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A házinyulak nagyüzemi tartásának minimális állatvédelmi követelményei – a WRSA Magyar Tagozatának ajánlása

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ABSTRACT **- Minimum requirements of welfare for housing of rabbits on large-scale farms – recommendations of the Hungarian Branch of WRSA**
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The Hungarian Branch of WRSA formulated the minimum requirements of welfare for housing of rabbits in large-scale farms. These are as follows.

General recommendations
- The farmers are obliged to keep the animals in accordance with the scientific knowledge and experience and the genotype, age, physiological status and behavioural needs. Attention needs to be paid on: provision of adequate housing system to meet the environmental requirements of the animals; provision of sufficient space, feed and water for the animals; care them according to welfare requirement; with attention to the behavioural and social needs of the animals. Any person working with the animals has to receive instructions and guidance on the relevant provisions and complete appropriate training in particular focus on welfare aspects.
- Animal accommodation should be designed and managed to meet species-specific needs, and can not be contrary to any of the five freedoms of the animals.
- The buildings have to be constructed and maintained so that the risk of fire is minimised. The materials used should be fire-resistant.
- Avoid causing unnecessary pain, suffering or injury of the animals.
- Animals and any technological equipment used in rabbit farm should be inspected at least once a day, and any malfunctions should be eliminated immediately.
- The cages or pens of rabbits should be designed to meet the breed, age, sex, and physiological state.
- The cages or pens should be constructed of durable, impervious materials and designed and continuously maintained so that they do not cause injury of animals, and the animals are free from pain and injuries whilst taken in and out of the cage.
- The cages or pens have to be clearly arranged, and easy to control.
- Floors of cages must ensure that the droppings fall out of the cage, and the animals do not contact with manure.
- Feeding and watering systems have to be constructed, placed, operated and maintained to meet the physiological needs of the animals; reduce feed waste, leakage or contamination of the water, each animal should have sufficient access opportunities, so avoiding competition among animals for feed or water; do not cause injury of the animals; the feed and water consumption can be controlled.
- Permanent access to a sufficient quantity of fresh water has to be provided. The feeders and drinking systems have to be checked daily.
- Before the introduction of a new stock of the rabbits, the house/cages should be thoroughly cleaned and disinfected.

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- All-in all-out management is recommended to improve rabbit health, where rabbits are produced in one batch on a 6 week (or similar) cycle allowing the rabbit building to be vacated for a short period for intensive cleaning and disinfection.
- Only skilled persons are permitted to work with direct contact to the rabbits. Care of the rabbits can only be carried out by a person who has adequate theoretical and practical knowledge of that breed, age group and the used management in order to be able to detect the cause of behavioural changes in the animals and their health status.
- If it is suspected that the animal is not healthy or the behaviour appears to change adversely, the farmer has to eliminate the cause without delay, and if necessary, call a veterinarian. If necessary, the sick or injured animal has to be separated.
- The farmer has to register the number of rabbits, the animal losses, the treatments, disinfection, production and stock change (selling and buying) in the record keeper in a daily basis.
- Keeping rabbits in buildings with continuous high noise levels would be avoided. While rabbits can be scared by sudden noise, a continuous music can have a positive effect.

General rules for rabbit does, bucks, suckling and growing rabbits

- The air flow rate (maximum 0.4 m/sec), dust level, relative humidity (between 55 and 85%), the concentration of carbon dioxide (maximum 0.002 %Vol.) and ammonia (maximum 0.3%Vol.) should be at a level which is not harmful to rabbits.
- The air temperature should be between 10 °C and 28 °C. The minimum temperature for does at time of kindling is 15 °C.
- Rabbits are active from late afternoon till early morning; however, suitable lighting is necessary for them (minimum 30 and 10 lux for breeding does and growing rabbits, respectively), but protection against bright light should be provided for them. At least a daily continuous 8-hour dark period should be provided.
- Keeping rabbits in cages with solid walls without visual connection is prohibited, except bucks mainly at the time of mating and semen collection.
- Floors of cages and pens must be smooth but not slippery to prevent injuries of the rabbits and so designed, constructed and maintained as not to cause injuries or suffering of the animals.
- The dead rabbits must be removed immediately from the cage.
- Rabbits should have permanent access to a sufficient quantity of material, such as wood, straw, hay, or other material, which does not compromise the health of the animals, to enable proper investigation and manipulation activities and gnaw it. Gnawing sticks or other chewable materials are recommended for rabbit does, bucks and growing rabbits.
- Using a dual system in rabbit farms is recommended to decrease the stress at weaning. These systems are used for does with their kits, and after weaning the growing rabbits stay in the original cage and does are moved into a clean and disinfected cage before kindling.
- Environmental pollution of the rabbit farms have to be decreased.

The minimum requirements for housing of rabbit does

- Future breeding female rabbits have to be housed individually after the age of 12 weeks because of the high incidence of aggressiveness, fighting and injuring.
- At present, all cages are accepted which are produced by large cage factories in Europe, these cages can be found in several European rabbit farms, if its floor size is minimum 38x87 cm and its height is minimum 32 cm). The minimum requirement is that the floor area allows the does to turn around easily and lie in stretched position.
- When using a wire-mesh floor, it is necessary to provide at least a 25×40 cm sized plastic-mesh footrest for the does for preventing the sore hocks.
- Elevated platforms in cages or pens of rabbit does are recommended because they give larger possibility for moving than the flat-deck cages.

- Group-housing of rabbit does is not recommended, because of aggressiveness, fighting, injuries and stress.
- A nest box or nesting part in the cage has to be provided for rabbit does. The basic area size of the nest box for a medium-sized doe is at least 800 cm^2 with a shorter side of minimum 38 cm.
- The nest box or nest tray has to be given at least three days before the expected parturition to have enough time for does to prepare a good quality nest.
- For the nest-building suitable nesting material (e.g. wood shavings, hay, strax) in sufficient quality and quantity must be given.
- The possibility of closing the nest box should be provided by a door for using controlled nursing.
- The nest should be clearly arranged, and the kits in it have to be checked daily.

The minimum standards for housing of bucks
- Future breeding male rabbits have to be housed individually after the age of 10 weeks to avoid aggressiveness, fighting and castration.
- The minimum requirement is that the floor area allows the bucks to easily turn around and lie in stretched position. The minimum floor size is 50x64 cm, and 40 cm height.
- Plastic-mesh floor is recommended; however, when using a wire-mesh floor, it is necessary to provide at least a 25×40 cm sized plastic-mesh footrest for preventing the sore hocks.

The minimum standards for housing of growing rabbits
- The cages or pens for growing rabbits can be up to maximum three-storey for the proper checkability of the animals.
- No individual housing of growing rabbits is allowed, except aggressive or sick animals.
- The smallest group size is 2-3 rabbits per cage. In case of larger group, it is not recommended to house animals from more than one litter (8-10 rabbits) in a cage or pen, because in large groups the risk of infection, stress and aggressiveness is higher.
- The stocking density at the end of the fattening period should not exceed 16 rabbits/m^2 or 45 kg rabbit/m^2.
- The height of 30-35 cm for fattening cages/pens is acceptable because rabbits prefer covered places.
- Deep litter floor is not recommended because growing rabbits refuse staying on it, animals can consume from the litter material mixed with manure and urine, and the risk of diseases and mortality is higher.
- Wire-mesh and plastic-mesh floors are accepted.
- When growing rabbits are kept in groups they are fed using a system which ensures that each individual can obtain sufficient food even when competitors for the food are present. A 10 cm wide feeder is enough for 10 growing rabbits. If the size of the group is larger, the size of the feeder should be increased proportionally.
- When growing rabbits are kept in groups such a drinking system should be used which ensures that each individual can obtain sufficient water even when competitors for the water are present. Ten growing rabbits should reach at least two nipple drinker. If the group size is larger, the number of drinkers should be increased.

We agree with the text of European Parliament resolution of 14 March 2017 on minimum standards for the protection of farm rabbits (2016/2077(INI)). It pointed out that "a balance must be kept between the various aspects to be taken into consideration, as regards animal welfare and health, the financial situation and working conditions of farmers, sustainability of production, environmental impact and consumer protection; points out also that account must be taken of consumer needs for affordable, high-quality rabbit meat." Namely, changing housing system is high cost for the farmer, so a financial assistance is necessary to farmers by Commission to support the rabbit farming sector in future EU budgets.

***Keywords*:** rabbit, housing, welfare, requirements

BEVEZETÉS

Az európai fogyasztók egyre nagyobb hangsúlyt helyeznek az állatok jóllétére. Az elvárások megfogalmazásában jelentős szerepe van az állatvédő mozgalmaknak, amelyek egy része szakmai ismeretek, mások viszont érzelmi benyomások alapján fogalmazzák meg véleményüket. Sajnos ritkán jutnak el a kutatási eredmények a kereskedőkhöz és a fogyasztókhoz, az állatvédők viszont nagyon eredményesen gyakorolnak nyomást a kereskedelemre, a politikusokra, és végül a fogyasztók választását is hatékonyan befolyásolják.

Ugyanakkor az állatjólléti előírásoknak kutatási eredményeken kellene alapulniuk, valóban az állatok jóllétét kellene szolgálniuk, figyelemmel a termelőre és a fogyasztói árakra is. A nyulak védelmére vonatkozó minimális követelményekről szóló, az Európai Parlament 2017. március 14-i állásfoglalásában (2016/2077 (INI)) rámutattak arra, hogy „számos szempont mérlegelésével egyensúlyt kell tartani, figyelemmel az állatok jóllétére és egészségére, a gazdálkodók pénzügyi helyzetére és a munkakörülményekre, a termelés fenntarthatóságára, a környezeti hatásra és a fogyasztóvédelemre, továbbá a fogyasztók megfizethető, jó minőségű nyúlhús iránti igényére".

A házinyulak tartásával és jóllétével kapcsolatban számos tudományos cikk, irodalmi összefoglaló és tanulmány jelent meg (*EFSA, 2005; Hoy és mtsai, 2006; Trocino és Xiccato, 2006; Verga és mtsai, 2009; Szendrő és Dalle Zotte, 2011; Szendrő és McNitt, 2012; Szendrő és mtsai, 2016; Hoy és Matics, 2016; González-Mariscal és mtsai, 2017; Turner és mtsai, 2017; Szendrő és mtsai, 2019*).

A fogyasztók igényeinek kielégítése érdekében az Európai Unió Tanácsa (EU Tanácsa) irányelveket adott ki a tenyésztés céljából tartott állatok védelméről (*Council Directive 98/58/EC of 20 July 1998*), a tojótyúkok, (*Council Directive 1999/74/EC of 19 July 1999*), a hústermelés céljából tartott csirkék (*Council Directive 2007/43/EC of 28 June 2007*), a borjak (*Council Directive 2008/119/EC of 18 December 2008*), valamint a sertések védelmére vonatkozó minimális követelményekről (*Council Directive 2008/120/EC of 18 December 2008*). Az EU Tanácsa azonban még nem tett közzé semmilyen előírást a tenyésztett nyulak védelméről.

Jelen cikk célja, hogy tudományos eredményekre alapozva foglalja össze a házinyulak nagyüzemi tartásának minimális követelményeit. Nemcsak a követelményeket írjuk le, hanem ahol van, röviden összefoglaljuk az adott terület irodalmi eredményeit, vagyis a javaslatok magyarázatát is.

A minimális követelmények „A mezőgazdasági haszonállatok tartásának állatvédelmi szabályairól" szóló *32/1999. (III. 31.) FVM rendeletre*, illetve annak

módosított változataira (*20/2002. (III. 14.) FVM rendelet, 72/2004. (IV. 29.) FVM rendelet, 178/2009. (XII. 29.) FVM rendelet*) épülnek. Ugynakkor független a sokkal szigorúbb állatkísérletekről szóló *40/2013. (II. 14.) Korm. rendelettől*.

A WRSA MAGYAR TAGOZATÁNAK AJÁNLÁSAI

1. Általános ajánlások

- Az előírásokat csak a legalább 120 anyanyulat vagy 1000 hízónyulat tartó telepekre kell alkalmazni.

- A különböző alternatív (márkázott) tartási rendszerek magasabb szintű elvárásokat fogalmazhatnak meg, mint amit a minimális követelményekben írnak elő.

- A gazdáknak megfelelő ismeretekkel és tapasztalattal kell rendelkezniük a nyulak fajtájával, korával, fejlettségével, élettani állapotával és viselkedési szükségleteivel kapcsolatban. Bármely más, az állatokkal foglalkozó személynek is ismerni kell a vonatkozó rendelkezéseket, különös tekintettel az állatjólléti szempontokra.

- A nyulakat az állatfaj igényének megfelelően kell elhelyezni, és a jó gazda gondosságával kell őket kezelni.

- Nagyüzemi (nem alternatív) tartás esetén a nyulakat beltéri, zárt helyiségben kell tartani.

- Az épületeket úgy kell megépíteni és karbantartani, hogy minimális legyen a tűzveszély, továbbá legyenek felszerelve tűz– vagy füstriasztóval.

- Csökkenteni kell a nyúltelep által okozott környezeti terhelést.

- A tartás helyén a levegő hőmérsékletének 10°C és 28°C között kell lennie. A fialó anyanyulak, szopósnyulak, valamint a választott növendéknyulak tartási helyén viszont legalább 15°C-ot kell biztosítani. A megfelelő, az ideálishoz közeli hőmérséklet biztosításához télen és nyáron az istállók klimatizációjáról gondoskodni kell.

> *Megjegyzés: Az újszülött nyulaknak, az elletőládában 38°C körül alakul az optimális hőmérsékletigénye, ami 14 napos korra 30°C-ra csökken (*Hull és mtsai, 1986*), majd tovább csökkenve, a hizlalás végére 15 és 25 °C közé esik (*Cervera és Fernández Carmona, 2010*), ami az anyanyulak igényénél kissé alacsonyabb. A magas hőmérséklet jelent nagyobb gondot, mert a bundában levő nyulaknak alig van izzadtságmirigyük (*Brewer és Cruise, 1994*), elsősorban a fül vérerei segítségével szabályozzák a test hőmérsékletét (*Lukefahr és Ruiz-Feria, 2003*).*

- Az istállóban a légáramlás sebessége legfeljebb 0,4 m/sec, a relatív páratartalom 55 és 85% között, a szén-dioxid legfeljebb 0,002 térfogatszázalék, az ammónia 0,30 térfogatszázalék, az egyéb gázok és a por koncentrációja olyan mértékű legyen, amely nem káros a nyulakra.

Megjegyzés: A légáramlás sebessége függ a hőmérséklettől: 10–15 °C között 0,1–0,15 m/sec, 15–19 °C között 0,15–0,20 m/sec, 19–23 °C között 0,2–0,3 m/sec, 23–26 °C között 0,3–0,4 m/sec. Ugyanezekhez a hőmérséklet-tartományokhoz tartozó levegő szükséglet, sorrendben 1–2; 2–3, 3–3,5 és 3,5–4 m³/óra/testsúly kg. Az ideális és az elfogadható relatív páratartalom tenyésztistállóban 65–70% illetve 55–85%, hizlaló istállóban 55–70%, illetve 50–85%. Hoy és Lange (1997) *legfeljebb 0,002 térfogatszázalék ammónia és 0,30 térfogatszázalék széndioxid szintet javasol. Érdemes figyelembe venni, hogy más állatfajokhoz viszonyítva a házinyúl nem érzékeny a magas széndioxid szintre, mert az üregi nyúl az üregrendszerben magas széndioxid tartalmú levegőben tartózkodik, ami toleránssá tette a széndioxiddal szemben (*Hayward és Lisson, 1978*).*

- Gondoskodni kell az állatok igényének megfelelő megvilágításról: anyanyulaknak legalább 30 lux, növendéknyulaknak 10 lux fényerősség szükséges, az erős fénnyel szemben ugyanakkor védelmet kell nyújtani. Naponta legalább 8 óra folyamatos sötét időszakot kell az állatok számára biztosítani.

*Megjegyzés: Az üregi és a házinyulak sötétben aktívak, nappal pihennek (*Prud'hon és mtsai, 1975; Villafuerte és mtsai, 1993; Diez és mtsai, 2005*). Több szakkönyv 30–50 lux fényintenzitást javasol (*Lebas és mtsai, 1997; Schlolaut, 1998; EFSA 2005*).*

- Ne tartsák a nyulakat túl zajos helyen. Mivel a hirtelen zajtól megijedhetnek, ezért ennek hatását csökkenthetjük, ha az állatok például állandóan zenét hallhatnak.

- Kerülni kell az állatok szükségtelen fájdalmát, szenvedését vagy sérülését.

- A ketreceket vagy fülkéket úgy kell megtervezni, hogy megfeleljenek a fajta, az életkor, az ivar és az élettani szükségleteknek, és könnyen ellenőrizhetők, tisztíthatók legyenek.

- A ketreceket vagy fülkéket úgy kell kialakítani és folyamatosan karbantartani, hogy azok ne okozhassanak sérülést az állatoknak, emellett az állatok fájdalom- és sérülésmentesen legyenek kivehetők a ketrecből vagy fülkéből.

- Lemez, vagy más átláthatatlan oldalfalú ketrecben nyulat tartani – kivéve bakok, különösen fedezéskor és ondóvételkor – tilos. Biztosítani kell az állatok számára, hogy láthassák fajtársaikat.

*Megjegyzés: Az üregi nyúl számára a túlélés miatt fontos, hogy jól belássák a környezetüket és időben vegyék észre a ragadozót. A nem túl sűrű bozótos területet kedvelik (*Lombardini és mtsai, 2003*). Ha a ketrec fala tömör, ez akadálya a vizuális kapcsolatnak és megijedhetnek, ha az épületben levő személyt csak a ketrec fölé érkezéskor látják meg (*Szendrő és Dalle Zotte, 2011*). Drótrácsoldalfal esetén látják, és jobban érzik a szomszédos ketrecekben levő nyulak szagát, emellett – a fal két oldalán feküdve – a szociális kapcsolat szempontjából fontos test-kontaktus is megfigyelhető.*

- A ketrecek és fülkék padozata legyen sima, csúszásmentes, ne okozzon talpfekélyt, és úgy alakítsák ki, hogy ne érintkezzenek az állatok az ürülékkel.

- Rácspadozat alkalmazásakor a hosszanti huzalok távolsága nem haladhatja meg a 20 mm-t, a kereszthuzalok távolsága az 55 mm-t, a dróthuzal átmérője legalább 2 mm legyen.

- Az állatok új helyre telepítése előtt a tartási helyeket alaposan ki kell takarítani és fertőtleníteni.

- Az egészség védelme, a betegségek terjedésének megelőzése céljából az egyszerre telepítés, egyszerre ürítés (all-in all-out) rendszer ajánlott.

*Megjegyzés: Kettős használatú ketrecben (dual purpose cage) választáskor a kisnyulak helyben maradnak, és a vemhes anyanyulak kerülnek egy másik épület tiszta ketrecébe. Ezáltal kisebb a választási stressz és betegség fellépésének kockázata. Vágáskor kiürülnek a ketrecek és ekkor kerül sor – az all-in all-out rendszernek megfelelően – a teljes épület és a berendezési tárgyak alapos takarítására és fertőtlenítésére (*Hoy és Matics, 2016*).*

- Az állományt naponta kell ellenőrizni. Gondoskodni kell a beteg állatok kezeléséről, lehetőség szerint az elkülönítéséről. Az elhullott egyedeket azonnal el kell távolítani.

- Az etetőket és itatókat olyan méretben és számban kell a ketrecbe helyezni, hogy a nyulak könnyen, egymást nem zavarva férjenek hozzájuk. Az esetleges meghibásodás miatt minden ketrecbe két itatószelepet kell tenni. Az etetők és az itatók kialakítása olyan legyen, hogy a takarmány és az ivóvíz ne szennyeződjön, a takarmány- és ivóvízveszteség minimális legyen.

- Az etetőket és az itatókat naponta kell ellenőrizni. A meghibásodást lehetőleg azonnal meg kell szüntetni.

- Rágófa vagy más rágható anyag használata ajánlott, különösen csoportos tartás esetén.

*Megjegyzés: A nyulaknak lehetőleg állandó hozzáférést kell biztosítani olyan anyaghoz, mint pl. rágófa, szalma, széna vagy más anyag, amellyel foglalkozhatnak, amit megrághatnak, sőt el is fogyaszthatnak, és nem veszélyezteti az állatok egészségét. Környezetgazdagítással csökkenthető az unatkozás, javulnak a nyulak életkörülményei (*Verga és mtsai, 2009*). A rágófának általában nincs negatív hatása a termelésre (*Verga és mtsai, 2004; Princz és mtsai, 2007, 2009*). Legnagyobb előnye, hogy csökken az agresszív viselkedés gyakorisága és a sérülések száma, különösen, ha puhafából készülnek a rágófák (*Princz és mtsai, 2008a*)*

2. Az anyanyulak tartásának minimális követelményei

- 12 hetes kortól a tenyésznövendék-, majd az anyanyulakat egyedileg kell elhelyezni, hogy megelőzhetőek legyenek az agresszív viselkedés miatti verekedések és sérülések.

Megjegyzés: Mirabito és mtsai (2005) *négyesével neveltek együtt nőivarú nyulakat, de – sérülés miatt – tenyésztésbevételig a nyulak harmadát kellett selejtezni.*

- Jelenleg valamennyi nagy európai ketrecgyártó által forgalmazott tenyészketrec mérete elfogadható, ha az alapterülete legalább 38x87 cm és 32 cm magas.

*Megjegyzés: Kis ketrecben korlátozott az anyanyulak mozgási lehetősége, ami frusztrálhatja őket és sztereotip viselkedés (pl. fémrács rágása) fordulhat elő (*Verga és mtsai, 2007*). Bár a legtöbb kísérlet szerint a ketrecméret nem befolyásolja az anyanyulak termelését (*Mirabito és mtsai, 2005; Rommers és Meijerhof, 1998*); a nagyobb ketrec több mozgási lehetőséget és kényelmesebb elhelyezést nyújt (*Szendrő és McNitt, 2012*). Véleményünk szerint az eddig elfogadott minimális, elletőládán kívül legalább 3000 cm² terület szűk, de ennél nagyobb alapterületű ketrec cseréjéhez támogatás szükséges. Amennyiben az EU bármilyen a jelenleginél nagyobb minimális ketrecméretet határozna meg, a kertecek cseréjéhez elegendő átmeneti időt kell hagyni és támogatást kellene nyújtani. Az Európában használt ketrecek méretét az 1. táblázat mutatja.*

- Az anyanyulak ketrecébe polc behelyezése javasolt.

*Megjegyzés: A polc nagyobb mozgási lehetőséget, fel- és leugrást biztosít. Elvileg az anyanyúl el tud menekülni az elletőládát már elhagyó, szopni akaró kisnyulak elől (*Mirabito és mtsai, 1999*), de 3 hetes kortól a szopósnyulak is fel tudnak menni a polcra (*Mirabito és mtsai, 1999, 2004; Mikó és mtsai, 2014*). Emiatt mégsem csökken a szopás miatti zavarások száma (*Mirabito és mtsai, 2004*). Ugyanakkor* Mikó és mtsai (2014) *kísérletében az egyszintes ketrechez képest nőtt a 3 hetes alomsúly. Legjobb, ha a polc műanyag rácsból készül, és az alsó szinten is van pihenőlap.*

1. táblázat

Az európai nagy nyúltelepeken jelenleg használt egyedi tenyészketrecek méretei

Ketrec típusa	Szélesség (cm)	Hosszúság (cm)	Magasság (cm)	Alapterület (cm²)
Fiatal és nem vemhes anya	38	43	35	1600
Szoptató anyanyúl kisnyulakkal (standard és kettős célra)	38	87*	32	3300
Szoptató anyanyúl kisnyulakkal (kettős célra)	38	95*	35	3600
Szoptató anyanyúl kisnyulakkal (gazdagított ketrec) 20 cm széles fémrácspolccal	38/46	102,5*	61	4650/ 5600**
Szoptató anyanyúl kisnyulakkal, műanyagrács polccal (kettős célra)	52,5	102,5*	97	6400**

*Fészek beleszámítva. **A polc alapterülete is beleszámítva. Forrás: *Szendrő és mtsai, 2019*

Table 1. *The dimensions of the cage which are used in the European large rabbit farms at the moment *calculated with the basic area of nest box; ** calculated with the basic area of elevated platform. Source:* Szendrő et al, 2019

- Az agresszív viselkedés, verekedés, sérülés és stressz miatt az anyanyulak csoportos tartása nem javasolt.

*Megjegyzés: Ha az anyanyulak folyamatosan együtt vannak, gyakori a verekedésekre visszavezethető sérülés és az álvemhesség miatti gyenge vemhesülés, két anyanyúl azonos elletőládába fialhat, részben emiatt nő a szopóskori elhullás, a folyamatos stressz miatt az anyanyulak rövidebb ideig élnek (*Mirabito és mtsai, 2005; Rommers és mtsai, 2006; Andrist és mtsai, 2013; Szendrő és mtsai, 2013*). Ezek a problémák a csoportban élő üregi anyanyulaknál is megfigyelhetők: gyakori a verekedés (*Southern, 1948; von Holst és mtsai, 1999*), a másik anya újszülött nyulainak elpusztítása, nő az alárendelt nyulakban a stresszhormonszint, valamint kevesebb nyulat fialnak és nevelnek fel (*von Holst és mtsai, 2002; Rödel és mtsai, 2004, 2008*).*

*Félig csoportos tartás esetén, az anyanyulak a fialás előtti néhány naptól 3 hétig egyedi ketrecekben vannak, majd az oldalfalak kivételével a négy anyanyúl és a kisnyulak 3 hétig ugyanabban a fülkében vannak csoportosan (elválasztás után a növendéknyulakat itt nevelik fel). Bár a termelési eredmények hasonlóak lehetnek az egyedi tartáshoz (*Maertens és mtsai, 2011; Maertens és Buijs, 2015*), de minden csoportosítás után – rövidebb vagy hosszabb ideig – heves verekedés és sok sérülés*

*fordul elő. Az agresszív viselkedés és a sérülések gyakoriságának csökkentése érdekében több módszert próbáltak ki (polc, PVC-csövek betétele, szalma, sötét folyosó, a csoport-kialakítás a korábbi vagy új fülkében, illatanyag permetezése az állatokra stb.), de nem, vagy alig tudták csökkenteni az agresszivitást, 40–60%-ban találtak sérült nyulakat (*Graf és mtsai, 2011; Andrist és mtsai, 2012, 2013, 2014; Rommers és mtsai, 2011, 2013*). Mindez ellentétes az állatjólléttel, az állatok öt szabadságjogával. A 70-es években Franciaországban épp emiatt szüntették meg az anyanyulak csoportos tartását (*Mirabito és mtsai, 2005*).*

*Nem szabad megfeledkezni arról, hogy az üregi nyulak – több más állatfajhoz hasonlóan – azért élnek csoportban, mert így nagyobb a ragadozókkal szembeni túlélési esélyük (több szem, többet lát, hátulsó lábukkal dobbantva figyelmeztetik a társakat a veszélyre, cikk-cakkban futva menekülnek be a közösen kiásott üregrendszerbe) (*Cowan, 1987; König, 1997*). A csoportban élésnek tehát több az előnye, mint a hátránya (*Ebensperger és Wallem, 2002*). Az istállókban viszont nincs ragadozó, csak a csoportban élés hátrányai jelentkeznek.*

- A fialó anyanyulaknak elletőládát kell betenni, vagy fialó részt kell kialakítani. Az elletőláda mérete középtestű anyanyulak esetén legalább 800 cm^2, egyik oldala legalább 38 cm hosszú legyen.

*Megjegyzés: Az üregi anyanyulak egy kisméretű, gömb alakú, kb. 25 cm átmérőjű fialóüreget készítenek (*Lloyd és McCowan, 1968*). Az elletőládának (fészeknek) elég nagynak kell lennie, hogy ne legyen túlzsúfolt, ugyanakkor elég kicsinek ahhoz, hogy az összebújt kisnyulak melegítsék egymást. Nagyméretű elletőládában kihűlhetnek, elpusztulhatnak a fészekből kimászó kisnyulak.*

- Az elletőládát, illetve a fészektálcát a várható fialás előtt legalább három nappal kell az állatok számára biztosítani, hogy az anyanyúlnak elég ideje maradjon a jó minőségű fészek elkészítésére.

*Megjegyzés: A fészekkészítési viselkedést (fialóüreg kiásása, alomanyag behordása, szőr tépése a testről) hormonok szabályozzák (*González-Mariscal és mtsai, 1994, 1996; Negatu és McNitt, 2002*). Az anyanyulak 2-3 nappal a fialás előtt kezdik el behordani az alomanyagot, vagyis építeni a fészket (*Zarrow és mtsai, 1963; González-Mariscal és mtsai, 1996*).*

- Az anyanyulaknak a fészeképítéshez megfelelő alomanyagot (pl. faforgácsot, szénát, szalmát) kell biztosítani.

*Megjegyzés: A csupaszon, gyenge hőszabályozással született kisnyulak megfelelően kialakított és szőrrel takart fészekben, összebújva, egymást melegítve – a néhány perces szopást kivéve – alszanak. Az üregi anyanyulak száraz füvet és más anyagokat gyűjtenek, és ezekből készítik el a fészket (*Hudson és mtsai, 2000*). Megfelelő fészek hiányában az üregi és a házinyulaknál is megemelkedik a szopósnyulak elhullása (*Canali és mtsai, 1991*). A nyúltelepeken leggyakrabban faforgácsot, szalmát*

*vagy szénát használnak fészekanyagnak (*Blumetto és mtsai, 2010*). Bár szabad választásnál a nyulak nem részesítik előnyben a faforgácsot (*Farkas és mtsai, 2017*), de a gyakorlatban jól bevált.*

- Zárható búvónyílású elletőláda javasolt.

*Megjegyzés: A nyúltelepeken korábban főként szabad-, az utóbbi években napi egyszeri szoptatást végeznek. A két szoptatási mód összehasonlításakor ellentmondó eredményeket kaptak (*Pizzi és Crimella, 1985; Costantini és mtsai, 1986; Coureaud és mtsai, 1998; Szendrő és mtsai, 1999*). Mivel a kisnyulak életük első 12 napjában csak szopnak és alszanak, ezért, a megijedt, elletőládába ugró anyanyúl megzavarhatja, szétszórhatja, megsebesítheti, akár agyon is taposhatja őket, ezért az egyszeri szoptatás állatjólléti oldalról előnyös lehet (*Szendrő és mtsai, 1999*). A napi egyszeri szoptatás nem természetellenes, mert az üregi és a házinyúl is leggyakrabban 24 óránként, egyszer szoptatja kicsinyeit, aminek időtartama 3-4 perc (*Zarrow és mtsai, 1965; Hudson és Distel, 1982; Hoy és mtsai, 2000; Hoy és Selzer, 2002*). Az üregi nyulaknál megfigyelték, hogy fialás és minden szoptatási alkalom után az anyanyúl fűvel, levelekkel és földdel tömi be az üreg bejáratát, hogy elrejtve azt csökkentse a ragadozók kártételét (*Zarrow és mtsai 1965; Lloyd és McCowan, 1968*).*

- Naponta ellenőrizni kell az elletőládát és benne a szopósnyulakat.

*Megjegyzés: Fialáskor el kell távolítani a halva született és az életképtelen kisnyulakat. Alom-kiegyenlítést célszerű elvégezni úgy, hogy az először fialó anyanyulak alatt legfeljebb 8, az idősebbeknél 10, lehetőleg azonos súlyú kisnyúl maradjon. Ezzel az elhullás mellett az almon belüli súly szóródását is csökkenteni lehet (*Bautista és mtsai, 2008, 2015*). Ha az anyanyúl nem tép elég szőrt, a testéről kitépett szőrrel kell az almot betakarni.*

- Fémrácspadozat esetén, a talpfekély kialakulásának megelőzése céljából, legalább 25×40 cm nagyságú, perforált műanyag pihenőlap szükséges. Hasonló célt szolgál a műanyag rácsból készült polc.

*Megjegyzés: Az anyanyulaknál – mivel viszonylag nagy a testsúlyuk és hosszú ideig termelnek – fémrácspadozaton gyakran alakulhat ki a talpfekély enyhébb vagy súlyosabb formája (*De Jong és mtsai, 2008; Rosell és de la Fuente, 2009*). Pihenőlap behelyezése esetén csökken a talpfekély előfordulási gyakorisága és annak súlyossága is (*De Jong és mtsai, 2008; Rosell és de la Fuente, 2009, Rommers és De Jong, 2011; Mikó és mtsai, 2014*). Hasonló pozitív eredmény érhető el, ha a ketrecben műanyagrács-polc található (*Mikó és mtsai, 2014*).*

3. A baknyulak tartásának minimális követelményei

- A hímivarú tenyésznövendék-nyulakat 10 hetes kortól – agresszió, verekedés, sérülés és egymás kasztrálásának megelőzése céljából – egyedileg kell elhelyezni.

- A baknyulaknak legalább 50x64 cm alapterületű és 40 cm magas ketrec javasolt.

- Fémrácspadozat esetén, a talpfekély kialakulásának megelőzése céljából, legalább 25×40 cm nagyságú perforált műanyag pihenőlapot kell behelyezni, de a műanyagrács-padozat alkalmazása megfelelőbb.

4. A növendéknyulak tartásának minimális követelményei

- Az állatok megfelelő ellenőrzése érdekében a növendéknyulakat legfeljebb háromszintes ketrecsorban szabad elhelyezni.

- Nem engedhető meg a hízónyulak egyedi tartása, kivéve az agresszív és a beteg egyedeket.

> *Megjegyzés: Bár egyedi elhelyezés esetén érhető el a legjobb termelés (*Maertens és De Groote, 1984; Xiccato és mtsai, 1999, 2013*), de ebben korlátozott a szociális kapcsolat, és emiatt a stressz élettani tünetei (*Held és mtsai, 1995; Chu és mtsai, 2004*), unatkozás, bizonyos sztereotip viselkedés, mint a rács nyaldosása vagy rágása, figyelhető meg (*Podberscek és mtsai, 1991*).*

- A hizlaló ketrecbe legalább 2-3 növendéknyulat kell tenni. Több nyúl esetén nem javasolt, ha fülkénként egy alomnál (8-10 nyúlnál) több egyedet nevelnek együtt.

> *Megjegyzés: Minél nagyobb a csoport létszáma, annál gyengébb termelés várható (*Szendrő és Dalle Zotte, 2011*). Legnagyobb probléma, hogy minél nagyobb a csoport, annál nagyobb az esély arra, hogy a fülkében agresszív egyed is legyen, ezért a csoportnagyság növekedésével párhuzamosan gyakoribb a sérült egyedek előfordulása (*Bigler és Oester, 1996; Szendrő és mtsai, 2009*). A gyakoribb verekedés miatti krónikus stressz a takarmányfogyasztás és az ellenállóképesség csökkenését, a megbetegedések gyakoribb fellépését okozhatja. Rágófa behelyezésével, különösen, ha puhafából készül, csökkenthető az agresszivitás és a sérülések előfordulása.*

- A növendéknyulaknál legfeljebb 16 nyúl/m^2 a megengedhető telepítési sűrűség, ami a hizlalás végén 40–45 kg nyúl/m^2-nek felel meg.

> *Megjegyzés: Számos kísérlet eredménye igazolja, hogy 16–17 nyúl/m^2 telepítési sűrűség felett romlik a termelés (*Szendrő és Dalle Zotte, 2011*).* Maertens és De Groote (1985)*, valamint* Aubret és Duperray (1992) *igazolták, hogy az 1 m^2-re eső nyúl súlya pontosabban meghatározza az ideális telepítési sűrűséget, mint az egyedek száma. Bár többen ennél kisebb telepítési sűrűséget ajánlanak, de ennek előnyét még nem bizonyították.*

- A hízónyulaknak legalább 30–35 cm legyen a ketrec vagy fülke magassága.

*Megjegyzés: Többen úgy gondolják, hogy a nyulaknál fontos a hátulsó lábakra állás, a felegyenesedő figyelő testtartás. Az üregi nyulaknál ez életbevágó, mert az álló testhelyzet (upright alert position) segíti a ragadozó időben történő észrevételét (*Gunn és Morton, 1995*). Az istállóban – ragadozó híján – nincs gyakorlati jelentősége ennek a viselkedésnek. Nyitott tetejű ketrecben a nyulak idejük 0,7%-át töltötték ebben a testhelyzetben (*Martrenchar és mtsai, 2001), Finzi (2005; cit. Princz és mtsai, 2008b) *még ennél is kisebb gyakoriságot figyelt meg. Amikor a növendéknyulak szabadon választhattak, lengőajtókon keresztül mozoghattak a 20, 30, 40 cm magas és a felül nyitott ketrecek között, fele annyi nyúl tartózkodott a felül nyitottban, mint bármelyik más ketrecben (*Princz és mtsai, 2008c*). Amikor polc volt a fülkében a legtöbb nyúl a "védelmet nyújtó" polc alatt vagy a polcon tartózkodott és nem a tető nélküli részen (*Szendrő és mtsai, 2011*). A 20 cm magas fülke viszont már túl alacsony, mert ebben gyakoribb volt az agresszív viselkedés és a sérülés. Ezek a megfigyelések összhangban vannak az üreginyulak viselkedésével. Azok is a felülről takarást, védelmet nyújtó bokros területet részesítik előnyben, pihenésre és alvásra pedig az igen szűk üregrendszert választják (*Villafuerte és Moreno, 1997; Lombardini és mtsai, 2003*). A fentiek alapján megalapozott a 30-35 cm magas ketrec ajánlása.*

- Nem javasolt a növendéknyulak mélyalmon történő tartása.

*Megjegyzés: Szabad helyválasztásos kísérletekben – a hőmérséklettől és a telepítési sűrűségtől függetlenül – a növendéknyulak gyakrabban tartózkodtak műanyag- vagy fémrácspadozaton, mint mélyalmon (*Morisse és mtsai, 1999; Bessei és mtsai, 2001; Orova és mtsai, 2004; Gerencsér és mtsai, 2014*). A mélyalom kényelmesnek tűnik, de a bundában levő nyulak nem tudnak megszabadulni az emésztés során keletkező hőtől, ezért szívesebben választják a kevésbé kényelmes, de jó hővezető, hűvösebb padozatot (*Bessei és mtsai, 2001*). A nyulak esznek a bélsárral és vizelettel kevert alomanyagból (*Maertens és Van Oeckel, 2001; Jekkel és mtsai, 2008*), ezért kevesebb takarmányt fogyasztanak (*Kustos és mtsai, 2003*), csökken a súlygyarapodásuk és gyakoribb az emésztőszervi megbetegedés és az elhullás előfordulása, mint fémrács padozaton (*Dal Bosco és mtsai, 2000, 2002; Lambertini és mtsai, 2001; Gerencsér és mtsai, 2014*).*

- A fémrács- és a műanyagrács-padozat egyaránt elfogadott.

*Megjegyzés: Nincs vagy elhanyagolható a különbség a különböző padozatokon (műanyag rács, fémrács, léc) tartott nyulak termelésében és viselkedésében (*Petersen és mtsai, 2000; Trocino és mtsai, 2008; Princz és mtsai, 2008b, 2009*). A fém- és műanyagrács nagy előnye, hogy a trágya lehullik, nem érintkeznek vele az állatok, ezért csökken a kokcidiózis előfodulása, és szabad helyválasztás esetén gyakrabban tartózkodnak rajtuk a nyulak, mint mélyalmon (*Morisse és mtsai, 1999; Bessei és mtsai, 2001; De Jong és mtsai, 2008*). Melegben és idősebb korban a növendéknyu-*

*lak még a műanyagrácsnál is gyakrabban választják a fémrácsot, mert jobb hővezető és hidegebb, emiatt a nyulak könnyebben szabadulnak meg a hőterheléstől (*Gerencsér és mtsai, 2014*). Fontos azonban a lyuk és a padozat anyagának aránya. Ha a felülethez képest kevés a lyuk, felboltozódik a trágya és nő a fertőzés esélye (*Masthoff és mtsai, 2016*). Fémrács esetében legalább 2 mm átmérőjű huzalt és 55 x 20 mm-es rácsközt ajánlanak.*

- Csoportos tartásban tíz nyúl számára legalább 10 cm széles etető ajánlott. Nagyobb létszám esetén a nyulak számával arányosan növelni kell az etető méretét.

*Megjegyzés: 13 nyúl számára 10, 20, 30 vagy 40 cm széles etetőt tettek a fülkébe (*Orova, 2008, *személyes közlés). 10 cm-es etető esetében sem találtak különbséget termelésben és agresszív viselkedésben. Vagyis ad libitum takarmányozásnál tíz nyúl számára elegendő a 10 cm széles etető.*

- Csoportos tartásban annyi itató legyen a fülkében, hogy a nyulak egymás zavarása, versengés nélkül tudjanak inni. Tíz nyúl számára legalább két súlyszelepes itató szükséges.

Megjegyzés: Orova (2008*, személyes közlés) szerint 13 nyúl számára elég egy itatószelep. Biztonság kedvéért jobb, ha két itató van egy ketrecben.*

ZÁRÓ GONDOLATOK

A házinyulak nagyüzemi tartásának minimális követelményeit nem érzelmi alapon, hanem kutatási eredményekre támaszkodva foglaltuk össze. A bevezetésben olvasható, hogy magyar vezetéssel vagy közreműködéssel eddig már több összefoglaló mű született. Ezzel a közleménnyel egyidőben jelenik meg az anyanyulak tartásával kapcsolatban a legújabb kutatási eredményeket összefoglaló cikk, de hamarosan elkészül a növendéknyulakkal foglalkozó írásunk is, angol nyelven. Jelen cikkel hármas célunk teljesülhet. Egyrészt a készülő állatvédelmi szabályozásról szóló FM-rendelet megalkotását szeretnénk segíteni, másrészt az angol nyelvű összefoglalóból a világon bárki láthatja, hogy Magyarországon milyen előírások vannak, illetve várhatók a házinyulakkal kapcsolatban. Harmadrészt, a követelmények mellett ezek indoklását bárki elolvashatja magyarul. Jó lenne, ha ezzel az érdekeltek jobban tudnák érvényesíteni – a nyulak jóllétének szem előtt tartásával – a magyar érdekeket.

A világon kevés kutató foglalkozik a nyulak viselkedésével és jóllétével. Erre vezethető vissza, hogy több olyan kérdés van, amit alaposan vizsgálni kellene. Jóllehet számos esetben egyértelmű következtetés vonható le a kutatási eredményekből, ennek ellenére még mindig van támogatója a növendéknyulak mélyalmos vagy az anyanyulak csoportos tartásának. Pedig ezek több ponton

ellentétesek az állatok öt szabadságjogával (*Farm Animal Welfare Council, 1992*).

Úgy tűnik, hogy az EU is foglalkozik a nyulak tartási követelményeinek megfogalmazásával és elfogadásával. Több állatfaj példáját látva nem lehetünk biztosak abban, hogy az összes előírás a nyulak jóllétét fogja szolgálni, mert az állatvédők mindent elkövetnek, hogy elképzelésükből minél több kerüljön be a törvénybe. Sajnos a kutatási eredmények sokszor még a legérintettebb termelőkig sem jutnak el, és érdekeiket sem tudják megfelelően érvényesíteni. A kutatóknak pedig nem feladatuk, hogy az EU-törvényhozókig hallassák szavukat, ők tudományos és szakmai cikkekben, nemzetközi és hazai konferenciákon ismertetik eredményeiket. Nemcsak a nyúltenyésztőknek, hanem minden gazdasági állatfaj tenyésztőjének szüksége lenne az összefogásra, valamint arra, hogy a helyes állattartást olyan modern hírközlő eszközök használatával mutassák be, mint a fiatalok által általánosan használt YouTube, az Instagram, vagy a Facebook.

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IRODALOM

Andrist C.A., Bigler L.M., Würbel H., Roth B.A. 2012. Effects of group stability on aggression, stress and injuries in breeding rabbits. Appl. Anim. Behav. Sci., 142, 182–188. DOI: 10.1016/j.applanim.2012.10.017

Andrist C.A., van den Borne B.H.P., Bigler L.M., Buchwalder T., Roth B.A. 2013. Epidemiologic survey in Swiss group-housed breeding rabbits: Extent of lesions and potential risk factors. Prev. Vet. Med., 108, 218–224. DOI: 10.1016/j.prevetmed.2012.07.015

Andrist C.A., Bigler L.M., Würbel H., Roth B.A. 2014. Masking odour when regrouping rabbit does: Effect on aggression, stress and lesions. Livest. Sci., 170, 150–157. DOI: 10.1016/j.livsci.2014.10.017

Aubret J.M., Duperray J. 1992. Effect of cage density on the performance and health of growing rabbit. J. Appl. Rabbit Res., 15, 660–665.

Bautista A., García-Torres E., Martínez-Gómez M., Hudson R. 2008. Do newborn domestic rabbits *Oryctolagus cuniculus* compete for thermally advantageous positions in the litter huddle? Behav. Ecol. Sociobiol. 62, 331–339. DOI: 10.1007/s00265-007-0420-4

Bautista A., Zepeda J.A., Reyes-Meza V., Martínez-Gómez M., Rödel H.G., Hudson R. 2015. Contribution of within-litter interactions to individual differences in early postnatal growth in the domestic rabbit. Anim. Behav., 108, 145–153. DOI: 10.1016/j.anbehav.2015.07.028

Bessei W., Tinz J., Reiter K., 2001. Die Präferenz von Mastkaninchen für Kunststoffgitter und Tiefstreu bei unterschiedlichen Temperaturen. Proc. 12th Arbeitstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere, Celle. Germany, 133–140.

Bigler L., Oester H. 1996. Group housing for male rabbits. Proc. 6th World Rabbit Congress, Toulouse, France, Vol. 2, 411–415.

Blumetto O., Olivas I., Torres A.G., Villagrá A. 2010. Use of straw and wood shavings as nest material in primiparous does. World Rabbit Sci. 18, 237–242. DOI: 10.4995/wrs.2010.776

Brewer N.R., Cruise L.J. 1994. Physiology. In: Manning P.J., Ringler D.H., Newcomer C.E. (Eds.), The Biology of the Laboratory Rabbit. Academic Press, New York, 63–70.

Canali E., Ferrante V., Todeschini R., Verga M., Carenzi C. 1991. Rabbit nest construction and its relationship with litter development. Appl. Anim. Behav. Sci., 31, 259–266. DOI: 10.1016/0168-1591(91)90010-U

Cervera C., Fernández Carmona J. 2010. Nutrition and the climatic environment. In: de Blas C. and Wiseman J. (eds) Nutrition of the Rabbit, 2nd Edition, CAB International, Oxfordshire, 267–284.

Chu L., Garner J.P., Mench J.A. 2004. A behavioral comparison of New Zealand White rabbits (*Oryctolagus cuniculus*) housed individually or in pairs in conventional laboratory cages. Appl. Anim. Behav. Sci., 85, 121–139. DOI: 10.1016/j.applanim.2003.09.011

Costantini F., Panella F., Castellini C. 1986. Management of rabbit breeding. Rivista di Coniglicoltura, 23, 44–46.

Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes (OJ L 221, 8.8.1998, p. 23).

Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens (OJ L 203, 3.8.1999, p. 53).

Council Directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production (OJ L 182, 12.7.2007, p. 19).

Council Directive 2008/119/EC of 18 December 2008 laying down minimum standards for the protection of calves (OJ L 10, 11.1.2009, p. 7).

Council Directive 2008/120/EC of 18 December 2008 laying down minimum standards for the protection of pigs (OJ L 47, 18.2.2009, p. 5).

Coureaud G., Schaal B., Orgeur P., Coudert P. 1998. Le controle de l'acces au nid chez la lapine: conséquences sur la mortalité des lapereaux. Proc. 7émes Journ. Rech. Cunicole, France, Lyon, 245–249.

Cowan, D.P.,1987. Groupliving in the European rabbit (*Oryctolagus cuniculus*): mutual benefit or resource localization? J. Anim. Ecol., 5, 779–795. DOI: 10.2307/4948

Dal Bosco A., Castellini C., Bernardini M. 2000. Productive performance and carcass and meat characteristics of cage- or pen-raised rabbits. World Rabbit Sci. 8, Suppl. A, 579–583.

Dal Bosco A., Castellini C., Mugnai D, 2002. Rearing rabbits on a wire net floor or straw litter: behaviour, growth and meat quality traits. Livest. Prod. Sci., 75, 149–156. DOI: 10.1016/S0301-6226(01)00307-4

De Jong I.C., Reimert H., Rommers J.M.. 2008. Effect of floor type on footpad injuries in does: a pilot study. Proc. 9*th* World Rabbit Congress, Verona, Italy, 1171–1175.

Díez C., Pérez J.A., Prieto R., Alonso M.E., Olmedo J.A. 2005. Activity patterns of wild rabbit (*Oryctolagus Cuniculus*, L.1758), under semi-freedom conditions, during autumn and winter. Wildl. Biol. Pract., 1, 41–46. DOI: 10.2461/wbp.2005.1.6

Ebensperger L.A., Wallem P.K. 2002. Grouping increased the ability of the social rodent, Octodon degus, to detect predators when using exposed microbhabitats. Oikos, 98, 491–497. DOI: 10.1034/j.1600-0706.2002.980313.x

EFSA, 2005. The impact of the current housing and husbandry systems on the health and welfare of farmed domestic rabbits. The EFSA Journal, 267, 1–140.

Farkas T. P., Szendrő Zs., Matics Zs., Radnai I., Nagy I., Gerencsér Zs. 2017. Preference of rabbit does among different nest materials. World Rabbit Sci., 26, 81–90. DOI: *10.4995/wrs.2018.7373*

Farm Animal Welfare Council (FAWC) 1992. FAWC updates the five freedoms. Vet. Rec., 131, 357

Gerencsér Zs., Szendrő K., Szendrő Zs., Odermatt M., Radnai I., Nagy I., Dal Bosco A., Matics Zs. 2014. Effect of floor type on behavior and productive performance of growing rabbits. Livest. Sci., 165, 114–119. DOI: *10.1016/j.livsci.2014.04.022*

González-Mariscal, G., Rosenblatt, J. S. 1996. Maternal behavior in rabbits: A historical and multidisciplinary perspective. Adv. Study Behav., 25, 333–359. DOI: *10.1016/S0065-3454(08)60337-X*

González-Mariscal G., Díaz-Sánchez V., Melo A.I., Beyer C., Rosenblatt J.S. 1994. Maternal behavior in New Zealand white rabbits: Quantification of somatic events, motor patterns, and steroid plasma levels. Physiol. Behav., 55, 1081–1089. DOI: *10.1016/0031-9384(94)90391-3*

González-Mariscal G., Melo I.A., Jimenez P., Beyer C., Rosenblatt S.J. 1996. Estradiol, progesterone, and prolactin regulate maternal nest-building in rabbits. J. Neuroendocrinol., 8, 901–907. DOI: *10.1111/j.1365-2826.1996.tb00818.x*

González-Mariscal G., Burt A.S., Nowak R. 2017. Behavioral and neuroendocrine indicators of well-being in farm and laboratory mammals. In: Pfaff D.W., Joëls M. (eds): Hormones, Brain and Behavior (Third Edition*)*. Elsevier Inc., Amsterdam, 453–485.

Graf S., Bigler L.M., Failing K., Würbel H., Buchwalder T. 2011. Regrouping rabbit does in a familiar or novel pen: Effects on agonistic behaviour, injuries and core body temperature. Appl. Anim. Behav. Sci., 135, 121–127. DOI: *10.1016/j.applanim.2011.10.009*

Gunn D., Morton D.B. 1995. Inventory of the behaviour of New Zealand White rabbits in laboratory cages. Appl. Anim. Behav. Sci., 45, 277–292. DOI: *10.1016/0168-1591(95)00627-5*

Hayward J., Lisson P. 1978. Carbon dioxide tolerance of rabbits and its relation to burrow fumigation. Wildlife Res., 5, 253–261. DOI: *10.1071/WR9780253*

Held S.D.E., Turner R.J., Wootton R.J. 1995. Choice of laboratory rabbits for individual or group-housing. Appl. Anim. Behav. Sci., 46, 81–91. DOI: *10.1016/0168-1591(95)00632-X*

Holst D. von, Hutzelmeyer H., Kaetze P., Khashei M., Schönheiter R. 1999. Social rank, stress, fitness and life expectancy in wild rabbits. Naturwissenschaften, 86, 388–393. DOI: *10.1007/s001140050638*

Holst D. von, Hutzelmeyer H., Kaetze, P., Khashei M., Rödel H.G., Schrutka H. 2002. Social rank, fecundity and life time reproductive success in wild European rabbits (*Oryctolagus cuniculus*). Behav. Ecol. Sociobiol., 51, 245–254. DOI*: 10.1007/s00265-001-0427-1*

Hoy St., Lange K. 1997. Ergebnisse kontinuierlicher Ammoniak- und Kohlendioxidmessungen in der Kaninchenhaltung. Proc. 10. Arbeitstagung über Haltung und Krankheiten der Kaninchen, Pelztiere und Heimtiere. Celle, Germany, 298–303.

Hoy St., Selzer D. 2002. Frequency and time of nursing in wild and domestic rabbits housed outdoors in free range. World Rabbit Sci., 10, 77–84. DOI: *10.4995/wrs.2002.479*

Hoy St., Seitz K., Selzer D., Schüddemage M. 2000. Nursing behaviour of domesticated and wild rabbit does under different keeping conditions. Proc. 7th World Rabbit Congress, Valencia, Spain, 537–543.

Hoy St., Matics Zs. 2016. Alternative housing systems for rabbit does. Proc. 11th World Rabbit Congress, Qingdao, China, 637–651.

Hoy St. ed. 2006. Housing of rabbits in conformity with animal welfare and protection criteria. In: Maertens L., Coudert P. Recent advances in rabbit sciences. ILVO, Melle, Belgium, 69–130.

Hudson R., Distel H. 1982. The pattern of behaviour of rabbit pups in the nest. Behaviour, 79, 255–271. DOI: *10.1163/156853982X00292*

Hudson R., Schaal B., Martínez-Gómez M., Distel H. 2000. Mother-young relations in the European rabbit: physiological and behavioural locks and keys. World Rabbit Sci., 8, 85–90. DOI: *10.4995/wrs.2000.424*

Hull D, Hull J, Vinter J. 1986. The preferred environmental temperature of newborn rabbits. Biol. Neonate, 50, 323–330. DOI: *10.1159/000242616*

Jekkel G., Milisits G., Biró-Németh E., Radnai I., Matics Zs., Princz Z., Gerencsér Zs. 2008. Comparison on the slaughter characteristics of growing rabbits reared on wire net or combined (wire net/straw) floor. Proc. 9th World Rabbit Congress, Verona, Italy, 1365–1369.

König, B. 1997. Cooperative care of young in mammals. Naturwissenschaften, 84, 95–104. DOI: *10.1007/s001140050356*

Kustos K., Tóbiás G., Kovács D., Eiben Cs., Szendrő Zs. 2003. A telepítési sűrűség, a padozat és a takarmányozás hatása a növendéknyulak termelésére. 15. Nyúltenyésztési Tudományos Nap, Kaposvár, 123–128.

Lambertini L., Vignola G., Zagnini G. 2001. Alternative pen housing system for fattening rabbits: Effect of density and litter. World Rabbit Sci., 9, 141–147. DOI: *10.4995/wrs.2001.457*

Lebas F., Coudert P., de Rochambeau H., Thébault R.G. 1997. The Rabbit. Husbandry, health and production. FAO Animal Production and Health. Rome, Series No 21.

Lloyd H.G., McCowan D. 1968. Some observations on the breeding burrows of the wild rabbit *Oryctolagus cuniculus* on the island of Skokholm. J. Zool. (London), 156, 540–549. DOI: *10.1111/j.1469-7998.1968.tb04376.x*

Lombardini L., Fernández N., Moreno S., Villafuert R. 2003. Habitat-related differences in rabbit (*Oryctolagus cuniculus*) abundance, distribution, and activity. J. Mammal., 84, 26–36. DOI: *10.1644/1545-1542(2003)084<0026:HRDIRO>2.0.CO;2*

Lukefahr S.D., Ruiz-Feria C.A. 2003. Rabbit growth performance in a subtropical and semi-arid environment: effects of fur clipping, ear length, and body temperature. Livest. Res. Rural Develop., 15, 1–10.

Maertens L., Buijs S. 2015. Production performances of semi-group housed rabbit does. Proc. 19th International Symposium on Housing and Diseases of Rabbits, Furproviding Animals and Pet Animals, Celle, Germany, 22–31.

Maertens L., De Groote G. 1984. Influence of the number of fryer rabbits per cage on their performance. J. Appl. Rabbit Res., 7, 151–155.

Maertens, L., De Groote, G., 1985. L'influence de la densité d'occupation sur les résultats d'engraissement des lapins de chair. Rev. Agric., 38, 463–471.

Maertens L., Van Oeckel M.J. 2001. Effet du logement en cage on en parc et de son enrichment sur les performances et la couleur de la viande des lapins. Proc. 9émes Journ. Rech. Cunicole, Paris, France, 31–34.

Maertens L., Rommers J., Jacquet M. 2011. Le logement des lapins en parcs, une alternative pour les cages classiques dans un système "duo"? Proc. 14èmes Journ. Rech. Cunicole, Le Mans, France, 85–88.

Martrenchar A., Boilletot E., Cotte J.P., Morisse J.P. 2001. Wire-floor pens as an alternative to metallic cages in fattening rabbits: influence on some welfare traits. Anim. Welfare, 10, 153–161.

Masthoff T., Lang C., Hoy St. 2016. Effect of group size on fattening performance and of various types of slatted floor on dirtiness and occurrence of pododermatitis in growing rabbits. Proc. 11th World Rabbit Congress, Qingdao, China, 715–718.

Mikó A., Matics Zs., Gerencsér Zs., Odermatt M., Radnai I., Nagy I., Szendrő K., Szendrő Zs. 2014. Performance and welfare of rabbit does in various caging systems. Animal, 8, 1146–1152. *DOI: 10.1017/s1751731114001244*

Mirabito L., Buthon L., Cialdi G., Galliot P., Souchet C. 1999. Effect du logement des lapines en cages rehausse´ es avec plat-forme: Premiers re´ sultats. Proc. 8émes Journ. Rech. Cunicole, Paris, France, 67–70.

Mirabito L., Galliot P., Souchet C. 2004. Effet de la surface disponible et de l'ame´nagement des cages sur les performances zootechniques et le comportement des lapines et des jeunes. Proc. Journ. Nationale de l'élevage du lapin de chair, 40–52.

Mirabito L., Galliot P., Souchet C., Dumont F., Thomeret F. 2005. Logement collectif des lapines reproductrices: Conséquences zootechniques. Proc. 11émes Journ. Rech. Cunicole, Paris, France, 53–56.

Morisse J.P., Boilletot E., Martrenchar, A. 1999. Preference testing in intensively kept meat production rabbits for straw on wire grid floor. Appl. Anim. Behav. Sci., 64, 71–80. DOI: *10.1016/S0168-1591(99)00023-4*

Negatu Z., McNitt J. I. 2002. Hormone profiles and nest-building behavior during the periparturient period in rabbit does. Anim. Reprod. Sci., 72, 125–135. DOI: *10.1016/S0378-4320(02)00070-2*

Orova Z. 2008. Személyes közlés

Orova Z., Szendrő Zs., Matics Zs., Radnai I., Biró-Németh E., 2004. Free choice of growing rabbits between deep litter and wire net floor in pens. Proc. 8th World Rabbit Congress, Puebla City, Mexico, 1263–1265.

Pizzi F., Crimella C. 1985. Allattamento controlato in coniglicultura. Influenze sugli asorescimenti ed incidi conversione alimentara dallo svezzamento all eta di macellezione. Atti-della-Societa Italiana-delle-Scienze Veterinarie, 39, 467–470.

Podberscek A.L., Blackshaw J.K., Beattie A.W. 1991. The behaviour of group penned and individually caged laboratory rabbits. Appl. Anim. Behav. Sci., 28, 353–363. DOI: *10.1016/0168-1591(91)90167-V*

Princz Z., Orova Z., Nagy I., Jordan D., Štuhec I, Luzi F., Verga M., Szendrő Zs. 2007. Application of gnawing sticks in rabbit housing. World Rabbit Sci., 15, 29–36. DOI: *10.4995/wrs.2007.607*

Princz Z., Nagy I., Biró-Németh E., Matics Zs., Szendrő Zs. 2008a. Effect of gnawing sticks on the welfare of growing rabbits. Proc. 9th World Rabbit Congress, Verona, Italy, 1221–1224.

Princz Z., Dalle Zotte A., Radnai I., Biró-Németh E., Matics Zs., Gerencsér Zs., Nagy I., Szendrő Zs. 2008b. Behaviour of growing rabbits under various housing conditions. Appl. Anim. Behav. Sci., 111, 342–356. DOI: *10.1016/j.applanim.2007.06.013*

Princz Z., Radnai I., Biróné Németh E., Matics Zs., Gerencsér Zs., Nagy I., Szendrő Zs. 2008c. Effect of cage height on the welfare of growing rabbits. Appl. Anim. Behav. Sci., 114, 284–295. DOI: *10.1016/j.applanim.2008.01.006*

Princz Z., Dalle Zotte A., Metzger Sz., Radnai I., Biró-Németh E., Orova Z., Szendrő Zs. 2009. Response of fattening rabbits reared under different housing conditions. 1. Live performance and health status. Livest. Sci., 121, 86–91. DOI: *10.1016/j.livsci.2008.05.018*

Prud'hon M., Chérubin M., Goussupoulos J., Charles Y. 1975. Évolution, aucours de la croissance, des caractéristiques de la consommation d'aliments solide et liquide du lapin domestique nourri ad libitum. Ann. Zootech., 24, 289–298.

Rommers J.M., Jong de I.C. 2011. Technical note: Plastic mats prevent footpad injuries in rabbit doe. World Rabbit Sci., 19, 233–237. DOI: *10.4995/wrs.2011.868*

Rommers J.M., Meijerhof R. 1998. La dimensions de la cage influence-t-elle la productivitè et la bien-èntre des lapins. *Cuniculture, 25, 67–72.*

Rommers J.M., Boiti C., de Jong I., Brecchia G. 2006. Performance and behaviour of rabbit does in a group-housing system with natural mating or artificial insemination. Reprod. Nutr. Dev., 46, 677–687. DOI: 10.1051/rnd:2006038

Rommers J.M., Gunnink H., Klop A., de Jong I.C. 2011. Dynamics in aggressive behaviour of rabbit does in a group-housing system: a descriptive study. Proc. 17th International Symposium on Housing and Diseases of Rabbits, Fur Providing Animals and Pet Animals, Celle, Germany, 75–85.

Rommers J.M., Gunnink H., de Jong I.C. 2013. Effect of different types of places on aggression among does in a group-housing system: A pilot study. Proc. 18th International Symposium on Housing and Diseases of Rabbits, Fur Providing Animals and Pet Animals, Celle, Germany, pp. 59–68.

Rosell J.M., de la Fuente L.F. 2009. Effect of footrests on the incidence of ulcerative pododermatitis in domestic rabbit does. Anim. Welfare, 18, 199–204.

Rödel H.G., Bora A., Kaiser J., Kaetzke P., Khaschei M., von Holst D. 2004. Density-dependent reproduction in the European rabbit: a consequence of individual response and age-dependent reproductive performance. Oikos, 104, 529–539. DOI: 10.1111/j.0030-1299.2004.12691.x

Rödel G.H., Starkloff A., Bautista A., Friderich A.C., von Holst D. 2008. Infanticide and maternal offspring defence in European wild rabbits under natural breeding condition. Ethology, 114, 22–31. DOI: 10.1111/j.1439-0310.2007.01447.x

Schlolaut W. 1998. Das große Buch vom Kaninchen. DLGVerlag, Frankfurt am Main.

Southern H.N. 1948. Sexual and aggressive behaviour of the wild rabbit. Behaviour, 1, 173–194. *DOI: 10.1163/156853948x00092*

Szendrő Zs., Dalle Zotte A. 2011. Effect of housing conditions on production and behaviour of growing meat rabbits: A review. Livest. Sci., 137, 296–303. DOI: 10.1016/j.livsci.2010.11.012

Szendrő Zs., McNitt I.J. 2012. Housing of rabbit does: Group and individual systems: A review. Livest. Sci., 150, 1–10. DOI: 10.1016/j.livsci.2012.09.017

Szendrő Zs., Gyarmati T., Lévai A., Radnai I., Biróné Németh E., Milisits G. 1999. Comparison of once-daily, free and combined forms of suckling in rabbits. Acta Agr. Kapos., 3, 155–163.

Szendrő Zs., Princz Z., Romvári R., Locsmándi L., Szabó A., Bázár Gy., Radnai I., Biró-Németh E., Matics Zs., Nagy I. 2009. Effect of group size and stocking density on productive, carcass and meat quality traits and aggression of growing rabbits. World Rabbit Sci., 17, 153–162. DOI: 10.4995/wrs.2009.655

Szendrő Zs., Matics Zs., Odermatt M., Gerencsér Zs., Nagy I., Szendrő K., Dalle Zotte A. 2011. Use of different areas of pen by growing rabbits depending on the elevated platforms' floor-type. Animal, 6, 650–655. DOI: 10.1017/S1751731111001819

Szendrő Zs., Mikó A., Odermatt M., Gerencsér Zs., Radnai I., Dezséry B., Garai É., Nagy I., Szendrő K., Matics Zs. 2013. Comparison of performance and welfare of single-caged and group-housed rabbit does. Animal, 7, 463–468. DOI: 10.1017/s1751731112001760

Szendrő Zs., McNitt I.J., Matics Zs., Mikó A., Gerencsér Zs. 2016. Alternative and enriched housing systems for breeding does: A review. World Rabbit Sci., 24, 1–14. DOI: 10.4995/wrs.2016.3801

Szendrő Zs., Trocino A. , Hoy St., Xiccato G., Villagrá A., Szendrő K., Maertens L. 2019. A review of recent research outcomes about the housing of farmed domestic rabbits: Reproducing does. Wold Rabbit Sci., 27, 1–14. DOI: 10.4995/wrs.2019.10599

Trocino A., Xiccato G. 2006. Animal welfare in reared rabbits: A review with emphasis on housing systems. World Rabbit Sci., 14, 77–93. DOI: 10.4995/wrs.2006.553

Trocino A., Xiccato G., Majolini D., Fragkiadakis M. 2008. Effect of cage floor and stocking density on growth performance and welfare of group-housed rabbits. Proc. 9th World Rabbit Congress, Verona, Italy, 1251–1255.

Turner V.P., Buijs S., Rommers J., Tessier M. 2017. Code of practice for the care and handling of rabbits: Review of scientific research on priority issues. National Farm Animal Care Committee, Québec, Canada

Verga M., Zingarelli I., Heinzl E., Ferrante V., Martino P.A., Luzi F. 2004. Effect of housing and environmental enrichment on performance and behaviour in fattening rabbits. Proc. 8th World Rabbit Congress, Puebla, Mexico, 1283–1288.

Verga M., Luzi, F., Carenzi C. 2007. Effects of husbandry and management systems on physiology and behaviour of farmed and laboratory rabbits. Horm. Behav., 52, 122–129. DOI: *10.1016/j.yhbeh.2007.03.024*

Verga M., Luzi F., Petracci M., Cavani C. 2009. Welfare aspects in rabbit rearing and transport. Ital. J. Anim. Sci., 8, Suppl. 1, 191–204. DOI: *10.4081/ijas.2009.s1.191*

Villafuerte R., Moreno S. 1997. Predation risk, cover type, and group size in European rabbits in Donana (SW Spain). Acta Theriol., 42, 225–230. DOI: *10.4098/AT.arch.97-23*

Villafuerte E.R., Kufner M.B., Delibes M., Moreno S. 1993. Environmental factors influencing the seasonal daily activity of European rabbit (*Oryctolagus cuniculus*) in a Mediterranean area. Mammalia, 57, 341–347. DOI: *10.1515/mamm.1993.57.3.341*

Xiccato G., Verga M., Trocino A., Ferrante V., Queaque P.I., Sartori A. 1999. Influence de l'effectif et de la densité par cage sur les performances productives, la qualité bouchère et le comportement chez le lapin. Proc. 8émes Jour. Rech. Cunicole, Paris, France, 59–62.

Xiccato G., Trocino G., Majolini D., Tazzoli M., Zuffellato A. 2013. Housing of growing rabbits in individual, bicellular and collective cages: growth performance, carcass traits and meat quality. Animal, 7, 627–632. DOI: *10.1017/S175173111200198X*

Zarrow M.X., Farooq A., Denenberg V.H., Sawin P.B., Ross S. 1963. Maternal behaviour in the rabbit: endocrine control of maternal nest building. J. Reprod. Fertil., 6, 375–383.

Zarrow M.X., Denenberg V.H., Anderson C.O. 1965. Rabbit: frequency of suckling in the pup. Science, 150, 1835–1836.

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Historical overview of the selection indices applied in pig breeding

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ABSTRACT - Authors summarized the different selective animal breeding methods used in the pig breeding sector in the last few decades. Advantages and disadvantages of the conventional selection index procedures were summarized. The superiority of BLUP breeding values over the mass selection procedure was clarified. The economic aspects of the different traits were also examined and the most up to date economic BLUP indices were introduced as well.

Keywords: pig, selection index, blup, economic methodology

INTRODUCTION

Selective animal breeding is the artificial process where specific traits are improved by choosing superior males and females in order to create offspring generation (*Oldenbroek and Waaij, 2014*). Different methods of selection have been used in the pig industry throughout the history. The general methods of selection are: random selection, tandem selection, independent culling levels, total score method (index selection), selection index, estimated breeding value (EBV), expected progeny difference (EPD), best linear unbiased prediction (BLUP). Selection based on a selection index is the most commonly used method in genetic improvement programs for pigs (*Stas, 2017*). In pig industry measuring the important traits are accomplished through two types of performance tests which are station test and field test, respectively (*Csató et al., 2002*). Station test is more precise and accurate but currently field test have been becoming more and more frequent. The advantage of such an assessment, compared with the use of stations test, is that the assessment is a significantly cheaper. After collecting all required information measurements of different traits are combined to one score called selection index. Thus breeders can perform selection simultaneously for several traits. Structure and form of indices can differ among countries (*McPhee, 1981; Morris et al., 1982; Visser, 2004; Nagy et al., 2008; Csató et al., 2002*) based on the varying interest in the breeding objective.

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Conventional Selection index

Index selection is a method where values of all traits of the selection criteria are combined into a single index value (*Sangsuriya et al., 2002*). Throughout many generations and centuries selective breeding of domestic animals and plants were based on the phenotypic evaluation of the individuals. Smith introduced the so called index selection in 1936 (*Kang, 2002*). The index is a numerical expression of the genetic merit of a plant or animal for its further use as a parent for the production of a new generation. Smith presented an index of the form:

$$I = b_1x_1 + b_2x_2 + \ldots b_mx_m$$

Where I is an index of merit of an individual and $b_1 \ldots b_m$ are weights assigned to phenotypic trait measurements represented as $x_1 \ldots x_m$.. The selection index was first used for selection among inbred lines of a self-pollinated plant species (*Kang, 2002*).

Jay L. Lush who is known as the father of modern animal breeding published his work in the book called 'Animal Breeding Plans' in 1937, in this book he advocated that instead of subjective appearance, animal breeding should be based on a combination of quantitative statistics and genetic information. By this publication he greatly influenced animal breeding around the world scientists, one of them was Hazel (*Robinson, 1991*). In 1943 Hazel adapted methods originally used in plant production (*Graser et al., 2006*).

The main problem of Hazel with constructing an index was how to combine information in an optimal way on different individuals **Y** into a single number I on which selection can be based. Hazel chosen a linear approach (*Weaber, 2005*):

$$I=b' (y-\hat{y})$$

Where b' is a column vector of weightings which need to be calculated and y a row vector of observations. Note that in this very general form the vector y can include single observations on one trait from different animals, or single observations on different traits of one or more animals, as well as means of groups, e.g. mean of all progeny (*Weaber, 2005*). The Hazel selection index (*De Vries, 1989*) defines economic merit as:

$$H_i = a_1 BV_{i1} + a_2 BV_{i2} + \ldots + a_n BV_{in}$$

where,

H_i = the aggregate economic merit of an animal, i, as a parent,

a_j = the relative economic weight of trait j, j = 1…n, where n = the total number of traits
BV_{ij} = the breeding value of animal i for trait j.

During last 80 years since it has been introduced the theory of index selection has been improved. In general, before constructing a selection index, its purpose needs to be determined. The next step is to identify the traits involved in the breeding goal, then economic importance of every trait has to be calculated. The problem with conventional selection index is that unless the phenotypic measurements are pre-corrected by the influential environmental factors, these factors can highly modify the results. Unfortunately apart from conventional selection of body weight indices do not use data correction (Csató et al., 2002). Besides, conventional index methodology is based on the phenotype rather than the genetic merit of the animals in the different traits.

BLUP METHODOLOGY

Best Linear Unbiased Prediction, or BLUP, is a technique for estimating genetic merits. In general, it is a method of estimating random effects. This is a method of selection and genetic evaluation of animals. It was created and development by scientist Henderson in the 1950s but because of complexity of mathematical calculations and the computer power was too limited to be able to calculate the breeding values using the animal model, the practical implementation thus had to wait until the later 1980's. As we mentioned previously the so called estimated breeding value was developed also by Henderson, it has given possibilities to breeders to rank the animals according to their estimated genetic potential, which resulted in more accurate selection results and thus a faster genetic improvement through generations (*Robinson, 1991*).

The Estimated Breeding Value (EBV) provides an estimate of the genetic potential of the animal which is expressed relative to the population average. The true breeding value (TBV) is the real value of the animal for breeding. The perfect EBV would be equal the TBV. The EBV provides the BEST estimate of the breeding value of an animal. The accuracy indicates the risk of a difference between EBV and TBV, where the TBV may be higher or lower with equal probability (*Oldenbroek and van der Waaij, 2014*). The correlation between the true breeding value and the predicted breeding value is maximized and estimates realized values for a random variable using unbiased statistical methods. (*Stas, 2017*).

If we compare effectiveness of both methods in practice based on done research, it is possible to make a conclusion that BLUP methodology more appropriate than conventional selection index based exclusively on phenotype. Response would be greater from selection using BLUP than from selection using conventional index (*Keele et al., 1988*). The BLUP evaluation officially was introduced in Hungary in 2007 and quickly replaced the conventional indices, and pig breeding companies conducting breeding also started to pay more attention to progeny tests based on slaughter-house data than they had done previously (*Houška et al., 2010*). In literature review of thesis work of *Stas (2017)* was reviewed experiment of comparison selection based on phenotype performance (conventional index) with selection based on best linear unbiased prediction (BLUP) of breeding value selection for one trait of interest at varying levels of heritability. Genetic improvement was greater for BLUP selection compared to conventional index selection; but, with increasing of heritability, the difference between the two methods decreased. Selection based on BLUP had a relative advantage compared to conventional selection index by 55% for traits of low heritability and by 10% for traits of moderate heritability. The level of inbreeding increased faster with selection based on BLUP. Nevertheless, selection based on BLUP will help to improve selection accuracy and efficiency (*Stas, 2017*).

BLUP advantages are as follows (*Csató et al., 2002*):

- the most accurate division of criteria that determine the productivity of the animal: the impact of the environment; genetics (heredity)
- the possibility of simultaneous comparison of parameters that were obtained in different environmental conditions from different genotypes, as well as from animals of different generations;
- mathematically accurate records of all documented family ties
- Adjustment of all values of breeding value in relation to each other (for example accounting for genetic competition and the level of mating)
- very high accuracy of tribal assessment, which allows achieving high selection efficiency
- More accurate prediction of breeding values through the use of information on all the relationships
- More accurate comparison of animals at different times or under different management systems by correcting environmental factors
- More direct comparison among animals by using different levels of relevant information and/or by allowing comparisons across different generations

However, it is also necessary to mention also the limits of using BLUP in the Hungarian pig sector. There is no doubt that BLUP simultaneously corrects the phenotypes for systematic effects, and it estimates breeding values while making use of the additive genetic relationships between the animals with the help of matrix algebra. But there is a critical issue in correcting for systematic effects. It only works well if genotypes are sufficiently spread across systematic environmental influences (*Oldenbroek and van der Waaij, 2014*). That is not the case in Hungarian pig breeding sector yet. There are no sufficient genetic links between Hungarian breeding farms, because of the lack of AI with the semen of the same sires used in many farms, and because breeding animals are rarely brought to various breeding farm locations, they are mainly sold to commercial farms. So the lack of exchange of animals between farms results in poor genetic links between farms and often it is not possible to estimate systematic farm effects accurately.

As mentioned previously one of the main goal which is possible to achieve by BLUP is to quantify and eliminate the influence of the environmental factors. Because different genotype in different environment can give different feedback of productivity, this is called genotype environment interaction. In different literature sources different classifications of genotype x environment interaction exist. For example *Merks (1986)* defined as a change in relative performance of two or more genotypes measured in two or more environments. James (2009) classified interactions as being either of rank-type or of scale-type. He defined rank-type interactions as those in which genotype 1 may be superior over genotype 2 in the first environment, however, the reverse may be true when tested in the second environment. *Merks (1986)* made a similar classification for rank-type interactions. In addition to the rank-type classification, *James (2009)* defined scale-type interactions as those in which the differences between genotypes change in magnitude, but not in sign, with changes in environment. The implications of these interactions have to be considered when developing a breeding program. Although the magnitude of the performance differences may change with a scale-type interaction, the ranking of the genotypes for performance will stay the same; however, with rank-type interactions, the ranking of the genotypes for performance will change with a change in environment. Therefore, rank-type interactions are potentially of more practical importance.

ECONOMIC METHODOLOGY

After choosing all of appropriate traits and performing the procedures related to construction of selection index, breeders meet yet another problem of the economic value that will be more beneficial for certain traits compared to other traits. For this purposes were created the so called economic weights. However, when constructing an index based on economic values, only one of the traits may be included in the index in order to avoid double counting and recovering one by another for example such traits as lean meat content and back fat thickness (*Houška et al., 2010*).

Different approaches are available for construction and calculation economic weights, it depends on many factors. First citation that we found in literature describing methodology of calculating economic weights dates back to 1966. Authors Moav and Moav proposing idea about using profit equations to integrate the cost and returns of a production system to compare the profitability of lines and crosses. Main idea was that equations could be non-linear and outcome then depends on the levels of performance for the different traits. In 1973 Moav used the profit equation, and the economic weights derived from the interests on national level or producers (*Brascamp et al., 1985., Moav and Moav, 1966*)

The economic value of an individual in animal husbandry is expressed by the profit. Breeder is a main person who makes strategy of development of future selection programs; usually such activity is carried out in four stages:

- monitoring of production conditions and situation in the product market;
- analysis of profit and production costs;
- definition of important features;
- calculation of economic weight coefficients.

Main task of breeders to decide on and choose from numerous traits based on their economic importance, which means trait or traits must be improved in accordance with the objective of market demand. Selection always concentrates on improving specific traits of animals, in our case pigs, to increase specific productivity of interest and together with this increase financial welfare of industry. In general, a selection index as a function of the predicted breeding values of economically relevant traits and marginal economic values is used for example in such countries like Czech Republic and Hungary. The marginal economic values of the traits are calculated as change in predicted profit, holding all other traits constant. As mentioned previously, by reviewing literature,

it is possible to make a conclusion that huge differences can be observed between countries, but also between different farms with different environment. Also can be observed a small effect of specific selection methods on reduction in production costs in different markets. Therefore, diversity in genetic lines of pigs is not directly needed, just need adaptation of lines to specific environments or farming system. This finding is in accordance with *Hanenberg et al. (2010), Stewart and Neal S.M (1999).*

Pig breeding programs generally focusing on improving traits that are responsible for production and reproduction because they are more clear and important. Of course they are short – term goals because always need to be focusing on market and customer demand and predicting future needs on time. But recently animal associations start to pay attention not only to economic importance but also to animal welfare (*Kanis et al., 2005*).

Breeders generally applying two approaches for calculating economic weights for pig breeding based on the models of *De Vries (1989)* or *Stewart et al. (1990)*. The first approach applies to an integrated commercial production system buying their female replacements from the superior production tier. The second approach relates to a commercial sow herd producing their own replacement gilts and selling weaned piglets to the growing–finishing enterprise (*Houška et al., 2004*).

On reviewing of two countries Hungary and Czech Republic we will show some examples of approaches to calculating marginal economic values, economic weights and traits that are used for these purposes.

In Hungary first estimation of economic values and marginal economic values for traits was done in 2010. In table number 1 we can see the final result of the calculation for specific traits.

Basis of constant number of sows was used for the calculation of economic values for Hungary and therefore expressions of economic values were done per sow per year. Model describing by *De Vries (1989)* was used as approaches for calculating. Based on the data we can observe that best marginal economic values were the number of piglets born alive but based on standardized economic values the percentage of valuable cuts in the carcass was most economically important trait (*Houška et al., 2010*).

As authors mentioned in their conclusion for calculating economic weight we just simply need multiplied breeding values by the marginal economic values and we can build economic selection index (*Houška et al., 2010*).

In the research of *Houska et al. (2004)* it was showed that the production system in Czech Republic is similar to the Hungarian and therefore the model

of *De Vries (1989)* was used for calculating. Marginal economic value and economic value were also calculated. But for calculating economic weights the so called discounted expressions which were multiplied by marginal economic values were used. The discounted gene flow is expressed as a number of cumulative discounted expressions (CDE), as a consequence of one mating; "cumulative" refers to an accumulation of expressions over generations or years; and "discounted" implies to the fact that future return is discounted to today's values by a discounting factor (*Jiang et al., 1999*).

Table 1
Marginal economic values (MEW, in EUR per unit of trait, per sow and year when improving the trait level), genetic standard deviation (GSD), standardized economic values (SEW, in EUR/GSD)

Trait (unit)	MEW	GSD	SEW
Number of piglets born alive (piglets)	54.22	0.61	33.07
Age at slaughter (days)	2.71	15.02	40.70
Days in fattening	2.84	9.91	28.14
Lean meat content in the carcass (%)	22.45	1.62	36.37
Percentage of valuable cuts in carcass (%)	28.81	2.55	73.46

CONCLUSION

Conventional selection index resulted huge benefit in past, animals were selected based on phenotypic variation. However after some period of time when the methodology reached its maximum potential new procedures became widespread.

Best Linear Unbiased Prediction (BLUP) is a method that substitutes conventional phenotypic measurements in the selection index. It is more precise and accurate in prediction genetic potential of animals, taking into consideration the relationships among the animal and the influencing environmental factors. BLUP allows comparing animals merit within different farm with different environment, which is impossible to do with conventional methods.

Economic methodology in constructing selection index is the method by which we evaluate the economic value of each trait and get so called economic weights. This coefficient can be used to calculate aggregated breeding value thus profit can be maximized in the procedure of selection.

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REFERENCES

Brascamp E.W., Smith C., and Guy D.R., 1985. Derivation of economic weights from profit equations. Anim. Sci., *40*(1), 175-179. DOI: 10.1017/s0003356100031986

Cameron N.D., 1997. Selection indices and prediction of genetic merit in animal breeding. CAB international. URL: Link

Csató L., Nagy I., Farkas J., and Radnóczi L., 2002. Genetic parameters of production traits of Hungarian Pig populations evaluated in separate and joint (field and station) tests. Archives Ani. Breed., *45*(4), 375-386. DOI: 10.5194/aab-45-375-2002

De Vries A.G., 1989. A model to estimate economic values of traits in pig breeding. Lives. Prod. Sci., *21*(1), 49-66. DOI: 10.1016/0301-6226(89)90020-1

Graser H., James I., and Van der Werf I., 2006. Optimal designs of breeding programs.

Hanenberg E., Mathur P.K., and Knol E.F., 2010. Marginal economic values for pig production in different countries. In 9th World congress on genetics applied to livestock production Leipzig, Germany.

Houška L., Wolfová M., and Fiedler J., 2004. Economic weights for production and reproduction traits of pigs in the Czech Republic. Lives. Prod. Sci., 85(2-3), 209-221. DOI: 10.1016/s0301-6226(03)00128-3

Houška L., Wolfová M., Nagy I., Csörnyei Z., and Komlósi I., 2010. Economic values for traits of pigs in Hungary. Czech Journal of Ani. Sci., *55*(4), 139-148. DOI: 10.17221/100/2009-cjas

James J.W., 2009. Genotype by environment interaction in farm animals. In Adaptation and Fitness in Animal Populations (pp. 151-167). Springer, Dordrecht. DOI: 10.1007/978-1-4020-9005-9_10

Jiang X., Groen A.F., and Brascamp E.W., 1999. Discounted expressions of traits in broiler breeding programs. Poul. Sci., 78(3), 307-316. DOI: 10.1093/ps/78.3.307

Kang M.S., (Ed.)., 2002. Quantitative genetics, genomics, and plant breeding. CABI.

Kanis E., De Greef K.H., Hiemstra A., and Van Arendonk J.A.M., 2005. Breeding for societally important traits in pigs. Journal of Ani. Sci., *83*(4), 948-957. DOI: 10.2527/2005.834948x

Keele J.W., Johnson R.K., Young L.D., and Socha T.E., 1988. Comparison of Methods of Predicting Breeding Values of Swine 1, 2. Journal of Ani. Sci., 66(12), 3040-3048. DOI: 10.2527/jas1988.66123040x

McPhee C.P. 1981. Selection for efficient lean growth in a pig herd. Australian Journal of Agr. Res., 32(4), 681-690. DOI: 10.1071/ar9810681

Merks J.W.M., 1986. Genotype and environment interactions in pig breeding programmes. I. Central test. Live. Prod. Science, *14*(4), 365-381. DOI: 10.1016/0301-6226(86)90015-1

Moav R., and Moav J., 1966. Profit in a broiler enterprise as a function of egg production of parent stocks and growth rate of their progeny. British Poul. Sci., *7*(1), 5-15. DOI: 10.1080/00071666608415600

Morris C.A., Goss B.M., and Wolskel R.H. 1982. Offspring-sire regressions for production traits of pigs in a West Australian selection herd. Australian Journal of Expe. Agric., *22*(115), 47-53. DOI: 10.1071/ea9820047

Nagy I., Csato L., Farkas J., Gyovai P., Radnoczi L., and Komlosi, I., 2008. Genetic parameters of direct and ratio traits from field and station tests of pigs. Archives Ani. Breed., *51*(2), 172-178. DOI: 10.5194/aab-51-172-2008

Oldenbroek K., and van der Waaij L., 2014. Textbook animal breeding: animal breeding and genetics for BSc students. Centre for Genetic Resources and Animal Breeding and Genomics Group, Wageningen University and Research Centre.

Robinson G.K., 1991. That BLUP is a good thing: the estimation of random effects. Stat. Sci., 15-32. DOI: *10.1214/ss/1177011933*

Sangsuriya P., Reodecha C., and Chavananikul V., 2002. Selection Index for Reproductive Traits in Purebred Swine.

Schüler L., Swalve H., and Götz K.U., 2001. Grundlagen der quantitativen Genetik*: 94 Tabellen*. Ulmer.

Stas N.M., 2017. Effect of sire line and selection index category on pig growth performance from weaning to harvest and carcass characterisitics (Doctoral dissertation).

Stewart T.S., and Neal S.M., 1999. Multiple trait selection for pork improvement. NSIF-FS10. URL: *Link*

Stewart T.S., Bache D.H., Harris D.L., Einstein M.E., Lofgren D.L., and Schinckel A.P., 1990. A bioeconomic profit function for swine production: application to developing optimal multitrait selection indexes 1. Journal of Ani. Breed. and Gen., 107(1-6), 340-350. DOI: *10.1111/j.1439-0388.1990.tb00043.x*

Visser D.P., 2004. Structuring of breeding objectives in the pork supply chain in South Africa (Doctoral dissertation, University of Pretoria). URL: *Link*

Weaber R.L.B., 2005. Introduction to Indexes. 37th BIF Annual Research Symposium and Meeting.

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Recent trends in fish feed ingredients – mini review

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ABSTRACT - This review has highlighted - without claiming to be exhaustive - the recent trends in fish feed ingredients, started with the problems of fish meal and fish oil. The possible alternatives were presented and evaluated as replacement of FM in finfish diets. Current alternative feed ingredients are complimentary. Feeds that include several ingredients are more balanced and reduced the ANFs of each separate ingredient, what leads to better growth rates and animal health. Currently, vegetable-based sources like soy are dominating issue of the fishmeal replacement. By high level of processing and using novel techniques the digestibility and nutrient availability can be enhanced. There is room, however, for certain novel ingredients, such as algae- and insect-based feeds to gain a larger share of feed ratios in aquaculture. Yeasts and their derivatives are used by choice as alternatives for antibiotics. Processing of wood raw materials into a protein-rich components are also very promising ingredients for fish feeds. Many times, we have to use pre- and probiotic compounds, feed additives to enhance the utilization, palatability of the alternative ingredients. Maintaining good health and welfare of fish is vital to be sustainable and cost effective.

Keywords: nutrition, alternative, ingredients, aquaculture

INTRODUCTION

Fish farming is commonly described as being extensive, semi-intensive or intensive. Continuous growing of aquaculture sector (around 8 % per year) led systems to be more and more intensive. In intensive farming, the fish are kept at high stocking density, that's why the fish are dependent on the feed provided. Last decades aquaculture became more and more effective, but it has not been without concern for natural resource use, environmental impact and social judgement. Increasing fishmeal (FM) cost, irregular supply, decreasing availability, and poor quality of FM have put forward highlighting on its partial or complete substitution with other alternative protein sources. The fish feed industry has to be very responsible about to use FM alternatives. Scientists have to share on this burden to achieve sustainable, cost-effective aims. Indicators of sustainability in aquaculture could be energy efficiency, use of water, nutrient utilisation efficiency and production costs. The climate change is also affecting the sector, such as the predicted changes in ocean circulation pattern might also have a negative influence on the reliability of small pelagic stocks that being utilized for FM production. Appearance of mycotoxins or other dis-

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eases on terrestrial plants can affect negatively the potential alternative ingredients. Due to lots of investment and research in feed formulation promotion of non-marine ingredients resulted that these goods can provide the adequate nutrients for fish to achieve successful growth and still remain healthy. Significant progress has been made over the past decade in reducing levels of FM in commercial feeds for farmed fish. Some obstacles associated with plant-based protein diets are: amino acid imbalances and deficiencies, high levels of indigestible carbohydrates present in certain grain products, and varying antinutritive factors (ANFs) that negatively affect fish growth and health. Animal protein ingredients are also shown as possible replacements.

In this study the recent trends in alternative fish feed ingredients had been reviewed shortly. In conclusion, complete replacement of fishmeal in fish feeds is more difficult and will require further research efforts to achieve the expected goals, but there are many auspicious alternatives, what are illustrated here.

History of fishmeal-based diets

Fish meal and oil are easily digestible products used in fertilizers (*López-Mosquera et al., 2011*) and animal feed, ranging from livestock to aquaculture. Fish meal is a coarsely ground powder made from the cooked flesh of fish (*Miles and Chapman, 2015*). Though formerly important as a fertilizer, fish meal is now primarily used in animal feed. Certain species of oily fish, such as menhaden (*Brevoortia and Ethmidium*), anchovy (*Engraulidae*), herring (*Clupeidae*) and pilchard (*Sardinae*) are the main source of FM and its companion product, fish oil.

The first fish feed factories were established and started to developin the end of the 19th century. In the 1940's, feed producers started to make complete semi-dry diet mixtures, what contained FM. They had problems with carry over disease contaminations, what effected fish stocks as well. Cooking extrusion is the most recent development in pelleted fish feed manufacture. These pellets are formed by extrusion of a moist mixture (20-24 %) followed by drying to reduce the moisture content to 10% or less. In the development of modern aquaculture, starting in the 1970s, FM and fish oil were key components of the feeds. These feeds are formulated nutritionally balanced to promote rapid growth, good health and welfare, according to the farmed species' needs.

Concerns are being raised about the negative consequences on world fishmeal production of overfishing, and negative effects caused by climate change (*Soliman et al., 2017*). Fish feed accounts could be over 50 % of the total fish

production cost (*Rana et al., 2009*). FM price in 2030 in real terms would increase by 29 % (Figure 1) (*OECD/FAO, 2017; The World Bank, 2013*). If aquaculture consumes wild fish in the form of fishmeal and fish oil at higher amounts than what is produced, then aquaculture is a net consumer of fish, not a net producer, what is not sustainable (*Hardy, 2010*). Currently, a lot of studies have been conducted to assess the partial or complete substitution of FM in fish feeds (*Kaushik et al., 1995; Montero et al., 2003 and 2005; Moutinho et al., 2017; Webster et al., 1992*).

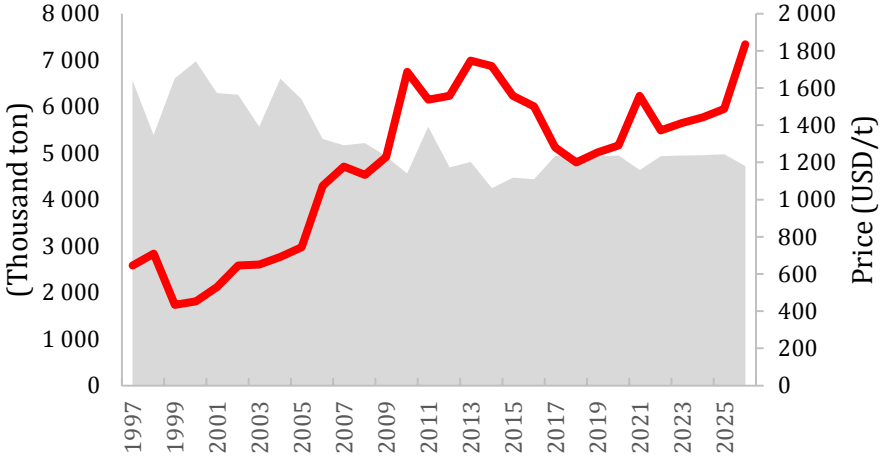

Figure 1. Prediction of worldwide Fish meal production
(Source: *OECD/FAO, 2017*)

Perspective fish feed alternative ingredients

Terrestrial plant products and by-products

The most important protein supplements of plant origin are the oilseed meals, produced from the cake remaining after oil has been extracted from soybeans, cottonseed, canola, peanuts, sunflower seeds (*Halver and Hardy, 2002*). Plant protein sources with a low degree of processing are inexpensive and readily available, but their use for carnivorous fish is limited by the presence of starch and structural carbohydrates, and a wide variety of antinutritional factors (ANFs) (*Øverland et al., 2009*). The most dominant ANFs in terrestrial plant products are protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins, allergens, tannins, gossypol, glucosinolates (*Francis et al., 2001*).

Currently soybean meal (SBM) is the most used alternative of fish meal due to its competitive nutrient composition, relatively good amino acid profile, easy accessibility and low price (*Gatlin et al., 2007*). However, SBM based diets causes in many fish species negative effects on digestibility, nutrient utilization, growth performance (*Booman et al., 2018; Kaushik et al., 1995; Urán et al., 2008; Zhang et al., 2018*). The ANFs in soybean meals caused enteritis in such cases (*Knudsen et al., 2008; Krogdahl et al., 2015; Marković et al., 2016; Nayak, 2010; Sørensen et al., 2011*). SBM has been the predominant form of soybean used and is available either as de-hulled (~ 48% crude protein) or with hulls added (~ 44% crude protein) (*NRC, 2001*).

Cottonseed meal (CSM) is a by-product of the cotton fiber and cottonseed oil industries. CSM has approximately 41.7 % crude protein content and this is the third leading seed by weight used (*Gatlin et al., 2007*). However, the major problem associated with CSM use is the toxicity of the gossypol (*Rinchard et al., 2002*). On the other hand there are other concerns of CSM as a protein source, because of its low levels of lysine and methionine, and high crude fiber level (*Cheng and Hardy, 2002b*). In case of channel catfish (*Ictalurus punctatus*) researchers reported that gossypol is a strong natural antioxidant and had received much attention due to its biological activities, such as it improved immune responses and disease resistance (*Yildirim et al., 2003*).

Canola and rapeseed are both names on the plants *Brassica napus* and *Brassica campestris*. Canola meals, resulting from oil extraction processes, contains about 35% crude protein and 12% crude fiber (*Sørensen et al., 2011*). Because of a relatively high content of crude fiber and phytate, canola has a limited use for carnivorous fish (*Drew et al., 2007*). The use of rapeseed meal as an animal feed is limited by the presence of ANFs (*Davies et al., 1990*).

Peanut meal (PM) is a by-product obtained from oil extraction of the whole or broken peanut seeds and it has variable chemical composition with an average content of 45.6 % crude protein (*Batal, et al., 2005*). For many fish species PM is deficient in lysine (*Lim, 1997*). Peanuts often contaminated with the fungus, *Aspergillis flavis*, which produces aflatoxin (*Bezerra da Rocha et al., 2014; Marroquín-Cardona et al., 2014; Richard, 2007*).

Sunflower meal (SFM) is produced from the oil cake after oil extraction from dehulled sunflower seed. SFM is highly palatable for fish and it has low antinutritional factors (*Sørensen et al., 2011*). It has low levels of lysine and it has high levels of fibre (18–23%) and lignin (*Mérida et al., 2010*). Sunflower meal showed good digestive utilization of protein, even though the digestible energy was low due to the carbohydrate fraction (*Sanz et al., 1994*).

DDGS (Dried Distiller's Grain with Solubles), a by-product from bioethanol production, has high amounts of energy, medium protein (~30%), digestible fibre and accessible phosphorous enable the preparation of sustainable fish feeds with a high nutritional value (*Gatlin et al., 2007*). Moreover, DDGS has an additional advantage over other plant feed ingredients, namely its lack of ANFs (*Makkar, 2012*).

Protein concentrates from various sources such as soybean, pea, rapeseed, sunflower, as well as corn and wheat gluten can be competitive alternatives of fish meal (*Collins et al., 2013; Escaffre et al., 1997; Øverland et al., 2009; Torstensen et al., 2011; Thiessen et al., 2004; Wu et al; 1995*). These advanced products have reduced ANF content, increased digestibility than their origin plant.

Micro and macro algae

Algae can be differentiated (by the body size and structure) either to microalgae (with algal bodies that need microscope to be observed) or to macroalgae (large enough to be seen with unaided eye) (*Yakoob et al., 2011*). Microalgae can grow in both fresh and marine water as well as in almost every environmental condition on earth from frozen lands of Scandinavia to hot desert soils of the Sahara (*Safi et al., 2014*). Algae have been considered potentially viable alternative feed ingredients for aquaculture. Macro and micro algae have been used as dietary supplements to enhance the health and nutritional performance of a range of farmed fish species (*Güroy et al., 2011*). Algae have attractive properties as a candidate to replace meals, because they are rich in protein and carbohydrates, which are necessary components in human and animal diets. Algae also contain a high percentage of lipids, which are crucial in aquaculture diets (Table 1) (*Maisashvili et al., 2015*). Algae can produce a number of biomolecules including astaxanthin, lutein, beta-carotene, chlorophyll, phycobiliprotein, Polyunsaturated Fatty Acids (PUFAs), beta-1,3-glucan, and pharmaceutical and nutraceutical compounds (*Yaakob et al., 2011*). Omega 3-fatty acids like eicosapentanoic acid (EPA) and docosahexaenoic acid from microalgae have therapeutic importance. This is found in fish oil and microalgae. In microalgae it is found in the classes of *Bacillariophceae* (diatoms) *Chlorophyceae, Chrysophyseae, Cryptophyceae, Eustigamatophyceae and Prasinophyceae*. This product from algae is superior over fish oil in not having off flavors, is more pure, has a low cholesterol content and is inexpensive (*Belarbi et al., 2000*). The protein content of marine algae differs according to the species. Generally, it is low for brown seaweeds (3 - 15% of dry weight), moderate for green algae (9 - 26% of dry weight), and high for red seaweeds (maximum 47% of dry weight) (*Fleurence, 1999*). Thus, among the different species of

macro algae, the red algae seems to be the most suitable source for animal nutrition because of their relatively high protein content and structurally diverse bioactive compounds with great pharmaceutical and biomedical potential (*Fleurence et al., 2018*).

Table 1
General composition of different algae (% of dry matter)

Species	Protein	Carbohydrates	Lipids
Anabaena cylindrica	43-56	25-30	4-7
Aphanizomenon flos-aquae	62	23	3
Chlamydomonas rheinhardii	48	17	21
Chlorella pyrenoidosa	57	26	3
Chlorella vulgaris	51-58	12-17	14-22
Dunaliella salina	57	32	6
Euglena gracilis	39-61	14-18	14-20
Porphyridium cruentum	28-39	40-57	9-14
Scenedesmus obliquus	50-56	10-17	12-14
Spirogyra sp.	6-20	33-64	11-21
Arthrospira maxima	60-71	13-16	6-7
Spirulina platensis	46-63	8-14	4-9
Synechococcus sp.	63	15	11

Source: *Maisashvili et al. 2015*

Single cell proteins

The term single-cell protein (SCP) is used to describe protein derived from cells of microorganisms such as yeast, fungi, algae and bacteria which are grown on various carbon sources for synthesis (*Ritala et al., 2017*). The production of SCP has important advantages over other sources of proteins, such as its considerably shorter doubling time, the small land requirement, and the fact that it is not affected by the weather conditions (*García-Garibay et al., 2015*). Studies have shown that yeasts, like *Saccharomyces cerevisiae*, *Candida utilis* and *Kluyveromyces marxianus* have favourable amino acid composition and good protein source (40-50 %) (*Shurson, 2018*). Besides this, yeasts have numerous beneficial effects on fish, such as these ingredients are improving weight gain, stimulating the antioxidant defence system and digestive enzymes (*Carvalho et al., 1997; Kiron, 2012; Pohlenz and Gatlin, 2014*). The common sources of β-glucan are derived from the cell wall of baker's yeast *S. cerevisiae* and the most important among all are β-1,3 and 1,6 glucan (*Meena et al., 2013*). The commonly used prebiotics, the mannanoligosaccharides (MOS) are

also derived from *S. cerevisae*, what have beneficial effects on gut health (*Merrifield et al., 2010*).

Yeast derived from processing of low-value and non-food lignocellulosic biomass is a potential sustainable source of protein in fish diets (*Kumar et al., 2008; Øverland and Skrede, 2017*). SCP can be produced using the residual stream from the forest industry. This offers an attractive concept of turning forest raw material into a protein-rich component in fish feed (*Alriksson et al., 2014*). Microbes can be used to ferment some of the waste materials, such as vegetable and fruit wastes, food-processing wastes, and residues from alcohol production (*Wadhwa and Bakshi, 2016*).

Animal by-products

Animal by-products are derived from the meat-packing, poultry processing and rendering industries (*Abdel-Warith et al., 2001*). Since the bovine spongiform encephalopathy (BSE) crisis meat and bone meal (category 1-2) cannot be used as feed material. The protein content of these products after drying ranges from 50 to over 85% (*Halver and Hardy, 2002*). These animal protein ingredients are good, but even not enough sufficient sources of amino acids with high protein content, total digestible dry matter and energy similar to fish meal, and besides this are comparatively less expensive (*Badillo et al., 2014; Fowler, 1991; Sealey et al., 2011; Shapawi et al., 2007*).

Processed animal protein (PAP) is a complete feed material with a high nutritional value produced from animal by-products (category 3), i.e. the part of animals (bones, offals, etc.) coming from non-ruminant animals controlled as fit for human consumption at the point of slaughter. Supplemented rendered animal protein for fish, can be an appropriate alternative protein source to replace partially the fishmeal (*El-Sayed, 1998; Havasi et al., 2015; Kumar et al., 2016*).

Poultry by-product meal (PBM) is also considered as proper replacement for FM. PBM has lower ash content such as FM, what is desirable in fish feeds, because it contributes to Phosphorous levels in fish farm effluents (*Cheng and Hardy, 2002a*). High inclusion of PBM is also able to reduce the growth performance (*Nengas et al., 1999; Abdel-Wraith and Davies, 2001*), but positive effects also had been reported (*Yones and Metwalli, 2015*).

Whole blood meal (WBM) and haemoglobin meal (HM) are very good protein sources (WBM ~ 80%; HM ~ 95%) with high level of lysine. High levels of histidine and low quantity of isoleucine may be limiting factors of blood meal inclusion (*NRC, 2011*). Due to haemoglobin, the high iron content in blood meal

limits inclusion, because of oxidation of astaxanthin and/or overload of iron in the fish (*Rørvik et al., 2003; Sørensen et al., 2011*).

Due to technological improvements feather meal became more and more digestible for fish with ~ 77% crude protein, what made it to be alternative ingredient (*Bureau et al., 2000; Davies et al., 2009; Sugiura et al., 1998*).

Insect meals

About 70–75% of all animal species living on earth are insects and, together, they play an important role in recycling materials in the terrestrial biosphere (*Katayama et al. 2008*). They grow and reproduce easily, have high feed conversion efficiency (since they are poikilotherm) and can be reared on biowaste streams (*Makkar et al., 2014*). Their further benefit is that they could serve as a more environmentally friendly alternative for the production of animal protein from the perspective of greenhouse gases and NH_3 emissions compared to the conventional livestock (*Oonincx et al., 2010*). However, the commercialisation of this resource has surprisingly just started in the last decade and is still in its very infancy (*Tschirner and Kloas, 2017*).

Insects' pupae, larvae, or adults can be consumed by other farm animals such chickens, cattle, fish, etc. (*Katayama et al., 2008*). One of the most intensively investigated species for fish feed production is *Hermetia illucens* (Diptera: *Stratiomyidae*) or Black Soldier Fly (BSF) (*Henry et al., 2015; Rumpold et al., 2016; Tschirner and Kloas, 2017*). Although insects generally present some characteristics that do not match with the fish meal, the amino acid profile of the *Diptera* shows that this group of insects could be a possible alternative protein source to be used in aquaculture (*Barroso et al., 2014*). Two other promising candidates in term of fish nutrition are mealworms and maggots (*Henry et al., 2015*).

From the nutritional point of view, depending on species and/or stage, insects are rich in protein and lipids; nevertheless, the presence of chitin *a priori* indicates a negative characteristic. However, chitin also is present in crustacean, which are widely consumed by fish (*Barroso et al., 2014*). Its potential as fish meal replacement is furthermore limited by its fatty acid composition that was of minor value compared to fish meal (*Rumpold et al., 2016*). This limitation could be reduced by supplementing the fly larvae with omega-3 fatty acids via fish offal (*St-Hilaire et al., 2007*). However, when insects (mealworm, maggots, BSF) were fed whole to fish, they usually compared positively with control fish usually fed low quality commercial pellets. The partial replacement with insect meal seems possible, mainly for herbivorous/omnivorous species,

but also for some carnivorous fish (black carp (*Mylopharyngodon piceus*), rainbow trout (*Oncorhynchus mykiss*), Japanese sea bass (*Lateolabrax japonicus*), chum salmon (*Oncorhynchus keta*), gilthead seabream *(Sparus aurata)* and european seabass *(Dicentrarchus labrax)*) (*Henry et. al., 2015*).

Nevertheless, more studies are needed to know the digestibility, chitin content and digestive effect, presence of toxic, meal treatments (such as degreasing), adequate mixtures of different insect species or to modify the nutritional value of insects by changing their diet or rearing condition. The great variety of insect species, habitats, development stages, feeding habits and other characteristics most likely affects insect nutritional value and makes insect meal very interesting to study as an alternative to fish meal (*Barroso et al., 2014*).The palatability of the insect meals containing diets is good and that these alternate feed resources can replace soybean and fishmeal in the diets of livestock and fish species (*Makkar et al., 2014*).

Conventional selection index resulted huge benefit in past, animals were selected based on phenotypic variation. However after some period of time when the methodology reached its maximum potential new procedures became widespread.

Best Linear Unbiased Prediction (BLUP) is a method that substitutes conventional phenotypic measurements in the selection index. It is more precise and accurate in prediction genetic potential of animals, taking into consideration the relationships among the animal and the influencing environmental factors. BLUP allows comparing animals merit within different farm with different environment, which is impossible to do with conventional methods.

Economic methodology in constructing selection index is the method by which we evaluate the economic value of each trait and get so called economic weights. This coefficient can be used to calculate aggregated breeding value thus profit can be maximized in the procedure of selection.

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Table 2.

Proximate and essential amino acid composition of selected fish feed ingredients

Sources									*Halver, 1995*	*Moutinho et al., 2017*	*Halver, 1995*			*Makkar et al., 2014*
	Fish meal (Anchovy)	Soybean meal	Cottonseed meal	Rapeseed meal	Peanut meal	Sunflower meal	DDGS	Algae meal*	Brewer's yeast	Meat bone meal	Poultry by-product meal	Blood meal	Larvae**	Mealworm
Crude protein (% DM)	71.20	47.70	44.30	40.60	52.00	44.10	29.80	65.00	46.90	54.40	62.80	93.00	42.10	52.80
Crude fat (% DM)	9.60	2.00	3.00	2.70	2.10	2.20	11.10	6.10	2.40	14.50	12.60	3.00	26.00	36.10
Ash (% DM)	14.90	6.00	7.40	7.70	6.80	7.10	5.30	8.90	7.00	25.50	14.40	1.75	7.00	3.10
Essential amino acids (% protein)														
Arginine	4.11	3.41	4.51	2.26	5.46	4.52	1.20	-	2.35	7.01	4.03	3.88	5.60	4.80
Histidine	1.76	1.26	1.15	1.09	1.17	1.18	0.66	-	1.17	2.04	1.08	5.59	3.00	3.40
Isoleucine	3.38	2.92	1.56	1.48	1.83	2.58	1.31	3.90	2.37	1.90	2.54	0.98	5.10	4.60
Leucine	5.43	4.02	2.50	2.74	3.26	3.23	2.76	5.20	3.45	4.94	4.28	11.86	7.90	8.60
Lysine	5.49	3.10	1.73	2.18	1.62	2.15	0.99	3.00	3.33	4.83	3.10	8.04	6.60	5.40
Methionine	2.16	0.72	0.62	0.78	0.53	1.72	0.52	0.91	0.79	1.42	1.13	0.95	2.10	1.50
Phenylalanine	3.03	2.45	2.35	1.55	2.53	2.58	1.36	3.25	1.96	2.98	1.97	6.36	5.20	4.00
Threonine	3.00	1.92	1.44	1.72	1.34	1.72	1.08	3.00	2.27	3.17	2.08	3.93	3.70	4.00
Valine	3.81	2.53	2.05	1.96	2.24	2.58	1.46	4.22	2.52	3.31	3.06	8.13	8.20	6.00

**Spirulina spp.*; **Black soldier fly*

REFERENCES

Abdel-Warith, A. A., Russell, P. M., & Davies, S. J. (2001). Inclusion of a commercial poultry by-product meal as a protein replacement of fish meal in practical diets for African catfish *Clarias gariepinus* (Burchell 1822). *Aquaculture Research*, *32* (Burchell 1822), 296–305. DOI: 10.1046/j.1355-557x.2001.00053.x

Alriksson, B., Hörnberg, A., Eirikur Gudnason, A., Knobloch, S., Arnason, J., & Johannsson, R. (2014). Fish Feed From Wood. *Cellulose Chemistry and. Technology*, *48* (910), 843–848. Retrieved from Link

Badillo, D., Herzka, S.Z., & Viana, M.T., (2014). Protein Retention Assessment of Four Levels of Poultry By-Product Substitution of Fishmeal in Rainbow Trout (*Oncorhynchus mykiss*) Diets Using Stable Isotopes of Nitrogen (d 15 N) as Natural Tracers. *PLOS One*, 9, 1–8. DOI: 10.1371/journal.pone.0107523

Barroso, F. G., de Haro, C., Sánchez-Muros, M. J., Venegas, E., Martínez-Sánchez, A., & Pérez-Bañón, C. (2014). The potential of various insect species for use as food for fish. *Aquaculture*, *422–423*, 193–201. DOI: 10.1016/j.aquaculture.2013.12.024

Batal, A., Dale, N., & Café, M., (2005). Nutrient composition of peanut meal. *Journal of Applied Poultry Research*. 14, 254–257. DOI: 10.1093/japr/14.2.254

Belarbi, E.H., Molina, E., & Chisti, Y., (2000). A process for high yield and scaleable recovery of high purity eicosapentaenoic acid esters from microalgae and fish oil. *Enzyme and Microbial Technology*. 26, 516–529. DOI: 10.1016/S0141-0229(99)00191-X

Bezerra da Rocha, M.E., Freire, F. da C.O., Erlan Feitosa Maia, F., Izabel Florindo Guedes, M., & Rondina, D., (2014). Mycotoxins and their effects on human and animal health. *Food Control* 36, 159–165. DOI: 10.1016/j.foodcont.2013.08.021

Booman, M., Forster, I., Vederas, J.C., Groman, D.B., & Jones, S.R.M., (2018). Soybean meal-induced enteritis in Atlantic salmon (*Salmo salar*) and Chinook salmon (*Oncorhynchus tshawytscha*) but not in pink salmon (*O. gorbuscha*). *Aquaculture* 483, 238–243. DOI: 10.1016/j.aquaculture.2017.10.025

Bureau, D.P., Harris, A.M., Bevan, D.J., Simmons, L.A., Azevedo, P.A., & Cho, C.Y., (2000). Feather meals and meat and bone meals from different origins as protein sources in rainbow trout (*Oncorhynchus mykiss*) diets. *Aquaculture* 181, 281–291. DOI: 10.1016/S0044-8486(99)00232-X

Carvalho, a P., Escaffre, a, Teles, a O., & Bergot, P. (1997). First feeding of common carp larvae on diets with high levels of protein hydrolysates. *Aquaculture International*, *5*, 361–367. DOI: 10.1023/A:1018368208323

Cheng, Z. J., & Hardy, R. W. (2002a). Apparent digestibility coefficients and nutritional value of poultry by-product meals for rainbow trout (*Oncorhynchus mykiss*) measured *in vivo* using settlement. *Journal of the World Aquaculture Society*, *33*, 458–465. DOI: 10.1016/s0044-8486(02)00260-0

Cheng, Z.J., & Hardy, R.W., (2002b). Apparent digestibility coefficients and nutritional value of cottonseed meal for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 212, 361–372. DOI: 10.1016/S0044-8486(02)00260-0

Collins, S.A., Øverland, M., Skrede, A., & Drew, M.D., (2013). Effect of plant protein sources on growth rate in salmonids: Meta-analysis of dietary inclusion of soybean, pea and canola/rapeseed meals and protein concentrates. *Aquaculture* 400–401, 85–100. DOI: 10.1016/j.aquaculture.2013.03.006

Davies, S.J., Gouveia, A., Laporte, J., Woodgate, S.L., & Nates, S., (2009). Nutrient digestibility profile of premium (category III grade) animal protein by-products for temperate marine fish species (European sea bass, gilthead sea bream and turbot). *Aquaculture Research*. 40, 1759–1769. DOI: 10.1111/j.1365-2109.2009.02281.x

Davies, S.J., McConnell, S., & Bateson, R.I., (1990). Potential of rapeseed meal as an alternative protein source in complete diets for tilapia (*Oreochromis mossambicus* Peters). *Aquaculture* 87, 145–154. DOI: 10.1016/0044-8486(90)90271-N

Drew, M.D., Borgeson, T.L., & Thiessen, D.L., (2007). A review of processing of feed ingredients to enhance diet digestibility in finfish. *Animal Feed Science Technology.* 138, 118–136. DOI: *10.1016/j.anifeedsci.2007.06.019*

El-Sayed, A.-F.M., (1998). Total replacement of fish meal with animal protein sources in Nile tilapia, *Oreochromis niloticus* (L.), feeds. *Aquaculture Research*. 29, 275–280. DOI: *10.1046/j.1365-2109.1998.00199.x*

Escaffre, A.M., Zambonino Infante, J., Cahu, C.L., Mambrini, M., Bergot, P., & Kaushik, S.J., (1997). Nutritional value of soy protein concentrate for larvae of common carp (*Cyprinus carpio*) based on growth performance and digestive enzyme activities. *Aquaculture* 153, 63–80. DOI: *10.1016/S0044-8486(97)00010-0*

Fleurence, J., (1999). Seaweed proteins: Biochemical, nutritional aspects and potential uses. *Trends in Food Science and Technology*. 10, 25–28. DOI: *10.1016/S0924-2244(99)00015-1*

Fleurence, J., Morançais, M., & Dumay, J., (2018). Seaweed proteins, *Proteins in Food Processing.* Elsevier Ltd. DOI: *10.1016/B978-0-08-100722-8.00010-3*

Fowler, L.G., (1991). Poultry by-product meal as a dietary protein source in fall chinook salmon diets. *Aquaculture* 99, 309–321. DOI: *10.1016/0044-8486(91)90251-2*

Francis, G., Makkar, H.P.S., & Becker, K., (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish, *Aquaculture*. DOI: *10.1016/S0044-8486(01)00526-9*

Garcia-Garibay, M., Gomezi-Ruiz, L., & Cruz-Guerrero, A. (2015). Single-cell protein. In *Biochemical Engineering and Biotechnology* (pp. 417–434). Elsevier. DOI: *10.1016/C2013-0-09819-2*

Gatlin, D.M., Barrows, F.T., Brown, P., Dabrowski, K., Gaylord, T.G., Hardy, R.W., Herman, E., Hu, G., Krogdahl, Å., Nelson, R., Overturf, K., Rust, M., Sealey, W., Skonberg, D., Souza, E.J., Stone, D., Wilson, R., &W Wurtele, E., (2007). Expanding the utilization of sustainable plant products in aquafeeds: A review. *Aquaculture Research*. 38, 551–579. DOI: *10.1111/j.1365-2109.2007.01704.x*

Güroy, D., Güroy, B., Merrifield, D.L., Ergün, S., Tekinay, A.A., & Yiğit, M., (2011). Effect of dietary *Ulva* and *Spirulina* on weight loss and body composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum), during a starvation period. *Journal of Animal Physiology and Animal Nutrition*. 95, 320–327. DOI: *10.1111/j.1439-0396.2010.01057.x*

Halver, J. E. (1995). Use of INFIC data base for amino acid requirement studies. *Journal of Applied Ichthyology*, *11*(3–4), 129–140. DOI: *10.1111/j.1439-0426.1995.tb00014.x*

Halver, J.E., & Hardy, R.W., (2002). *Fish Nutrition*, 3rd ed. Academic Press. DOI: *10.1016/b978-012319652-1/50006-9*

Hardy, R.W., (2010). Utilization of plant proteins in fish diets: Effects of global demand and supplies of fishmeal. *Aquaculture Research*. 41, 770–776. DOI: *10.1111/j.1365-2109.2009.02349.x*

Havasi, M., Kumar, S., Nagy, Z., Pál, L., Beliczky, G., Bercsényi, M., & Gál, D., (2015). Effect of total fish meal replacement with vegetal protein alone or combined with rendered animal protein on growth performance and tissue composition of european catfish (*Silurus glanis* L.). *Israeli Journal of Aquaculture - Bamidgeh* 67.

Henry, M., Gasco, L., Piccolo, G., & Fountoulaki, E. (2015). Review on the use of insects in the diet of farmed fish: Past and future. *Animal Feed Science and Technology*, *203* (1), 1–22. DOI: *10.1016/j.anifeedsci.2015.03.001*

Katayama, N., Ishikawa, Y., Takaoki, M., Yamashita, M., Nakayama, S., Kiguchi, K., Kok, R., Wada, H., & Mitsuhashi, J. (2008). Entomophagy: A key to space agriculture. *Advances in Space Research*, *41*(5), 701–705. DOI: *10.1016/j.asr.2007.01.027*

Kaushik, S. J., Cravedi, J. P., Lalles, J. P., Sumpter, J., Fauconneau, B., & Laroche, M. (1995). Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or

antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. *Aquaculture*, *133* (3–4), 257–274. DOI: 10.1016/0044-8486(94)00403-B

Kiron, V. (2012). Fish immune system and its nutritional modulation for preventive health care. *Animal Feed Science and Technology*, *173* (1–2), 111–133. DOI: 10.1016/j.anifeedsci.2011.12.015

Knudsen, D., Jutfelt, F., Sundh, H., Sundell, K., Koppe, W., & Frøkiær, H., (2008). Dietary soya saponins increase gut permeability and play a key role in the onset of soyabean-induced enteritis in Atlantic salmon (*Salmo salar* L.). *British Journal of Nutrition*. 100, 120–129. DOI: 10.1017/S0007114507886338

Krogdahl, A., Gajardo, K., Kortner, T.M., Penn, M., Gu, M., Berge, G.M., & Bakke, A.M., (2015). Soya Saponins Induce Enteritis in Atlantic Salmon (*Salmo salar* L.). *Journal of Agricultural and Food Chemistry*. 63, 3887–3902. DOI: 10.1021/jf506242t

Kumar, R., Singh, S., & Singh, O. V. (2008). Bioconversion of lignocellulosic biomass: Biochemical and molecular perspectives. *Journal of Industrial Microbiology and Biotechnology*, *35* (5), 377–391. DOI: 10.1007/s10295-008-0327-8

Kumar, S., Sándor Zs, J., Nagy, Z., Fazekas, G., Havasi, M., Sinha, A.K., De Boeck, G., & Gál, D., (2016). Potential of processed animal protein versus soybean meal to replace fish meal in practical diets for European catfish (*Silurus glanis*): growth response and liver gene expression. *Aquaculture Nutrition*. 1–11. DOI: 10.1111/anu.12487

Lim, C., (1997). Replacement of Marine Animal Protein with Peanut Meal in Diets for Juvenile White Shrimp, *Penaeus vannamei*. *Journal of Applied Aquaculture*. 7, 67–78. DOI: 10.1300/J028v07n03

López-Mosquera, M. E., Fernández-Lema, E., Villares, R., Corral, R., Alonso, B., & Blanco, C. (2011). Composting fish waste and seaweed to produce a fertilizer for use in organic agriculture. *Procedia Environmental Sciences*, *9*, 113–117. DOI: 10.1016/j.proenv.2011.11.018

Maisashvili, A., Bryant, H., Richardson, J., Anderson, D., Wickersham, T., & Drewery, M., (2015). The values of whole algae and lipid extracted algae meal for aquaculture. *Algal Research*. 9, 133–142. DOI: 10.1016/j.algal.2015.03.006

Makkar, H. P. S., Tran, G., Heuzé, V., & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, *197*, 1–33. DOI: 10.1016/j.anifeedsci.2014.07.008

Marković, Z., Stanković, M., Rašković, B., Dulić, Z., Živić, I., & Poleksić, V., (2016). Comparative analysis of using cereal grains and compound feed in semi-intensive common carp pond production. *Aquaculture International*. 24, 1699–1723. DOI: 10.1007/s10499-016-0076-z

Marroquín-Cardona, A.G., Johnson, N.M., Phillips, T.D., & Hayes, A.W., (2014). Mycotoxins in a changing global environment - A review. *Food and Chemical Toxicology*. 69, 220–230. DOI: 10.1016/j.fct.2014.04.025

Meena, D. K., Das, P., Kumar, S., Mandal, S. C., Prusty, A. K., Singh, S. K., … & Mukherjee, S. C. (2013). Beta-glucan: An ideal immunostimulant in aquaculture (a review). *Fish Physiology and Biochemistry*, *39* (3), 431–457. DOI: 10.1007/s10695-012-9710-5

Mérida, S.N., Tomás-Vidal, A., Martínez-Llorens, S., & Cedrá, M.J., (2010). Sunflower meal as a partial substitute in juvenile sharpsnout sea bream (*Diplodus puntazzo*) diets: Amino acid retention, gut and liver histology. *Aquaculture* 298, 275–281. DOI: 10.1016/j.aquaculture.2009.10.025

Merrifield, D. L., Dimitroglou, A., Foey, A., Davies, S. J., Baker, R. T. M., Bøgwald, J., … & Ringø, E. (2010). The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture*, *302*(1–2), 1–18. DOI: 10.1016/j.aquaculture.2010.02.007

Miles R.D., & Chapman F. (2015). The Benefits of Fish Meal in Aquaculture Diets. *University of Florida IFAS Extension*, (May 2006), 1–6. Retrieved from Link

Montero, D., Kalinowski, T., Obach, A., Robaina, L., Tort, L., Caballero, M. J., & Izquierdo, M. S. (2003). Vegetable lipid sources for gilthead seabream (*Sparus aurata*): Effects on fish health. *Aquaculture*, *225*(1–4), 353–370. DOI: 10.1016/S0044-8486(03)00301-6

Montero, D., Robaina, L., Caballero, M. J., Ginés, R., & Izquierdo, M. S. (2005). Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: A time-course study on the effect of a re-feeding period with a 100% fish oil diet. *Aquaculture*, *248*(1–4), 121–134. DOI: 10.1016/j.aquaculture.2005.03.003

Moutinho, S., Martínez-Llorens, S., Tomás-Vidal, A., Jover-Cerdá, M., Oliva-Teles, A., & Peres, H. (2017). Meat and bone meal as partial replacement for fish meal in diets for gilthead seabream (*Sparus aurata*) juveniles: Growth, feed efficiency, amino acid utilization, and economic efficiency. *Aquaculture*, *468*, 271–277. DOI: 10.1016/j.aquaculture.2016.10.024

Nayak, S.K., 2010. Role of gastrointestinal microbiota in fish. *Aquaculture Research*. 41, 1553–1573. DOI: 10.1111/j.1365-2109.2010.02546.x

Nengas, I., Alexis, M. N., & Davies, S. J. (1999). High inclusion levels of poultry meals and related byproducts in diets for gilthead seabream Sparus aurata L. *Aquaculture*, *179*(1–4), 13–23. DOI: 10.1016/S0044-8486(99)00148-9

NRC - Committee on the Nutrient Requirements of Fish and Shrimp. (2011). *Nutrient Requirements of Fish and Shrimp*, National Academies Press. DOI: 10.17226/13039

OECD/FAO. (2017). *OECD-FAO Agricultural Outlook 2017-2026*. Paris: OECD Publishing. Link

Oonincx, D. G. A. B., van Itterbeeck, J., Heetkamp, M. J. W., van den Brand, H., van Loon, J. J. A., & van Huis, A. (2010). An exploration on greenhouse gas and ammonia production by insect species suitable for animal or human consumption. *PLoS ONE*, *5*(12), 1–7. DOI: 10.1371/journal.pone.0014445

Øverland, M., & Skrede, A., (2017). Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture. *Journal of the Science of Food and Agriculture*. 97, 733–742. DOI: 10.1002/jsfa.8007

Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å., & Skrede, A., (2009). Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (*Salmo salar*)-Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. *Aquaculture* 288, 305–311. DOI: 10.1016/j.aquaculture.2008.12.012

Pohlenz, C., & Gatlin, D. M. (2014). Interrelationships between fish nutrition and health. *Aquaculture*, *431*, 111–117. DOI: 10.1016/j.aquaculture.2014.02.008

Rana, K. J., Siriwardena, S., & Hasan, M. R. (2009). Impact of rising feed ingredient prices on aquafeeds and aquaculture production. *FAO Fisheries and Aquaculture Technical Paper* (Vol. 541).

Richard, J.L., (2007). Some major mycotoxins and their mycotoxicoses-An overview. *International Journal of Food Microbiology*. 119, 3–10. DOI: 10.1016/j.ijfoodmicro.2007.07.019

Rinchard, J., Mbahinzireki, G., Dabrowski, K., Lee, K.-J., Garcia-Abiado, M.-A., & Ottobre, J., (2002). Effects of dietary cottonseed meal protein level on growth, gonad development and plasma sex steroid hormones of tropical fish tilapia *Oreochromis sp*. *Aquaculture International*. 10. DOI: 10.1023/A:1021379328778

Ritala, A., Häkkinen, S.T., Toivari, M., & Wiebe, M.G., (2017). Single cell protein-state-of-the-art, industrial landscape and patents 2001-2016. *Frontiers in Microbiology*. 8. DOI: 10.3389/fmicb.2017.02009

Rørvik, K.A., Dehli, A., Thomassen, M., Ruyter, B., Steien, S.H., & Salte, R. (2003). Synergistic effects of dietary iron and omega-3 fatty acid levels on survival of farmed Atlantic salmon, *Salmo salar* L., during natural outbreaks of furunculosis and cold water vibriosis. *Journal of Fish Diseases*. 26, 477–485. DOI: 10.1046/j.1365-2761.2003.00482.x

Rumpold, B. A., Klocke, M., & Schlüter, O. (2017). Insect biodiversity: underutilized bioresource for sustainable applications in life sciences. *Regional Environmental Change*, *17*(5), 1445–1454. DOI: 10.1007/s10113-016-0967-6

Safi, C., Zebib, B., Merah, O., Pontalier, P.-Y., & Vaca-Garcia, C. (2014). Morphology, composition, production, processing and applications of Chlorella vulgaris: A review. *Renewable and Sustainable Energy Reviews*. 35, 265–278. DOI: 10.1016/j.rser.2014.04.007

Sanz, A., Morales, A.E., de la Higuera, M., & Gardenete, G., (1994). Sunflower meal compared with soybean meals as partial substitutes for fish meal in rainbow trout (*Oncorhynchus mykiss*) diets: protein and energy utilization. *Aquaculture* 128, 287–300. DOI: 10.1016/0044-8486(94)90318-2

Sealey, W.M., Hardy, R.W., Barrows, F.T., Pan, Q., & Stone, D.A.J., (2011). Evaluation of 100% Fish Meal Substitution with Chicken Concentrate, Protein Poultry By-Product Blend, and Chicken and Egg Concentrate on Growth and Disease Resistance of Juvenile Rainbow Trout, *Oncorhynchus mykiss*. *Journal of the World Aquaculture Society*. 42, 46–55. DOI: 10.1111/j.1749-7345.2010.00442.x

Shapawi, R., Ng, W.K., & Mustafa, S. (2007). Replacement of fish meal with poultry by-product meal in diets formulated for the humpback grouper, *Cromileptes altivelis*. *Aquaculture* 273, 118–126. DOI: 10.1016/j.aquaculture.2007.09.014

Shurson, G. C. (2018). Yeast and yeast derivatives in feed additives and ingredients: Sources, characteristics, animal responses, and quantification methods. *Animal Feed Science and Technology*, *235*, 60–76. DOI: 10.1016/j.anifeedsci.2017.11.010

Soliman, N. F., Yacout, D. M. M., & Hassaan, M. A. (2017). Responsible Fishmeal Consumption and Alternatives in the Face of Climate Change. *International Journal of Marine Sciences*, *7* (15), 130–140. DOI: 10.5376/ijms.2017.07.0015

Sørensen, M., Berge, G.M., Thomassen, M., Ruyter, B., Hatlen, B., & Ytrestøyl, T. (2011). *Today's and tomorrow's feed ingredients in Norwegian aquaculture*. ISBN: 9788272519437

Sørensen, M., Penn, M., El-Mowafi, A., Storebakken, T., Chunfang, C., Øverland, M., &D Krogdahl, Å. (2011). Effect of stachyose, raffinose and soya-saponins supplementation on nutrient digestibility, digestive enzymes, gut morphology and growth performance in Atlantic salmon (*Salmo salar*, L). *Aquaculture* 314, 145–152. DOI: 10.1016/j.aquaculture.2011.02.013

St-Hilaire, S., Cranfill, K., McGuire, M. A., Mosley, E. E., Tomberlin, J. K., Newton, L., … & Irving, S. (2007). Fish Offal Recycling by the Black Soldier Fly Produces a Foodstuff High in Omega-3 Fatty Acids. *Journal of the World Aquaculture Society*, *38*(2), 309–313. DOI: 10.1111/j.1749-7345.2007.00101.x

Sugiura, S.H., Dong, F.M., Rathbone, C.K., & Hardy, R.W., (1998). Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. *Aquaculture* 159, 177–202. DOI: 10.1016/S0044-8486(97)00177-4

The World Bank. (2013). Fish to 2030: Prospects for fisheries and aquaculture. *Agriculture and Environmental Services*, *3* (83177), 1–102.

Thiessen, D.L., Maenz, D.D., Newkirk, R.W., Classen, H.L., & Drew, M.D., (2004). Replacement of fishmeal by canola protein concentrate in diets fed to rainbow trout (*Oncorhynchus mykiss*). *Aquaculture Nutrition*. 10, 379–388. DOI: 10.1111/j.1365-2095.2004.00313.x

Torstensen, B.E., Espe, M., Stubhaug, I., & Lie, Ø. (2011). Dietary plant proteins and vegetable oil blends increase adiposity and plasma lipids in Atlantic salmon (*Salmo salar* L.). *British Journal of Nutrition*. 106, 633–47. DOI: 10.1017/S0007114511000729

Tschirner, M., & Kloas, W. (2017). Increasing the sustainability of aquaculture systems: Insects as alternative protein source for fish diets. *Gaia*, *26*(4), 332–340. DOI: 10.14512/gaia.26.4.10

Urán, P.A., Gonçalves, A.A., Taverne-Thiele, J.J., Schrama, J.W., Verreth, J.A.J., & Rombout, J.H.W.M., (2008). Soybean meal induces intestinal inflammation in common carp (*Cyprinus carpio* L.). *Fish Shellfish Immunology*. 25, 751–760. DOI: 10.1016/j.fsi.2008.02.013

Wadhwa, M., & Bakshi, M. P. S. (2016). Application of Waste-Derived Proteins in the Animal Feed Industry. *Protein Byproducts: Transformation from Environmental Burden Into Value-Added Products*. Elsevier Inc. DOI: 10.1016/B978-0-12-802391-4.00010-0

Webster, C. D., Yancey, D. H., & Tidwell, J. H. (1992). Effect of partially or totally replacing fish meal with soybean meal on growth of blue catfish (*Ictalurus furcatus*). *Aquaculture*, *103*(2), 141–152. DOI: *10.1016/0044-8486(92)90408-D*

Wu, Y.V., Rosati, R.R., Sessa, D.J., & Brown, P.B., (1995). Evaluation of Corn Gluten Meal as a Protein Source in Tilapia Diets. *Journal of Agricultural Food Chemistry*. 43, 1585–1588. DOI: *10.1021/jf00054a032*

Yaakob, Z., Ali, E., Zainal, A., Mohamad, M., & Takriff, M. S. (2014). An overview: Biomolecules from microalgae for animal feed and aquaculture. *Journal of Biological Research (Greece)*, *21* (1), 1–10. DOI: *10.1186/2241-5793-21-6*

Yildirim, M., Lim, C., Wan, P.J., & Klesius, P.H. (2003). Growth performance and immune response of channel catfish *(Ictalurus puctatus)* fed diets containing graded levels of gossypol-acetic acid. *Aquaculture* 219, 751–768. DOI: *10.1016/S0044-8486(03)00062-0*

Yones, A., & Metwalli, A. (2015). Effects of Fish Meal Substitution with Poultry By-product Meal on Growth Performance, Nutrients Utilization and Blood Contents of Juvenile Nile Tilapia (Oreochromis niloticus). *Journal of Aquaculture Research & Development*, *07* (01), 1–6. DOI: *10.4172/2155-9546.1000389*

Zhang, C., Rahimnejad, S., Wang, Y. ru, Lu, K., Song, K., Wang, L., & Mai, K. (2018). Substituting fish meal with soybean meal in diets for Japanese seabass (*Lateolabrax japonicus*): Effects on growth, digestive enzymes activity, gut histology, and expression of gut inflammatory and transporter genes. *Aquaculture*, 483, 173–182. DOI: *10.1016/j.aquaculture.2017.10.029*

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PCR and qPCR-based applications in rumen microbiology research: a review

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ABSTRACT - The rumen and its microbial ecosystem play a central role in the overall nutrition and health of ruminant animals. However, development and homeostatic state of the entire gut system is influenced by different interrelated factors. Recent developments in molecular diagnostic tools by using amplicon sequencing of 16S ribosomal RNA and use of high-throughput data generated through applications of pyrosequencing is a promising approach to defining the rumen microbial genome. Several "omic"-based technologies based on PCR and real-time PCR (qPCR), are currently being used in gut microbiology in order to predict how the gut microbiota works. Such procedures include pyrosequencing, genome-wide shotgun sequencing, short metagenomics sequencing and metagenomics analysis, bacterial DNA integration for editing genomes of isolated microbes, etc. These tools are sensitive and precise in quantitation, identification and functional characterisation of the entire rumen microbiome. PCR/qPCR enables investigations of changes in the microbiome and microbiota with respect to age, diet, species and environmental variations thus providing new information about rumen microbial genome. In this review, we will highlight recent findings using PCR and qPCR-based procedures in investigating the complex nature of the rumen microbial population which has advanced our knowledge and understanding of the rumen microbial genome.

Keywords: Bacteria, Genome, Microbiome, Microbiota, Rumen, PCR/qPCR, 16S rRNA.

INTRODUCTION

One of the most widely used molecular diagnostic tool since its invention in the twentieth century is the polymerase chain reaction, PCR. PCR amplifies a segment of the DNA, such that millions or even billions of copies of a DNA molecule are generated in a very short time. PCR has been described as a versatile tool with diverse applicability to many different situations in molecular diagnostic research (*Barlett and Stirling, 2003*). It has also been used to detect DNA sequences, in forensic investigations, DNA fingerprinting, detection of bacteria and viruses to diagnose genetic diseases as well as cloning. PCR is the key that

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unlocked several advances made in our understanding of the genome of human, bacteria and other species (*Mullis et al., 1986; Innis and Galfand, 1990*).

Bacteria, protozoa and anaerobic fungi have been implicated as key degraders of polysaccharides in the rumen (*Schofield, 2000*). They are found in all the four compartments of ruminants gut (rumen, reticulum, omasum and abomasum). Microbiologically, the rumen is said to be made up of a complex ecosystem which is composed of cells/ml concentrations of about 10^{10}, 10^{6} and 10^{4} for bacteria, fungi and protozoa respectively, which interact in synergy and are active throughout the process of digestion and fermentation. This synergistic interaction also augment enzymatic digestion process in the animal for energy generation and yields volatile fatty acids (VFAs): acetate, butyrate, propionate, as well as other compounds such as ammonium, formic acid, methane gas, H_2, as well as CO_2 as end products of rumen fermentation (*Krause et al., 2003; Pitta et al., 2010; Stewart and Bryant, 1988*). Biomass-degrading enzymes encoded in the rumen microbiome and proteins belonging to bacterial and archaeal genomes have been associated with carbohydrate metabolism (*Stewart et al., 2018*).

Different bacterial groups function as one unit and assist the host (ruminants) degrade and utilize fibrous feed stuff in order to make volatile fatty acids as a source of energy from plant structural and nonstructural carbohydrates and proteins. Noteworthy, due to their relative abundance and metabolic diversity, *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* (*Hungate, 2013; Zhou et al., 2015*) are main cellulolytic bacteria associated with ruminal digestion of plant cell wall materials whereas *Ruminobacter amylophilus* or *Prevotella ruminicola* dominates the degradation of starch (*Steward and Bryant, 1988; Mobashar et al., 2019*). Amino acids, soluble proteins and insoluble, particulate proteins are primarily degraded by bacteria and protozoa respectively (*Cotta, 1998; Hino and Russel, 1987; Foroozandeh et al., 2009; Russell, 2009*) while protozoa were reported to be limited in their ability to assimilate peptides (or amino acids) (*Hino and Russel, 1987*).

Classical culture-based procedures which are applied in isolation, identification and quantitation of rumen microbes have been reported to account for less than 30% of the rumen microbial population. This is because several rumen microbes have shown tendencies of bypassing or escaping conventional culturing procedures and this necessitates the application of enhanced and more sensitive techniques (*Nathani et al., 2013*). However, recent developments in molecular diagnostic tools and use of high-throughput data generated

through applications of next-generation sequencing, pyrosequencing of bacterial 16S ribosomal RNA gene has provided wider understanding of the operations and activities of rumen resident microbes. Additionally, genome-wide shotgun sequencing for metagenomic data generation have revealed new information and innovative research areas about the function, characteristics and diversity of rumen microbiota. This has provided linkage between functional gene activity, metabolic pathways and rumen metabolites (*Nathani et al., 2013; McCann et al., 2014; Roehe et al., 2016; Denman et al., 2018; Stewart et al., 2018*). Research in rumen microbiome is quite complicated and consists of different areas which provides a wide range of research possibilities. *Figure 1* shows some of these areas that are PCR/qPCR-based procedures in analysing, characterising or quantitating the microbial diversity in the rumen.

Development of Rumen Microbiota

Microbial genomes remain unchanged for life while the microbiome profiles tends to change as the new born animal grows (*Goodrich et al., 2017*). The rumen and its microbial ecosystem play a central role in the overall nutrition and health of the animal. However, development and homeostatic state of the entire gut system is influenced by different interrelated factors. These factors include genetic, physiological and environmental factors which may include dietary variations (quality and quantity), age, and maternal factors among others, are central in determining how the microbial population carryout their functions in supplying energy and nutrient to the animal as well as providing information about the variation in microbial diversity within the rumen microbiome (*Callaway et al., 2010; Callaway et al., 2011; De Menezes., et al., 2011; Tymensen et al., 2012; Han et al., 2015; Dang et al., 2017*).

Microbiologically, the rumen could generally be said to be made up of a complex ecosystem which is composed of cells/ml concentrations of about 10^{10}, 10^{6}, and 10^{4} for bacteria, fungi and protozoa respectively (*Hungate, 1966; Skillman et al., 2006; Jewell et al., 2015*), these microbes interact in synergy and are active throughout the process of digestion. Their synergistic interaction and effects tend to influence immunological responses, gut health and also augment enzymatic digestion process in the animal. As such, they are active in generating volatile fatty acids (VFAs): acetate, butyrate, propionate, and other compounds such as formic acid, Methane gas, H_2, as well as CO_2 for utilisation by the animal in energy generation for cellular and metabolic processes. These groups function as one unit and assist the host (ruminants) in degrading and utilising fibrous feed stuff in a symbiotic relationship in order to make volatile

fatty acids as a source of energy from plant structural and nonstructural carbohydrates and proteins. The significance of the rumen microbiota in ruminant and human nutrition necessitates careful research in order to elucidate their roles and mode of action (*Krause et al., 2003; Kamara, 2005; Pitta et al., 2010; Taschuk and Griebel, 2012; Rodríguez et al., 2015; Roehe et al., 2016*).

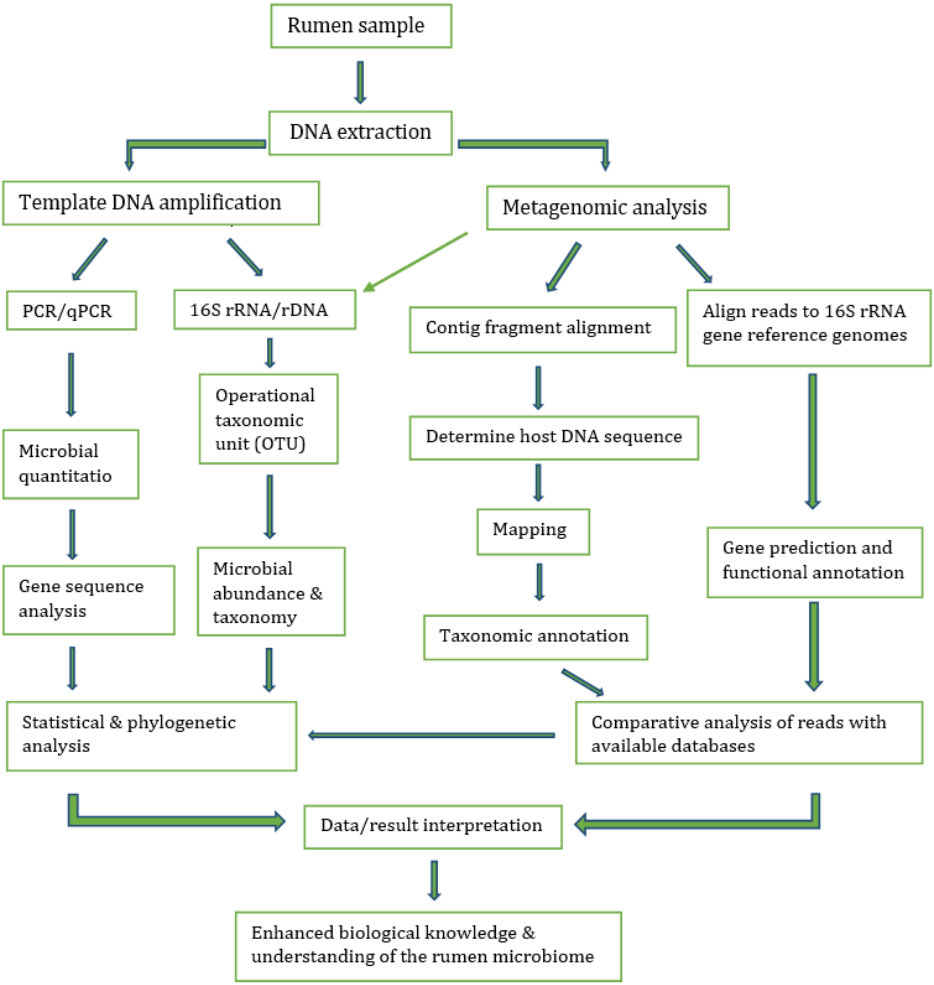

Figure 1: Rumen microbiome project workflow chart
(Modified from *McCann et al., 2014*)

Microbial Diversity in the Rumen

Rumen Bacteria

Rumen development has a significant effect on the microbial diversity of the entire gut system. Bacteria are the most dominant and diverse microbial community in the rumen. *Flint et al.* (2008) reported that bacteria are the most diversified groups of microbes in the rumen, approximately 95% of total microbiota. Subsequent studies involving rumen microbiology revealed a very large number of bacteria present in the rumen (up to about 10^{11} viable cells/ml) (*Kim et al., 2011; Jami and Mizrahi, 2012b*). Since new born ruminants generally have no functional rumen at birth, development and establishment of the ruminal microbiota still has various controversies by so many researchers (*Hungate, 1966; Jewell et al., 2015*). As the young ruminant grows, the species and bacterial community also experience a shift in composition (*Li et al., 2012*). *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* were quantified using real-time PCR targeting 16S rDNA and the results showed that *R. flavefaciens* was slightly more dominant compared to *F. succinogenes* with the population of *R. flavefaciens* and *F. succinogenes* are significantly more than *R. albus* species (*Mosoni et al., 2007*).

Rumen Protozoa

Protozoa play a key role in the hydrogen transfer between microbial species and methane production within the rumen microbiome (*Salonen et al., 2014*). Ciliates have been reported to be the most abundant protozoa resident in the rumen of both domesticated and wild ruminants, contributing to fiber digestion, control of CHO fermentation process and the possibility of having a negative effect on protein metabolism. Until the application of PCR in rumen microbiological studies, rumen protozoa was quantified based on a microscopic count and this has shown that the most abundant genus accounting for about 95% of the total protozoal population in the rumen, is the genus *Entodinium*. However, comparing microscopic analysis with PCR based protocols has shown that there are several species and genera of protozoans which are resident in the rumen and are yet to be fully characterised (*Regensbogenova et al., 2004; Skillman et al., 2006; Sylvester et al., 2009; Tymensen et al., 2012*). This necessitates the need to intensively investigate protozoal populations in the rumen because they play significant role in fiber digestion and have negative effect on microbial protein turnover. However, they have been reported to have variation in the number of their rDNA copies per cell during a life cycle and also their cell sizes vary inter and intra-protozoal specie (*Prescott, 1994;*

Dehority, 2003; McSwansea et al., 2007). A major limitation of real-time PCR in microbial quantitation as well as protozoal analysis is that qPCR-based approach is more expensive than microscopic counts procedures. However, qPCR is more sensitive and more accurate with the ability to detect 1 to 10 million protozoal cells using genus specific primers that was developed by Skillman and co-workers for detection of *Entodinium* (*Skillman et al., 2006*).

Fungi

Fungi initiate mechanical and enzymatic break down of plant fiber to allow access for secondary metabolisers (*Boots et al., 2013*). Anaerobic rumen filamentous fungi form extensive interlocking rhizoidal systems. Accurate quantification and identification within this group of microbes by direct count using culture-based protocols are difficult, especially when considering that environmental samples contain both polycentric and monocentric species (*Denman and McSweeney, 2006*). Six genera, *Neocallimastix*, *Piromyces*, *Anaeromyces*, *Caecomyces*, *Orpinomyces*, and more recently *Cyllamyces*, have been recognized, while 18 species of anaerobic rumen fungi have been described on the basis of their thallus morphology and their zoospore ultrastructure (*Liggenstoffer et al., 2010; Chaucheyras-durand and Ossa, 2014*). However, investigation using PCR-based high-throughput sequencing technology has revealed several uncultured taxa and the relative abundance of the rumen fungal populations estimated to be 10% of the total microbial biomass, with large variations according to diet and individual (*Krause et al., 2013*).

Rumen Viromes and Bacteriophages

The occurrence of bacteriophages in the rumen has previously been documented at 10^7 to 10^9 particles per ml. About 28,000 different viral genotypes were identified using PCR-based protocols. Despite the possibility of isolating and storing several rumen phages in culture collections, particularly in the 1970's and 1980's, it was reported that only those phages with applicability in genetic engineering and phage therapy were successfully characterised beyond an initial assessment on the basis of morphology (*Gilbert and Klieve, 2015*). DNA sequencing and the advent of metagenomic studies to comprehensively sequence phage particle fractions obtained from rumen fluid as well as the full extent of viral diversity within the rumen based on "omic" applications is gradually being revealed (*Berg Miller et al., 2012*). Thus, diverse groups of rumen resident phages were found to have a high tendency of infecting various

species of rumen bacteria through their tendency for exhibiting lytic replication (*Gilbert et al., 2017*). However, little information is available about the genetics of phages while their genome has remained largely uncharacterised. *Gilbert et al. (2017)* reported that bacterial genome sequencing project is a powerful tool which is revealing the presence and activity of phages and their ability to integrate their DNA into the genome of their host to form stable, lysogenic associations. Thus, rumen phages could infect and replicate within the host and the release of progeny phage particles (*Gilbert et al., 2017*).

Exploring the rumen microbiome using PCR/qPCR

End point PCR

Polymerase chain reaction, PCR, is a step-by-step in vitro primer interposing DNA enzymatic amplification process. It is a technique of making millions of copies of a particular DNA target of interest that is being replicated during PCR cycles over a period of time (*Ginzinger, 2002; Sluijter et al., 2006*). At the last cycle of the reaction, PCR products are detected and analysed using electrophoresis. The amplification process (detection and identification of target DNA) occurs in three stages; denaturation, annealing and elongation stages. Identification is made through visual methods based on size the amplified DNA piece (*Freeman et al., 1999; Ginzinger, 2002; Sluijter et al., 2006*). Microbial PCR procedures have been optimised using primers targeting V2 – V4 regions of the 16 rRNA gene. Recently, pathophysiological examination in sheep by comparing resident microbiome in the upper aerodigestive and lower respiratory tracts of lambs, PCR was used to amplify the V2-V3 region of the 16S rRNA gene and subsequently sequenced via Illumina Miseq which revealed oropharyngeal swabs were either dominated by bacteria commonly associated with the rumen or by bacteria commonly associated with the upper aerodigestive tract (*Glendinning et al., 2017*). Similarly, PCR procedures have been optimised using primers targeting the V4 and V3-V4 region of the 16S rRNA gene under 20 and 28 PCR identical cycle conditions for amplicon sequencing to generate a relatively accurate representation of rumen microbiome (*McGovern et al., 2018*).

RT-PCR; qPCR

Real time PCR is also known as quantitative PCR (qPCR), depending on the application. The greatest advantage real-time PCR reaction compared to endpoint PCR is the possibility to determine concentration of the targeted DNA fragment as the template DNA is being synthesized. This makes data to be collected throughout the PCR process providing the possibility to view the entire

reaction and product generation, also combining the amplification and detection in one step (*Suzuki et al., 2000; Rasmussen, 2001; Wong and Medrano, 2005; Sluijter et al., 2006; Yibing, 2012; Alvarez and Doné, 2014*). In qPCR, a major feature is the point during amplification at which PCR product accumulation is first detected instead of quantitation of PCR product after a defined number of cycles. Results from real-time PCR can be both quantitative and qualitative. In the latter case, it is possible to visualise the melting curve of a DNA of interest after amplification, which can prove the presence or absence of the gene of interest and specificity of the reaction and not the amount of DNA present in a given sample (*Sluijter et al., 2006; VanGuilder et al., 2008; Malmuthuge et al., 2014; Glendinning et al., 2017*).

Rumen microbial composition consist of bacteria, archaea, ciliate protozoa, fungi, bacteriophage and viruses. Over 200 species of bacteria (approximately 10^{10} - 10^{11} cells/ml) and 100 species of protozoa and fungi are found in the rumen. However, despite their abundance and significant roles in fermentation and microbial digestion in the rumen, it was difficult to have them cultured, identified or characterised using conventional culture-based methods. There are many several species and groups of bacteria, protozoa and fungi which tend to influence different physiological processes such as *Fibrobacter succinogens*, *Lactobacillus ruminis*, *Escherichia coli*, anaerobic *chytridiomycete* fungi, *Peste des petits ruminants*, rinderpest, etc in ruminants (*Chaucheyras-durand and Ossa, 2014; Bainbridge et al., 2016; Mathew et al., 2018*). These identified microbial species could vary with diet, genetics and environment (*Benson et al., 2010; Uyeno, 2010; Kim et al., 2012; Henderson et al., 2015*). The use of PCR and qPCR in microbial investigations have evinced that the abundance of rumen bacteria is up to about 1012 cell/ml (*Kim et al., 2011; Jami and Mizrahi, 2012b*). With real-time PCR, the number of isolated bacterial species has been significantly increased and was found to play salient roles in the rumen (*Denman et al., 2018*). Similarly, 16S rRNA-gene-targeted specific primers for analysis of caecal microbial community using qPCR revealed how dietary supplements and age influenced amount of the copy number of each bacterium (total bacteria, *Bacteroides* and *Clostridia*) found in the caecum (*Bagóné Vántus et al., 2018*). A peculiar feature of the real-time PCR is that the bacterial and total microbial populations can be measured concurrently, this is important when dealing with heterogeneous rumen samples (*Skillman et al., 2006*).

Application of optimised and sensitive protocols based on the use of PCR/qPCR has identified and characterised several rumen microbes which

have shown tendencies of by-passing or escaping conventional culturing procedures thus providing accurate and more detailed information about the nature of microbial diversity and interaction that exist within and between rumen microbial populations (*Nathani et al., 2013*).

Bacterial Diversity:

Phylogenetic analysis of the bacterial communities that colonise the gut system of pre-weaned calves by *Malmthuge et al., (2014)*, revealed that a total of 83 genera belonging to 13 phyla with *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* predominating the gastro intestinal tract of the calves under investigation. Subsequently, qPCR analysis of the selected dominant bacterial genera (*Prevotella*, *Bacteroides*, *Lactobacillus*, and *Faecalibacterium*) in the same study revealed that, the occurrence of the identified bacterial genera varies significantly with different locations of the gastro intestinal tract as well as between bacteria found on the mucosa and in the rumen digesta (*Malmthuge et al., 2014*). These findings were consistent with *Henderson et al. (2015)*, who used a qPCR-based sequencing procedures and reported that *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* constitute the most dominant bacterial phyla in the rumen while subsequent phylogenetic analysis of the genomic sequences showed that *Prevotella*, *Butyrivibrio*, and *Ruminococcus*, as well as unclassified *Lachnospiraceae*, *Ruminococcaceae*, *Bacteroidales*, and *Clostridiales* are a "core bacterial microbiome" at the genus level (*Henderson et al., 2015*). Similarly, data from pyrosequencing of the bovine rumen bacterial 16S ribosomal rDNA gene sequences evince that the average composition of the rumen bacterial community consisted mainly of the phyla *Bacteroidetes* and *Firmicutes*, 51% and 43% of all reads, respectively while *Proteobacteria* accounted for 5.455% of the reads (*Kim et al., 2011; Jami and Mizrahi, 2012*). Molecular diversity of methanogens in the rumen of goats investigated with 16S rRNA gene clone libraries which consist of rumen fluid-associated methanogens (FAM) and rumen particle-associated methanogens (PAM) using methanogen-specific primers. The data obtained from the qPCR results shows that there is a significant difference in the concentration of methanogens in the two investigated groups, PAM (8.97 log 10) > FAM (7.57 log 10) (*Gu et al., 2011*). Recently, several bacteria has been identified which are significantly more abundant in lung fluids than in the upper aerodigestive tract swabs in sheep; *Staphylococcus equorum* was reported to be most predominant (*Glendinning et al., 2017*).

Protozoal Diversity:

Real-time PCR is more sensitive than microscopic count when it comes to protozoal detection and quantification because with qPCR, upto about 1-106 cells/ml protozoa is detectable (*McSwasea et al., 2007*). Cell counts and real-time PCR has been applied in quantifying total rumen protozoa and different genera of rumen protozoa on the basis of morphology (microscopy) or primer choice (real-time PCR) (*Skillman et al., 2006*). Diversity of the rumen protozoa using protozoa-specific PCR primers revealed that major protozoal species found in the rumen includes *Epidinium caudatum*, *Entodinium caudatum*, and *Isotricha prostoma* (*Sylvester et al., 2004*). *Tymensen et al. (2012)* compared protozoan communities from hay-fed cattle and silage-grain-fed cattle using T-RFLP analysis and the outcome yielded similar overall results to data obtained from microscopic analysis; it was concluded that *Entodinium* was the predominant genus of rumen protozoa present in all cattle regardless of diet, accounting for 94.2% of protozoa in silage/grain-fed cattle versus 57.4% for hay-fed cattle (*Skillman et al., 2006; Tymensen et al., 2012*). Apart from *Entodinium*, which has been reported to be dominant in the rumen, large phylogenetic diversity was found from a few numbers of sequenced clones. Protozoa that inhabit the rumen were detected by PCR using protozoan-specific primers based on the 18S rDNA sequences in the rumen contents of cow and the predominant protozoan genus identified in the whole rumen belonged to the *Entodinium* group (81.1%) (*Karnati et al., 2003; Shin et al., 2004*). *Entodinium* accounted for about 98% of the total protozoa, and populations within the same sheep were relatively stable, but greater variation occurred among individual sheep (10^0 and 10^6 *Entodinia* per gram of rumen contents).

Fungal Diversity:

Earlier attempts using the PCR/qPCR in order to quantitate rumen fungi has shown that there is high level of conservation within fungal 18S rDNA gene sequences and a more appropriate target for identification known as the internal transcribed spacer 1 region (ITS1) has been proposed to be targeted. ITS1 is located between the 18S rDNA and 5.8S rDNA genes and this region. It was identified as containing high levels of sequence variation, and it is used for the phylogenetic identification of anaerobic rumen fungi using qPCR-based techniques (*Bowman et al., 1992; Li and Heath, 1992; Brookman et al., 2000; Edwards et al., 2008*). Phylogenetic diversity of the gut anaerobic fungi was investigated in 30 different herbivore species by *Liggenstoffer et al. (2010)* using the

internal transcribed spacer region 1 rRNA in addition to a total of 267,287 sequences representing all known anaerobic fungal genera were obtained. The study revealed that sequences related to the genus Piromyces are predominant in rumen, which is about 36% of the total sequences obtained. Other sequences were related to the genera *Cyllamyces* and *Orpinomyces* and were categorised as the least abundant, representing 0.7 and 1.1% of the total sequences obtained, respectively. Subsequently, 38.3% of the sequences obtained did not cluster with previously identified genera and formed 8 phylogenetically distinct novel anaerobic fungal lineages (*Liggenstoffer et al., 2010*). qPCR also revealed significant reduction in the relative abundance of fungi with respect to diet and dietary additives (*Tapio et al., 2017*).

Rumen Bacteriophage:

Bacteriophages have significant role in maintaining rumen microbial balance (*Berg Miller et al., 2012*) and assisting the progress of horizontal gene transfer in the rumen microbiome (*Rohwer et al., 2009; Berg Miller et al., 2012*). There is an association between bacteriophage and microbial populations in the rumen evinced by sequence similarities that between the rumen viromes and the rumen microbiome. The application "omic"-based procedures have shown that most abundant rumen bacteriophage and prophage have been reported to be closely associate with several members of the rumen dominant bacterial phyla, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* (*Brulc et al., 2009; Berg Miller et al., 2012*). *Berg Miller et al., (2012)* reported that total DNA isolation from a phage-enriched fraction of rumen fluid and subsequent pyrosequencing of the total rumen virus-enriched metagenome (virome) revealed that rumen microbial genomes tend to share some sequence similarity to rumen viruses (phages) and prophage which also agrees with earlier findings which suggests transfer of genetic information between the microbial and viral populations within the rumen (*Brulc et al., 2009*) while prophages have been reported to be more numerous than lytic phages by approximately 2:1(*Berg Miller et al., 2012*).

Taxa Specific Oligonucleotide (Primer) Sequences for PCR

Application of real-time PCR in rumen microbiology using species-specific bacterial PCR primers (*Table1*) have been developed. Each pair of PCR primer sequence targets the 16S rRNA gene for detection and quantification of bacteria from diverse species within the rumen microbiome (*Khayalethu, 2013*). Also, these primer sets have been applied in studying the bacterial variation of microbial population based on DNA copy numbers in relation to changes in diet,

gut physiology of the animal as well as environmental conditions (*Tajima et al., 2001; Kamra, 2005*). The most frequently used primers for PCR/qPCR-based investigations of various bacteria, fungi, protozoa and archea species as shown by several studies are presented in *Table 1* and *2*.

Application of "omic" procedures with PCR/qPCR to rumen microbial diversity

Application of PCR/qPCR "omic"-based procedures has enhanced investigation of rumen microbiome over the past few years. Whole-genome sequencing, pyrosequencing, proteomics (metagenomic-proteomic), transcriptomics has provided a clearer insight into composition, functionality and diversity of each rumen microbial species (*Morrison et al.,* 2003; *Roesch et al.,* 2007; *Dowd et al.,* 2008; *Brulc et al.,* 2009; *Callaway et al.,* 2010; *Iakiviak et al.,* 2011; *Ransom-Jones et al.,* 2012; *Krause et al.,* 2013). Associations between microbial genes and the host animal could provide information on microbiome genotype-environment interaction using genome wide association studies, GWAS, in order to predict genetic predisposition to disease or performance among species (*Goodrich et al.,* 2017). Such DNA technologies are the basis of current rumen microbiological research and are revealing information about the true nature of the rumen microbiome. Basic concept underlying analysis of rumen microbiome on the basis of taxonomic structure, diversity, function, and sequence analysis methodology has been described by the rumen microbiome project workflow shown on *Figure 1* (*Di Bella et al.,* 2013; *McCann et al.,* 2014).

Metagenomics and Metatranscriptomics

Estimation of functional and fractional potentials of DNA and RNA can be achieved through the application of metagenomic and metatranspriptomic analysis. Such analysis are carried out using bioinformatics tools in order to study genetic materials from uncultured microorganisms. These tools have proven to be efficient in characterising the rumen microbiota according to function on the basis of their genomes as well as high throughput analysis of amplified taxonomic marker genes (*Li et al.,* 2017). In metagenomics, the template DNA is sequenced without prior amplification of specific genes which results in a snapshot of the gene pool and functional potential of the microbiome while in transcriptomics, mRNA is analyzed to provide a measure of gene expression within the intestinal microbiome (*Suchodolski,* 2012). A deeper and clearer insight into taxonomic and functional characteristics of the rumen mi-

crobiome and its interaction with the environment offers researchers the opportunity to optimize the digestion process in the rumen for enhanced and efficient sustainable utilization of dietary nutrients (*Walsh et al.,* 2017).

Metagenomic and metatranscriptomic inventions are considered more accurate and are widely used with conventional PCR and qPCR based protocols for routine identification, for result justifications and taxonomic assessment of the microbiota (*Urich et al.,* 2008; *Hong et al.,* 2009; *Huber et al.,* 2009; Ross *et al.,* 2012). The use of 16S rDNA sequences generated in metagenomics datasets sequencing procedures based on specific targeted marker genes and transcripts using 16S bacterial and archaeal rDNA/rRNA, 18S rDNA/rRNA for protozoa, and internal transcribed spacer (ITS) gene/transcript for fungi has been reported to be the basis of classification and phylogenetic analysis of rumen microbiome (*Deng et al.,* 2007; *Ellison et al.,* 2014).

Metagenomics and metatranscriptomics are fast, reliable, less expensive with diverse applicability. It has been applied in soils, water and animal-based samples. However, as a result of the low fraction of 16S rDNA reads present in metagenomics datasets, most metagenomic studies rely on analogous DNA amplicon sequence to characterise microbial populations (*Urich et al.,* 2008; *Baker et al.,* 2013; *Martínez et al.,* 2013 *Franzosa et al.,* 2014; *Logares et al.,* 2014; *Mason et al.,* 2014; *Rooks et al.,* 2014; *Tveit et al.,* 2014; *Li et al.,* 2016). To characterise biomass-degrading genes and genomes, 268 gigabases of metagenomic DNA from rumen microbes (which are viscid to plant fiber incubated in cow rumen), *Hess et al.,* identified 27,755 putative carbohydrate-active genes and 90 expressed candidate proteins, of which 57% were enzymatically active against cellulosic substrates (*Hess et al.,* 2011). However, recent studies reported that the rumen microbes are under-represented in the public databases (*Li et al* 2017; *Stewart et al.,* 2017; *Stewart et al.,* 2018). From these recent studies, 220 high quality bacterial and archaeal genomes assembled directly from 768 gigabases of rumen metagenomic data were presented and the comparative analysis of these sequences against current publicly available genomes shows that over 90% of these sequences represent previously non-sequenced strains and species of bacteria and archaea. The genomes contain over 13,000 proteins predicted to be involved in carbohydrate metabolism in the cow rumen. Furthermore, rumen virome has been explored using metagenomics and metatranscriptomics in dairy cattle and the results have shown that the rumen virome is composed of highly diversified and vast number of phages (*Berg Miller et al.*, 2012).

Table 1
PCR Primer sequences for detection of rumen bacteria, arachea and methanobacteriales

Target bacterium	Primer sequence (5′–3′)	AT (°C)*	Ps (bp)**	References
Total bacteria	Forward: CCTACGGGAGGCAGCAG Reverse: ATTACCGCGGCTGCTGG	60	194	(*Muyzer et al., 1993; Mosoni et al., 2007*)
Streptococcus bovis	Reverse: CTAATACCGCATAACAGCAT Forward: AGAAACTTCCTATCTCTAGG	57	869	(*Tajima et al., 2001*)
Eubacterium ruminantium	Forward: GCTTCTGAAGAATCATTTGAAG Reverse: TCGTGCCTCAGTGTCAGTGT	57	671	(*Tajima et al., 2001*)
Fibrobacter succinogenes	Forward: GGTATGGGATGAGCTTGC Reverse: GCCTGCCCCTGAACTATC	60	446	(*Tajima et al., 2001; Wanapat & Cherdthong, 2009*)
Ruminococcus albus	Forward: CCCTAAAAGCAGTCTTAGTTCG Reverse: CCTCCTTGCGGTTAGAACA	60	175	(*Koike & Kobayashi, 2001; Khampa et al., 2006*)
Ruminococcus flavefaciens	Forward: GGACGATAATGACGGTACTT Reverse: GCAATCYGAACTGGGACAAT	62	835	(*Tajima et al., 2001*)
Ruminobacter amylophilus	Forward: CAACCAGTCGCATTCAGA Reverse: CACTACTCATGGCAACAT	57	642	(*Tajima et al., 2001*)
Prevotella bryantii	Forward: AGTCGAGCGGTAAGATTG Reverse: CAAAGCGTTTCTCTCACT	68	540	(*Tajima et al., 2001; Wanapat & Cherdthong, 2009*)
Prevotella ruminicola	Forward: GGTTATCTTGAGTGAGTT Reverse: CTGATGGCAACTAAAGAA	53	485	(*Tajima et al., 2001; Wanapat & Cherdthong, 2009*)
Anaerovibrio lipolytica	Forward: TGGGTGTTAGAAATGGATTC Reverse: CTCTCCTGCACTCAAGAATT	57	597	(*Tajima et al., 2001; Wanapat & Cherdthong, 2009*)
Ruminobacter amylophilus	Forward: CAACCAGTCGCATTCAGA Reverse: CACTACTCATGGCAACAT	57	642	(*Tajima et al., 2001; Wanapat & Cherdthong, 2009*)
Selenomonas ruminantium	Forward: TGCTAATACCGAATGTTG Reverse: TCCTGCACTCAAGAAAGA	57	513	(*Tajima et al., 2001; Wanapat & Cherdthong, 2009*)
Treponema bryantii	Forward: ACTGCAGCGCGAACTGTCAGA Reverse: ACCTTACGGTGGCAGTGTCTC	57	412	(*Tajima et al., 2001; Wanapat & Cherdthong, 2009*)
Total arachea	Forward: GYGCAGCAGGCGCGAAA Reverse:GGACTACCSGGGTATCTAAT		415–420	(*Takai and Horikoshi 2000*)
Methanobacteriales	Forward: CGAAGGGAAGCTGTTAAGTC Reverse: TACCGTCGTCCACTCCTTC		343	(*Yu et al., 2005*)

*Annealing Temp., **Product size

Table 2
PCR primer sequences for detection of fungi and protozoa

Target microbe	Primer sequence (5′–3′)	Ps (bp)*	References
General anaerobic Fungi	Forward: GAGGAAGTAAAAGTCGTAACAAGGTTTC Reverse: CAAATTCACAAAGGGTAGGATGATT	120	(*Denman et al., 2006*)
Neocallimastigales-specific primer	Forward: TTGACAATGGATCTCTTGGTTCTC Reverse: GTGCAATATGCGTTCGAAGATT	-	(*Edwards et al., 2008*)
General Protozoa	Forward: CAYGTCTAAGTATAAATAACTAC Reverse: CTCTAGGTGATWWGRTTTAC	-	(*Sylvester et al., 2004*)
Ciliate Protozoa	Forward: TGTCTGGTTAATTCCGA Reverse: GTGATRWGRTTTACTTRT	-	(*Isaq et al., 2014*)

*Product size

Additionally, cow rumen metatranscriptomic datasets revealed distinct taxonomic and functional signatures in a study by (*Jiang et al.,* 2016) with enzymes involved in amino acid, energy and nucleotide metabolism and also identified microbiome-specific pathways such as phosphonate metabolism and glycan degradation pathways in the rumen. Rumen metagenomic profiling has been used to investigate relative abundance of microbial genes in the gastrointestinal tract of ruminants and could be applied to different species in order to predict influence of microbial composition on traits. Roehe and coworkers (2016) investigated the link between microbial genes, methane emissions and feed conversion efficiency in ruminants using metagenomic analysis and concluded that host microbial composition is an additional reliable basis for selection in animals since the host animal controls its own microbiota (*Roehe et al.,* 2016).

Bacterial 16S rRNA and Next Generation Sequencing (NGS) applications

Bacterial 16S rRNA gene is common and conversed among bacteria species but vary between taxa and as such, PCR/qPCR-based protocols target the 16S rRNA for identification, classification or characterisation of bacteria since decades of PCR application in molecular microbiological procedures (*Gutell et al.,* 1985; *Noller,* 1984; *Ginzinger et al.,* 2002; *Khayalethu,* 2013). Next-generation sequencing (NGS) provides high-throughput data for precise and accurate analysis, characterisation and an in-depth examination of the complex rumen microbiome (*Shokralla et al.,* 2012). NGS-based 16S rRNA sequencing is considered cost-effective technique for identifying microbial strains that may not be easily characterised using culture-based methods. Early studies by *Gray et al.,* (1984) highlighted that the bacterial 16S gene is composed of about nine hypervariable regions (V1-V9) that ranged from about 30-100 base pairs long

that are involved in the secondary structure of the small ribosomal subunits. However, the degree of conservation varies widely between hypervariable regions, with more conserved regions correlating to higher-level taxonomy and less conserved regions to lower levels, such as genus and species. Consequently, amplicon sequencing revealed diet specific taxa abundance variations revealing significant differences in protozoal and fungal composition within the rumen of a mature cow (*Tapio et al.,* 2017).

Several studies have explored the sequencing of the 16S rRNA gene in evaluating the rumen epithelial bacterial diversity during the development of the rumen and the most dominant phyla detected in the rumen epithelium were found to belong to *Proteobacteria*, *Firmicutes*, and *Bacteroidetes* (*Steveson & Waimer*, 2007; *Li et al.,* 2009; *Petri et al.,* 2013; *Chaucheyras-durand and Ossa,* 2014; *Jiao et al.,* 2015). Additionally, *Bacteroidetes* has also been reported to be the most abundant phylum in the rumen microbiota, representing upto about 74.8% of the 16S sequences, followed by *Firmicutes* (12.0%), *Proteobacteria* (10.4%), *Verrucomicrobia* (1.2%) and *Synergistetes* (1.1%) and the researchers concluded that the four most abundant phyla in the rumen of matured bull calves which were fed a solid diet are *Bacteroidetes* (52.0%), *Firmicutes* (42.7%), *Spirochaetes* (2.3%) and *Fibrobacteres* (1.9%)(*Li et al.,* 2012).

Pyrosequencing, Illumina Sequencing (MiSeq) and Massively Parallel Signature Sequencing (MPSS)

Pyrosequencing procedure also enables comparison between and within bacterial species in a given rumen microbiome using 16S rRNA hypervariable regions (approx. 1500 base pairs long) as standard and reliable markers for the taxonomic classification and phylogenetic analysis (*Yang et al.,* 2016). Pyrosequencing is a well optimized procedure for phylogenetic analysis of the complex rumen microbiome (*Kim et al.,* 2011; *Klindworth et al.,* 2013; *Yang et al.,* 2016; *Fuks et al.,* 2018; *McGovern et al.,* 2018). *Tewari et al.* (2011), showed that utilisation of sequence data from 2 variable regions within the 16S rRNA gene: V1 and V6 identified about 80% of microbes isolated from animals up to the genus level. Similarly, pyrosequencing of hypervariable V3-V5 regions of the 16S rRNA gene and whole-genome shotgun approach to rumen microbiota in pre-ruminant (42-day-old) calves has identified fifteen bacterial phyla in a study conducted by *Li et al.* (2012). Previously, it has been shown by *Tewari et al.* (2011) that the differences among different bacterial strains were identified using pyrosequencing. From the strain differential studies, they pointed out that *Staphylococcus aureus* differs from other *Staphylococci* while *Rhodococcus*

equi also differs from the closely related genus *Dietzia spp*. Subsequently, pyrosequencing of *S. bovis* identified and differentiated the species from *Enterococcus cecorum* (*Tewari et al.,* 2011). These findings are consistent with other studies which evinced that deep amplicon sequencing of the 16S ribosomal RNA gene have significant differences within the bacterial communities on dietary basis and between rumen solid and liquid contents (*Callaway et al.,* 2010; *Callaway et al.,* 2011; *De Menezes., et al.,* 2011).

Similarly, taxonomic analysis of metagenomic reads from 16S rRNA sequences during investigation of the establishment of gut microbial community indicated that the predominant phyla were distinct at different growth stages (*Han et al.,* 2015). They show that phyla *Firmicutes* and *Synergistetes* were predominant in rumen samples taken from 80 to 100-day-old goats. The age of the animals has shown to have significant effect on the abundance of bacterial species. Goats on day 100 showed *Bacteroidetes* and *Firmicutes* as most abundant phyla while relative abundance of *Firmicutes* and *Synergistetes* was found to be reduced after weaning and *Bacteroidetes* and *Proteobacteria* increased with increase with age (from 80 to 110 days). Similarly, Illumina MiSeq platform was used for amplification of the V4 hypervariable region of the 16S rRNA gene from rumen fluid of goats fed the same diet for 20 days. Analysis of the gene sequences using the quantitative insights into microbial ecology pipeline software shows that *Bacteroidetes, Firmicutes, Tenericutes* were the predominant phylum in both groups, and their relative abundance was 60.63%, 29.48%, 2.24% respectively. *Prevotella* being the most abundant shared genus between the control and experimental groups (*Wang et al.,* 2018).

Massive parallel signature sequencing (MPSS) procedure is a tool that is used for an in-depth profiling of gene expression pattern through sequencing mRNA transcripts. Bioinformatics tools sort out sequence signature of about 16-20 base pairs generated and identified from each bead by counting the number of individual mRNA molecules produced from each gene (*Reinartz et al.,* 2002). Thus the level of expression of each gene is directly proportional to the number signatures for the gene's mRNA counted. It is highly specific, sensitive (with a level of sensitivity as low as a few molecules of mRNA per sample), and transcripts are captured on individual microbeads through a complementary DNA signature sequence without prior identification and characterization of the gene. This provides wide-range analysis of DNA templates transcribed into an RNA of an entire genome (Brenner *et al.,* 2000; *Reinartz et al.,* 2002*; Jongeneel et al.,* 2005; *Rédei*, 2008).

Application of MPSS has also provided deeper insight into rumen microbiological research in recent years. It has possibility of providing accurate predictions that are based on the host genome for traits which are largely influenced by the gut microbiota such as digestive tract disorders, metabolic functions, body mass index (BMI), inflammatory bowel disorder (IBD) and enteric methane production in cattle (Leahy *et al.,* 2013; *Ross et al.,* 2013a). It could also be applied in genomic predictions of complex phenotypes in humans and animals (*Ross et al.,* 2013a). Diversity of the rumen virome in dairy cattle using MPSS revealed strong similarities at functional level between rumen viral samples, which were highly distinct from the rumen microbiome samples. Significant variation between animals living in different housing while animals housed together presented similar viromes (*Bathe et al.,* 2003). Ross and coworkers also observed large effects of feed additives on the rumen microbiome profiles using MPPS (*Ross et al.,* 2013b). Recently, *Earl et al.,* (2018), analysed the 16S rRNA microbiome surveys using massively parallel DNA sequencing technologies and reported an increased taxonomic and phylogenetic resolution with species-level classification of >90% of taxa and relative abundances microbial population have been reported. They concluded that application of MPSS to marker genes could help enhance taxonomic assignments of microbial species and reference databases and subsequently increase the specificity of relationship between microbial communities (*Earl et al.,* 2018).

Non-PCR based next generation sequencing technology

With recent advancements in NGS, several PCR-independent NGS technologies have evolved which does not require prior DNA amplification using PCR. Some of these emerging sequencing technologies include single molecule sequencing with Heliscope single molecule sequencer, PacBio single molecule real-time (PacBio SMRT) sequencer and Oxford nanopore sequencer systems which need no pre-amplification steps. Pacific biosciences single molecule real-time (SMRT) sequencing technology is another molecular microscope with no requirement for template amplification step. It enables single molecule detection using sensitive optics which could spot individual fluorescently labeled nucleotide (*Buermans and den Dunnen,* 2014). These technologies are reliable, fast and sensitive enough to detect signal and read the DNA sequence of individual molecule template extensions. Thus, providing extension of unamplified molecules with greater flexibility in the kinetics of the sequencing chemistry (*Pushkarev et al.,* 2009; *Zhou et al.,* 2013). llumina shotgun sequencing (such as HiSeq, MiSeq), is another PCR-independent sequencing procedure

that enables estimation of species composition without PCR as a pre-step (*Zhou et al.,* 2013).

RFLP/T-RFLP and DGGE/TGGE

Restriction fragment length polymorphism (RFLP), or terminal-restriction fragment length polymorphism (T-RFLP), (allows profiling, but also quantitation of microbiota). Denaturing gradient gel electrophoresis (DGGE), or temperature gradient gel electrophoresis (TGGE), are commonly used PCR based molecular fingerprinting techniques for evaluating microbial community and diversity. T-RFLP uses fluorescent-labelled primer for amplification of bacterial DNA fragments during PCR and this allows profiling as well as quantitation of microbial communities using capillary electrophoresis of PCR products fragmented by size with sequence-specific restriction enzymes. During DGGE analysis, a gel containing a linear gradient of DNA denaturants is used, while TGGE a temperature gradient is used for separation. DGGE/TGGE has short shorter bands (20 to 40 bands) though many bacterial phylotypes will have similar or the same melting characteristics. This is a key factor that limits visualisation and evaluation of changes in only predominant bacterial groups. There are possibilities to resect and sequence individual band of interest. These techniques separate the PCR amplicons that were generated with either universal or group-specific primers to yield a profile (fingerprint) of the bacterial community (*Suchodolski,* 2012).

RFLP has been applied in combination with PCR procedures in comparing the genetic diversity and phylogenetic experiments. *Avguštin et al.* (1994). aimed to evaluate the nature of diversity that exist among 29 strains of *Prevotella ruminicola* from the rumen has identified signature oligonucleotides based on 16S ribosomal DNA sequences which distinguished the strains related to strains 23T, B(1)4, GA33, and M384, as well as an oligonucleotide that specifically recognised all but one of the *Bacteroides* and *Prevotella* strains tested. 5 ammonia hyper-producing (HAP) bacteria, using RFLP of 16S rDNA indicated that isolates differed from the previously described HAP bacteria and 16S rDNA PCR-RFLP-based investigations suggested that ruminal *Strptoccocu. bovis* diversity may be a diet-dependent phenomenon (*Kleive et al.,* 1999; *Jarvis et al.,* 2001). *Regensbogenova et al.,* (2004b) reported that restriction fragment length polymorphism (RFLP) analysis of PCR amplified 18S rDNA sequences could be applied in identifying different rumen protozoa species. However, researchers were of the opinion that since 18S rDNA sequences among different protozoa are found to be closely related, the phylogenetic resolution of such RFLP analysis remains unclear (*McSweeney et al.,* 2007).

The crucial element of RFLP as a procedure is the selection of an efficient and suitable restriction enzymes for the analysis and is applied in exploring the composition of individual bacterial species (*Dang et al.*, 2007). *Schlegel et al.,* (2003), used two strains of streptococcus, *S. bovis* and *S. equinus* which are important intestinal bacterial isolates from human and animal specimens. *Schlegel et al.* (2003), investigated the nature of the diversity of large bacterial complex including different species frequently isolated from infections of humans (*Streptococcus gallolyticus, Streptococcus infantarius*) or animals (*S. bovis, S. equinus, S. alactolyticus*). The diversity of strains of *S. bovis* biotype II was analysed, and it was confirmed that they belong to different species, either *S. equinus* or *S. infantarius*. *Tymensen et al.* (2012), used T-RFLP to analyse rumen protozoa communities. The protozoa communities from hay-fed and silage/grain-fed cattle were different while multivariate analysis indicated that cattle fed the same diet and housed together (pen mates) tended to have similar protozoa community types.

Analysis of ruminal methanogenic *Archaea* communities in the rumen using DGGE is an efficient PCR-based technique in revealing the diversity and complexity of the rumen microbial community. It separates PCR products of the same length but differing in sequence since different sequences possess different melting temperatures resulting in contrasting migration behaviour. Additionally, analysis of PCR-DGGE fingerprint and phylogenetic analysis 16S rDNA sequences in DGGE profiles were combined to reveal the dominant bacterial communities and compared the variations of such bacterial communities in cattle breed. *Leng et al.* (2011) (consistent with *Yang et al.,* 2010) shows that the dominant bacteria in the rumen phyla are *Firmicutes*, *Bacteroidetes and Actinobacteria* with significant variation in bacterial specie according to the cattle breeds investigated. *Székely et al.* (2009), compared the accuracy of DGGE and T-RFLP using bacterial diversity in mature compost bacterial community and cloning of environmental 16S rDNA and reported that DGGE shows less diversity (15–22 bands per sample) than T-RFLP (20–59 peaks greater than or equal to 1% of total peak area per sample). However, principal component analysis (PCA), of the DGGE and T-RFLP profiles showed that both molecular fingerprinting techniques gave a similar clustering of the samples.

CONCLUSION

The development and thorough application of PCR/qPCR DNA-based technologies in rumen microbiology research have provided a deeper insight into the biological understanding of the rumen microbiota. It is precise, more

reliable and more efficient than traditional, culture-based identification and characterisation of the rumen microbiota. Through the use of PCR/qPCR-based procedures, quantitation of different rumen microbiota as well as analysis of phylogenetic relationship between and within these various microbial constituents in the rumen is achievable. However, it is advisable to optimise these procedures and other "omic"-based technologies used with PCR/qPCR protocols for accurate analysis of the complex rumen microbiome.

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REFERENCES

Alvarez, M. L., & Doné, S. C. (2014). SYBR® green and TaqMan® quantitative PCR arrays: Expression profile of genes relevant to a pathway or a disease state. Methods Mol. Biol., 1182, 321–359.DOI: *10.1007/978-1-4939-1062-5_27*

Avguštin, G., Wright, F., & Flint, H. J. (1994). Genetic diversity and phylogenetic relationships among strains of Prevotella (Bacteroides) ruminicola from the rumen. Int. J. Syst. Bacteriol., 44, 246–255.DOI: *10.1099/00207713-44-2-246*

Bagóné Vántus, V., Dalle Zotte, A., Cullere, M., Bónai, A., Dal Bosco, A., Szendrő, Z., Tornyos, G., Pósa, R., Bóta, B., Kovács, M., & Zsolnai, A. (2018). Quantitative PCR with 16S rRNA-gene-targeted specific primers for analysis of caecal microbial community in growing rabbits after dietary supplementation of thyme (Thymus vulgaris) and spirulina (Arthrospira platensis). Ital. J. Anim. Sci., 17, 657–665. DOI: *10.1080/1828051X.2017.1400413*

Bainbridge, M. L., Cersosimo, L. M., Wright, A. D. G., & Kraft, J. (2016). Rumen bacterial communities shift across a lactation in Holstein, Jersey and Holstein × Jersey dairy cows and correlate to rumen function, bacterial fatty acid composition and production parameters. FEMS Microbiol. Ecol., 92. DOI: *10.1093/femsec/fiw059*

Baker, B. J., Sheik, C. S., Taylor, C. A., Jain, S., Bhasi, A., Cavalcoli, J. D., & Dick, G. J. (2013). Community transcriptomic assembly reveals microbes that contribute to deep-sea carbon and nitrogen cycling. ISME J., 7, 1962–1973. DOI: *10.1038/ismej.2013.85*

Bartlett, J. M. S.; Stirling, D. (2003). "A Short History of the Polymerase Chain Reaction". PCR Protocols. Methods Mol. Biol., 226, 3–6. DOI: *10.1385/1-59259-384-4:3*

Bathe, B., Bartels, D., Bischoff, N., Bott, M., Burko, A., Dusch, N., Wiegra, I. (2003). The complete Corynebacterium glutamicum ATCC 13032 genome sequence and its impact on the production of L -aspartate-derived amino acids and vitamins. J. Biotechnol., 104, 5–25. DOI: *10.1101/gr.120618.111*

Benson, A. K., Kelly, S. A., Legge, R., Ma, F., Low, S. J., Kim, J., Pomp, D. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc. Natl. Acad. Sci., 107, 18933–18938. DOI: *10.1073/pnas.1007028107*

Berg Miller, M. E., Yeoman, C. J., Chia, N., Tringe, S. G., Angly, F. E., Edwards, R. A., … White, B. A. (2012). Phage-bacteria relationships and CRISPR elements revealed by a metagenomic survey of the rumen microbiome. Environ. Microbiol., 14, 207–227. DOI: *10.1111/j.1462-2920.2011.02593.x*

Boots, B., Lillis, L., Clipson, N., Petrie, K., Kenny, D. A., Boland, T. M., & Doyle, E. (2013). Responses of anaerobic rumen fungal diversity (phylum Neocallimastigomycota) to changes in bovine diet. J. Appl. Microbiol., 114, 626–635. DOI: *10.1111/jam.12067*

Brenner, S., Johnson, M., Bridgham, J., Golda, G., Lloyd, D. H., Johnson, D., … Corcoran, K. (2000). Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. Nat. Biotechnol. DOI: *10.1038/76469*

Brulc, J. M., Antonopoulos, D. A., Berg Miller, M. E., Wilson, M. K., Yannarell, A. C., Dinsdale, E. A., White, B. A. (2009). Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. Proc. Natl. Acad. Sci., 106, 1948–1953. DOI: *10.1073/pnas.0806191105*

Buermans, H. P. J., & den Dunnen, J. T. (2014). Next generation sequencing technology: Advances and applications. *Biochimica et Biophysica Acta - Molecular Basis of Disease*. DOI: *10.1016/j.bbadis.2014.06.015*

Callaway, T. R., Dowd, S. E., Edrington, T. S., Anderson, R. C., Krueger, N., Bauer, N., Nisbet, D. J. (2010). Evaluation of bacterial diversity in the rumen and feces of cattle fed different levels of dried distillers grains plus solubles using bacterial tag-encoded FLX amplicon pyrosequencing. J. Anim. Sci., 88, 3977–3983. DOI: *10.2527/jas.2010-2900*

Chaucheyras-Durand, F. F., & Ossa, F. (2014). The rumen microbiome: Composition, abundance, diversity, and new investigative tools. The Prof. Anim. Sci., 30(1), 1–12. DOI: *10.15232/s1080-7446(15)30076-0*

Chaucheyras-Durand, F., Chevaux, E., Martin, C., & Forano, E. (2012). Use of Yeast Probiotics in Ruminants: Effects and Mechanisms of Action on Rumen pH, Fibre Degradation, and Microbiota According to the Diet. In Probiotic Anim. (pp. 119–162). DOI: *10.5772/50192*

Cotta, M. A. (1988). Amylolytic activity of selected species of ruminal bacteria. *Appl. Environ. Microbiol*.

Dang, H. A., Zsolnai, A., Kovacs, M., Bors, I., Bonai, A., Bota, B., & Szabóoo-Fodor, J. (2017). In vitro interaction between fumonisin B1and the intestinal microflora of pigs. Pol. J. Microbiol., 66, 245–250. DOI: *10.1002/jps.2600791210*

De Menezes, A. B., Lewis, E., O'Donovan, M., O'Neill, B. F., Clipson, N., & Doyle, E. M. (2011). Microbiome analysis of dairy cows fed pasture or total mixed ration diets. FEMS Microbiol. Ecol., 78, 256–265. DOI: *10.1111/j.1574-6941.2011.01151.x*

Dehority, B. A. (2003). Rumen Microbiology. Nottingham, UK. Nottingham University Press.

Denman, S. E., & McSweeney, C. S. (2006). Development of a real-time PCR assay for monitoring anaerobic fungal and cellulolytic bacterial populations within the rumen. FEMS Microbiol. Ecol., 58, 572–582. DOI: *10.1111/j.1574-6941.2006.00190.x*

Di Bella, J. M., Bao, Y., Gloor, G. B., Burton, J. P., & Reid, G. (2013). High throughput sequencing methods and analysis for microbiome research. J. Microbiol. Methods, 95, 401–414. DOI: *10.1016/j.mimet.2013.08.011*

Dowd, S. E., Callaway, T. R., Wolcott, R. D., Sun, Y., McKeehan, T., Hagevoort, R. G., & Edrington, T. S. (2008). Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). BMC Microbiol, 8. DOI: *10.1186/1471-2180-8-125*

Earl, J. P., Adappa, N. D., Krol, J., Bhat, A. S., Balashov, S., Ehrlich, R. L., … Mell, J. C. (2018). Species-level bacterial community profiling of the healthy sinonasal microbiome using Pacific Biosciences sequencing of full-length 16S rRNA genes. *bioRxiv*, 338731. DOI: *10.1101/338731*

Edwards, J. E., Kingston-Smith, A. H., Jimenez, H. R., Huws, S. A., Skøt, K. P., Griffith, G. W., … Theodorou, M. K. (2008). Dynamics of initial colonization of nonconserved perennial ryegrass by anaerobic fungi in the bovine rumen. FEMS Microbiol. Ecol., 66, 537–545. DOI: *10.1111/j.1574-6941.2008.00563.x*

Foroozandeh, A. D., Rezaeian, M., Balaly, G. R., & Alikhani, M. (2009). Relative contributions of Rumimil Bacteria, Protozoa and Fungi to degradation of forage fiber fractions. *J. Anim. Vet. Adv.,*

Franzosa, E. A., Morgan, X. C., Segata, N., Waldron, L., Reyes, J., Earl, A. M., Huttenhower, C. (2014). Relating the metatranscriptome and metagenome of the human gut. Proc. Natl. Acad. Sci., 111, E2329–E2338. DOI: *10.1073/pnas.1319284111*

Freeman, W. M., Walker, S. J., & Vrana, K. E. (1999). Quantitative RT-PCR: Pitfalls and potential. BioTechniques, DOI: *10.2144/99261rv01*

Fuks, G., Elgart, M., Amir, A., Zeisel, A., Turnbaugh, P. J., Soen, Y., & Shental, N. (2018). Combining 16S rRNA gene variable regions enables high-resolution microbial community profiling. Microbiome, 6. DOI: *10.1186/s40168-017-0396-x*

Gilbert, R. A., & Klieve, A. V. (2015). Ruminal viruses (bacteriophages, archaeaphages). In Rumen Microbiology: From Evolution to Revolution. DOI: *10.1007/978-81-322-2401-3_9*

Gilbert, R. A., Kelly, W. J., Altermann, E., Leahy, S. C., Minchin, C., Ouwerkerk, D., & Klieve, A. V. (2017). Toward understanding phage: Host interactions in the rumen; complete genome sequences of lytic phages infecting rumen bacteria. Front. Microbiol., 8. DOI: *10.3389/fmicb.2017.02340*

Ginzinger, D. G. (2002). Gene quantification using real-time quantitative PCR: An emerging technology hits the mainstream. *Experimental Hematology*, 30, 503–512. DOI: *10.1016/S0301-472X (02)00806-8*

Glendinning, L., Collie, D., Wright, S., Rutherford, K. M. D., & McLachlan, G. (2017). Comparing microbiotas in the upper aerodigestive and lower respiratory tracts of lambs. *Microbiome*, *5*, 145. DOI: *10.1186/s40168-017-0364-5*

Goodrich, J. K., Davenport, E. R., Clark, A. G., & Ley, R. E. (2017). The Relationship Between the Human Genome and Microbiome Comes into View. Annu. Rev. Genet., 51, 110711-155532. DOI: *10.1146/annurev-genet-110711-155532*

Gray, M. W., Sankoff, D., & Cedergren, R. J. (1984). On the evolutionary descent of organisms and organelles: A global phylogeny based on a highly conserved structural core in small subunit ribosomal RNA. Nucleic Acids Res., 12, 5837–5852. DOI: *10.1093/nar/12.14.5837*

Gu, M. J., Alam, M. J., Kim, S. H., Jeon, C. O., Chang, M. B., Oh, Y. K., Lee, S. S. (2011). Analysis of methanogenic archaeal communities of rumen fluid and rumen particles from Korean black goats. Anim. Sci. J., 82, 663–672. DOI: *10.1111/j.1740-0929.2011.00890.x*

Gutell, R. R., Weiser, B., Woese, C. R., & Noller, H. F. (1985). Comparative Anatomy of 16-S-like Ribosomal RNA. Prog. Nucleic Acid Res. Mol. Biol., 32, 155–216. DOI: *10.1016/S0079-6603(08)60348-7*

Han, X., Yang, Y., Yan, H., Wang, X., Qu, L., & Chen, Y. (2015). Rumen bacterial diversity of 80 to 110-day- Old goats using 16s rRNA sequencing. PLoS One., 10. DOI: *10.1371/journal.pone.0117811*

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., & Janssen, P. H. (2016). Erratum: Erratum: Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci. Rep., 5, 14567. DOI: *10.1038/srep19175*

Hess, M., Sczyrba, A., Egan, R., Kim, T. W., Chokhawala, H., Schroth, G., Rubin, E. M. (2011). Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Sci., 331, 463–467. DOI: *10.1126/science.1200387*

Hino, T., & Russell, J. B. (1987). Relative contributions of ruminal bacteria and protozoa to the degradation of protein in vitro. J. Anim. Sci., DOI: 10.2527/jas1987.641261x

Huber, J. A., Morrison, H. G., Huse, S. M., Neal, P. R., Sogin, M. L., & Mark Welch, D. B. (2009). Effect of PCR amplicon size on assessments of clone library microbial diversity and community structure. Environ. Microbiol., 11, 1292–1302. DOI: *10.1111/j.1462-2920.2008.01857.x*

Hungate, R. E. (2013). The Rumen Bacteria. In; The Rumen and its Microbes. DOI: 10.1016/b978-1-4832-3308-6.50005-x

Iakiviak, M., Mackie, R. I., & Cann, I. K. O. (2011). Functional analyses of multiple lichenin-degrading enzymes from the rumen bacterium Ruminococcus albus 8. Appl. Environ. Microbiol., 77, 7541–7550. DOI: *10.1128/AEM.06088-11*

Ishaq, S. L., & Wright, A.-D. G. (2014). Design and validation of four new primers for Next-Generation Sequencing to target the 18S rRNA genes of gastrointestinal ciliate Protozoa. Appl. Environ. Microbiol., 80, 5515–5521. DOI: *10.1128/aem.01644-14*

Jami, E., & Mizrahi, I. (2012). Composition and similarity of bovine rumen microbiota across individual animals. PLoS One, 7. DOI: *10.1371/journal.pone.0033306*

Jarvis, G. N., Kurtovic, A., Hay, A. G., & Russell, J. B. (2001). The physiological and genetic diversity of bovine Streptococcus bovis strains. FEMS Microbiol. Ecol., 35, 49–56. DOI: 10.1016/S0168-6496(00)00110-0

Jiang, Y., Xiong, X., Danska, J., & Parkinson, J. (2016). Metatranscriptomic analysis of diverse microbial communities reveals core metabolic pathways and microbiomespecific functionality. Microbiome, 4. DOI: *10.1186/s40168-015-0146-x*

Jiao, J., Huang, J., Zhou, C., & Tan, Z. (2015). Taxonomic identification of ruminal epithelial bacterial diversity during rumen development in goats. Appl. Environ. Microbiol., 81, 3502–3509. DOI: *10.1128/AEM.00203-15*

Jongeneel, C. V., Delorenzi, M., Iseli, C., Zhou, D., Haudenschild, C. D., Khrebtukova, I., … Vasicek, T. J. (2005). An atlas of human gene expression from massively parallel signature sequencing (MPSS). Genome Res. DOI: *10.1101/gr.4041005*

Kamra, D. N. (2005). Rumen microbial ecosystem. *Current Science*.

Karnati, S. K. R., Yu, Z., Sylvester, J. T., Dehority, B. A., Morrison, M., & Firkins, J. L. (2003). Technical note: Specific PCR amplification of protozoal 18S rDNA sequences from DNA extracted from ruminai samples of cows. J. Anim. Sci., 81, 812–815. DOI: *10.2527/2003.813812x*

Khayalethu, N. (2013). Identifying bacteria and studying bacterial diversity using the 16S ribosomal RNA gene-based sequencing techniques: A review. African J. Microbiol. Res., 7, 5533–5540. DOI: *10.5897/AJMR2013.5966*

Kim, M., Morrison, M., & Yu, Z. (2011). Evaluation of different partial 16S rRNA gene sequence regions for phylogenetic analysis of microbiomes. J. Microbiol. Methods., 84, 81–87. DOI: *10.1016/j.mimet.2010.10.020*

Klieve, A. V., Heck, G. L., Prance, M. A., & Shu, Q. (1999). Genetic homogeneity and phage susceptibility of ruminal strains of Streptococcus bovis isolated in Australia. Lett. Appl. Microbiol., 29, 108–112. DOI: *10.1046/j.1365-2672.1999.00596.x*

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., & Glöckner, F. O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res., 41. DOI: *10.1093/nar/gks808*

Krause, D. O., Denman, S. E., Mackie, R. I., Morrison, M., Rae, A. L., Attwood, G. T., & McSweeney, C. S. (2003). Opportunities to improve fiber degradation in the rumen: Microbiology, ecology, and genomics. FEMS Microbiol. Rev., DOI: *10.1016/S0168-6445(03)00072-X*

Krause, D. O., Nagaraja, T. G., Wright, A. D. G., & Callaway, T. R. (2013). Board-invited review: Rumen microbiology: Leading the way in microbial ecology. J. Anim. Sci., DOI: *10.2527/jas.2012-5567*

Leahy, S. C., Kelly, W. J., Ronimus, R. S., Wedlock, N., Altermann, E., & Attwood, G. T. (2013). Genome sequencing of rumen bacteria and archaea and its application to methane mitigation strategies. Intl. J. Anim. Biosci., DOI: *10.1017/S1751731113000700*

Leng, J., Xie, L., Zhu, R., Yang, S., Gou, X., Li, S., & Mao, H. (2011). Dominant bacterial communities in the rumen of Gayals (Bos frontalis), Yaks (Bos grunniens) and Yunnan Yellow Cattle (Bos taurs) revealed by denaturing gradient gel electrophoresis. Mol. Biol. Rep., 38, 4863–4872. DOI: *10.1007/s11033-010-0627-8*

Li, F., Neves, A. L. A., Ghoshal, B., & Guan, L. L. (2017). Mining metagenomic and metatranscriptomic data for clues about microbial metabolic functions in ruminants 1. J. Dairy Sci., DOI: *10.3168/jds.2017-13356*

Li, M., Penner, G. B., Hernandez-Sanabria, E., Oba, M., & Guan, L. L. (2009). Effects of sampling location and time, and host animal on assessment of bacterial diversity and fermentation parameters in the bovine rumen. J. Appl. Microbiol., 107. 1924–1934. DOI: *10.1111/j.1365-2672.2009.04376.x*

Li, R. W., Connor, E. E., Li, C., Baldwin Vi, R. L., & Sparks, M. E. (2012). Characterization of the rumen microbiota of preruminant calves using metagenomic tools. Environ. Microbiol., 14, 129–139. DOI: *10.1111/j.1462-2920.2011.02543.x*

Liggenstoffer, A. S., Youssef, N. H., Couger, M. B., & Elshahed, M. S. (2010). Phylogenetic diversity and community structure of anaerobic gut fungi (phylum Neocallimastigomycota) in ruminant and non-ruminant herbivores. ISME J., 4, 1225–1235. DOI: *10.1038/ismej.2010.49*

Logares, R., Sunagawa, S., Salazar, G., Cornejo-Castillo, F. M., Ferrera, I., Sarmento, H., Acinas, S. G. (2014). Metagenomic 16S rDNA Illumina tags are a powerful alternative to amplicon sequencing to explore diversity and structure of microbial communities. Environ. Microbiol., 16, 2659–2671. DOI: *10.1111/1462-2920.12250*

Malmuthuge, N., Griebel, P. J., & Guan, L. L. (2014). Taxonomic identification of commensal bacteria associated with the mucosa and digesta throughout the gastrointestinal tracts of preweaned calves. Appl. Environ. Microbiol., 80, 2021–2028. DOI: *10.1128/AEM.03864-13*

Martínez, A., Ventouras, L. A., Wilson, S. T., Karl, D. M., & DeLong, E. F. (2013). Metatranscriptomic and functional metagenomic analysis of methylphosphonate utilization by marine bacteria. Front. Microb., 4. DOI: *10.3389/fmicb.2013.00340*

Mason, O. U., Scott, N. M., Gonzalez, A., Robbins-Pianka, A., Bælum, J., Kimbrel, J., Jansson, J. K. (2014). Metagenomics reveals sediment microbial community response to Deepwater Horizon oil spill. ISME J., 8, 1464–1475. DOI: *10.1038/ismej.2013.254*

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P. W., & Cotter, P. D. (2018). The rumen microbiome: a crucial consideration when optimising milk and meat production and nitrogen utilization efficiency. Gut Microbes, 1–18. *10.1080/19490976.2018.1505176*

McCann, J. C., Wickersham, T. A., & Loor, J. J. (2014). High-throughput methods redefine the rumen microbiome and its relationship with nutrition and metabolism. Bioinf. Biol. Insights., *10.4137/BBIi.Ss15389*

McGovern, E., Waters, S. M., Blackshields, G., & McCabe, M. S. (2018). Evaluating established methods for Rumen 16S rRNA amplicon sequencing with mock microbial populations. Front. Microbiol., 9(JUN). DOI: *10.3389/fmicb.2018.01365*

McSweeney, C. S., Denman, S. E., Wright, A. D. G., & Yu, Z. (2007). Application of recent DNA/RNA-based techniques in rumen ecology. Asian-Aust. J. Anim. Sci., 20, 283–294. DOI: *10.5713/ajas.2007.283*

Mobashar, M., Hummel, J., Blank, R., & Südekum, K. H. (2019). Contribution of different rumen microbial groups to gas, short-chain fatty acid and ammonium production from different diets—an approach in an in vitro fermentation system. J. Anim. Physiol. Anim. Nutr. DOI: *10.1111/jpn.12996*

Morgavi, D. P., Kelly, W. J., Janssen, P. H., & Attwood, G. T. (2013). Rumen microbial (meta) genomics and its application to ruminant production. In Anim., 7, 184–201. DOI: *10.1017/S1751731112000419*

Morrison, M., K. E. Nelson, D. Antonopoulos, B. Cheng, E. Devillard, D. E. Goodheart, H. S. Jun, V. Parisi, J. S. Park, S. M. Qi, C. Reveneau, I. K. O. Cann, S. Daugherty, C. W. Forsberg, I. Hance, R. I. Mackie, W. C. Nelson, J. B. Russell, B. A.

White and D. B. Wilson. (2003). New and emerging approaches to improve herbivore nutrition: Rumen microbiology in the genomics era. In: Proc.s 6th Intl. Symp. Nutr. Herbivores, Merida, Mexico. 6, 309–320.

Mosoni, P., Chaucheyras-Durand, F., Béra-Maillet, C., & Forano, E. (2007). Quantification by real-time PCR of cellulolytic bacteria in the rumen of sheep after supplementation of a forage diet with readily fermentable carbohydrates: Effect of a yeast additive. J. Appl. Microbiol., 103, 2676–2685. DOI: *10.1111/j.1365-2672.2007.03517.x*

Muyzer, G., de Waal, E., & Uitterlinden, A. G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain. Appl. Environ. Microbiol., 59, 695–700. DOI: *0099-2240/93/030695-06$02.00/0*

Nathani, N. M., Patel, A. K., Dhamannapatil, P. S., Kothari, R. K., Singh, K. M., & Joshi, C. G. (2013). Comparative evaluation of rumen metagenome community using qPCR and MG-RAST. AMB Express., 3, 1–8. *10.1186/2191-0855-3-1*

Petri RM, Schwaiger T, Penner GB, Beauchemin KA, Forster RJ, McKinnon JJ, McAllister TA. 2013. Changes in the rumen epimural bacterial diversity of beef cattle as affected by diet and induced ruminal acidosis. Appl. Environ. Microbiol., 79, 3744 –3755. DOI: *10.1128/AEM.03983-12*

Pitta, D. W., Pinchak, W. E., Dowd, S. E., Osterstock, J., Gontcharova, V., Youn, E., & Malinowski, D. P. (2010): Rumen bacterial diversity dynamics associated with changing from bermudagrass hay to grazed winter wheat diets. Microb. Ecol., 59, 511–522. DOI: 10.1007/s00248-009-9609-6.

Prescott, D. M. (1994). The DNA of ciliated protozoa. Microbiol. Rev., 58, 233–267. DOI: *10.1016/0022-2836(72)90095-2*

Pushkarev, D., Neff, N. F., & Quake, S. R. (2009). Single-molecule sequencing of an individual human genome. Nat. Biotechnol. DOI: *10.1038/nbt.1561*

Ransom-Jones, E., Jones, D. L., McCarthy, A. J., & McDonald, J. E. (2012). The Fibrobacteres: An Important Phylum of Cellulose-Degrading Bacteria. Microb. Ecol., DOI: *10.1007/s00248-011-9998-1*

Rasmussen, R. (2001). Quantification on the LightCycler. In Rapid Cycle Real-Time PCR. 21–34. DOI: *10.1007/978-3-642-59524-0_3*

Rédei, G. P. (2008). MPSS (multiple parallel signature sequencing). In Encycloped. Gen. Genom. Prot. and Inform. DOI: 10.1007/978-1-4020-6754-9_10815

Regensbogenova M., McEwan N. R., Javorsky P., Kisidayova S., Michalowski T., Newbold C. J., et al. (2004b). A re-appraisal of the diversity of the methanogens associated with the rumen ciliates. FEMS Microbiol. Lett., 238, 307–313. DOI: *10.1111/j.1574-6968.2004.tb09771.x*

Regensbogenova, M., Kisidayova, S., Michalowski, T., Javorsky, P., Moon-Van Der Staay, S. Y., Moon-Van Der Staay, G. W. M., Pristas, P. (2004). Rapid identification of rumen protozoa by restriction analysis of amplified 18S rRNA gene. Acta Protozoologica, 43, 219–224.

Reinartz, J., Bruyns, E., Lin, J. Z., Burcham, T., Brenner, S., Bowen, B., … Woychik, R. (2002). Technique review massively parallel signature sequencing (MPSS) as a tool for in-depth quantitative gene expression profiling in all organisms. Briefings Funct. Genomics Proteomics. DOI: *10.1093/bfgp/1.1.95*

Roehe, R., Dewhurst, R. J., Duthie, C. A., Rooke, J. A., McKain, N., Ross, D. W., Wallace, R. J. (2016). Bovine Host Genetic Variation Influences Rumen Microbial Methane Production with Best Selection Criterion for Low Methane Emitting and Efficiently Feed Converting Hosts Based on Metagenomic Gene Abundance. PLoS Genetics, 12. DOI: *10.1371/journal.pgen.1005846*

Roesch, L. F. W., Fulthorpe, R. R., Riva, A., Casella, G., Hadwin, A. K. M., Kent, A. D., … Triplett, E. W. (2007). Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J., 1, 283–290. DOI: *10.1038/ismej.2007.53*

Rohwer, F., Prangishvili, D., & Lindell, D. (2009). Roles of viruses in the environment. Environ. Microbiol. DOI: *10.1111/j.1462-2920.2009.02101.x*

Rooks, M. G., Veiga, P., Wardwell-Scott, L. H., Tickle, T., Segata, N., Michaud, M., Garrett, W. S. (2014). Gut microbiome composition and function in experimental colitis during active disease and treatment-induced remission. ISME J., 8, 1403–1417. DOI: *10.1038/ismej.2014.3*

Ross, E. M., Moate, P. J., Bath, C. R., Davidson, S. E., Sawbridge, T. I., Guthridge, K. M., Hayes, B. J. (2012). High throughput whole rumen metagenome profiling using untargeted massively parallel sequencing. BMC Genet., 13. DOI: *10.1186/1471-2156-13-53*

Ross, E. M., Moate, P. J., Marett, L., Cocks, B. G., & Hayes, B. J. (2013b). Investigating the effect of two methane-mitigating diets on the rumen microbiome using massively parallel sequencing. J. Dairy Sci., 96, 6030–6046. DOI: *10.3168/jds.2013-6766*

Ross, E. M., Moate, P. J., Marette, L. C., Cocks, B. G., & Hayes, B. J. (2013a). Metagenomic predictions: from microbiome to complex health and environmental phenotypes in humans and cattle. *PLOSone*, 8, e73056. DOI: *0.1371/journal.pone.0073056*

Russell, J. B. (2009). Rumen. In Encyclopedia of Microbiology. DOI: 10.1016/b978-012373944-5.00061-4

Salonen A, Lahti L, SalojaÈ rvi J, Holtrop G, Korpela K, Duncan SH, et al. (2014). Impact of diet and individual variation on intestinal microbiota composition and fermentation products in obese men. The ISME J., 8. DOI: 10.1038/ismej.2014.63

Schlegel, L., Grimont, F., Ageron, E., Grimont, P. A. D., & Bouvet, A. (2003). Reappraisal of the taxonomy of the Streptococcus bovis/Streptococcus equinus complex and related species: Description of Streptococcus gallolyticus subsp. gallolyticus subsp. nov., S. gallolyticus subsp. macedonicus subsp. nov. and S. gallolyticus subsp. pasteurianus subsp. nov. Int. J. Syst. Evol. Microbiol., 53, 631–645. DOI: 10.1099/ijs.0.02361-0

Schofield, P. (2000). Gas production methods. In J. P. F. D´Mello (Ed.), Farm animal metabolism and nutrition (pp. 209–232). Wallingford, UK: CABI Publishing.

Shin, E. C., Cho, K. M., Lim, W. J., Hong, S. Y., An, C. L., Kim, E. J., … Yun, H. D. (2004). Phylogenetic analysis of protozoa in the rumen contents of cow based on the 18S rDNA sequences. J. Appl. Microbiol., 97, 378–383. DOI: 10.1111/j.1365-2672.2004.02304.x

Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. Mol. Ecology. DOI: 10.1111/j.1365-294X.2012.05538.x

Skillman L. C., Toovey A. F., Williams A. J., Wright A.-D. G. (2006). Development and validation of a real-time PCR method to quantify rumen protozoa and examination of variability between *Entodinium* populations in sheep offered a hay-based diet. Appl. Environ. Microbiol., 72, 200–206. DOI: 10.1128/AEM.72.1.200-206.2006

Sluijter, J. P. G., Pasterkamp, G., & De Kleijn, D. P. V. (2006). Quantitative real-time PCR. In *Cardiovascular Research:* New Technologies, Methods, and Applications (pp. 75–83). DOI: 10.1007/0-387-23329-6_4

Stevenson DM, Weimer PJ (2007). Dominance of Prevotella and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Appl. Microbiol. Biotechnol., 75: 165–174.

Stewart, C. S., & Bryant, M. P. (1988). The rumen bacteria. In P. N. Hobson (Ed.). The Rumen Microbial System (pp. 21–75). London: Elsevier Applied Science.

Stewart, R. D., Auffret, M. D., Warr, A., Wiser, A. H., Press, M. O., Langford, K. W., … Watson, M. (2018). Assembly of 913 microbial genomes from metagenomic sequencing of the cow rumen. Nat. Commun., DOI: 10.1038/s41467-018-03317-6

Stewart, R., Auffret, M. D., Warr, A., Snelling, T., Dewhurst, R., Walker, A. W., Watson, M. (2017). Assembly of hundreds of microbial genomes from the cow rumen reveals novel microbial species encoding enzymes with roles in carbohydrate metabolism. *bioRxiv*. DOI: 10.1101/162578

Suchodolski, J. S. (2012). Chapter 2: Gastrointestinal Microbiota. Canine and Feline Gastroenterology. DOI: 10.1016/B978-1-4160-3661-6.00002-X

Sylvester J. T., Karnati S. K. R., Dehority B. A., Morrison M., Smith G. L., St-Pierre N. R., et al. (2009). Rumen ciliated protozoa decrease generation time and adjust 18S ribosomal DNA copies to adapt to decreased transfer interval, starvation, and monensin. J. Dairy Sci. 92, 256–269. DOI: 10.3168/jds.2008-1417

Sylvester, J. T., Karnati, S. K. R., Yu, Z., Morrison, M., & Firkins, J. L. (2004). Development of an assay to quantify rumen ciliate protozoal biomass in cows using real-time PCR. J. Nutr., 134, 3378–3384. DOI: 10.1093/jn/134.12.3378

Székely, A. J., Sipos, R., Berta, B., Vajna, B., Hajdú, C., & Márialigeti, K. (2009). DGGE and T-RFLP analysis of bacterial succession during mushroom compost production and sequence-aided T-RFLP profile of mature compost. Microb. Ecol., 57, 522–533. DOI: 10.1007/s00248-008-9424-5

Tajima, K., Aminov, R. I., Nagamine, T., Matsui, H., Nakamura, M., & Benno, Y. (2001). Diet-Dependent Shifts in the Bacterial Population of the Rumen Revealed with Real-Time PCR. Appl. Environ. Microbiol., 67, 2766–2774. DOI: 10.1128/AEM.67.6.2766-2774.2001

Tapio, I., Fischer, D., Blasco, L., Tapio, M., Wallace, R. J., Bayat, A. R., Vilkki, J. (2017). Taxon abundance, diversity, co-occurrence and network analysis of the ruminal microbiota in response to dietary changes in dairy cows. PLoS ONE, 12. DOI: 10.1371/journal.pone.0180260

Tewari, D., Cieply, S., & Livengood, J. (2011). Identification of bacteria recovered from animals using the 16s ribosomal RNA gene with pyrosequencing and Sanger sequencing. J. Vet. Diag. Invest., 23, 1104–1108. DOI: 10.1177/1040638711425583

Tveit, A. T., Urich, T., & Svenning, M. M. (2014). Metatranscriptomic analysis of arctic peat soil microbiota. Appl. Environ. Microbiol., 80, 5761–5772. DOI: 10.1128/AEM.01030-14

Tymensen L., Barkley C., McAllister T. A. (2012b). Relative diversity and community structure analysis of rumen protozoa according to T-RFLP and microscopic methods J. Microbiol. Methods., 88, 1–6. DOI: 10.1016/j.mimet.2011.09.005v

Tymensen, L., Barkley, C., & McAllister, T. A. (2012). Relative diversity and community structure analysis of rumen protozoa according to T-RFLP and microscopic methods. J. micorbiol. Methods., 88, 1–6. DOI: *10.1016/j.mimet.2011.09.005*

Urich, T., Lanzén, A., Qi, J., Huson, D. H., Schleper, C., & Schuster, S. C. (2008). Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. PLoS One, 3. DOI: *10.1371/journal.pone.0002527*

VanGuilder, H. D., Vrana, K. E., & Freeman, W. M. (2008). Twenty-five years of quantitative PCR for gene expression analysis. BioTechniques. DOI: *10.2144/000112776*

Walsh, P., Palu, C., Kelly, B., Lawor, B., Wassan, J. T., Zheng, H., & Wang, H. (2017). A metagenomics analysis of rumen microbiome. In Proceedings - 2017 IEEE International Conference on Bioinformatics and Biomedicine, BIBM 2017. 2077–2082. DOI: *10.1109/BIBM.2017.8217980*

Wanapat, M., & Cherdthong, A. (2009). Use of real-time PCR technique in studying rumen cellulolytic bacteria population as affected by level of roughage in swamp buffalo. Curr. Microbiol., 58, 294–299. DOI: *10.1007/s00284-008-9322-6*

Wang, L., Wu, D., Yan, T., & Wang, L. (2018). The impact of rumen cannulation on the microbial community of goat rumens as measured using 16S rRNA high-throughput sequencing. J. Anim. Physiol. Anim. Nutr., 102, 175–183. DOI: *10.1111/jpn.12676*

Yang, B., Wang, Y., & Qian, P. Y. (2016). Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. BMC Bioinf., 17. DOI: *10.1186/s12859-016-0992-y*

Yang, L. Y., Chen, J., Cheng, X. L., Xi, D. M., Yang, S. L., Deng, W. D., & Mao, H. M. (2010). Phylogenetic analysis of 16S rRNA gene sequences reveals rumen bacterial diversity in Yaks (Bos grunniens). Mol. Biol. Rep., 37, 553–562. DOI: *10.1007/s11033-009-9794-x*

Yu, Y., Lee, C., Kim, J., & Hwang, S. (2005). Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol. Bioeng., 89, 670–679. DOI: *10.1002/bit.20347*

Zhou, M., Chen, Y., & Guan, L. L. (2015). Rumen bacteria. In Rumen Microbiology: From Evolution to Revolution. DOI: *10.1007/978-81-322-2401-3_6*

Zhou, X., Li, Y., Liu, S., Yang, Q., Su, X., Zhou, L., … Huang, Q. (2013). Ultra-deep sequencing enables high-fidelity recovery of biodiversity for bulk arthropod samples without PCR amplification. GigaScience. DOI: *10.1186/2047-217X-2-4*

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The Development of Vegetation Indices: a Short Overview

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ABSTRACT - Vegetation indices computed from remote sensing data became key components of agricultural monitoring and assessment. With the help of these indices, the difference of vegetational and other land covers can be contrasted and many useful and applicable data can be gathered ranging from vegetation health to growth dynamics among others. In recent decades, starting from the first Landsat satellite, a huge number of VIs were developed in order to be able to effectively monitor vegetation – the reason for the immense number is due to the fact that every sensor, topographic, geographic, vegetative and atmospheric feature is different, and more so are their combinations. This is the reason why there is no unified spectral band mathematical formula. The aim of this short overview is to provide the reader insight of the main vegetation indices (VIs) that have been used in scientific literature and their development over the last 40 years.

Keywords – vegetation indices, NDVI, VI, VIs, soil line, SAVI, ARVI, GEMI, VDVI

INTRODUCTION

Monitoring and assessment of vegetation were always distinctive fields in agriculture. With the help of passive (meaning the source of radiation is the Sun, thus with diffused sunlight) remote sensing, experts and even farmers gain the ability to get information about the plant cover without using excessive destructive methods. In this context, the expression of remote sensing (RS) is used in measuring reflectance in different spectral ranges (bands). Other than that, RS is the term of gathering and processing information from objetcs and phenomena without direct physical contact (*Balázsik*, 2010).

The usual spectral ranges used in RS of vegetation are bands in the red part of the visible spectrum (wavelength of appr. 0.6-0.7 μm) and near infrared (NIR, appr. 0.7-1.4 μm), because they contain about 90% of the information relating to vegetation (*Baret et al.*, 1989). The biophysical aspect of this is contributed to the fact that visible radiation (appr. 0,4 μm to 0,7 μm) is absorbed by leaf pigment cells (chlorophyll-a, chlorofill-b and carotinoids), while radiation in NIR (0,7 μm to 1,4 μm) is strongly reflected by inner leaf cellular structures. Therefore vegetative covers can be distinguished by their spectral behavior in relation to ground elements and soils by measuring and

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quantifying the difference of the two spectral bands, and that is the basis of a VI (*Major et al.*, 1990; *Bannari et al*, 1995).

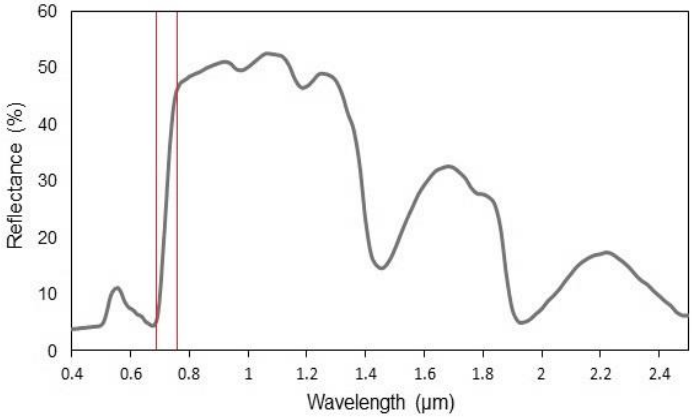

Figure 1: Typical spectral response of plants with indication of Red Edge region
(*source:* Introduction to Remote Sensing, Humboldt State University)

A vegetation index is usually a mathematical combination of a red and near infrared channel which are detected by a sensor. The reason behind the usual usage of these two bands is the fact that the biggest difference in reflectance is comprised between them (the inbetween region is also called Red Edge, i.e. the inflection point between 0,65 µm and 0,75 µm, as seen on *Figure 1*). The formula of such an index is the separating factor between VIs and their usability, and the exact computation is done with the digital numbers (or relative reflectances) of image pixels. An index is a number measuring the intensity of a phenomenon that is often too complex to be broken down into easily understandable factors. This is the case for remote sensing of vegetation, because it is generally difficult to make the decomposition for signals observed on RS images. (*Bannari et al*., 1995) Yet, even a well documented vegetation index is usually hard to interpret (questionable correlation with vegetative cover) and to compare (due to sensor, atmospheric and tomographic differences).

It is worth mentioning that satellite-based remote sensing has been operational since the first Landsat mission (1972). Nowadays, there is a huge number of satellites that are constantly watching the globe, and among them there are a handful of examples which are open access – examples are the

latest multispectral Landsat satellites and ESA's Sentinel missions. Furthermore, aerial photography and more specifically unmanned aerial vehicles (UAVs) extended with multi- and hyperspectral cameras (also called Unmanned Aerial Systems, UASs) are becoming more numerous and affordable each year giving access to temporal and spatial resolutions not seen before compared to satellites based on today's technology.

The aim of this article is to give an overview of significant, mostly ratio-based vegetation indices found in scientific literature in historical order and in a short manner, and to assess their development throughout the last four decades. Nowadays, classification of VIs is affected by technological advancement as modern sensors allow us to gather more precise data. Thus, narrow band (meaning the data is less scattered but also of lesser amount) indices are distinguished from broad band ones. In fact, narrow bands (such as bands at the red edge positions) can be used singularly to acquire applicable information of vegetation. The main differentiating factor between broad and narrow bands lies in their applicability: while high spectral resolution means very precies and compact data, the range of interpretation is also smaller, due to the fact that the gathered information is very specific. On the other hand, broad band indices can be more universally applied to plant assesment but their specificallity – because of the bulk information – is low. The choice between them has to be made every time, whether it be scientific purpose or general farm management. In the article, we avoid the differentiatiation of narrow and broad bands, thus we use a more traditional approach of discerning vegetation indices.

Indices mentioned here are checked and referenced in the online database, https://www.indexdatabase.de/.

HISTORY OF VEGETATION INDICES

One of the first researches conducted in the topic of reflective properties of plants was done by *Wilstatter and Stoll* in 1913. However, the creation of the first vegetation index was prior to the launch of the first Landsat satellite as stated by *Mróz and Sobieraj*, (2004). The first two VIs were simple ratios of bands Red and NIR (Near Infrared), the Ratio Vegetation Index (RVI) and the Vegetation Index Number (VIN). VIN is also called the Simple Ratio (SR).

$$\mathrm{RVI} = \frac{\mathrm{RED}}{\mathrm{NIR}} \tag{1}$$

$$\mathrm{VIN} = \frac{\mathrm{NIR}}{\mathrm{RED}} \tag{2}$$

In spite of the simplicity of the formulas, sources differ on their origins. However, the earliest mention was by *C.F. Jordan* in 1969, according to *Xue and Su* (2017).

The sole purpose of them is to contrast the difference between vegetation and other ground objects, and they are still used even today (mostly in comparisons, such as in *Huete and Jackson*, 1987) as they are easy to compute and interpret. Ratio-based indices have the huge benefit of eliminating disturbing factors that affect all bands in the same way (such as topography, *Baret and Guyot*, 1991). However, if the vegetation is sparse and soil reflectances come in action, they become near useless and these indices do not have normal distribution either (*Bannari et al.*, 1995).

One of the oldest, yet even nowadays among the most used indices wordwide is the Normalised Difference Vegetation Index (NDVI) which was created by *Rouse et al*. in 1974 (a). It could be viewed as the enhanced and normalised version of VIN (SR), as it is its linear function (*Perry and Lautenschlager*, 1984).

$$\mathrm{NDVI} = \frac{\mathrm{NIR} - \mathrm{RED}}{\mathrm{NIR} + \mathrm{RED}} = \frac{VIN - 1}{VIN + 1} \tag{3}$$

The success of NDVI is through its normalization (index range is from -1 to 1) process that enables the user to easily explain and compare the results. Beside that, as it is also a ratio-based index the same benefits apply here as well. However, it does not correct atmospheric effects, and has a short dynamic range – underestimates sparse vegetation because of soil effects and rapidly saturates at very high vegetation density because it weighs the red and near-infrared bands the same in its formula. The NDVI has been successfully applied in a great number of studies in the last four decades, ranging from vegetation monitoring (*Santos and Negri*, 1996; *Radoslaw*, 2010; *Yengoh et al*., 2014), classification (*Julien et al*., 2011; *Havasi and Benő*, 2012;) to estimation of different vegetational traits (i.e. chlorophyll and nitrogen content, plant height; *Prince and Tucker*, 1986; *Deblonde and Cihlar*, 1993; *Payero et al*. 2004; *Ambrus et al*, 2015).

The fact that NDVI lacks normal distribution and has negative values, in the same year of the developement of NDVI (1974, b), *Rouse et al*. created the

Transformed Vegetation Index (TVI) to eliminate the aforementioned problems.

$$\mathrm{TVI} = \sqrt{\mathrm{NDVI} + 0.5} \tag{4}$$

Obviously, if NDVI < -0.5 (very low NDVI values) then it cannot be computed. According to *Perry and Lautenschlager* (1984), TVI could still be negative so they improved it (some sources list it as TVI2).

$$\mathrm{TVI2} = \frac{\mathrm{NDVI} + 0.5}{|\mathrm{NDVI} + 0.5|} * \sqrt{|\mathrm{NDVI} + 0.5|} \tag{5}$$

In 1976, *Kauth and Thomas*, based on the processing of Landsat images, developed the method of Tasseled Cap Transformation (TCT). It is different from the aforementioned indices in the fact that it converses and compacts the original bands into fewer ones leaving only the useful information in them The results of the transformation are four computed bands (as outputs), namely the Soil Brightness Index (SBI), Green Vegetation Index (GVI). Yellow Vegetation Index (YVI) and the Non-Such Index (NSI, which contains the noise from the image) (*Xue and Su,* 2017). It is worth mentioning that in 2017, *Nedokov* successfully applied the TCT on Sentinel-2 images and reported remarkable results.

One of the main problems of ratio-based indices is the fact that they are not taken into account noise coming from sparsely vegetated areas (i.e. soil reflectance). In light of this, after prior research of soil effects, *Richardson and Wiegand* proposed the Perpendicular Vegetation Index (PVI) in 1977 which was perfected by the work of *Jackson et al*. in 1980 (*formula 6 and 7* accodingly).

$$\mathrm{PVI} = \sqrt{(\mathrm{RED}_{\mathrm{soil}} + \mathrm{RED}_{\mathrm{veg}})^2 + (\mathrm{NIR}_{\mathrm{soil}} + \mathrm{NIR}_{\mathrm{veg}})^2} \tag{6}$$

$$\mathrm{PVI} = \frac{(\mathrm{NIR} - \mathrm{aRED} - \mathrm{b})}{\sqrt{\mathrm{a}^2 + 1}} \tag{7}$$

Where 'a' and 'b' are the slope and intercept of the 'soil line' (*Figure 2*). *Richardson and Wiegand* (1977) noted that pixels representing soils tend to align in RED-NIR space on the same line, and they named it the Soil Background Line (SBL) which came to be known as the soil line. Vegetation indices that utilize

the soil line parameters represent a different category of ratio- (or slope-) -based indices. The gerenal formula of the soil line concept can be seen on *Formula 8* (*Bannari et al.*, 1995).

$$NIR = aR + b \tag{8}$$

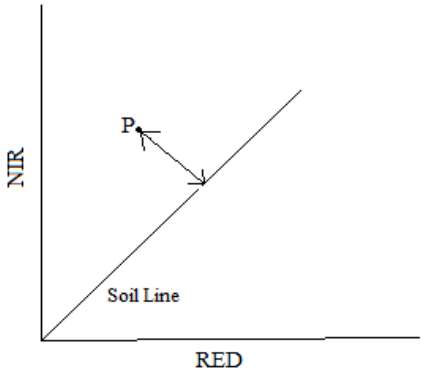

Figure 2: PVI index and the soil line
(*source:* http://a-a-r-s.org)

The sole purpose of PVI is to measure the prependicular distance of vegetation pixels from the soil line (*Figure 2*) as *Richardson and Wiegand* also noted that pixels representing vegetation are always on the left side of the SBL (and pixels representing water bodies are on its right side). However, studies showed that PVI is still very prone to miscalculations due to variable soil effects, and the determination the exact parameters of a given soil line is challenging (i.g. *Major et al.*, 1990; *Huete*, 1985). Yet, it is used quite often in comparisons, classifications and biomass-estimations (such as in: *Huete et al.*, 1985; *Major et al.*, 1990; *Garey et al.*, 2004, *Payero et al.*, 2004; *Wenlong*, 2009).

One of the most straightforward vegetation indices is the simple difference of the bands RED-NIR, which is called the Differenced Vegetation Index (DVI).

$$DVI = NIR - RED \tag{9}$$

As with RVI and VIN, sources differ on the origins of DVI, but most of them agree on the fact that it was first mentioned with Landsat-bands in the works of *Richardson and Wiegand* in 1977. It is still used nowadays due to its simplicity (faster computational times) mainly in comparisons and vegetation monitoring (*Bannari et al.*, 1995; *Barzegar et al.*, 2015).

In 1988, mostly because of the deficiencies of NDVI and PVI in evaluating the soil reflectances, *Huete* proposed the Soil Adjusted Vegetation Index (SAVI, *Formula 10*). It is a compromise between the two main types of VIs (slope-based and orthogonal indices) as it contains a non-linear factor (L) which correlates with vegetation density (*Huete, 1988*).

$$\mathrm{SAVI} = \frac{\mathrm{NIR} - \mathrm{RED}}{\mathrm{NIR} + \mathrm{RED} + \mathrm{L}}(1 + L) \tag{10}$$

It is apparent if L equals 0, then SAVI equals NDVI. According to *Huete*, when L is 0.5 (L can range from 0 to 1) it permits the best adjustment against canopy backscattering, but the general rule is that the denser the vegetation, the closer L is to 1. So in order to have adequate results, the user needs to evaluate the density of vegetation on a given area and pair it with the correct L value from literature or prior research. However, in many cases the density of vegetation is the one that we are aiming to determine with VIs. So in order to address this problem *Qi et al*. proposed the Modified Soil Adjusted Vegetation Index (MSAVI) in 1994 that varies with the amount of vegetation present on the picture (*Formula 11*).

$$\mathrm{MSAVI} = \frac{2\mathrm{NIR} + 1 - \sqrt{(2\mathrm{NIR} + 1)^2 - 8(\mathrm{NIR} - \mathrm{R})}}{2} \tag{11}$$

There are many variations of SAVI, but one of the most influential is the Transformed Soil Adjusted Vegetation Index (TSAVI) created by Baret et al. in 1989 (and perfected it in 1991). It uses the parameters from the soil line (Formula 8) which makes it robust according to Baret.

$$\mathrm{TSAVI} = \frac{(\mathrm{a}(\mathrm{NIR} - \mathrm{aR} - \mathrm{b}))}{(\mathrm{R} + \mathrm{aNIR} - \mathrm{ab})} \tag{12}$$

Abdou and Huete published of the usability of SAVI and especially TSAVI compared to NDVI as they are independent from the sensor used, and they give better results in differentiating vegetative cover from bare soil (*Bannari et al.*, 1995).

As it has been already mentioned, one of the main problems of NDVI is its narrow dynamic range or small sensitivity. In 1991, *Escadafal and Huete* addressed this shortcoming by correcting NDVI (and also SAVI) with a factor

that correlates with soil color. This factor is the Redness Index (RI) and the VI used was corrected with the slope ('k') of the linear correlation of RI and the given VI (*Formula 12-13*; *Escadafal and Huete*, 1991). Based on the results of the authors, this correction can enhance the sensitivity of NDVI and SAVI.

$$\mathrm{RI} = \frac{\mathrm{RED} - \mathrm{GREEN}}{\mathrm{RED} + \mathrm{GREEN}} \tag{13}$$

$$\mathrm{VI}_{\mathrm{corr}} = \mathrm{VI} - \mathrm{kRI} \tag{14}$$

When the vegetation is sparse, the VI used is greatly influenced by the soil parameters. However, atmospherical factors always play a remarkable role, and researchers started to create vegetation indices which deal with this issue starting from the 1990's. One of the first and most significant was the Atmospherically Resistant Vegetation Index (ARVI) in 1992 by *Kaufman and Tanré*. Their explanation is that in general the atmosphere increases the reflectance of the red bands, and decreases it on the near-infrared channels, therefore they implemented a self-correcting factor for the former into the formula.

$$\mathrm{ARVI} = \frac{\mathrm{NIR} - \mathrm{RB}}{\mathrm{NIR} + \mathrm{RB}} \tag{15}$$

$$RB = R - \gamma(R - B) \tag{16}$$

$$\gamma = \frac{\rho_{a-r}}{\rho_{a-b} - \rho_{a-r}} \tag{17}$$

RB is the corrected difference of the red and blue channel, and γ is the self-correcting factor ($\rho_{a\text{-}b}$ and $\rho_{a\text{-}r}$ are components of the atmospherical reflectance in the blue and red channel accordingly). Of course, if γ=0, then ARVI equals NDVI. According to *Kaufman and Tanré*, if no atmospherical data is present a priori, than γ=1 is a good choice for better adjustments (*Kaufman and Tanré*, 1992). There are many versions of ARVI, and the RB factor in its formula inspired a number of researchers to create new VIs with it (or to implement it into former ones). However, precisely defining the value of γ can be difficult, hence ARVI is mainly used in comparisons, and not as a main VI in researches (*Xue and Su*, 2017).

Based on the analisation of satellite images, *Pinty and Verstrate* proposed a new vegetation index in the same year as ARVI (1992) that – according to the

authors – deals with atmospherical effects, and is easier to compute than ARVI. It is called the Global Environment Monitoring Index (GEMI), and as its name suggests, it is designed for global monitoring of vegetation.

$$\mathrm{GEMI} = \frac{\eta(1 - 0.25\eta) - (\mathrm{RED} - 0.125)}{(1 - \mathrm{RED})} \tag{18}$$

$$\eta = \frac{(2(\mathrm{NIR}^2 - \mathrm{RED}^2) + 1.5\mathrm{NIR} + 0.5\mathrm{RED})}{\mathrm{NIR} + \mathrm{RED} + 0.5} \tag{19}$$

GEMI is aimed to be more sensitive than NDVI, however many studies showed that (i.e.: *Bannari et al*., 1994) sparse vegetation can seriously alter the index values due to reflectance coming from soils. In spite of it, GEMI is still widely used today in studies that need information of vegetation on a global scale.

One of the first VIs that combined the elimination of atmospherical and soil effects in one single vegetation index was the Transformed Soil Atmospherically Resistant Vegetation Index (TSARVI). As the name suggest, TSARVI is the combination of TSAVI and ARVI, and as such, in the formula of TSAVI the red channel was replaced by RB (*Formula 16*; according to *Rondeaux et al*, 1996). In scientific literature there is little mention of TSARVI, and *Myneni and Asrar* in 1994 showed that the combination of TSAVI and ARVI does not reach the same level of correction as their individual parts.

In 1995, *Liu and Huete* created a vegetation index that – similarly to TSARVI – tries to eliminate disturbing factors coming both from soil and atmospheric effects. The Enhanced Vegetation Index (EVI) is so called because the aim was to enhance NDVI. The formula was designed with Landsat bands, but the authors give a generalised version of it as well (*Formula 20-21*).

$$\mathrm{EVI} = 2.5 * \frac{(\mathrm{TM}_4 - \mathrm{TM}_3)(1 + \mathrm{L})}{\mathrm{TM}_4 - (\mathrm{C}_1 * \mathrm{TM}_3) + (\mathrm{C}_2 * \mathrm{TM}) + \mathrm{L}} \tag{20}$$

$$\mathrm{EVI} = 2.5 * \frac{\mathrm{NIR} - \mathrm{RED}}{\mathrm{NIR} + (\mathrm{C}_1 * \mathrm{RED}) - (\mathrm{C}_2 * \mathrm{BLUE}) + \mathrm{L}} \tag{21}$$

TM (Thermatic Mapper) stands for the Landsat band used, factor L is the same concept as in SAVI, and the two constant values (C_1, $C_{2)}$ are 6 and 7.5

respectively. Unlike TSARVI, EVI has ample mention in scientific literature, and is still used nowadays in various studies.

Another widely used index (created in 1996 by Gao) is the Normalised Difference Water Index (NDWI) that uses two infrared bands (a NIR band at around 840-860 nm, and an infrared band at 1630-1660 nm) in a formula very simliar to NDVI.

$$\mathrm{NDWI} = \frac{\mathrm{NIR} - \mathrm{IR}}{\mathrm{NIR} + \mathrm{IR}} \tag{22}$$

It is a measure of liquid water molecules in vegetation canopy that interact with solar radiation, thus it indicates and enhances water content of canopy making the user be able to assess canopy health and – for example – stress-levels.

The Chlorophyll Absorption Ratio Index (CARI) has many different variations, but all rely on the fact that spectral response of chloropyll content of leaves is constant in spite of variable leaf attributes (*Kim et al*, 1994). In 2000, *Daughtry et al*. improved CARI to Modified Chlorophyll Absorption Ratio Index, (MCARI) which – according to them – is more sensitive to leaf chlorophyll content.

$$\mathrm{MCARI} = \frac{1.5[2.5(\mathrm{NIR} - \mathrm{RED}) - 1.3(\mathrm{NIR} - \mathrm{GREEN})]}{\sqrt{(2NIR + 1)^2 - (6NIR - 5RED) - 0.5}} \tag{23}$$

Another approach of improving the NDVI can be seen in 2004 by *Gitelson*. When the LAI (Leaf Area Index) is high, NDVI loses its accuracy due to diminishing NIR reflectance. Thus, he proposed a correctional factor (α) for NIR that correlates with LAI or VF (Vegetation Fraction) and created a new vegetation index, the Wide Dynamic Range Vegetation Index (WDRVI).

$$\mathrm{WDRVI} = \frac{\alpha\mathrm{NIR} - \mathrm{RED}}{\alpha\mathrm{NIR} + \mathrm{RED}} \tag{24}$$

Starting around from the year 2000, the emergence of vegetation indices using the other visible spectrum bands (mostly green) is apparent. *Gitelson* in 1996 reasons with the fact that in prior years, researchers were only focusing on the difference between the red and NIR bands to identify vegetation, not on evolution's choice of identification of plants, the green channel. Novel examples of this are GARI (or GARVI, Green Atmopherically Resistant

Vegetation Index developed with the subtitution of red with green channel in ARVI) and the Green NDVI (using a green channel instead of red one in the formula of NDVI). The authors claim that using the green channel (that highly correlates with chlorophyll content) can make these formulas at least five times more sensitive to existing chlorophyll in vegetation, and can make them have a wider dynamic range (*Gitelson et al*, 1996). Another example of this is the Visible Atmospherically Resistant Vegetation Index (VARI) by *Gitelson et al* in 2002.

$$\mathrm{VARI} = \frac{\mathrm{GREEN} - \mathrm{RED}}{\mathrm{GREEN} + \mathrm{RED} - \mathrm{BLUE}} \tag{25}$$

According to the authors, the red/NIR ratio is less effective to evaluate the vegetation fraction when the VF is high (because NIR reflectance saturates at higher fractions of vegetation), in contrast the green/red ratio moves with the vegetation fraction in very high correlation. Besides, they realised that by adding a blue channel to the formula as a self-correcting factor for the atmosphere, they could increase the correlation of the index with VF.

Another example of the emerge of visible vegetation indices is the usage of the Green Difference Vegetation Index (GDVI) which was developed in 1979 by *Tucker*. It is very similar to DVI (*Formula 9*), however it uses a green channel instead of a red one. The GDVI has sparse mention prior to the publication of *Sripada et al*. (2005) who successfully created a model of optimal nitrogen fertilisation using the GDVI.

GDVI (1979) is not to be confused with the GDVI (Generalised Difference Vegetation Index) of *Wu* (2014), where the latter is the improvement of NDVI through exponentiation.

$$\mathrm{GDVI} = \frac{\mathrm{SR}^{\mathrm{n}} - 1}{\mathrm{SR}^{\mathrm{n}} + 1} = \frac{\mathrm{NIR}^{\mathrm{n}} - \mathrm{RED}^{\mathrm{n}}}{\mathrm{NIR}^{\mathrm{n}} + \mathrm{RED}^{\mathrm{n}}} \tag{26}$$

'n' is an integer from 1 to n. GDVI (2014) is proven to be a lot more sensitive than NDVI, SAVI, WDRVI and SARVI, and has a greater range as well.

One of the latest and most promising indices is the Visible Band-Difference Vegetation Index (VDVI, *Wang et al*., 2015).

$$\mathrm{VDVI} = \frac{2(\mathrm{GREEN} - \mathrm{RED} - \mathrm{BLUE})}{2(\mathrm{GREEN} + \mathrm{RED} + \mathrm{BLUE})} \tag{27}$$

Compared to other visible light indices (NGRDI, NGBDI, RGRI]), it performs much better when using UAV imagery, reported accuracy is over 90% (*Xue and Su*, 2017). Thus, it holds great promise for agriculture and for – for example – farmers using simple, visible light unmanned aerial systems.

SUMMARY

According to *Jackson et al*. (1983), the ideal vegetation index can be described as an index which is 'particularly sensitive to vegetative covers, insensitive to soil brightness, insensitive to soil color, little affected by atmospheric effects, environmental effects and solar illumination geometry and sensor viewing conditions'. Unfortunately, the ideal one is still to be created as every above mentioned circumtence is different when a vegetation index is used.

If we take a look at the timeline of VIs, the first ones created (which are very much used nowadays due to their simplicity, i.e.NDVI) were the staples of the other later vegetation indices, as they originate from plant biophysics without correcting with any disturbing features. Thus, these simple and mostly slope-based (as they practically measure the slope of vegetation isolines) indices are still the basis of every research done in the topic.

Starting from the 1980's, the attention of remote sensing scientists were directed at the problem of soil reflectance. Thus, the soil line concept was born, and with it angular vegetation indices (such as the PVI). Later that decade, soil adjusted NDVI-based indices were also created (SAVI, MSAVI, TSAVI).

However, there was still an untended problem, the atmospheric disturbances. In the 1990's, the focus shifted, and it lead to atmospherically corrected indices, such as the ARVI, EVI, GEMI and other derivates. As we were moving towards the next millenium more complex attempts to create the ultimate index (soil and atmopherical corrections in one VI) were conducted, not reaching the perfect solution yet.

Today, the perfection of older indices are still in work, and the emergance of VIs that are utlising the visible spectrum broadens the possibilities of creating effective vegetation indices for every user, even for those that do not possess multi- and hyperspectral cameras. This is supported by the fact that commercial cameras and drones are emerging to be more available for everybody.

Also, due to the above mentioned technical advancements and ongoing researches, our knowledge of vegetation spectrums are widening. This led to various narrow bands that try to reflect core information about vegetation. The future of vegetation monitoring may lie in these narrow bands, thus in hyperspectral cameras, as data-gathering can be tailored according to the

types and phenological stages of plants based on prior research. However, as already stated above, usage of broader bands means a higher range of interpretation as well.

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REFERENCES

Baret, E., Guyot, G., Major, D. J., (1989): TSAVI: A vegetation index which minimizes soil brightness effects on LAI and APAR estimation. Proceedings of the 12th Canadian Symposium on Remote Sensing, Vancouver, Canada, p. 1355-1358.

Major, D. J., Baret, F., Guyot, G., (1990): A ratio vegetation index adjusted for soil brightness. International Journal of Remote Sensing 11 (5), p. 727-740.

Sripada, R. P., Ronnie W., Heiniger, J., White, G., Meijer, A.D., (2006): Aerial Color Infrared Photography for Determining Early In-Season Nitrogen Requirements in Corn. Agronomy Journal 98 (4), p. 968–77. DOI: *10.2134/agronj2005.0200*

Ambrus, A., Burai, P., Lénárt, Cs., Enyedi, P., Kovács, Z., (2015): Estimating biomass of winter wheat using narrowband vegetation indices for precision agriculture, Journal of Central European Green Innovation 3 (2) p. 13-22.

Balázsik, V., (2010): Fotogrammetria 1. : A távérzékelés fogalma, a fotogrammetria és a távérzékelés kapcsolata – Nyugat-magyarországi Egyetem Geoinformatikai Kar, p. 1-18.

Bannari, A., Morin, D., Bonn, F., Huete, A.R., (1995): A Review of Vegetation Indices; Remote Sensing Reviews, Vol. 13, p. 95-120.

Bannari, A., Morin, D., He, D. C., (1994): High spatial and spectral resolution remote sensing for the management of the urban environment. First International Airborne Remote Sensing Conference and Exhibition, Strasbourg, France, vol. III, p. 247-260.

Baret, E., Guyot, G., (1991): Potentials and limits of vegetation indices for LAI and APAR assessment. Remote Sensing of Environment 35, p. 161-173.

Baret, E., Guyot, G., Major, D. J., (1989): TSAVI: A vegetation index which minimizes soil brightness effects on LAI and APAR estimation. Proceedings of the 12th Canadian Symposium on Remote Sensing, Vancouver, Canada, p. 1355-1358.

Barzegar, M., Hamid, E., Abbas, K., (2015): Comparison of Different Vegetation Indices for Very High-Resolution Images, Specific Case UltraCam-D Imagery. International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences - ISPRS Archives 40 (1W5), p. 97–104. DOI: *10.5194/isprsarchives-XL-1-W5-97-2015*

Daughtry, C.S.T., Walthall, C.L., Kim, M.S., De Colstoun, E.B., McMurtrey, J.E. III, (2000): Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance, Remote Sensing of Environment, vol. 74, no. 2, p. 229–239.

Deblonde, G., Cihlar, J., (1993): A multiyear analysis of the relationship between surface environmental variables and NDVI over the Canadian landmass, Remote Sensing Reviews 7, p. 151-177.

Escadafal, R., Huete, A. R., (1991): Étude des propriétés spectrales des sols arides appliquée à l'amélioration des indices de végétation obtenus par télédétection, CR Acad. Sci. Paris 312 (2), p. 1385-1391.

Gao, B. C., (1996): NDWI - A normalized difference water index for remote sensing of vegetation liquid water from space. Remote Sensing of Environment, 58(3), p. 257–266. DOI: *10.1016/S0034-4257(96)00067-3*

Garey, A., Sabbagh, G.J., Searcy, S.W., Yang, C., (2004): An Automated Soil Line Identification Routine for Remotely Sensed Images, Soil Science Society of America Journal 68 (4), p. 1326. DOI: *10.2136/sssaj2004.1326*

Gitelson, A. A., Kaufman, Y. J., Merzlyak, M. N., (1996): Use of a green channel in remote sensing of global vegetation from EOS-MODIS. Remote Sensing of Environment, 58(3), p. 289–298. DOI: *10.1016/S0034-4257(96)00072-7*

Gitelson, A. A., Kaufman, Y. J., Stark, R., Rundquist, D., (2002): Novel algorithms for remote estimation of vegetation fraction. Remote Sensing of Environment, 80(1), p. 76–87. DOI: *10.1016/S0034-4257(01)00289-9*

Gitelson, A.A., (2004): Wide dynamic range vegetation index for remote quantifcation of biophysical characteristics of vegetation, Journal of Plant Physiology, vol. 161, no. 2, p. 165–173.

GSP 216: Introduction to Remote Sensing, Humboldt State University, Available online at: *http://gsp.humboldt.edu/OLM/Courses/GSP_216_Online/lesson2-1/reflectance.html*

Havasi I., Benő D., (2012): Hagyományos és Fuzzy nem felügyelt osztályozás összehasonlítása vegetációs index példáján, Tájökológiai lapok, 10(1), p. 115–123.

Huete, A. R., Jackson, R.D., Post, D.F., (1985): Spectral response of a plant canopy with different soil backgrounds. Remote Sensing of Environment, Volume 17(1), p. 37-53.

Huete, A. R., (1988): A soil-adjusted vegetation index (SAVI). Remote Sensing of Environment 25, p. 295-309.

Huete, A. R., Jackson, R. D., (1987): Suitability of Spectral Indices for Evaluating Vegetation Characteristics on Arid Rangelands, 232, p. 213–232.

Jackson, R. D., Pinter, P. J., Paul, J., Reginato, R.J., Robert, J., Idso, S. B., (1980): Hand-held radiometry. Agricultural Reviews and Manuals ARM-W-19. Oakland, California: U.S. Department of Agriculture, Science and Education Administration.

Jackson, R. D., Slater, P. N., Pinter, P. J., (1983): Discrimination of growth and water stress in wheat by various vegetation indices through clear and turbid atmospheres. Remote Sensing of Environment 13, p. 187-208.

Julien, Y., Sobrino, J. A., Mattar. C., Ruescas, A. B., Jimenez-munoz, J. C., Soria, G., Hidalgo, V., Atitar, M., Franch, B., Cuenca, J., (2011): Temporal analysis of normalized difference vegetation index (NDVI) and land surface temperature (LST) parameters to detect changes in the Iberian land cover between 1981 and 2001. International Journal of Remote Sensing, Vol. 32, No. 7, 10 April 2011, p. 2057–2068.

Kaufman, Y. J., Tanré, D., (1992): Atmospherically Resistant Vegetation Index (ARVI) for EOS-MODIS. IEEE Transactions on Geoscience and Remote Sensing 30 (2), p. 261-270.

Kauth, R.J., Tomas, G., (1976): The tasselled cap-a graphic description of the spectral-temporal development of agricultural crops as seen by landsat, in Proceedings of the LARS Symposia, p. 159.

Kim, M.S., Daughtry, C., Chappelle, E., McMurtrey, J., Walthall, C., (1994): The use of high spectral resolution bands for estimating absorbed photosynthetically active radiation (a par), in Proceedings of the 6th International Symposium on Physical Measurements and Signatures in Remote Sensing, CNES, Phoenix, Ariz, USA, January

Liu H.Q., Huete A., (1995): Feedback based modifcation of the NDVI to minimize canopy background and atmospheric noise, IEEE Transactions on Geoscience and Remote Sensing, vol. 33, no. 2, p. 457–465.

Major, D.J., Baret, F., Guyot, G., (1990): A ratio vegetation index adjusted for soil brightness, International Journal of Remote Sensing, vol. 11, no. 5, p. 727–740.

Mróz, M., Sobieraj, A., (2004): Comparison of several vegetation Indices calculated on the basis Of a seasonal spot xs time series, And their suitability for land cover And agricultural crop identification. Technical Sciences, 7, p. 40-68.

Myneni, R. B., Asrar G., (1994): Atmospheric effects and spectral vegetation indices, Remote Sens. Environ. 47, p. 390-402.

Nedokov, R., (2017): Orthogonal Transformation of Sentinel-2, Comptes rendus de l'Acad´emie bulgare des Sciences, 70(5), p. 687–692.

Payero, J.O., Neale, C.M.U., Wright. J.L., (2004): Comparison of eleven vegetation indices for Estimating plant height of alfalfa and grass. Applied Engineering in Agriculture Vol. 20(3), p. 385–393.

Perry, C. R., Lautenschlager, L. F., (1984): Functional equivalence of spectral vegetation indices. Remote Sensing of Environment 14, p. 169-182.

Pinty, B., Verstraete, M. M., (1992): GEMI: a non-linear index to monitor global vegetation from satellites. Vegetatio 101, p. 15-20.

Prince, S. D., Tucker, C. J., (1986): Satellite remote sensing of rangelands in Botswana. II. NOAA AVHRR and herbaceous vegetation. International Journal of Remote Sensing 7, p. 1555-1570.

Qi, J., Chehbouni, A ., Huete, A.R., Kerr, Y.H., Sorooshian, S., (1994): A modified soil adjusted vegetation index. Remote Sensing of Environment 47, p. 1-25.

Quan, Z., Xianfeng, Z., Miao, J., (2011): Eco-environment variable estimation from remote sensed data and eco-environment assessment: models and system. Acta Botanica Sinica, vol. 47, p. 1073–1080.

Radoslaw, G, (2010): Comparison of vegetation indices to determine their accuracy in predicting spring phenology of Swedish ecosystems. Master Thesis in Physical Geography and Ecosystem Analysis Department of Earth and Ecosystem Sciences, Lund University.

Richardson, A. J., Wiegand, C. L., (1977): Distinguishing vegetation from soil background information. Photogrammetric Engineering and Remote Sensing 43 (12), p. 1541-1552.

Rondeaux, G., Michael S,, Baret, F., (1996): Optimization of Soil-Adjusted Vegetation Indices. Remote Sensing of Environment 55 (2), p. 95–107. DOI: *10.1016/0034-4257(95)00186-7*

Rouse, J. W. Jr., Haas, R., Schell, J., Deering, D., (1974, a): eune systems in the great plains with erts. NASA Special Publication 351, p. 309.

Rouse, J. W., Haas, R. W., Schell, J. A., Deering, D. W., Harlan, J. C., (1974, b): Monitoring the vernal advancement and retrogradation (Greenwave effect) of natural vegetationa NASA/GSFCT Type III Final Report, Greenbelt, MD, USA

Santos, P., Negri, A., (1996): A Comparison of the Normalized Difference Vegetation Index and Rainfall for the Amazon and Northeastern Brazil. Journal of Applied Meteorology, vol. 36., p. 958-966.

Sripada, R. P., Heiniger, R. W., White, J. G.,, Meijer, A. D., (2006). Aerial color infrared photography for determining early in-season nitrogen requirements in corn. Agronomy Journal, 98(4), 968–977. DOI: *10.2134/agronj2005.0200*

Tucker, C. J., (1979): Red and Photographic Infrared Linear Combinations for Monitoring Vegetation. Remote Sensing of Environment 8 (2), p. 127–50. DOI: *10.1016/0034-4257(79)90013-0*

Wang, X., Wang, M., Wang, S., Wu, Y., (2015): Extraction of vegetation information from visible unmanned aerial vehicle images. Nongye Gongcheng Xuebao/Transactions of the Chinese Society of Agricultural Engineering, vol. 31, no. 5, p. 152–159.

Wenlong, X. D. L., (2009): Vegetation index controlling the influence of soil reflection. *http://www.paper.edu.cn/releasepaper/content/200906-376*

Wu, W., (2014): The Generalized Difference Vegetation Index (GDVI) for Dryland Characterization. Remote Sensing 6 (2), p. 1211–33. DOI: *10.3390/rs6021211*

Xue, J., Su, B., (2017): Significant Remote Sensing Vegetation Indices: A Review of Developments and Applications. Journal of Sensors, Volume 2017, p. 17.

Yengoh, G.T., Dent, D., Olsson, L., Tengberg, A.E., Tucker, C.J., (2014): The use of the Normalized Difference Vegetation Index (NDVI) to assess land degradation at multiple scales: a review of the current status, future trends, and practical considerations. Final Report, Lund University Center for Sustainability Studies (LUCSUS), and The Scientific and Technical Advisory Panel of the Global Environment Facility (STAP/GEF)