Animal Health

Original

Molecular Modelling of Five Flavonoids as Antagonists of the Aryl Hydrocarbon Receptor. Potentialities for Health and Animal Production

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ABSTRACT

Background: The Aryl hydrocarbon receptor (AHR) plays a significant role in the development of the mammary gland, as it is related to the transforming growth factor β1 (TGF-β1), which regulates several cellular processes. Hence, its overexpression may lead to pathological processes in the animals, and affect their health and production. **Materials and methods:** The 3-methyl luteolin, kaempferol, resveratrol, myricetin, and quercetin flavonoid molecules were studied. Modelling relied on the AHR:ARNT structures obtained from Swiss Model software for coupling, program MOE 2019.01, and to determine the protein-protein interactions (PPI). The Cocomaps (bioComplexes Contact MAPS) servers, and Robetta and Rosetta Backruband were used for determining the mutations of alanine. **Results:** The flavonoids studied associated with contact interfaces at the bHLH, PAS-A domain level and the bHLH/PAS-A and PAS-A/PAS-B of AHR interfaces, and they can undergo an antagonistic behavior due to the interactions at the

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contact surface level to block or modulate the protein-protein interactions between AHR and ARNT. **Conclusions**: The five flavonoids can interact at different AHR superficial interfaces to modulate the formation of the functional heterodimer, acting as antagonist agents. The order of occurrence probability of these actions is higher with 3-methyl luteolin, kaempferol, resveratrol, and lower with myricetin, and quercetin. Feed supplementation using foliage rich in these flavonoids might improve animal health and production.
Keywords: Animal nutrition, Aryl hydrocarbon receptor, flavonoids, breeding, transcription factors (*Source*: *DeCS*)

INTRODUCTION

The one-health concept was introduced in the early 2000 to name a notion known for over a century: human health and animal health are interdependent, and are linked to the ecosystems where they exist. One health is associated with food safety, sustainable agriculture, food security, antimicrobial resistance, nutrition, animal and plant health, fishing, and living means, among others (Collado, Álvarez, and Martínez, 2022).

Accordingly, the AHR interrelation in animal health and production is given by the relevance of this sector for the restoration of the damage caused by xenobiotics. It also includes the damage caused by pathogens or inappropriate diets of post-weaned pigs due to an increase in interleukin-22. It also aims to favor the reestablishment and stability of the intestinal microbiota using probiotics to promote positive responses in the production and health parameters (Collado, Rodríguez, and Barreto, 2022).

Animals and humans are exposed to multiple chemicals in the air, water, and feeds, on a daily basis. They have developed a set of enzymes and inducible transporters resulting from the activation of xenobiotic receptors, such as AHR. They act as transcription factors for the regulation of target genes that enable the biotransformation and removal of these compounds (Larigot *et al*., 2018). The aryl hydrocarbon receptor (AHR) is an ancient and highly-conserved protein that has evolved for over 600 million years. It belongs to the superfamily of transcription factors basic-helix-loop-helix-Per-ARNT-Sim (bHLH-PAS), and like the nuclear AHR translocator (ARNT), it has a basic-helix-loop-helix-Per-ARNT-Sim (bHLH-PAS). It plays a key role in the development of cellular homeostasis, the circadian rhythm, the capacity to promote or inhibit cell proliferation, and it contributes to adhesion processes that involve cell-cell and cell-extracellular matrix interactions. It has also been involved in regulating processes that affect the immune system, liver homeostasis, heart development, healing, apoptosis, tumor promotion, and metabolic diseases (Schulte *et al.*, 2017; Wright *et al*., 2017; Larigot *et al*., 2018; Han *et al*., 2021).

AHR expression is essentially ubiquitous in mammals, consisting in a broad range homeostatic role. However, the expression levels vary widely among tissues like the liver, thyme, lung, kidney, spleen, and placenta, with the highest expression. Besides, the expression of AHR is regulated by development; the most recent evidence shows a function for AHR in the

development process affecting hematopoiesis, the biology of the immune system, neural differentiation, and the architecture of the liver (Wright *et al*., 2017). The physiological and toxicological consequences of AHR ligands are mediated by the AHR signaling path. AHR without ligand is found in the cytosol, and it is associated with chaperone proteins (that is, Hsp90, p23, and protein 1, which interacts with the AHR). Upon the ligand binding, AHR moves to the nucleus, where the ligated AHR forms a heterodimeric complex with the nuclear AHR translocator (ARNT). The AHR-ARNT heterodimer then binds the akin dioxin-reactive elements (DRE) and induces the expression of a target gene variety that mediates a lot of physiological consequences, from xenobiotic metabolism and cytotoxicity to the immune function and the normal vascular function. The activation of AHR has a general anti-inflammatory and immunoregulator role in innate and adaptative immunity, both in stationary state and in inflammatory settings, such as self-immunity or infection (Xing *et al.*, 2012; De Juan and Segura, 2021; Collado, Álvarez and Martínez, 2022; Collado, Rodríguez and Barreto, 2022).

The interrelation between the transforming growth factor signaling β1(TGF-β1) with the AHR has been known for several years. Likewise, there are mutual regulatory mechanisms between AHR and the TGF-β1. AHR plays an important role in the development of the mammary gland, and its overexpression has been observed to induce malign transformation of the epithelial breast. TGF-β1 is expressed in the epithelial cells along the phases of mammary development, and it regulates cellular proliferation, differentiation, migration, invasion, and apoptosis processes. AHR exposure to agonist substances (Collado, Álvarez and Martínez, 2022) increases the migration, and activates the AHR membrane path, and other canonic and non-canonic TGF-β1 paths, which might be involved in the action mechanism of the toxic, in case of exposure to these agents. The environmental concentrations of these substances modulate the AHR and TGF-β1 signaling pathways. This might be the cause of alterations observed in the normal mammary morphogenesis, and to exacerbate a promigratory prototype in the normal epithelial cells and the neoplasia cells, creating greater malignity.

The polyphenols have aromatic rings containing one or more hydroxyl group. In this classification, there are more than 8000 compounds which are subdivided into several groups, including the phenolic acids, flavonoids, stilbenes, and lignans (Maury *et al*., 2020).

Flavonoids are AHR activity modulators (Desmet *et al*., 2021) that protect against a variety of AHR dependent effects (cancer, colitis), and due to their structural characteristics, they interact and may bind to several amino acids present in the protein structure of the AHR. Consequently, molecular coupling was performed to determine the binding of five flavonoids to AHR, to block the formation and cellular activity of the functional and modular heterodimer at the AHR and ARNT protein interaction level.

MATERIALS AND METHODS

The following flavonoids were selected for molecular modelling: 3-methyil luteolin, kaempferol, resveratrol, myricetin, and quercetin.

Molecular docking: the structure of the above flavonoids was used for modelling, an AHR receptor model designed by SwissModel Web Server, and a model of its heterodimer complex with ARNT, using the crystalline structure of ARNT (PDB: 4zp4), and the AHR sequence as a model. The molecular modelling, complex presentation, and interaction visualizations were performed through MOE 2019.01. The server program Cocomaps (bioCOmplexes Contact MAPS) was used for the determination of atomic contacts (≤ 6 Å), between the protein AHR:ARNT interfaces. (Vangone *et al*., 2011). The important amino acid residues for the protein-protein interaction (PPI), and the flexibility of alanine mutations were determined with the Robetta and Rosetta Backrub web servers, according to their respective protocols. The residues were considered significant when the ΔΔGbinding expected was greater or equal to 1.0 kcal mol^{-1} (Kortemme and Baker, 2002; Kortemme, Kim, and Baker, 2004).

RESULTS AND DISCUSSION

When the AHR:ARNT heterodimer formation is activated by a ligand (agonist), it takes place in AHR PAS-B domain. Figure 1 shows the interaction model of the structural architecture (3D) generated by SwissModel for the AHR domains, and its binding to the ARNT pleiotropic partner. The PPI interferences established by each monomer (AHR and ARNT), are relevant to modulate the formation of the functional heterodimer.

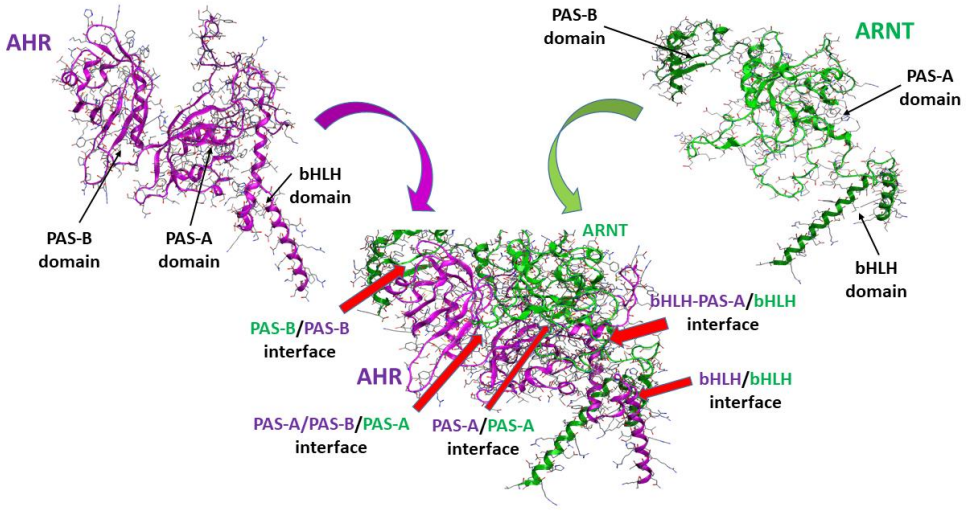


Figure 1. Interaction architecture at the structural level domains associated with the AHR and ARNT dimerization in the formation of the functional heterodimer AHR:ARNT. Magenta: AHR. Green: ARNT.

The five flavonoids studied by molecular modelling at the AHR surface bind the contact interfaces in the bHLH, PAS-A domains, and the bHLH/PAS-A and PAS-A/PAS-B interfaces (Figure 2). Consequently, these compounds may have some antagonistic behavior by interactions on the contact surfaces, to block PPI between AHR and ARNT.

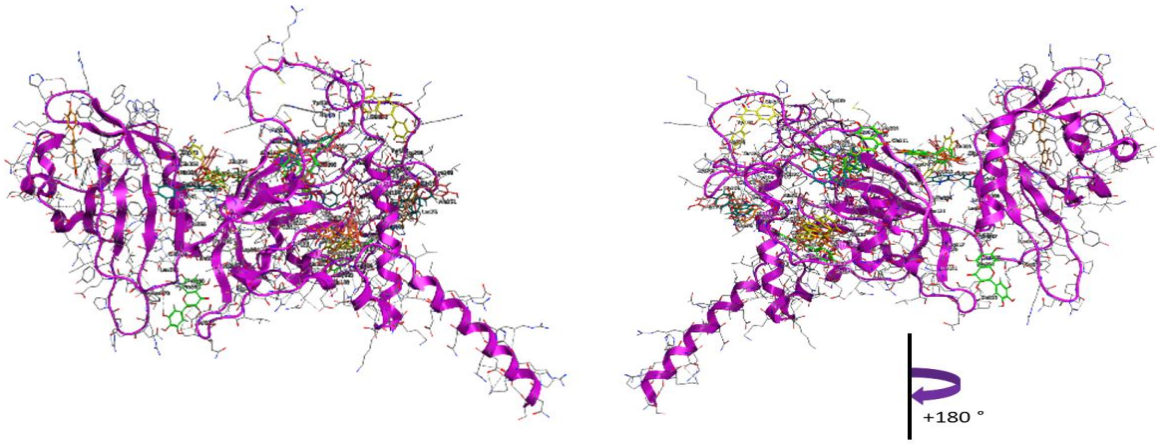


Figure 2. Graphic representation of the binding of five flavonoids in AHR (AHR SwissModel). Magenta.

The five flavonoids have different capacities of modulating the interactions at the AHR with the ARNT to form an AHR:ARNT functional heterodimer. The interference order is as follows 3-methyil luteolin, kaempferol, resveratrol, myricetin, and quercetin. It coincides with reports of the first four compounds as antagonists of AHR activity, and quercetin as agonist/antagonist (Figure 3) (Zhang, Qin and Safe, 2003).

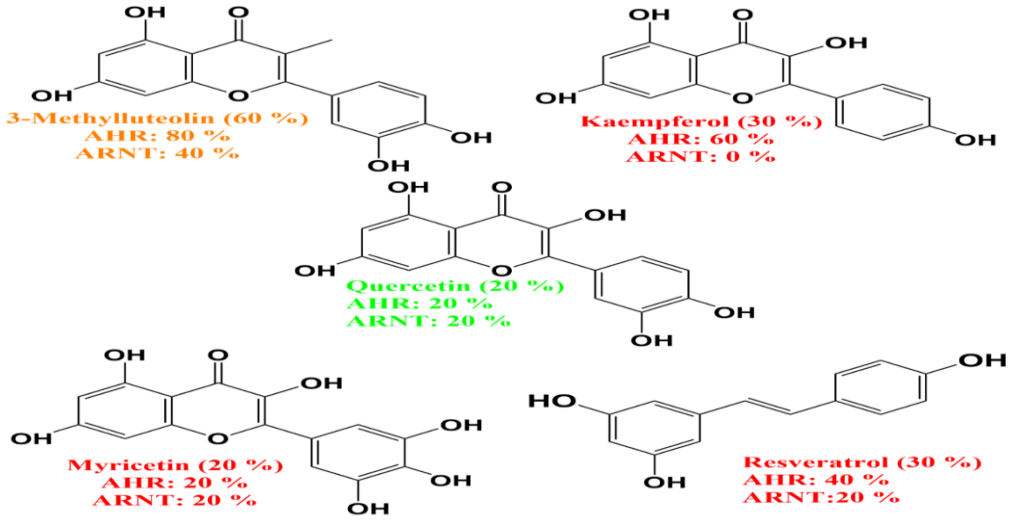
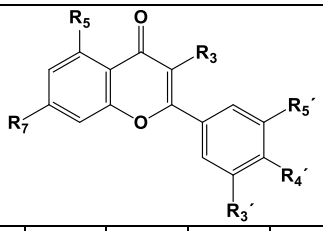


Figure 3. Structures of the five AHR-activity modulating flavonoids interference percentages in the formation of the AHR: ARNT functional heterodimer, and at the AHR and ARNT monomer level.

The structural analysis of the four flavonols is shown in Table 1, with the variations of these chemical structures in their binding/interaction mode with the AHR and ARNT monomers. The flavonol with the highest antagonistic activity to block the AHR:ARNT heterodimer complex was 3-methyl luteolin, which was the reference in relation to the other three flavonols.

Table 1. Structural analysis of antagonist flavonols at the PPI level to form the AHR:ARNT heterodimer.



Flavonols	R_3	R_5	R_7	$R_{3'}$	$R_{4'}$	$R_{5'}$	AHR binding	ARNT binding
3-Methylluteolin	CH_3	OH	OH	OH	OH	H	60 %	40 %
Kaempferol	OH	OH	OH	H	OH	H	60 %	0 % ↓
Quercetin	OH	OH	OH	OH	OH	H	20 % ↓	20 %
Myricetin	OH	OH	OH	OH	OH	OH	20 %	20 %

The analysis of the structure-activity ratio (SAR) of these antagonist compounds is important to characterize the binding to the receptor. Hence, to establish AHR surface interactions, the flavonols must have a hydroxyl group (OH) in the R_5, R_7, and $R_{3'}$ positions for the flavonoid ring. The substitution of a methyl group (CH_3) by a hydroxyl group (OH) in the R_3 position of the flavonoid ring, decreases AHR-related activity, so in general terms, quercetin is less active than 3-methyl luteolin. The substitution of a hydroxyl group (OH) by a hydrogen atom (H) in the $R_{3'}$ position of the flavonoid ring decreases ARNT-related activity, so a pure anti-AHR antagonist flavonoid can be obtained, making kaempferol less active than quercetin. On the contrary, the inclusion of a hydroxyl group (OH) in the $R_{5'}$ position of the flavonoid ring will not affect monomer binding. Similarly, to establish AHR surface interactions, the flavonols must have a hydroxyl group (OH) in the R_5, R_7, $R_{3'}$, and $R_{4'}$ positions of the flavonoid ring.

Quercetin shows possible interaction at the AHR PAS-A/PAS-B interface, so the logical response is to wait until a dual activity, such as agonist/antagonist is reported as the preferential binding at the PAS-B ligand binding domain (LBD) (Figure 4). The other four flavonoids have been described as AHR antagonists. As shown in figure 4, they can have specific binding to the AHR bHLH/bHLH, bHLH-PAS-A, PAS-A/PAS-A and PAS-A/PAS-B interfaces, and interfere with the AHR:ARNT heterodimer formation through this protein domain, which is linked to dimerization.

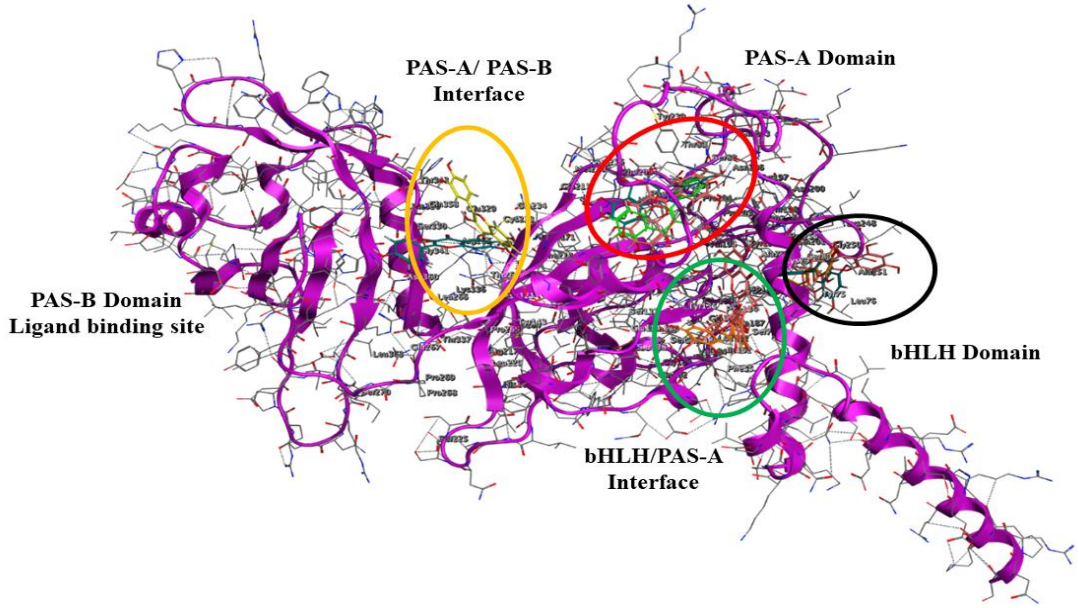


Figure 4. Active flavonoids that interfere with the AHR and ARNT PPI at the AHR bHLH domain. bHLH/bHLH interfaces in a black circle; bHLH-PAS-A, in a green circle; PAS-A/PAS-A, in the red circle; and PAS-A/PAS-B, in the yellow circle.

At the bHLH interface level in the AHR bHLH domain (figure 5), the flavonoids bind through hydrophobic interactions and hydrogen binding, so they can interfere with heterodimer formation, due to steric impediments for the ARNT partner binding.

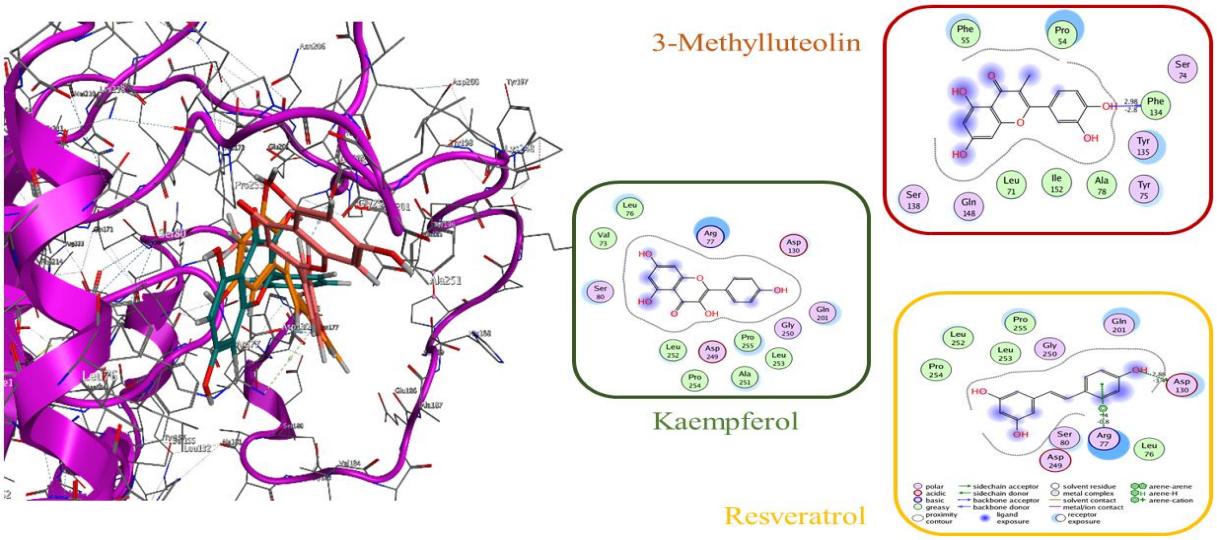


Figure 5. Active flavonoids that interfere with the AHR and ARNT PPI at the AHR bHLH/bHLH domain. Methyl luteolin (pink), kaempferol (olive green), resveratrol (orange). AHR (magenta).

In the bHLH/PAS-A internal interface composed of AHR bHLH and PAS-A domains, only two flavonoids bind through hydrogen methyl luteolin and resveratrol bonds (Figure 6).

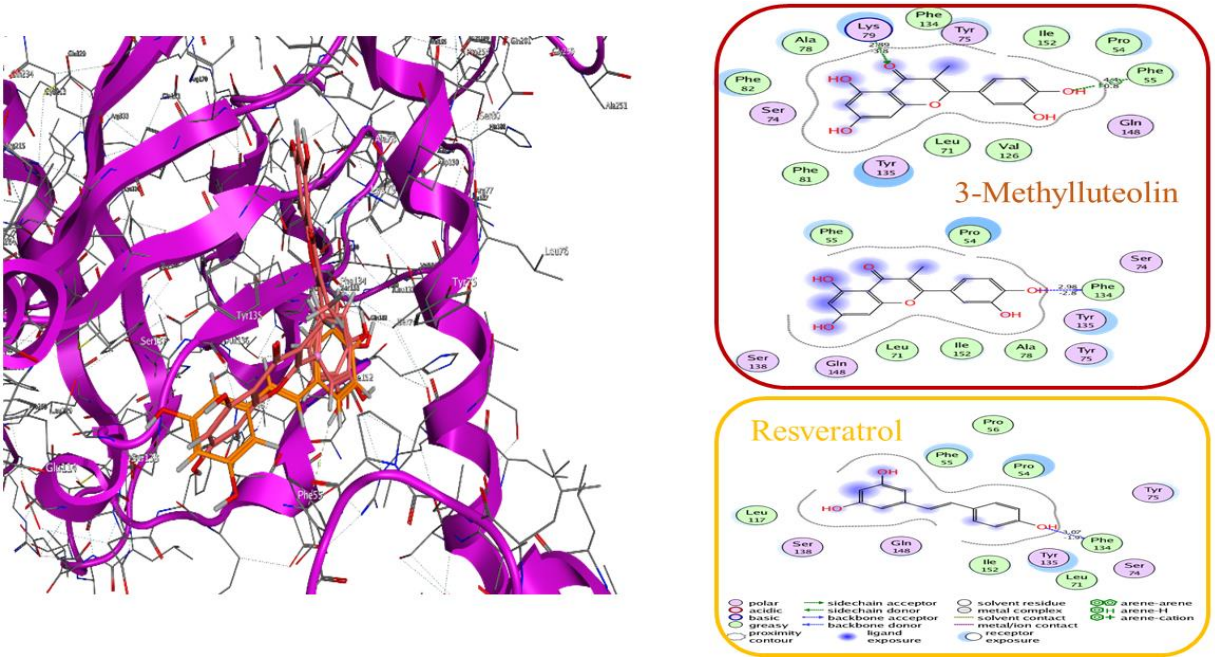


Figure 6. Active flavonoids that interfere with the AHR and ARNT PPI at the AHR bHLH/PAS-A interface. Methyl luteolin (pink), resveratrol (orange), AHR (magenta).

Resveratrol (3,4′,5-trihydroxy-trans-stilbene) is a natural polyphenol commonly found in the peels of grapes, with antioxidant activity, and free-radical catcher properties. Many AHR-CYP1A1-independent action mechanisms have been reported (non-AHR selective antagonist). Although it inhibits the adaptative path of AHR, it also permits its nuclear location and binding to alternative xenobiotic response elements. The activation of this alternative AHR path increases another set of genes associated with anti-inflammatory and antioxidant properties. Moreover, resveratrol has also been described as a weak CYP1A1 inhibitor, and protector of the toxic effects of environmental pollutants that can activate AHR in the lungs, thyme, testicles, prostate, and pancreas (Coelho *et al.*, 2022). Furthermore, may affect PPI flavonoids, 3-methyl luteolin, kaempferol, and myricetin at the structural PAS-A domain level (Figure 7).

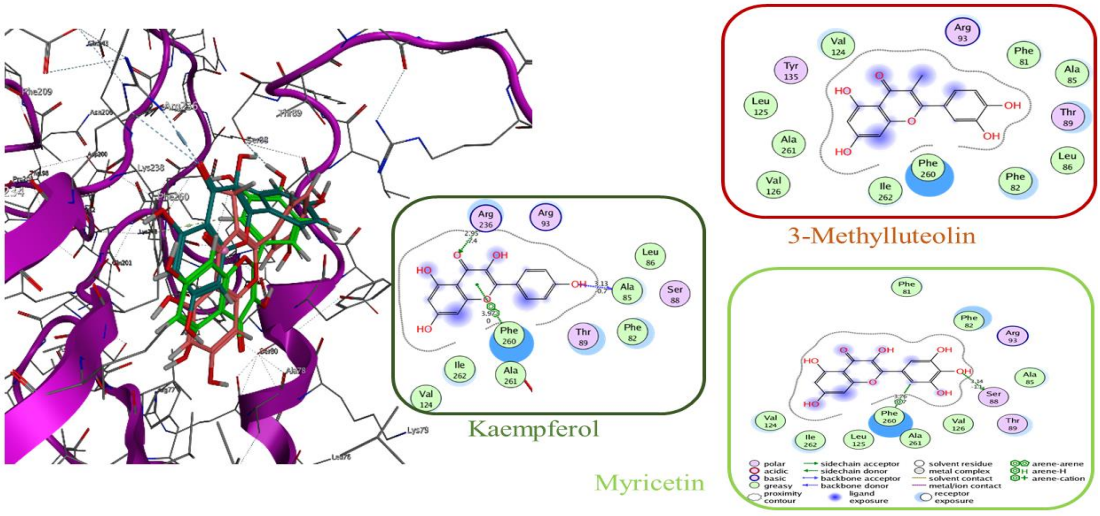


Figure 7. Active flavonoids that interfere with the AHR and ARNT PPI at the AHR PAS-A interfaces. Methyl luteolin (pink), kaempferol (olive green), myricetin (green). AHR (magenta).

The interface created between the AHR PAS-A and PAS-B domains is another flavonoid interference site to the formation of the AHR:ARNT heterodimer. At this level, quercetin and kaempferol could affect PPI (Figure 8). In the former, it is described as an agonist/antagonist modulator of AHR activity, due to the different roles according to the cell type and concentration (Ashida *et al.*, 2000; Jin *et al.*, 2018; Gasaly, Riveros and Gotteland, 2020; Desmet *et al.*, 2021; Han *et al.*, 2021). In this case, its preferential bond to the binding-ligand domain (LBD) is a contributor, whereas this paper suggests that its antagonist activity may be caused by surface PPI, just like for kaempferol and other previously shown flavonoids (Zhang, Qin and Safe, 2003).

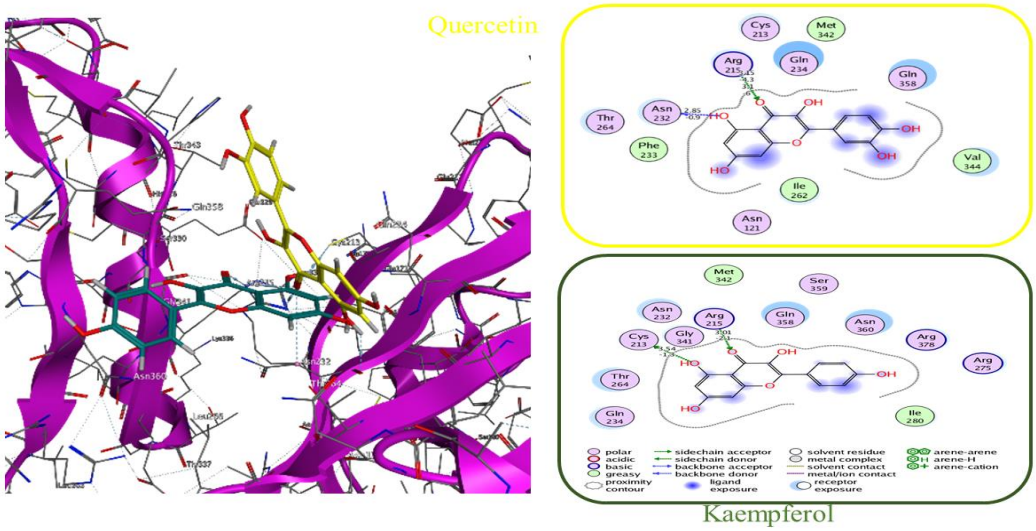


Figure 8. Active flavonoids that interfere with the AHR and ARNT PPI at the AHR PAS-A/PAS-B interface. Quercetin (yellow), kaempferol (olive green), myricetin (green). AHR (magenta).

Flavonoids are AHR ligands with proven therapeutic effects on several diseases (Park *et al*., 2019; Bungsu *et al.,* 2021). However, further studies of more accurate molecular action mechanisms through AHR signaling are required to unveil its potential roles as an immunological modulator (Bungsu *et al.,* 2021), and its participation in physiological and pathological processes. These elements are presented like limitations to reach conclusive results. Nevertheless, the evidence points to the fact that flavonoids are AHR selective modulators with agonist and antagonist activities, and different specific strengths of tissue/organ/species. The definition of "agonist" for AHR-activating ligands has been questioned, and the term "modulator" has been suggested as more appropriate, according to the previous data presented (Dolciami *et al.*, 2020), since it is hard to predict its agonist/antagonist selectivity (Bungsu *et al.,* 2021; Desmet *et al*., 2021).

Because the TGF-β1 signaling pathways are interrelated with AHR, they have common targets, such as the retinoblastoma protein and the E2F factor, which regulate cellular processes like the cell cycle, apoptosis, TEM, and Treg and TH17 cellular differentiation (Miret, 2018). When AHR is activated due to the presence of some toxic agent, at high doses (5 μM), the toxic element stimulates the AHR nuclear path, thus not only causing cell motility, but also cell cycle arrest (Miret, 2018).

The utilization of transgenic mice and powerful AHR ligands have been useful to understand if the alterations of AHR function affect the reproductive system of females. The intrinsic role of AHR in the female's reproductive system is linked to the following functions: i) regulation of ovarian function by controlling the number of antral follicles and their growth, as well as the capacity to produce steroids and reach ovulation; ii) the existence of an optimum environment for fertilization, embryo nurturing, and pregnancy maintenance; iii) regulation of fertility; and iv) control of reproductive senescence. However, it is still unknown if AHR is involved in embryo development in the uterus and oviducts. AHR involvement is also unknown in the regulation of reproductive senescence through the AHR path, or if it modifies genes related to longevity; if an AHR signaling pathway activation/removal is necessary to regulate the functions of the reproductive system of females; and the endogenous ligands that activate the AHR signaling pathway in the same reproductive system, then further studies are required to elucidate the complex mechanisms through which AHR can regulate reproduction in females. It will facilitate the establishment of prevention/treatment measures of reproductive disorders, such as infertility, miscarriages, and the reduction or absence of reproductive life (Hernandez-Ochoa *et al.*, 2009).

There is experimental evidence that AHR is present in all the ovarian cells obtained in the two phases of swine estrus. And it has been overexpressed in granulose cells of pre-ovulating follicles, compared to all the other remaining cell types (Hernandez-Ochoa *et al.*, 2009).

AHR and ARNT have been observed to be present in granulose cells, and the internal theca of mid-sized, pre-ovulating follicles, as well as in luteal cells from sows, suggesting that AHR also plays a role in the regulation of ovarian steroidogenesis, follicle development, and the formation

and maintenance of corpus luteum in the female pigs (Jablonska and Ciereszko, 2013). In another study, Pocar *et al.* (2020) reported that AHR activity inhibition was associated to the reduction of the oocyte capacity to advance in the restart of meiosis. On the contrary, the exposure to the AHR antagonist resveratrol, reduced both CYP1A1 expression and the oocyte maturation capacity, without affecting ERK1/2 signaling.

Furthermore, the relation between AHR and hormonal disorders was studied recently with the purpose of elucidating how the relation between endoplasmic reticulum stress in the ovarian granulose cells and AHR might cause the polycystic ovarian syndrome (POS). In a PCOS murine model induced by dehydroepiandrosterone (DHEA), no cyclicity or changes in the morphology of the ovary were observed during the estrus cycle. The subcutaneous administration of 10 mg/kg of CH-223191 (AHR antagonist) restored the loss of ovarian cyclicity and morphology, with a drop in the number of atretic antral follicles, which took place at the same time of a down regulation on the AHR-CYP1B1 axis of the granulose cells (Coelho *et al.*, 2022).

The TGF-β family causes a wide impact on the reproductive systems of several organisms. In humans, it is associated with normal women's reproductive function during pregnancy, and in several gynecological pathologies. In adult mammals, the TGF-β-related proteins rule somatic germinal cell growth and differentiation in the gonads. Additionally, the TGF-β ligands are closely involved in the control of ovulation and fertilization, and the establishment and maintenance of pregnancy in women. Several growth factors related to TGF-β also function as endocrine hormones to integrate the gonad's reproductive status into the body's physiological state. Translational studies of human tissue, cells, and studies of the whole genome also involve TGF-β abnormal signaling in cases of reproductive diseases in females, such as endometriosis, uterine fibromas, and preeclampsia in women (Monsivais, Matzuk and Pangas, 2017; Larigot *et al*., 2018).

Possible AHR implications in the health of females during animal reproduction and herd performance

In recent years, a multiple number of relevant AHR functions have been unveiled, beyond its original role as a xenobiotic sensor, and regulator of xenobiotic detoxication. In fact, AHR has been confirmed as an important signaling molecule that regulates and maintains homeostasis in different cells, tissues, and organs. New data refer to the activation of the AHR-CYP1A1 axis in the mechanisms of the disease, thus explaining the putative value of its therapeutical blockage. In that sense, further research and characterization of the pharmacological properties of the AHR-CYP1A1 axis blockers is needed, and therefore, the AHR-CYP1A1 blockers, which might be useful to treat chronic diseases (Coelho *et al.*, 2022).

The exposure to pollutants may have a negative effect on the reproductive function of animals, and probably, feeds are the most relevant point of entry for many or most species, especially those situated high in the food chain. However, risk assessment is difficult due to the fact that in the "real world" feeds contain many types of different contaminants at variable concentrations. Hence, their exposure is produced along their lifespan, even during the growth stages, when

animals are particularly sensitive to external agents. In short, feed contaminants constitute a risk factor for reproduction, but accurate quantification of these risks to people becomes extremely difficult to determine under the current knowledge (Rhind, 2008; Collado, Álvarez and Martínez, 2022).

AHR plays a significant role in the physiopathology of reproduction through the AHR/ARNT signaling pathway (Zao *et al.*, 2020). When the AHR is overexpressed, it can affect the signaling pathways directly or indirectly and the proteins associated with the physiology of reproduction. In veterinary practice, reproductive problems are common, and cause enormous economic losses; for instance, metritis lowers milk production, reproduction, and the survival of the female (Pérez-Báez *et al.*, 2021), like mastitis (Puerto *et al.*, 2021) in dairy cattle. According to Dallago *et al.* (2021), the reduction in involuntary wastes could cut down healthcare costs, increasing cow profitability throughout their reproductive lives, as well as the life quality of animals. It would contribute to a more sustainable dairy industry, while the resources of dairy farmers could be used more efficiently.

Feasibility of the treatment with supplements based on flavonoid-like functional active metabolites

Polyphenols are natural plant compounds, and the most abundant antioxidants in human diet (Ray and Mukherjee, 2021). Generally, antioxidants are defined as substances capable of preventing the oxidation of a substrate (proteins, lipids, carbohydrates, and DNA) at low concentrations. they are found in leafy vegetables, fruits, and grains consumed habitually as natural diets, since they are safe and offer a wide range of benefits to health (Yordi et al., 2012; Maury *et al.*, 2020). Because the gastrointestinal tract is the main organ that degrades the different components present in the feeds, the diet could be thought of as one of the essential factors in the functionality, integrity, and composition of the intestinal microbiota. Quite a few polyphenols remain unabsorbed, and may accumulate in the large intestine, where the intestinal microbiota metabolizes to a larger extent. By assuming primary roles to promote the host's wellbeing, this intestinal health setting is exposed to the effects of outer influences, including diet patterns (Ray and Mukherjee, 2021).

Around 400 B.C., Hippocrates said "death rests in the intestines", and that "bad digestion is the root of all evils", which suggests the essential role of the human intestines in health and disease. Hence, phenols and their metabolites have a positive influence on the intestinal health by promoting the production of beneficial microbiota and controlling the propagation of pathogenic bacteria (Ray and Mukherjee, 2021). The evidence compiled indicates that nutrition can modulate the immune system through metabolites, such as tryptophan derivatives, either produced by the host's digestion or the metabolism of the intestinal microbiota (IM) (Wheeler, Rothhammer and Quintana, 2017; Zhang, *et al*., 2020; De Juan and Segura, 2021; Modoux *et al*., 2021; Collado, Rodríguez and Barreto, 2022).

Flavonoids are found in glycosylated forms of plants. Although some of them could be de-glycosylated by the intestinal lactase, most are by the bacteria present in the colon. The diholosides and oligosides released in this process are quickly fermented, generating volatile fatty acids (VFA), whereas the aglycone fraction is also metabolized by the IM, resulting in the formation of several metabolites (Gasaly, Riveros and Gotteland, 2020).

Flavonoids are one of the main AHR ligand classes derived from the diet. They are a large polyphenolic secondary metabolite class widely spread in fruit and leafy vegetables. Quercetin, taxiphyllin, and robinetin act as agonists and may activate AHR, whereas luteolin acts an AHR antagonist (Ashida *et al.*, 2000; Zhang, Qin and Safe, 2003; Jin *et al*., 2018; Gasaly, Riveros and Gotteland, 2020; Han *et al*., 2021). Consequently, the results of molecular modelling coincide with the reports. Particularly, quercetin functions as a partial antagonist, and the luteolin derivative (3-methyl luteolin) functions as an antagonist (Zhang, Qin and Safe, 2003), through an action mechanism at the different superficial interfaces herein mentioned for every compound and monomer.

Moreover, the presence of flavonoids in cattle milk could be a source of natural antioxidants to consumers, which is a beneficial effect for the prevention of some pathological processes mediated by free radicals. The inclusion of forages with antioxidant substances like flavonoids, has been suggested as a supplementation in the diet of production cows. Apart from being bioavailable in the species, they are also excreted actively in the milk, and in concentrations equivalent to the plasmatic ones, thus providing antioxidant protection against possible milk souring (Cruz Carrillo and Lizarazo, 2016).

The supplementation based on active functional metabolites was evidenced through the results published when comparing the polyphenol contents in the milk of goats, which received *Sulla coronarium* L or barley grass hay. The animals showed a similar behavior as for the free polyphenols in the milk, but in the first group, the total polyphenol contents were higher, with an additional value in terms of oxidative status (Zapata and Mellado, 2021). It demonstrates the practical usefulness of supplementation with polyphenol-rich foliage for the production of high-quality milk.

CONCLUSIONS

The five flavonoids can interact at different AHR superficial interfaces to block or modulate the formation and the cellular activity of the functional heterodimer AHR:ARNT, according to the results of the molecular docking study. The order of occurrence probability of these actions was higher with 3-methyl luteolin, kaempferol, resveratrol, while it was lower with myricetin and quercetin. The Structure-Activity Ratio (SAR) for these compounds to be antagonists at the AHR surface interactions, requires flavanols with a hydroxyl group (OH) in the R_5, R_7, and $R_{3'}$ positions for the flavonoid ring. In ARNT, the flavanols must present a hydroxyl group (OH) in the same previous positions, but adding the $R_{4'}$ position of the flavonoid ring. Feed

supplementation using foliage rich in these flavonoids might improve animal health and production, according to the results of the molecular modeling.

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AUTHOR CONTRIBUTION STATEMENT

Author contribution was as follows: Research conception and design: OGCG, SJMS, JABV, HDW, EM, PC, redaction of the manuscript: OGCG, SJMS, JABV, HDW, EM, PC.

CONFLICT OF INTEREST STATEMENT

The authors declare the existence of no conflicts of interests.