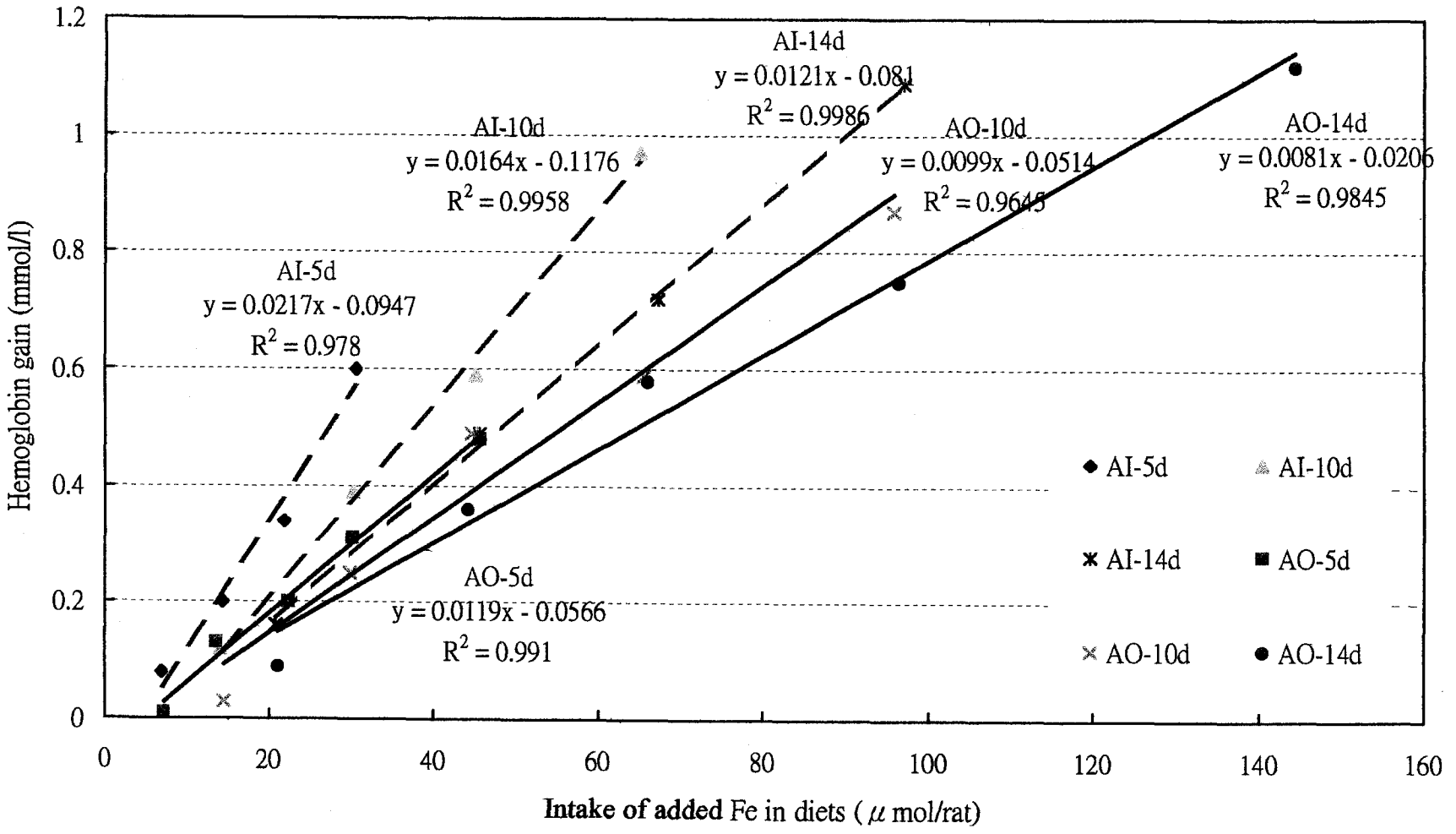

Fig.2.5 Regression analysis of hemoglobin gain on intakes of added iron from AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 not included in analysis)

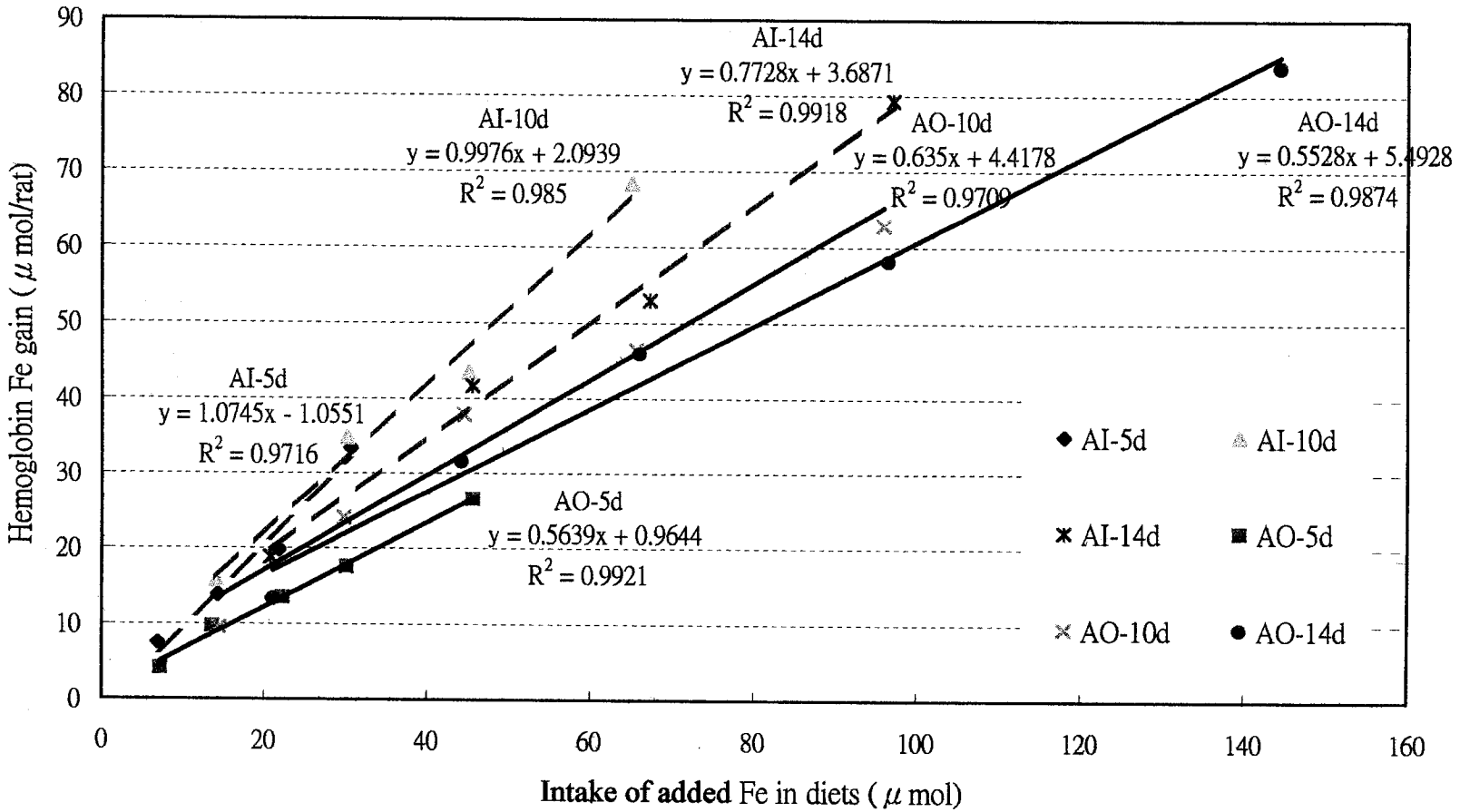

Fig.2.6 Regression analysis of hemoglobin Fe gain on intakes of added iron from AIN-76 (AI) and AOAC modified (AO) diets after 5, 10, and 14 days of iron repletion (group AI35 not included in analysis)

Bioavailability of Iron from Purple Laver (*Porphyra* spp.) Estimated in a Rat Hemoglobin Regeneration Bioassay

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Laver belongs to the genius of Porphyra and is the most valuable seaweed in the aqua-culture industry. It contains higher iron than many other plant foods. The bioavailability of iron from laver was evaluated in a rat hemoglobin regeneration assay. Reagent-grade ferrous sulfate was used as the reference standard, and the relative biological value (RBV) for laver was expressed as a percentage of the response to ferrous sulfate. RBV was calculated by two methods: slope-ratio and ratio of hemoglobin regeneration efficiency, and both yielded RBV of 26 for laver. Amount of available iron from laver estimated from RBV was comparable to many iron-fortified foods.

Keywords: *Iron bioavailability; laver; Porphyra spp.; relative biological value; hemoglobin regeneration bioassay*

INTRODUCTION

Laver is a type of red algae belonging to the genus *Porphyra*. Modern aqua-cultivation of *Porphyra* occurred in the 1960s as a result of the discovery of the *Conchocelis* phase of *Porphyra*, which allowed artificial seeding and maximizing production under controlled conditions. Laver has been a staple in the diet of Asian cultures, such as China and Japan, and dried laver appears to be the most widely eaten seaweed in the world. It contains various biologically active substances beneficial to human health, including significant amounts of nutrients such as protein and free amino acids, vitamins C, B groups, and A, and trace minerals such as zinc, copper, manganese, and selenium (Noda, 1993). Dried laver contains vitamin B_{12} in comparable amounts to animal foods (Watanabe et al., 1999; Yamada et al., 1996) and is an excellent source for strict vegetarians. In addition, components such as porphyran, a sulfated polysaccharide, porphyosin and pigments were reported to exhibit medical benefits including antitumor (Noda et al., 1990), antiulcer (Noda, 1983), and antimutagenic activities (Okai et al., 1996).

Dried laver also has higher iron concentration (>28 mg per 100 g dry matter) than most plant foods (FAO, 1972). Since iron deficiency still occurs in both developing and developed countries (United Nations ACC/SCN, 1992), natural foods rich in iron provide an alternative choice other than iron fortification for improving iron nutrition. However, plant foods usually have components that inhibit iron absorption (Hallberg, 1981), and availability of iron from laver was evaluated in a rat hemoglobin regeneration bioassay (AOAC, 1995; Fritz et al., 1974; Rotruck and Luhrsen, 1979) before its novel use as an iron source.

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MATERIALS AND METHODS

Diets. Dry purple laver (*Porphyra* spp.), purchased from a local supermarket, was oven dried at 65 °C for 6 h, ground to pass a 20-mesh screen, and stored in plastic bags at room temperature. Proximate analysis of dried laver resulted in compositions as follows: moisture 6.0%, crude protein 28.8%, crude fat 0.5%, crude fiber 2.8%, crude ash 15.5%, and nitrogen-free extract 46.5%. Measured iron concentration was 0.91 mg per gram dried laver.

The depletion diet was formulated according to AIN-76 with some modifications (American Institute of Nutrition, 1977). Ingredients (g/kg) were as follows: corn starch (CERESTAR, France), 621; casein (ICN, Ohio), 200; soybean oil, 100; cellulose (Vitacel M80 from IRS, Germany), 30; mineral mixture (AIN-76 with ferric salt omitted), 35; vitamin mixture (AIN-76), 10; choline chloride, 3; and L-methionine, 1. This depletion diet contained less than 3 mg of Fe per kg. For the standard regeneration diets, ferrous sulfate ($FeSO_4 \cdot 7H_2O$) was added to the depletion diet to supply 6, 12, 18, and 35 g of added iron per kilogram of diet. For laver regeneration diets, dried laver from 7.4 to 74 g was added to one kilogram of the depletion diet to provide 6, 12, 18, 24, 40, and 60 g of added iron per kilogram of diet and replaced equivalent amounts of corn starch and casein. Iron concentration in the diets was verified by analysis. Ingredients used in the mineral and vitamin mixtures were obtained from Sigma.

Animals and Bioassay. Weanling male Wistar rats (Laboratory Animal Center, College of Medicine, National Taiwan University) weighing 55 ± 7 g were housed individually in stainless steel cages with wire mesh floors in an animal room where temperature was controlled at a constant 25 °C with 12-h light/dark periods. Food and deionized water were freely available. Animal care and handling conformed to the NSC's *Guidelines for Use and Care of Laboratory Animals* (National Science Council, 1993). Body weight and feed intake corrected for spillage were recorded twice a week. In the depletion period, rats had free access to the depletion diet, and blood for monitoring hemoglobin concentration was drawn from the tail. The average hemoglobin concentration was sufficiently low (0.72 ± 0.11 mmol/L) by the 16th day, and average body weight was 125 ± 13 g.

In the beginning of the regeneration period, seven rats were allotted to each of 11 groups in such a manner that all groups had similar mean values of hemoglobin concentration and body

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Table 1. Changes of Body Weight and Feed Efficiency in Rats during the 14-Day Regeneration Period of the Rat Bioassay

diets	iron added (mg/kg)	final body wt (g/rat)	body wt gain (g/rat)	food intake (g/rat)	feed efficiency (g gain/g food)
depletion[a]	0	158 ± 21[b]	36 ± 11[b]	139 ± 17[b]	0.26 ± 0.06[b]
standard $FeSO_4$	6	199 ± 25	73 ± 11	187 ± 23	0.39 ± 0.04
	12	200 ± 18	82 ± 7	192 ± 17	0.43 ± 0.03
	18	209 ± 10	90 ± 8	215 ± 14	0.42 ± 0.06
	35	217 ± 9	87 ± 13	220 ± 11	0.39 ± 0.05
laver	6	182 ± 11	51 ± 9[c]	161 ± 10[c]	0.32 ± 0.05[c]
	12	193 ± 21	69 ± 15	184 ± 23	0.37 ± 0.05
	18	190 ± 29	65 ± 18	176 ± 33	0.37 ± 0.04
	24	205 ± 18	77 ± 8	188 ± 14	0.41 ± 0.02
	40	208 ± 15	83 ± 11	201 ± 19	0.41 ± 0.03
	60	212 ± 11	85 ± 10	206 ± 14	0.41 ± 0.05

[a] Iron concentration was <3 mg/kg. Values in this group are significantly lower than those of all other dietary groups by Duncan's multiple range test at $p < 0.05$. [b] Each value was mean ± SD of seven rats. [c] Significantly lower than the standard groups and other laver groups by Duncan's multiple range test at $p < 0.05$.

Table 2. Hemoglobin and Iron Responses in the Rat Hemoglobin Regeneration Assay of Laver Iron

diets	iron added (mg/kg)	intake of added iron (μmol/rat)	hemoglobin regenerated (mmol/L)	hemoglobin gain (nmol/rat)	hemoglobin Fe gain (μmol/rat)	HRE[a] (%)
depletion	0	0 ± 2[b]	0.57 ± 0.15[b]	0.4 ± 1.0[b]	1 ± 4[b]	
standard $FeSO_4$	6	20 ± 2	0.86 ± 0.11	5.4 ± 1.2	22 ± 5	110 ± 8
	12	41 ± 4	1.16 ± 0.12	9.8 ± 1.9	40 ± 7	97 ± 5
	18	69 ± 4	1.57 ± 0.13	16.3 ± 1.8	66 ± 7	96 ± 3
	35	138 ± 7	1.96 ± 0.07	22.5 ± 2.5	92 ± 10	67 ± 3[c]
laver	6	17 ± 1	0.64 ± 0.09	1.4 ± 1.0	6 ± 4	33 ± 9[d]
	12	39 ± 5	0.67 ± 0.03	2.4 ± 0.8	10 ± 3	25 ± 2[d]
	18	56 ± 11	0.76 ± 0.09	3.7 ± 1.1	15 ± 5	28 ± 4[d]
	24	81 ± 6	0.78 ± 0.07	4.5 ± 0.7	18 ± 3	23 ± 1[d]
	40	144 ± 14	1.05 ± 0.05	8.6 ± 1.2	35 ± 5	25 ± 1[d]
	60	221 ± 15	1.33 ± 0.08	12.9 ± 1.4	52 ± 6	24 ± 1[d]

[a] Ratio of hemoglobin Fe gain to intake of added Fe. [b] Each value was mean ± SD of seven rats. [c] Significantly lower than other three standard diets. [d] Significantly lower than all the standard diets, but no difference among the laver diets.

weight. One group of rats continued on the depletion diet. Four groups were fed the standard regeneration diets, and six groups were fed the laver regeneration diets. The regeneration period lasted for 14 days, and at the end rats were killed by carbon dioxide asphyxiation after body weight was recorded. Blood was collected from the abdominal vena cava into heparin-containing tubes for immediate analysis.

Analysis. Samples of dried laver, the depletion diet, the standard diets, and the laver regeneration diets were first digested by nitric acid in a microwave digestion oven (MLS-1200 MEGA, Milestone). Iron concentration was measured with an atomic absorption spectrophotometer (Model 3100, Perkin-Elmer Co.) with air-acetylene flame at 248.3 nm. Hemoglobin was determined colorimetrically by the cyanomethemoglobin method using Drabkin's reagent (Crosby and Munn, 1954).

Calculation. Intake of the added iron was calculated from food intake and the concentration of added iron in the diets. Total circulating hemoglobin and hemoglobin iron were calculated on the assumption that 6.7% of body weight is blood volume (Mahoney and Hendrick, 1982) and that hemoglobin contains 0.34% iron (Miller, 1982). The hemoglobin iron gain was calculated as the difference between total hemoglobin iron at the end and beginning of the regeneration period. The hemoglobin regeneration efficiency (HRE) was calculated as the percentage of added iron consumed that was retained in circulating hemoglobin (Mahoney and Hendrick, 1982, Forbes et al., 1989). The relative biological value (RBV) of iron in laver compared to that of ferrous sulfate was calculated by two methods: the slope-ratio procedure (Miller, 1977) and the ratio of HREs (Mahoney and Hendrick, 1982).

Statistical Analysis. Differences among all the dietary groups were tested by Duncan's multiple range test. Except for the standard regeneration group of 35 mg of Fe per kg diet, data from individual rats were used in the regression analysis of hemoglobin response on iron intake or dietary iron concentrations for each iron source in the regeneration period. Standard procedures for regression analysis were used with no assumption about the point of intersections. All statistics were carried out using the SAS System (version 6, SAS Institute, Cary, NC).

RESULTS

Growth and Food Consumption. Body weight gain and food intake increased as the levels of iron supply increased either as ferrous sulfate or as laver (Table 1). Rats fed on the depletion diet throughout had the lowest body weight, weight gain, and feed efficiency. Except for the group fed the laver diet at 6 mg Fe/kg diet, rats fed on laver diets had weight gain, food intake, and feed efficiency comparable to rats fed standard diets.

Hemoglobin and Iron Response. The regenerated hemoglobin concentrations and gains in total hemoglobin and in hemoglobin iron all increased as the iron supply increased in both standard and laver groups (Table 2). Rats fed on the depletion diet throughout had the lowest response for all these measures.

The HRE reached almost 100% in the standard groups at 6, 12, and 18 mg Fe/kg diet and averaged 101%, but it was significantly lower in the standard group of 35 mg Fe/kg diet (Table 2). All the laver groups had significantly lower HRE than the standard groups, and the average was 26.3%. The relative biological value of laver iron based on HRE was 26%

Regression Analysis. All of the dose–response lines in this experiment were essentially linear (r^2 ranged from 0.84 to 0.94, $p < 0.0001$; Figure 1), and intersections from the two dietary groups converged. The regression pattern met the requirements for the slope-ratio model, and relative biological values for laver iron

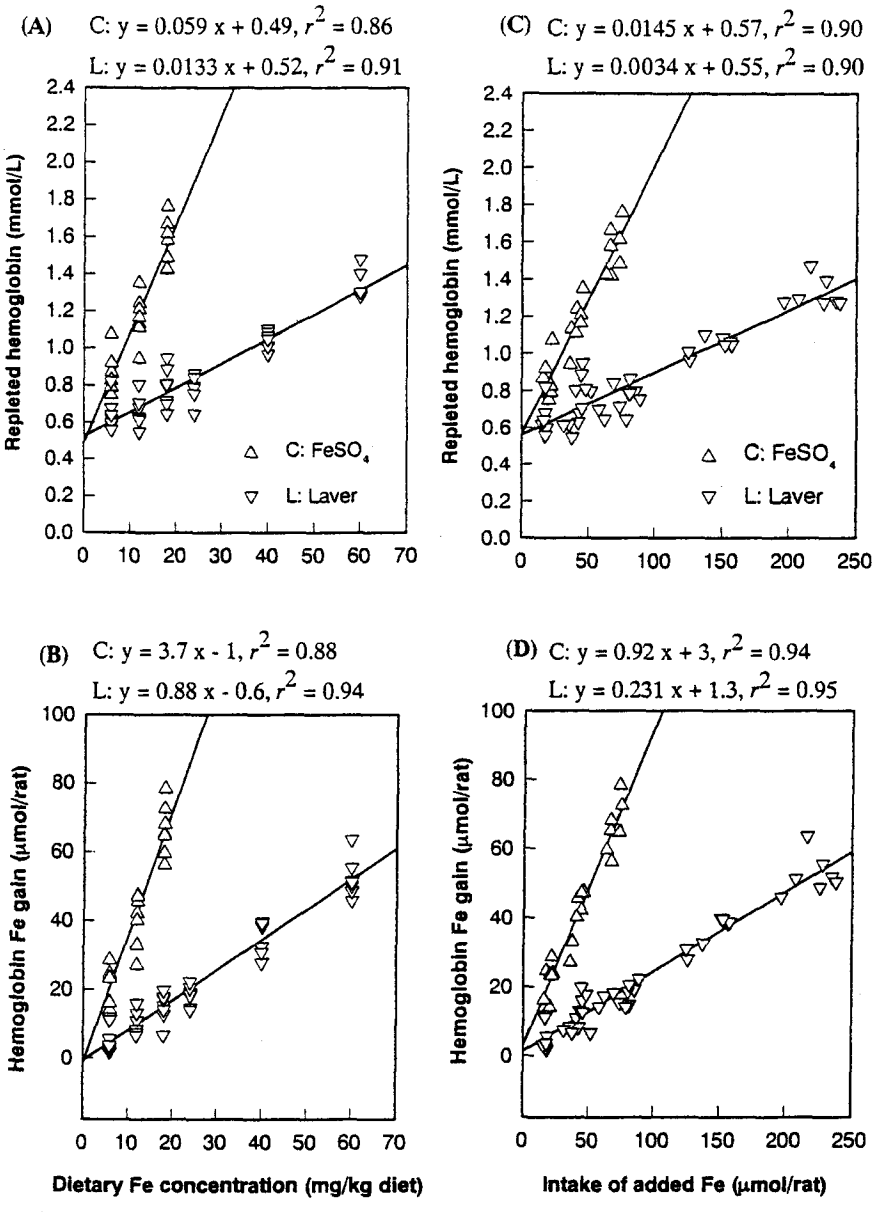

Figure 1. Regression analysis of hemoglobin responses on dietary iron from laver in a rat hemoglobin regeneration bioassay in comparison to ferrous sulfate standard diets. Data of individual rats were shown in each plot. Significance was set at $p = 0.0001$. Parts A and B are regenerated hemoglobin concentrations and hemoglobin Fe gain on dietary Fe concentration, respectively. Parts C and D are regenerated hemoglobin concentration and hemoglobin Fe gain on intake of added Fe, respectively.

thus calculated ranged from 24 to 26. The best correlation was obtained from the function of hemoglobin Fe gain versus intake of added Fe (Figure 1D).

DISCUSSION

In calculating the average HRE and regression analysis for the standard diets, data from the standard group at 35 mg Fe/kg were not included. This level of iron was above the sub-optimal iron requirement of the rat so as that response in body weight as well as in regenerated hemoglobin concentration tended to deviate from a linear relationship. This deviation was imposed by physiological homeostasis of iron metabolism and did not represent true availability of iron.

Johnson and Evans (1978) observed that absorption of ferrous sulfate in rapidly growing, iron-depleted rats approached 100%. In this study, HRE for the standard groups fed diets at suboptimal iron levels was near 100%, indicating that HRE in iron-deficiency anemic rats was equivalent to iron absorption rate and truly reflected iron availability. Therefore, the low and consistent HRE for the laver groups was a result of limited iron availability at low or high iron concentrations.

Our data fit the slope-ratio model well for analysis of RBV (Amine and Hegsted, 1974). Regression analysis of the linear dose–response relationship was highly significant, indicating that iron level was the major determinant of the hemoglobin response in the regeneration period for both standard and laver diets. Projections of the regression lines in Figure 1 to the zero iron intercept (Y axis intercept) yielded a value similar to that of the depletion group. For example, intercepts for regenerated hemoglobin concentrations ranged from 0.49 to 0.57 mmol/L and for hemoglobin Fe gains ranged from −0.6 to 3 μmol/rat, which were close to the mean of 0.57 ± 0.15 mmol/L and 1 ± 4 μmol/rat for the depletion group, respectively. Therefore, these hemoglobin responses were contributed solely by the added iron and can be taken as a quantitative indicator of the bioavailability of iron source.

Several authors (Miller, 1977; Rotruck and Luhrsen, 1979) have suggested that dose−response correlation can be improved with each mathematical adjustment for individual animal differences. Between the two measures of dietary Fe, iron intake consistently gave the better correlation (Figure 1) because variation in food intake among animals was corrected. Among the two measures of hemoglobin regeneration, hemoglobin Fe gain gave consistently better correlation than hemoglobin concentration for both dietary groups (Figure 1) because the former have taken into account the differences in weight gain, and thus in expansion of blood volumes during the regeneration period.

Both slope-ratio and HRE methods yielded consistent RBV for laver iron. Food with a RBV of 26% had iron availability lower than electrolytic iron, but similar to or higher than ferric orthophosphate or sodium iron pyrophosphate (Fritz et al., 1970; Forbes et al., 1989). However estimated, the amount of available iron from laver is comparable to those from many iron-fortified foods (Amine and Hegsted, 1974) when the high iron concentration (ranged from 0.28 mg to 0.91 mg Fe/g dry matter) had been considered. Iron in foods or diets comprises heme iron and non-heme iron pools (Hallberg, 1981). Laver can be used as a natural iron source in diets or as food ingredients to add to the non-heme iron pool.

ABBREVIATIONS USED

RBV, relative biological value; HRE, hemoglobin regeneration efficiency.

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